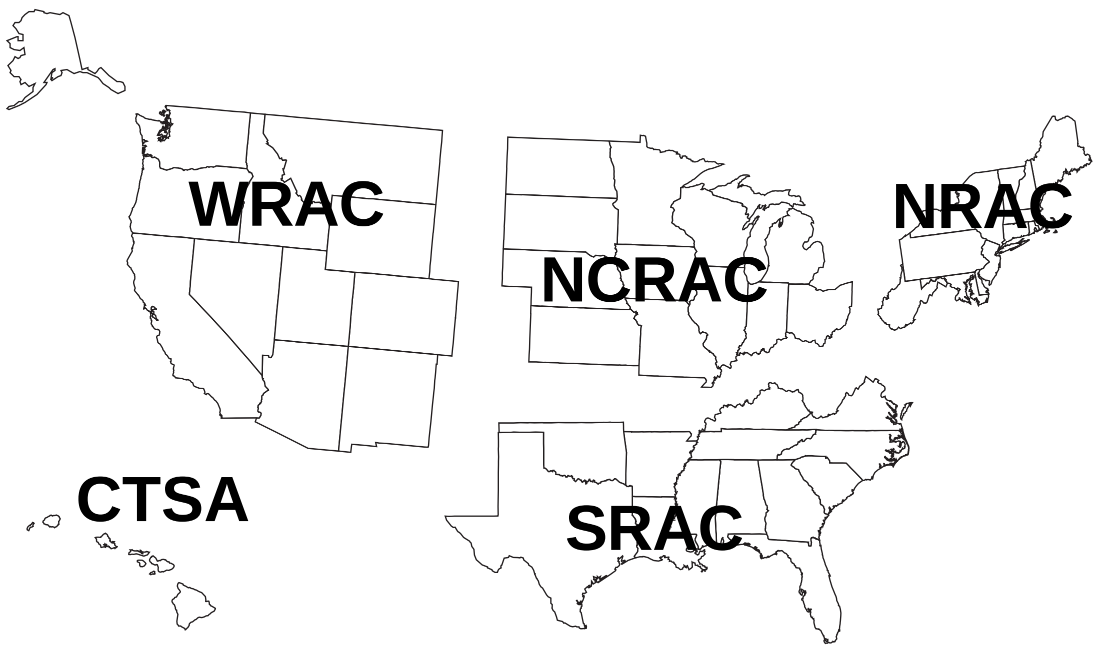
Compendium Report: 1989-1996

January 1998



Regional Aquaculture Centers

Funding has been supplied by
Cooperative State Research,
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(CSREES)

Compendium Report

Regional Aquaculture Centers

for the period
May 1, 1989 to August 31, 1996

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Compendium Report

Regional Aquaculture Centers

Center for Tropical and Subtropical Aquaculture
Compenium Report *for the Period May 1, 1989 to August 31, 1996*

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Executive Summary

Mission

The Center for Tropical and Subtropical Aquaculture, or CTSA, is one of five Regional Aquaculture Centers funded by the U.S. Department of Agriculture. The mission of CTSA is to support aquaculture research, development, demonstration, and extension education to enhance viable and profitable U.S. aquaculture. Research projects span the American Insular Pacific, using its extensive resource base to meet the needs and concerns of the tropical aquaculture industry.

The Center for Tropical and Subtropical Aquaculture is jointly administered by the University of Hawaii and The Oceanic Institute. The Center's offices and staff are located at The Oceanic Institute's Makapu‘u Point site on windward Oahu.

Organization

CTSA funds aquaculture research, development and demonstration projects. Each year's program is the result of several groups working together for many months. A Board of Directors oversees the Center's programmatic functions, and an Executive Committee is responsible for the Center's administrative policy and functions.

In addition, CTSA has two working groups. The Industry Advisory Council (IAC) comprises members from financial institutions, aquacultural and agricultural enterprises, government agencies, and other business concerns. The Technical Committee (TC) is made up of researchers, extension agents, and fisheries officers. The Board, the IAC, and the TC draw their members from American Samoa, the Commonwealth of the Northern Mariana Islands, the Federated States of Micronesia, Guam, Hawaii, the Republic of Belau, and the Republic of the Marshall Islands.

Program scope

The Center has funded 99 projects in its 9 years of operation. These projects fall into six categories:

- National Aquaculture Priorities;
- Information Dissemination;
- Extension Support to Further Industry Development;
- Marketing and Economics;
- Development of New Technologies; and
- Demonstration and Adaptation of Known Technologies.

A brief listing of the principal accomplishments of the active projects in these categories during 1996 is presented below. Details on each project's funding, participants, objectives, anticipated benefits, progress, and future plans are presented in individual sections on each project.

National aquaculture priorities

Effluent Discharge Program

Accomplishments

Under the sixth year of this program, investigators summarized results from the first 5 years of work. The summary pointed out that government regulation is a critical limitation to the success of the domestic aquaculture industry, that aquaculture effluent does not always negatively affect the environment, and that a comprehensive revision of aquaculture effluent discharge regulations offers an opportunity to develop new methods of resolving environmental issues. In addition, investigators assisted in planning a video titled “The Promise of Aquaculture,” which states the case for revising regulations regarding aquaculture effluent discharge.

National Coordinator for Aquaculture New Animal Drug Applications

Accomplishments

As a direct result of the project, the U.S. Food and Drug Administration (FDA) announced on February 12, 1996, that it will “defer regulatory enforcement against the unapproved sales and use of an approved Human Chorionic Gonadotrophin (HCG) product as a spawning aid in fish by or on the order of a licensed veterinarian.” This provides aquaculture farmers with a means of legally obtaining and using HCG until it is approved by the FDA.

Information dissemination

Library aquaculture workstation

Accomplishments

This project, known as the Pacific Regional Aquaculture Information Service for Education or PRAISE, established remote workstations equipped with modems. From these workstations, users can connect to the information service at the University of Hawaii to perform CD-ROM database searches 24 hours a day. Six remote sites were established in Hawaii and two were established in Guam. PRAISE entered a cooperative agreement with Pacific Education and Communications Experiment by Satellite (PEACESAT), a federally funded communications satellite, whereby residents at five Pacific Island sites can directly access the Aquatic Sciences and Fisheries Abstracts (ASFA) database through an Internet connection between the local PEACESAT station and the mainland vendor. In addition, PRAISE established a home page on the WorldWide Web. Search requests can be sent to PRAISE personnel via the web page.

Production of CTSA educational extension materials

Accomplishments

This project produced a video titled "The Promise of Aquaculture" for the Aquaculture Effluent Discharge Program. In addition, it published a practical manual titled "Starting a Successful Aquaculture Sponge Farm," and four extension fact sheets:

- "Shrimp Diseases;"
- "Chinese Catfish;"
- "Tilapia;" and
- "*Gracilaria* Gall Syndrome."

Publications

Accomplishments

This project produced a quarterly newsletter, biannual technical bulletins on each of the Center's active funded projects, and a video update on selected projects. In addition, the project published a practical manual and companion video titled "Clams to Cash: How to Make and Sell Giant Clam Shell Products."

Extension support to further industry development aquaculture extension and training support in the U.S.-affiliated Pacific Islands

Accomplishments

This project provides extension and training support to aquaculturists and to government fisheries and aquaculture staff throughout the region. This support included conducting aquaculture training courses at various locations, providing scientific advice to the FSM National Aquaculture Center and other private and public concerns, and assisting with reef surveys and reseeding programs for giant clams, sponges, and other species as requested by local authorities.

Gill discoloration in Penaeus stylirostris

Accomplishments

This project examined the primary and secondary causes of gill discoloration in market-sized *P. stylirostris* and developed recommendations to prevent the condition on aquaculture farms in Guam.

Disease management for Hawaiian aquaculture

Accomplishments

This project is identifying factors that may contribute to the occurrence of bacterial disease during growout of Chinese catfish (*Clarias fuscus*) and developing strategies to control those diseases. In addition, methods of decontaminating shrimp ponds infected with the IHHN virus are being tested, groups of imported freshwater tropical fish are being surveyed to document mortality patterns, portray environmental conditions and determine the presence and prevalence of certain parasites and bacterial pathogens, and the effects of ectoparasites on cultured tilapia and mullet are being assessed.

Development of new technologies

*Development of threadfin (*Polydactylus sexfilis*) fry production technology*

Accomplishments

This project is developing methods to produce threadfin fry for commercial production. This year's efforts focused on overcoming the high mortality rate of threadfin larvae during the first 15 days after hatching and developing a rearing technology with less than 1 percent mortality per day during the cannibalistic period (days 25 to 50).

Demonstration and adaptation of known technologies differential growth rate studies in cultured commercial sponges

Accomplishments

This project is conducting growth comparison studies to improve the production efficiency of commercial sponge culture. Studies are examining whether sponges with high growth rates retain those growth rates when divided into cuttings and whether cutting sponges with low growth rates stimulates their growth rates.

Improvement of Tilapia stocks in Hawaii

Accomplishments

This project is reviewing the international, national, and Hawaii state technical and regulatory status of tilapia strains in relation to improvement of the tilapia culture industry in Hawaii. Morphometrics and genetics are being used to assess the status of both farmed and wild tilapia stocks in Hawaii. Growth characteristics of farmed tilapia will be determined and compared with the data in relevant scientific literature. Finally, measures will be recommended either to improve existing stocks or to import new strains or species based on review of the information collected.

Ornamental aquaculture technology transfer

Accomplishments

This project is evaluating the culture potential and production economics of a number of ornamental fish species in Hawaii. In 1995, the project established ornamental fish in commercial production at a number of cooperating commercial aquaculture facilities in Hawaii, began reproducing Amazon Basin fish using reverse osmosis technology and produced an analysis of the feasibility of an ornamental culture business in Hawaii.

Mangrove crab as a model for development of a quarantine system to screen species for aquaculture in Guam

Accomplishments

This project established an aquatic animal quarantine area at the University of Guam Marine Laboratory, where captured local mangrove crabs will be examined for pathogens. A list of pathogens found in the animals will be compiled, and the economic feasibility of commercial scale mangrove crab production in Guam is being evaluated.

Introduction

During 1996 the Center for Tropical and Subtropical Aquaculture completed work on projects funded under its 6th Annual Plan of Work and continued work on projects funded under its 7th and 8th Annual Plans of Work. In addition, the Center initiated work on projects developed under its 9th Annual Plan of Work and began developing its 10th Annual Plan of Work.

Eleven projects were funded under the Center's ninth year program, which was approved by the Center's Board of Directors on January 18, 1996. Three projects were new and eight were continuations of projects begun under previous years' programs.

One sign of the effectiveness of the Center's program is the willingness of other agencies to provide supplemental funding for projects. Over the life of CTSA, other agencies provided $2,170,978 in additional support to projects.

The development of the year 10 program was initiated in March 1996 at the annual meeting of the Industry Advisory Council (IAC). The IAC reviewed the progress of funded projects and recommended year 10 research priorities that would aid industry development. Members identified 12 project areas; three were in new areas, and nine were continuations of projects funded under previous years. These were:

- Disease Management for Hawaiian Aquaculture — year 5;
- Library Aquaculture Workstation — year 10;
- Aquaculture Extension and Training Support — year 9;
- Differential Growth Rate Studies in Cultured Commercial Sponges — year 5;
- Diversification of Species for Aquaculture in Guam — year 3;
- National Aquaculture Priorities:
  Component I: National Coordinator for New Animal Drug Applications
  Component I: National Extension Workshop
- Expansion and Diversification of Freshwater Tropical Fish Culture — year 2;
- Development of Pacific Threadfin and Milkfish Growout Technology and Production of Live Feeds — year 2;

- Genetics of Giant Clam Color — year 1;
- Demonstration of Tilapia and Hydroponic Co-culture in the Commonwealth of the Northern Mariana Islands — year 1;
- Survey of Aquatic Ornamental Plant Culture Technology — year 1; and
- Publications.

In April 1996, the Technical Committee (TC), acting on the IAC's recommendations, drafted problem statements for new or expanded projects. Those formed the basis for the Preliminary Plan of Work, which was approved by the Board of Directors in June. The Center staff then solicited proposals for projects; 11 proposals were submitted.

In September the Center began its 3-month review process. New proposals were first subjected to external peer review by at least three experts in the project topic area; these experts were identified with the assistance of the directors of the other Regional Aquaculture Centers and the U.S.D.A. program administrators. Proposals for both new and continuing projects then underwent review by panels comprising members of the Industry Advisory Council and the Technical Committee. The final version of the proposals will be incorporated into the 10th Annual Plan of Work, which will be sent to the Center's Board of Directors for approval. Following Board approval, the plan will be submitted to the U.S. Department of Agriculture for final approval.

Since the inception of the Center for Tropical and Subtropical Aquaculture in 1988, it has funded 99 research, demonstration, development, and extension projects. Thirteen projects were active during 1996. These projects fall into six categories:

- National aquaculture ariorities;
- Information dissemination;
- Extension support to further industry development;
- Marketing and economics;
- Development of new technologies; and
- Demonstration and adaptation of known technologies.

Projects addressing national aquaculture priorities comprise:

- Aquaculture Effluent Discharge Program and
- National Coordinator for New Animal Drug Applications.

Projects addressing information dissemination comprise:

- Production of CTSA Educational Extension Materials;
- Library Aquaculture Workstation; and
- Publications.

Projects addressing extension support to further industry development comprise:

- Aquaculture Extension and Training Support in the U.S.-Affiliated Pacific Islands;
- Gill Discoloration in *Penaeus stylirostris*; and
- Disease Management for Hawaiian Aquaculture.

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Projects addressing development of new technologies comprise:

- Development of Threadfin *(Polydactylus sexfilis)* Fry Production Technology.

Projects addressing demonstration and adaptation of known technologies comprise:

- Differential Growth Rate Studies in Cultured Commercial Sponges;
- Improvement of Tilapia Stocks in Hawaii;
- Mangrove Crab as a Model for Development of a Quarantine System to Screen Species for Aquaculture in Guam; and
- Ornamental Aquaculture Technology Transfer.

Organizational structure

Title XIV of the Agriculture and Food Act of 1980 and the Food Security Act of 1985 authorized establishment of aquacultural research, development, and demonstration centers in the United States (Subtitle L, Sec. 1475[d]) in association with colleges and universities, state departments of agriculture, federal facilities, and non-profit private research institutions.

The five Regional Aquaculture Centers encourage cooperative and collaborative aquaculture research and extension education programs that have regional or national applications. Center programs complement and strengthen existing research and extension educational programs provided by the U.S. Department of Agriculture and by other public institutions. The Centers' objectives are to:

- promote aquaculture research, development, and demonstration for the enhancement of viable and profitable commercial aquaculture production in the United States for the benefit of producers, consumers, and the American economy;

- utilize the Regional Centers in a national program of cooperative and collaborative research, extension, and development activities among public and private institutions having demonstrated capabilities in support of commercial aquaculture in the United States.

Administrative Center

The Center for Tropical and Subtropical Aquaculture is co-administered by the University of Hawaii and The Oceanic Institute. CTSA's Administrative Center is located at The Oceanic Institute on the island of Oahu in Hawaii. The Administrative Center staff provides all necessary support services for the Executive Committee, the Board of Directors, the Industry Advisory Council, the Technical Committee, various project review panels, and delegations and project work groups. Dr. Kevan L. Main, Center Director, supervises operation of the Center.

Executive Committee

The Executive Committee is the legal entity responsible for the Center's overall administrative policy formulation, budget, and procedures. It also appoints the CTSA Director.

The members of the Executive Committee are:

Dr. Paul K. Bienfang, The Oceanic Institute, {Executive Committee Chairman} and
Dr. Dean Smith, University of Hawaii.

Board of Directors

The Board of Directors is responsible for the development and implementation of the Center's program policy, including concurrence on total budget issues. The Board is also responsible for development of ancillary agreements with other agencies and institutions.

The members of the Board of Directors represent educational, state, and non-profit private research institutions throughout the region. The Board of Directors:

- establishes initial guidelines for regional aquaculture research, development, and demonstration activities;
- appoints and removes members of the Industry Advisory Council and the Technical Committee;
- approves the proposed strategy for project selection;
- approves the priority areas and goals for industry development identified by the Industry Advisory Council and Technical Committee;
- approves the Annual Plan of Work, including budget allocations;
- approves the Annual Accomplishment Report for consistency with the goals and objectives of CTSA and the authorizing legislation; and
- develops ancillary agreements with other institutions.

The members of the Board of Directors are:

Dr. Jeff Barcinas, College of Agriculture and Life Sciences, University of Guam;
Dr. Paul K. Bienfang, The Oceanic Institute;
Mr. John Corbin, Hawaii State Aquaculture Development Program;
Dr. Charles Helsley, Sea Grant College Program, University of Hawaii;
Dr. Dean Smith, University of Hawaii, {Board Chairman};
Dr. Singeru Singeo, Land Grant Program, College of Micronesia; and
Dr. Harry Yamamoto, Hawaii Institute of Tropical Agriculture and Human Resources, University of Hawaii.

Industry Advisory Council

Members of the Industry Advisory Council include commercial aquaculture farmers, aquaculture suppliers, and members of government bodies and financial institutions. Members are appointed by the Board of Directors for 3-year, renewable terms. In the Industry Advisory Council's capacity as an advisory body, it provides an open information exchange forum for those involved in the aquaculture business. With the approval of the Board of Directors, contributions of the IAC can be incorporated into annual and ongoing plans for CTSA. The Industry Advisory Council:

- recommends research and development needs and priorities from the perspective of the aquaculture industry;
- participates as needed in the review of proposals, project progress reports, program review delegations, and other functions of the Center; and
- recommends to the Board actions regarding new and continuing proposals, proposal modifications, and terminations.

Members of the Industry Advisory Council are:

Mr. Bo Alexander, Hawaii Institute of Marine Biology, University of Hawaii;
Mr. David Barclay, Aquatic Culture and Design;

Mr. Dennis Bishop, Kona Mariculture;
Ms. Mary Brooks, Pacific Aquaculture;
Dr. Linden Burzell, Aquaculture International Inc.;
Mr. Steve Chaikin, Molokai Sea Farms;
Mr. Shinji Chibana, Palau Biotech Marine Tropicals;
Mr. Michael Crisostomo, Kurumaya SeaHorse Restaurant;
Mr. Richard Croft, Pohnpei Natural Products;
Mr. Craig Emberson, Makauu Aquafarm;
Mr. John Gourley, Micronesia Clam Company;
Ms. Linda Gusman, Island Aquaculture;
Mr. Steve Katase, Royal Hawaiian Sea Farms;
Mr. Jeff Koch, Mokuleia Aquafarm;
Mr. Ray Kosaka, Discus of Hawaii;
Mr. Andrew Kuljis, Amorient Aqua Farms;
Dr. Craig MacDonald, Hawaii State Ocean Resources Development;
Mr. Jerry B. Norris, Pacific Basin Development Council;
Mr. Ramsey Reimers, Robert Reimers Enterprises;
Dr. Rick Spencer, Hawaiian Marine Enterprises {Council Chairman};
Mr. John Taitano, Guam Aquafarms;
Mr. Ron Weidenbach, Hawaii Fish Company; and
Dr. Leonard Young, Hawaii State Aquaculture Development Program.

Technical Committee

The Technical Committee's members represent participating research institutions and state extension services, other state or territorial public agencies as appropriate, and non-profit private research institutions. The Technical Committee provides research expertise to address priorities set by the Industry Advisory Council. Members are appointed by the Board of Directors for 3-year, renewable terms.

The Technical Committee:

- prepares Problem Statements for priority areas identified by the Industry Advisory Council; and
- participates as needed in project review panels, Program Review Delegations, and other functions of the Center.

The members of the Technical Committee are:

Dr. Harry Ako, University of Hawaii, {Committee Chairman};
Mr. Richard Bailey, Sea Grant Extension Service, University of Hawaii;
Dr. James Brock, Hawaii State Aquaculture Development Program;
Mr. Mark Brotman, Northern Marianas College;
Dr. Christopher Brown, Hawaii Institute of Marine Biology, University of Hawaii;
Dr. John Brown, College of Agriculture and Life Sciences, University of Guam;
Mr. David Coleman, Hamilton Library, University of Hawaii;
Mr. David Crisostomo, University of Guam Cooperative Extension Service;
CTSA Regional Aquaculture Extension Agent, College of Micronesia;
Dr. Roger Fujioka, Water Resources Research Center, University of Hawaii;
Dr. Kevin Hopkins, University of Hawaii;
Dr. Robert D. Howerton, Cooperative Extension Service, University of Hawaii;
Mr. Tom Iwai, Anuenue Fisheries Research Center;
Dr. Christopher Kelley, The Oceanic Institute;
Dr. Andrew Kuniyuki, Cooperative Research and Extension, College of the Marshall Islands;
Mr. Obichang Orak, Palau Mariculture Demonstration Center;
Dr. Anthony Ostrowski, The Oceanic Institute;
Dr. Yung C. Shang, Department of Agricultural and Resource Economics, University of Hawaii;
Dr. Ilse Silva-Krott, College of Agriculture and Life Sciences, University of Guam;
Dr. James Szyper, Aquasearch;
Mr. Howard Takata, Sea Grant Extension Service, University of Hawaii; and
Dr. Clyde Tamaru, Sea Grant Extension Service, University of Hawaii.

Effluent Discharge Program

Dates of work, March 1988 through June 1996

Funding level

$520,020

Participants

Dr. Gary D. Pruder and Dr. David Ziemann, The Oceanic Institute
Dr. Jaw-Kai Wang, University of Hawaii

Reason for termination

This project was terminated because all objectives were completed.

Project objectives

The overall goal of this project was to reduce the uncertainty, time and cost of obtaining aquaculture effluent discharge permits, and the cost of satisfying aquaculture effluent discharge regulations.

Anticipated benefits

Hawaii's regulations regarding aquaculture effluent discharge form one of the most critical obstacles to growth and success of the aquaculture industry. This project laid the foundation for reducing the uncertainty, time, and expense involved in trying to satisfy these regulations, which would greatly benefit aquaculture farmers.

Progress and principal accomplishments

During the first year of the project, effluent discharge from commercial aquaculture facilities throughout Hawaii was characterized.

During the second year of the project, relevant scientific literature on zones of mixing was reviewed, and effluent discharge issues and the costs of various treatment processes were analyzed. In addition, a workshop was held to disseminate the results of the first year's work to commercial aquaculturists and other interested parties. At the World Aquaculture Society Meeting in 1990, investigators presented a paper on characterization of effluents in Hawaii, projected environmental impacts, and conventional treatment technologies. They also participated in a panel discussion on U.S. regulations of effluent. A list of organizations active in Hawaii environmental affairs was compiled.

During the third year of the project, case studies were developed documenting the experiences of three Hawaii facilities in obtaining National Pollution Discharge Elimination System (NPDES) permits. The case studies showed that the only commercial facility in Hawaii that got an NPDES permit spent more than $210,000 and 4 years to do so—an investment of time and money that exceeds the resources of most aquaculture producers. The effects of different feeds, feeding levels, water exchange rates, and continuous versus batch mixing regimes were studied.

In addition, investigators participated in a national conference titled "Water Quality and the Environment: Aquaculture" in Washington, D. C., which was attended by representatives of the Food and Drug Administration and Environmental Protection Agency, directors of all five Regional Aquaculture Centers, and researchers and commercial producers from throughout the country. Information on injection wells, trenches, offshore pipes, slant drilling, polyculture, particle removal, and recycling was collected and analyzed for their potential as alternatives to direct coastal discharge. The Hawaii state Department of Health's technical data and procedures for establishing Zones of Mixing were analyzed, and the quality of aquaculture effluent was compared with that of industrial and municipal waste-water facilities and state standards.

During the fourth year of the project, investigators helped launch an effluent discharge project that was undertaken cooperatively by the five Regional Aquaculture Centers. Interviews with recreational fishermen, divers, and beach users showed that they associate improved fishing and increased levels of marine life with aquaculture discharge.

During the fifth year of the project, studies were conducted to determine effluent's effect on the growth of shrimp and fish. Data from a previous shrimp study using intensive round pond technologies clearly showed a pond water enhancement of shrimp growth with both medium and high quality feeds. This led to a projection that exposure to aquaculture effluent would enhance the growth of desirable species. Growth comparison studies of shrimp and fish were conducted in replicate indoor tanks supplied with flow-through shrimp pond water, well water, or a mixture of half pond and half well water. The water was relatively poor quality when compared to the fully mixed waters of the intensive round pond used in the first study. In these trials, pond water suppressed shrimp growth rates compared to the shrimp growth rates achieved in well water. However, the pond water had no detrimental effect on mullet growth, which was essentially the same in pond water, well water, and the mixture of both.

During the sixth year of the project, a report summarizing the findings from the first 5 years of the program was prepared. It raised a number of points about current effluent discharge regulations.

- Government regulation is a critical limitation to the success of the domestic aquaculture industry.
- Aquaculture effluent discharges are extensively regulated by a number of agencies at all levels of government. These regulations, especially at the state level, have significantly affected the rate and character of aquaculture development in Hawaii. Further, the regulations tend to have more severe effects on small entrepreneurs.
- Aquaculture effluents, though different depending on the type of crop and culture system, share many similarities. They are distinctly different from domestic sewage in nature and potential environmental impacts. The most notable difference is that aquaculture effluents contain no pathogenic microorganisms, pesticides, toxic chemicals, or heavy metals, all of which are incompatible with successful production of aquaculture crops.
- Alteration of aquaculture diets and feeding practices is not a viable solution to the problem of effluent discharge regulations. Although changes in feeds and feeding practices can alter the amount of nitrogen, phosphorus, and other materials in aquaculture effluents, no dietary formulation or feeding strategy has been found that significantly improves the possibility of meeting current regulatory standards.
- Limited discharges of aquaculture effluents in appropriate locations do not adversely affect the environment. In fact, aquaculture effluent has been shown to have positive biological effects in some environments. Data suggest that populations of commercially valuable near-shore sport fishes have increased in areas of effluent discharge while no adverse effects were seen in other near-shore biological resources.
- Effluent effects vary depending on the nature of the coastal environment.
- The potential impact of aquaculture effluents on coastal waters varies considerably depending on the characteristics of the nearshore environments. The Hawaii state system of classifying waters as "AA" or "A" bears little correlation to the potential risk to specific nearshore environments from aquaculture effluent discharges.
- The susceptibility of the coastlines of each inhabited Hawaiian island to environmental damage from aquaculture effluent was evaluated. The results provide a rational basis for directing future aquaculture development based on the ability of the coastal environment to tolerate effluent discharges.
- Few, if any, viable alternatives to direct ocean discharge exist. Alternatives to ocean discharge, including slant drilling, discharge wells, and shallow dispersion ponds, were evaluated in terms of cost and efficacy. None of the alternatives were economically feasible.
- A broad spectrum of wastewater treatment technologies was evaluated for applicability to aquaculture effluents. None would bring aquaculture effluents within existing state discharge standards without a Zone of Mixing. The Zone of Mixing costs are beyond the reach of most commercial aquaculture enterprises.
- The comprehensive revision of aquaculture effluent discharge regulations represents a great opportunity to develop new methods to resolve environmental issues.

Past discussions with government officials led investigators to believe that changing the state regulations regarding effluent discharge would be near impossible but that the Department of Health's water quality standards could be amended to address aquaculture. However, at a September 1995 meeting with Department of Health officials, investigators learned that changing the standards would also be nearly impossible and that waivers will not be issued for certain industries. However, the Department of Health officials suggested that aquaculture industry members could use its new, streamlined administrative approach to Zones of Mixing, which relies on computerized models and minimal monitoring for industries that have best management practices plans in place.

In addition, the investigator prepared a termination report titled "Social and Political Aspects of the Development and Promulgation of Revised Water Quality Standards." It contains the following sections:
• Background;
• Roots of Resistance and Conflict: A Review;
• Risk and Culture;
• The Politics of Product Risks;
• Trashing the Planet;
• ECOSCAM: The False Profits of Ecological Apocalypse;
• Breaking the Vicious Circle: Toward Effective Risk Regulation;
• When You Speak, Why Don't They Listen?;
• Summary and Recommendations;
• Guidelines;
• The Activist Message;
• Guidelines for Working with Activists; and
• References Cited.

Impacts
This project compiled a large database of information on the impact and potential impact of aquaculture effluent on the coastal environment in Hawaii. The results will be used to educate and inform legislators, government regulators, environmentalists, scientists, farmers, and the general public. In addition, this project stimulated the development of a national inter-regional initiative on aquaculture effluent discharge research that was undertaken by several of the regional aquaculture centers in 1992/1993.

Recommended follow-up activities
The data gathered under this project could be used to develop a Best Management Practices Plan for commercial aquaculture in Hawaii and a computerized model of a Zone of Mixing that is general enough to include the various commercial aquaculture enterprises throughout the state. Those developments could be presented to the state Department of Health for use by commercial aquaculture facilities applying for Zone of Mixing permits. The aquaculture industry has been reluctant to fund this work and is waiting for a clearer directive regarding the Department of Health's position on future permitting requests.

Support
This project was funded by the Center for Tropical and Subtropical Aquaculture (CTSA).

Year	CTSA	Total Support
One	$180,000.00	$180,000.00
Two	$48,800.00	$48,800.00
Three	$127,120.00	$127,120.00
Four	$64,100.00	$64,100.00
Five	$65,000.00	$65,000.00
Six	$35,000.00	$35,000.00
Total	**$520,020.00**	**$520,020.00**

Publications, manuscripts, or papers presented
Pruder, G. D. 1991. An Overview of Aquaculture Water Quality Issues in the United States. In Proceedings of a Conference on Aquaculture: Water Quality and the Environment. April 9-10, 1991. Washington, D.C.

Wang, J. K. 1990. Managing Shrimp Pond Water to Reduce Discharge Problems. Aquacultural Engineering. p. 61-73.

Ziemann, D. A., G. D. Pruder, and J.K. Wang. 1990. Aquaculture Effluent Discharge Program: Year 1 Final Report. Center for Tropical and Subtropical Aquaculture Publication #101. Waimanalo, Hawaii.

Ziemann, D. A., W. A. Walsh, E. G. Saphore, and K. Fulton-Bennett. A Survey of Water Quality Characteristics of Effluent from Hawaiian Aquaculture Facilities. Journal of the World Aquaculture Society. 23 (3): 174-203.

National Coordinator for Aquaculture New Animal Drug Applications

Dates of work, May 1995 through August 1996

Funding level

$15,000

Participants

Rosalie Schnick

Objectives

The overall goal of this project is coordination of activities for investigational new animal drug exemptions (INADs) and new animal drug applications (NADAs) to expedite approval for the use of various drugs in aquaculture. Specific objectives related to that goal are to:

- serve as an information conduit between INAD and NADA applicants and the U.S. Food and Drug Administration's Center for Veterinary Medicine (CVM);
- identify and encourage prospective INAD participants to become involved in specific investigational studies and NADA approval-related research;
- seek the support and participation of pharmaceutical sponsors for INAD studies and NADAs and coordinate with INAD and NADA sponsors to achieve CVM approval more quickly;
- guide prospective and current INAD holders on the format for INAD exemption requests and related submissions to CVM;
- identify existing data and remaining data requirements for NADA approvals;
- review, record, and provide information on the status of INADs and NADAs;
- act as liaison and provide coordination among all the federal agencies involved in the INAD/NADA process; and
- provide public education related to training and guidance in obtaining INAD exemptions and pursuing NADA approval.

Impacts

As a direct result of the project, the U.S. Food and Drug Administration (FDA) announced that it will "defer regulatory enforcement against the unapproved sales and use of an approved Human Chorionic Gonadotrophin (HCG) product as a spawning aid in fish by or on the order of a licensed veterinarian." This provides aquaculture farmers with a means of legally obtaining and using HCG until it is approved by the FDA.

In July 1996, the Center for Veterinary Medicine stated that formalin could be used safely on all fish eggs to control and prevent fungal infections if a statement is added to the label concerning the need for a preliminary bioassay on a sub-sample before the entire group is treated.

Progress and principal accomplishments

As a result of a successful funding initiative, the coordinator's position has been increased from 3/4 to full time during the second year, which began May 12, 1996. The coordinator worked to obtain INADs, NADAs, and approvals for a number of drugs that are considered high priority by the public and private aquaculture community.

Antibacterials

The Coordinator obtained a sponsor for amoxicillin. Vetrepharm Limited of Fordingbridge, United Kingdom (UK), submitted an INAD/NADA letter of intent to the Center for Veterinary Medicine on January 5, 1996. On January 16, 1996, the company was granted INAD #9659, which named AquaFuture as the U.S. representative. The NADA Coordinator met with representatives of Vetrepharm in May 1996 to discuss an action plan for the development of the INAD/NADA on amoxicillin.

H & S Chemical Co. Inc. was given information on obtaining an INAD/NADA for Chloramine-T as a control for bacterial gill disease and flexibacteriosis. INADs for Chloramine-T were consolidated and coordinated the INADs, label claims were developed and pivotal study sites were identified. Akzo Nobel Chemicals Inc., the NADA sponsor of Chloramine-T, recently committed to provide the information necessary for the approval of their product in the United States and Europe. CVM concluded that para-toluene sulfonamide (p-TSA) is the major metabolite and that data necessary for calculating a tolerance for Chloramine-T in juvenile rainbow trout have been completed.

A June 14, 1996, letter to CVM's Office of Science requesting that the agency administer and monitor three required genotoxicity studies on p-TSA for the IAFWA Project has been withdrawn until studies identified by Akzo Nobel Chemicals Inc. can be acquired and evaluated. It may be possible that no funds will need to be expended on any genotoxicity studies on p-TSA.

The coordinator met with representatives of Vetrepharm Limited to discuss the possibility of the company becoming the erythromycin NADA sponsor. In follow-up efforts, the coordinator has been working with Dr. Christine Moffitt of the University of Idaho at Moscow to determine how Vetrepharm can become the NADA sponsor of erythromycin.

CVM indicated that INADs for the use of OTC as a marking agent will continue but that the agency is close to a decision about extending the NADA for that purpose to all fish.

The coordinator worked with UMSC staff to coordinate the IAFWA Project activities regarding OTC as an antibacterial, especially concerning the development of pivotal efficacy data. The HPLC analytical method was adapted for determining OTC levels in edible tissues of several species of fish. Bridging studies between microbiological and HPLC analytical methods will be initiated soon. INADs will be consolidated under the direction of the state of Texas.

The coordinator is assisting the efforts of the National Research Support Program #7 (NRSP-7) to complete the approval process for sarafloxacin to control enteric septicemia in channel catfish. Abbott Laboratories holds the NADA.

Microbicides

Copper sulfate INADs are being consolidated under the direction of the state of Nebraska. The week of July 8, 1996, CVM determined that it has no human food or environmental safety concerns over the use of copper sulfate as a microbicide, thus making approval relatively easy. The coordinator met with a potential representative of Phelps Dodge Refining Corporation to discuss the company's interest in sponsoring an INAD/NADA on copper sulfate, data requirements for approval, and coordination activities. The company will submit a letter of intent to begin the process toward an INAD/NADA on copper sulfate.

The NADA Coordinator sent a letter on July 2, 1996, suggesting a potential licensee for diquat to Zeneca Professional Products. Zeneca had determined in early April 1996 that it would not pursue an INAD/NADA package for diquat because the company is not in the aquaculture business.

On July 18, 1996, CVM stated that formalin could be used safely on all fish eggs to control and prevent fungal infections if a statement is added to the label concerning the need for a preliminary bioassay on a sub-sample before the entire group is treated. CVM will soon issue a notice in the Federal Register inviting NADA sponsors of formalin to amend their labels to include extended claims for both the fungicide and parasiticide uses. These extensions of the formalin NADAs to additional species will remove the need for INADs on formalin for these claims. The INADs on formalin's use as a fungicide on fish will remain in effect until data are submitted to cover this use.

The coordinator contacted several potential researchers about studies to determine the potential of Fumagillin to control or prevent Hamburger Gill Disease in catfish and whirling and proliferative kidney diseases in salmonids. The compound has the potential to test the "early life stage concept" because it would be used in starter feed of very young fish. The coordinator met and corresponded with a potential NADA sponsor of Fumagillin, Sanofi Sante Nutrition Animale, about the development of an INAD/NADA in the United States.

The coordinator worked with Eka Nobel Inc. to submit an INAD/NADA letter of intent for hydrogen peroxide as a fungicide. On January 19, 1996, CVM granted INAD #9671 to the company. In June, the coordinator met with Dr. David Lovetro of Eka Nobel Inc. to discuss the procedures for the INAD/NADA for hydrogen peroxide as a fungicide and the potential for its use to control and prevent external bacteria and parasites. A Canadian environmental and safety package on hydrogen peroxide will be submitted to a veterinary master file at CVM. The coordinator reviewed an Upper Mississippi Science Center petition to CVM requesting that the low regulatory priority ruling on hydrogen peroxide be increased to maximum levels of 1,000 ppm when used to control fungus on eggs.

Sea lice control
On September 9, 1996, the coordinator will attend a joint Canada-United States workshop on the jurisdiction of drugs and pesticides used to control and prevent sea lice on salmon. The coordinator consulted with John Pitts of Bellwether Consulting and Rob Armstrong of Sahnon Health Consortium in Canada on which chemicals will be pursued to control sea lice on salmon.

INADs for Cutrine-Plus™, a parasiticide, fungicide, and control for columnaris in cool and warm water fishes, are being consolidated under the direction of the state of Iowa. The coordinator communicated with Applied Biochemists about its interest in sponsoring Cutrine-Plus™. The company will decided after reviewing analyses of data on the compound's efficacy as a therapeutant.

Pet fish therapeutants
In June, the coordinator met with the American Pet Products Manufacturers Association to develop strategies and discuss progress toward approval of drugs of interest to the pet fish industry.

Anesthetics
Aqui-S™, which is approved in New Zealand, has a zero withdrawal time and offers a potential alternative to benzocaine. Because of the potential for gaining approval of an anesthetic with a zero withdrawal time in the United States, the coordinator and UMSC staff decided to evaluate the efficacy and overall performance of Aqui-S™ before committing additional funds under the IAFWA Project to gain approval of benzocaine. In June, the U.S. representative of Aqui-S met the coordinator and UMSC staff to discuss the potential for development of Aqui-S™ in the United States under the IAFWA Project.

Spawning and gender manipulation aids
As a follow-up to the common carp pituitary (CCP) meeting at CVM headquarters in April, CVM coordinated a May conference call that covered: (1) identification of researchers and design of target animal safety studies; (2) writing of the environmental assessment through the NRSP-7; and (3) potential funding sources for the target animal safety studies. A target animal safety study protocol on CCP using channel catfish written by Auburn University was reviewed. Efforts were made to find funding for the target animal safety studies needed to obtain approval of CCP.

The INAD holder for the use of 17a-methyltestosterone (MT) on yellow perch was assisted in implementing that portion of the MT INAD under the authorization of Auburn University. The coordinator reviewed a protocol written by Southern Illinois University for a target animal safety study on MT using walleye as a surrogate percid. The study has begun and is funded by the North Central Regional Aquaculture Center (NCRAC). The coordinator reviewed a proposal by Auburn University to write an environmental assessment of MT for a NADA submission to CVM. This project will be funded by the NCRAC.

Work planned

During the second year of this project, which began May 15, 1996, the coordinator will continue facilitating activities for investigational new animal drug exemptions and new animal drug applications to expedite approval of the use of various compounds in aquaculture.

Support

Fourteen public and private entities contribute funding for this project. They are the Center for Tropical and Subtropical Aquaculture (CTSA), Abbott Laboratories, American Pet Products Manufacturers Association (APPMA), American Veterinary Medical Association (AVMA), Catfish Farmers of American (CFA), the Center for Veterinary Medicine (CVM), Florida Tropical Fish Farms Association (FTFFA), IAFWA Project (IAWFA), Northeastern Regional Aquaculture Center (NRAC), North Central Regional Aquaculture Center (NCRAC), Western Regional Aquaculture Center (WRAC), Simaron Freshwater Fish Inc. (SFFI), Hybrid Striped Bass Producers Association (HSBPA), and the National Aquaculture Council (NAC).

Contributor	Year One Funding	Total Funding
1 CTSA	$5,000.00	$5,000.00
2 AVMA	$10,000.00	$10,000.00
3 CFA	$2,000.00	$2,000.00
4 CVM	$24,000.00	$24,000.00
5 FTFFA	$ 500.00	$ 500.00
6 NRAC	$5,000.00	$5,000.00
7 NCRAC	$5,000.00	$5,000.00
8 HSBPA	$ 500.00	$ 500.00
9 NAC	$1,000.00	$1,000.00
TOTAL	**$53,000.00**	**$53,000.00**

Year	CTSA	Total Other Funding (see table above for breakdown)	Total Funding
One	$5,000	$48,000	$53,000
Total	**$5,000**	**$48,000**	**$53,000**

Publications and presentations

Gingerich, W. H. and Schnick, R. A. (In review). Federal-state aquaculture drug approval partnership program. Abstract for special session titled "Partnerships for aquaculture drug approvals: models for success" to be held at World Aquaculture '97, Seattle, WA, February 19-23, 1997.

Schnick, R. A., W. H. Gingerich, and K. H. Koltes. 1996. Federal-State Aquaculture Drug Registration Partnership: A Success In the Making, Fisheries 21(5):4.

Schnick, R. A. 1996. Chemicals and Drugs. Pages 135-142. In R. C. Summerfelt, editor. The Walleye Culture Manual. NCRAC Culture Series #101, North Central Regional Aquaculture Center Publications Office, Iowa State University, Ames.

Schnick, R. A. 1996. Cooperative Fish Therapeutic Binding Initiative: States in Partnership with Federal Agencies to Ensure the Future of Public Fish Culture. Transactions of the 61st North American Wildlife and Natural Resources Conference. 61:6-10.

Schnick, R. A. and R. D. Armstrong. (In review). Aquaculture drug approval progress in the United States. Northern Aquaculture.

Schnick, R. A. (In review). Overview of partnerships for aquaculture drug approvals. Abstract for special session titled "Partnerships for aquaculture drug approvals: models for success" to be held at World Aquaculture '97, Seattle, WA, February 19-23, 1997.

Schnick, R. A. 1996. International regulatory aspects of chemical and drug residues. Presented at the International Conference on Fish Inspection and Quality, Arlington, VA, May 19-24, 1996.

Schnick, R. A. 1996. Aquaculture drug approval progress in the United States. Presented at Aquaculture Canada '96, 13th Annual Meeting of the Aquaculture Association of Canada, Ottawa, Ontario, June 2-5, 1996.

Understanding *Gracilaria* Gall Syndrome

Dates of work, May 1, 1995 through June 1, 1996

Participants

Dr. James Brock, Aquaculture Disease Specialist, Hawaii Department of Land and Natural Resources

Dr. Lynda Goff, Professor of Biology, University of California at Santa Cruz

Dr. Roger Lallone, Scientific Director, Brookwood Biomedical Laboratory

Dr. Michael Shintaku, Assistant Professor of Plant Pathology, University of Hawaii at Hilo

Project objectives

The objectives of this project are to:

- demonstrate under controlled laboratory conditions the transmission and infectivity of the *Gracilaria* Gall Syndrome (GGS) agent to various *Gracilaria* strains;
- describe in detail by light and electron microscopy the external and internal characteristics of *Gracilaria tikvahiae* affected by *Gracilaria* Gall Syndrome (GGS);
- characterize the bacterial communities associated with *Gracilaria tikvahiae* unaffected and affected by GGS, including an assessment of antibiotic sensitivities, and to survey *Gracilaria* tissues for the presence of viruses; and

• develop a probe for identification of biochemical markers of GGS and/or the GGS agent.

Anticipated benefits

A management strategy aimed at GGS is clearly needed. The rapid progression of GGS outbreaks coupled with the severity of GGS symptomology combine for high yield losses and the expenditure of much effort in tank dumping. Unfortunately, the GGS causal agent has not yet been identified. Thus, much of the continued efforts in the second half of the first year funding were directed toward that goal.

Besides the obvious and direct benefits to seaweed growers, this project offers the unusual opportunity to investigate a novel pathogenic interaction. This project can be extremely fruitful in terms of generating ecological and physiological information about marine plant-microbe interactions. This area of research has not been greatly explored, and these issues become more and more pressing as the aquaculture industry continues to diversify.

Progress and principal accomplishments

Objective: *Demonstrate under controlled laboratory conditions the transmission and infectivity of the* Gracilaria *Gall Syndrome (GGS) agent to various* Gracilaria *strains, and to characterize the bacterial communities associated with* Gracilaria tikvahiae *unaffected and affected by GGS, including an assessment of antibiotic sensitivities, and to survey* Gracilaria *tissues for the presence of viruses.*

Originally these were separate objectives. However, investigators are assaying bacteria originally isolated from GGS-affected tissue for infection potential and have merged the progress of these objectives.

Dr. Shintaku's laboratory collected normal and GGS-affected seaweed samples three times from an Oahu farm and 11 times from a Hawaii farm between November 1994 and May 1996. Small (2 to 5 mg) samples of galls and of the tips of healthy tissue were crushed and spread on a culture medium. Extracts from healthy tissue showed between 5 and 50 bacterial colonies, while extracts from diseased tissue showed between 100 and 1,000 bacterial colonies. Galled tissue is more heavily colonized with epiphytic bacteria.
Dr. Shintaku archived 110 bacterial isolates from diseased seaweed, attempting to gather a collection as diverse as possible in terms of colony morphology and growth habits. Bacterial isolates that were abundant on or specific to GGS-affected tissue were selected for identification and further characterization.

Identification of the isolated bacteria was attempted using the Biolog bacterial identification system. Unfortunately, the Biolog bacterial identification database is rather limited regarding marine bacteria and was able to identify only nine of the 30 isolates to species and five to genus (Table 1).

Table 1.

Isolate	Biolog identification	Similarity to database isolate (if no fit, closest species in database)
1Tb	*Vibrio alginolyticus*	0.824
3Tb	*V. carchariae*	0.956
141b	*V. tubiashii*	0.873
4b	*V. alginolyticus*	0.734
13a	*Vibrio sp.*	0.495 *(V. meditarreanei)*
13c	*Vibrio* sp.	0.429 *(V. anguillarum)*
407	*Vibrio* sp.	0.631 *(V. cholerae)*
23C	*Vibrio sp.*	0.429 *(V. anguillarum)*
401	*Vibrio sp.*	0.529 *(V. alginolyticus)*
402	*V. alginolyticus*	0.734
403	no i.d.	0.386 *(Acinetobacter radioresistens)*
404	no i.d.	0.616 *(Hydrogenophaga pseudoflava)*
405	no i.d.	0.212 *(Halomonas elongata)*
406	no i.d.	no pattern
407	no i.d.	0.153 (*Xanthomonas oryzae*)
409	no i.d.	0.110 *(Gilardi pink gram -)*
142b	*Kingella denitrificans*	0.793
1434	*V. alginolyticus*	0.794
1437	*V. alginolyticus*	0.718
1439	no i.d.	0.232 (CDC group EF-4)
1440	no i.d.	0.618 *(K. denitrificans)*
LRO1	*V. alginolyticus*	0.732
ORO1	no i.d.	0.477 *(V. carchariae)*
ORO2	no i.d.	0.460 *(V. parahaemolyticus)*
1201	no i.d.	0.126 *(Burkholderia picketii)*
1202	no i.d.	0.452 *(B. picketii)*
1203	no i.d.	0.397 *(B. picketii)*
1204	no i.d.	0.048 *(Pseudomonas syringae pv. phaseolicola)*
302	no i.d.	no pattern

Forty-eight of the bacterial cultures isolated from GGS-affected material have been inoculated on *Gracilaria* in small flask cultures. Four bacteria induced GGS in red but not green *Gracilaria* 6 to 7 days after inoculation. No galls were observed in the uninoculated control groups.

All four of the bacteria that produced GGS in this trial were gram-negative, and two were identified using the

Biolog system as *Vibrio alginolyticus*. Unfortunately, repeated experiments using these strains have not induced GGS, which may indicate an environmental factor that predisposes *Gracilaria* for GGS susceptibility.

Dr. Goff's laboratory conducted three 3-month inoculation trials with the same bacteria. These inoculation experiments failed to identify a bacterial isolate capable of inducing GGS on cultured algae, despite the demonstrated ability of these isolates to induce GGS symptoms in cultured algae immediately after isolation. Algal specimens from both sites failed to develop GGS symptoms during the 3-month incubation period. However, within a week of inoculation with cultured bacteria, several algal cultures revealed a minor degree of apical bleaching. This response, which was not universal and was not correlated with a particular bacterial culture, suggested nutrient depletion.

Dr. Shintaku has attempted to detect the presence of viruses by repeatedly isolating total nucleic acids from healthy and diseased seaweeds and assaying for the presence of double-stranded RNA using CF-11 chromatography. These attempts have not revealed double-stranded RNA. In addition, analysis of the nucleic acid extracts revealed no subgenomic species of DNA. Although this does not rule out the presence of viruses in these tissues, viruses are not abundant if they are present. In light of this, investigators are continuing their efforts to identify a bacterial causal agent.

In addition, the propagation of GGS-affected *G. tikvahiae* has not been successful under laboratory conditions. Diseased seaweeds brought in from the field and cultured under a variety of conditions seem to cure themselves within several weeks. Laboratory-cultured GGS-affected *G. tikvahiae* again manifested GGS symptoms in only one case, which could not be correlated with any obvious culture conditions and could not be repeated. In that case, the cuticle showed no obvious bacterial infestation.

The irregular occurrence of GGS on farms, which has not been related to any obvious physical or biological variables, has proven to be an additional obstacle to the investigation. This has made a consistent source of GGS affected algae extremely difficult to obtain and precluded intensive investigation of this syndrome.

Objective: *Describe in detail by light and electron microscopy the external and internal characteristics of* Gracilaria tikvahiae *affected by* Gracilaria *Gall Syndrome.*

Dr. Goff's laboratory conducted this work. More than 1,000 sections of GGS-afflicted and healthy seaweed have been extensively examined using glycol methacrylate resin (JB-4) and various cytochemical staining techniques. Healthy seaweed shows *Gracilaria's* normal morphology: three cell layers—epidermal, cortical, and medullary— are present; the relative sizes of the cells within each layer are normal. The cuticle of healthy seaweed 1 to 25 microns wide and is covered with a thin, extensive layer of assorted epiphytic bacteria.

In contrast, GGS-afflicted seaweed show extensively indented scalloped and extremely thickened (up to 105 um) cuticle areas. When stained with cytochemical dyes, these indentations appear tightly packed with small rods (1 x 25um) indistinguishable from bacteria. Despite extensive survey of afflicted tissues, these symptoms are the only indication of a pathology that may be afflicting these obviously abnormal algae. There appears to be no internal (inter- or intracellular) symbionts or pathogens (bacterial, fungal, algal, or animal), although several instances of bacteria-containing "scallops" within the cuticle of the GGS-afflicted thalli appeared to have penetrated the algal epidermis. Areas of GGS-afflicted thallus which demonstrate these symptoms also seem to be correlated with hyperplasia of the algal epidermis. This induction of epidermal cell division and thickening of the algal cuticle may be a host response to bacterial infection. Current work is focusing on serial sectioning and reconstruction of a GGS-afflicted algal gall.

In summary, these investigations have revealed extensive pitting of a thickened epithallial layer of GGS-affected cell wall material. This layer of material is clear in preparations for light microscopy and electron transparent in preparations for TEM. Within this thickened layer of putative polysaccharide there appears to be localized proliferation of densely packed bacterial aggregations. These bacteria are uniform in size, implying that they are of a single type.

Objective: *Identify biochemical markers of GGS and/ or the GGS agent.*

Dr. Lallone found that some preliminary experiments documented differences in protein and carbohydrate content of detergent extracts of normal and affected cultures of *G. tikvahiae*. Aqueous extracts from GGS positive and negative plants were made and concentrated 10X by ultrafiltration using 10K molecular weight cut-off membranes and analyzed by SDS-PAGE under reducing and non-reducing conditions. The slab gels were stained using Coomassie Blue or PAS to reveal either proteins or carbohydrates. Investigators detected a group of proteins, of low to intermediate molecular weight and sensitive to mreduction by 2-ME in extracts of GGS negative plants, which are either absent or degraded to smaller molecular weight fragments in GGS-positive material. In addition, investigators observed increased amounts of low (but not high) molecular weight, PAS positive carbohydrate material in affected plant extracts compared to non-affected plant extracts.

Investigators made several non-detergent extracts of infected plant material. These extracts were also analyzed by SDS-PAGE and were found to contain a variety of high and low molecular weight proteins. Diseased plants were ground in a blender, soluble material was extracted by aqueous incubation at 4°C, insoluble residue was removed by filtration, and soluble material was fractionated by sequential ultrafiltration. Soluble material was fractionated using a series of decreasing molecular weight cutoff membranes (300D, 100D, 30K, and 10K). Banding patterns reveal both similarities and differences between each fraction. In addition, similar extracts and fractionations were performed on apparently healthy plants taken from two distant culture sites (one of which has to date had no record similar disease symptoms). When examined in parallel clear differences could be found between each of the various fractions taken from plants collected from each of the various sites.

Based on these observations we immunized four groups of animals. Each group of three rabbits received injections of extracted material taken from stocks of ultrafiltration-fractionated extracts of diseased plants. Antibodies were also raised to healthy *Gracilaria*. Antisera collected from these animals was cross-reacted with healthy *Gracilaria* tissue, labeled, and used to probe a Western blot containing extracts (fractionated as described above) from diseased tissue. These probes react strongly with a compound approximately 30 kD in mass in GGS-affected tissue. Further, the reactive antigen does not appear as a prominent band when total protein is visualized. We do not know if this compound is produced by the diseased plant, perhaps as a stress response, or is a component of the pathogenic agent.

The antibodies were formatted into a sensitive immunoassay and used to test water samples taken at regular time periods from production tanks. Records were kept during the sampling period so that correlations could later be made between a positive immunoassay test and the appearance of disease. Surprisingly, the antibodies raised to diseased plants showed no immunoreactivity with any of the water samples despite appearance and disappearance of disease whereas the antibodies raised to healthy plants did. Furthermore the concentration of immunoreactive material in the water samples was highest days before the visual appearance of disease. In light of this surprising result plants showing early signs of disease were collected, crudely dissected, and separate detergent extracts were made of plant stems, healthy growing tips, and galled growing tips. The extracts were probed with the same antibodies used in the immunoassay. The highest concentration of immunoreactive material was found in healthy tips and the lowest concentration was found in diseased tips. This indicates that components of healthy plant tissue may be released into the water prior to appearance of disease. This needs to be verified by comparing the material found in water samples with material extracted from healthy growing tips. The destruction of normal plant tissue and release of critical components is likely to be due to proteolytic enzymes, either from a bacterial or plant source. Dr. Lallone is exposing normal plant tissue to various commercially available bacterial, plant, and animal derived enzymes, and is monitoring the release of immunoreactive material from the plants.

In addition, Dr. Lallone's group is screening the bacterial cultures obtained from Dr. Shintaku for reactivity with the above-mentioned antisera.

In summary, several lines of evidence implicate a bacterial causal agent in this disease interaction. These include a therapeutic response to ampicillin and the *in vitro* induction of GGS with several bacteria. However, the apparent induction of GGS by several different bacteria and the inability to consistently reproduce these results confounds our findings. Furthermore, there appears to be an intimate association between aggregations of surface bacteria found in tissue indentations and GGS-affected thalli, but very little internal tissue invasion. The outer layers of GGS-affected tissue become greatly thickened in addition to acquiring those bacteria-filled indentations. However, investigators have not yet elucidated the temporal relationship between heavy bacterial colonization, wall thickening, and GGS. An axenic culture system for *Gracilaria* culture is in place in Dr. Goff's laboratory, and work continues towards maintaining tissue in the galled state. The failure to reproduce the symptoms associated with GGS on cultured specimens of *G. tikvahiae* may be due to several factors. Although the bacterial cultures provided were derived from affected material, re-isolation of pure cultures by classical microbiological technique was necessary. During this process the GGS agent may have been lost. Another possibility is GGS may be due to the action of a microbial consortium which would be eliminated in single-culture inoculation of axenic algae. Dr. Lallone has had success in generating GGS-related antibodies, and these are being used to determine the mechanism of GGS induction, and to help ascertain which bacteria are unique to GGS-affected material.

Work planned

Much of the work in year 2 will continue as above. Dr. Shintaku's laboratory continues to isolate bacteria from galled *Gracilaria* and inoculate them on healthy tissue to assay for infectivity. With regard to the four bacterial isolates that appear to have induced galls in laboratory culture—samples of those cultures are now being re-examined in Dr. Goff's and Dr. Lallone's laboratories, as the first shipment of bacteria consisted of mixed cultures.

The preliminary microscopy work is now concluded, and the focus in Dr. Goff's laboratory has shifted towards developing an axenic culture system with which to further assay potential pathogens. Environmental conditions in that system are being adjusted to allow for the induction and maintenance of *Gracilaria* in the galled condition. This culture system will be used in inoculation studies involving water treatments, such as ultrafiltration, ultraviolet irradiation, temperature and chemical treatments. These experiments are aimed at providing some information on the physical nature of the pathogen, and will further yield important information on possible GGS control measures.

Antisera generated in Dr. Lallone's laboratory reacts strongly with a specific antigen in GGS-affected tissue. Our efforts are now directed at incorporating this into a practical method for assaying culture water and *Gracilaria* tissue for latent or impending GGS infections. This sera will also be used in imm unofluorescence microscopy, to perhaps determine the distribution of the reactive antigen in tissue and/or culture water. Further, the antisera is being used to screen the bacterial cultures thus far isolated. This will provide correlative data for use in conjunction with the inoculation trials.

Impacts

At this stage, no impacts have been made. However, investigators have made good progress toward the further characterization of this disease and toward developing tools for the management of this disease.

Support

This project received support from CTSA, the University of Hawaii at Hilo, University of California at Santa Cruz, and Brookwood Biomedical Laboratory.

Year	CTSA	Other Support: UH	Other Support: UCSC	Other Support: Brookwood	Total Other	Total Support
One	$60,000	$8,400	$12,000	$10,000	$30,400	$90,400
Two	$60,000	$8,400	$12,000	$10,000	$30,400	$90,400
Total	**$120,000**	**$16,800**	**$24,000**	**$20,000**	**$60,800**	**$180,800**

Publications, manuscripts, or papers presented

No publications or manuscripts were prepared during the reporting period.

Library Aquaculture Workstation: Pacific Regional Aquaculture Information Service for Education (PRAISE)

Dates of work, March 1988 through April 1996

Funding level

$166,600

Participants

David E. Coleman (project coordinator), Randall Buettner, Kristen Anderson, Rachel Hu, Jue Wang and Catherine Stewart Edington, Hamilton Library, University of Hawaii
Bin Zhang, Kapiolani Community College

Project objectives

The overall goal of this project is to make scientific information more accessible to the aquaculture community. Specific year 8 objectives related to that goal are to:

- expand the existing dial-up remote access information network in the Pacific Islands;
- evaluate the effectiveness of the dial-up remote access information network that was expanded to the Pacific Islands during year 6;
- increase and ensure the continued usefulness of the PRAISE program through the use of CD-ROM database searching, telecommunications and new technologies as they develop, and disseminate information products as needed by the industry;
- increase the efficiency of PRAISE through interaction with other information agencies;
- establish a cooperative project to gather and disseminate Pacific Islands “gray literature” on aquaculture; and
- increase the support base for the project through cooperative agreements with other agencies.

Progress and principal accomplishments

Years 1 through 7

In 1988-1989, the Center for Tropical and Subtropical Aquaculture provided funding to establish an aquaculture workstation operated and managed by the staff of Hamilton Library, University of Hawaii. That program is known as the Pacific Regional Aquaculture Information Service for Education, or PRAISE. The workstation is a computer equipped with a multi-disk CD-ROM player, fax and modem. The service subscribes to a number of CD-ROM databases, including Aquatic Sciences and Fisheries Abstracts (ASFA), CINAHL Nursing Index, AGRICOLA, and Biological Abstracts. These databases list articles on thousands of aquaculture topics from hundreds of scientific journals.

During the first 2 years of the project, those interested in conducting a search could either travel to Hamilton Library at the University of Hawaii Manoa campus or call David Coleman, who would then conduct the search and fax the results to the PRAISE patron. The patron then selected the desired articles, which Coleman photocopied and faxed or mailed to the patron. Initially, a limit of 10 articles were sent, but that proved too restrictive and was eliminated. An average of 15 articles were sent to patrons who could not otherwise obtain them.

During the third and fourth years of the project, investigators compiled and published “A Union List of Aquaculture Journals in Hawaii.” The catalog listed science journals held at seven key libraries that have a large collection of aquaculture literature. The catalog assisted the aquaculture community with locating journal literature. In addition, PRAISE exchanged journal holdings data with the Scripps Institute of Oceanography, the California Academy of Sciences, and the Pacific Island Marine Resources Information Service of the University of the South Pacific.

During the fifth year of the project, remote workstations were established at the CTSA office, the Hawaii Institute of Marine Biology, the Aquaculture Development Program office, the Sea Grant office, the Pacific Island Network office, and the University of Hawaii at Hilo. From these remote workstations equipped with modems, users can dial into PRAISE to perform database searches 24 hours a day, 365 days a year. The remote sites increased the efficiency of the service, which was demonstrated by the vastly increased numbers of searches that have been perform since their establishment.

During the sixth year of the project, additional remote workstations were established at The Oceanic Institute's site at Keahuolu on the island of Hawaii, at Anuenue Fisheries Research Center, and at the Hawaii Institute of Marine Biology computer lab. A breakthrough in the Pacific Islands' ability to access scientific information came in August 1993, when two remote workstations were established on Guam. Users at the site at the offices of the University of Guam's Cooperative Extension Service and at the Guam Department of Commerce gained access to PRAISE through a toll-free telephone line. The investigator conducted training sessions at both locations.

The establishment of two remote workstations, from which users dial into PRAISE via a toll-free telephone number, marked the first time a toll-free line was established from the Pacific Islands for CD-ROM data transmission. The vendor, MCI, experienced a number of problems before instituting reliable service. The cost of establishing the line and monthly charges totaled $2,000. A total of 54 calls— an average of 3.9 calls per month— were made. The average call lasted 20 minutes and cost $1.85 per minute or $37 per call. Providing ready access to aquaculture information has proven to be useful to the aquaculturists of Guam. However, the service was quite costly, and the system continued to experience problems.

During the seventh year of the project, PRAISE submitted data to the U.S. Department of Agriculture Science and Evaluation Study Working Committee on Aquaculture. Results of the study showed that aquaculturists were particularly interested in sources of aquaculture information from various government agencies and educational facilities. Based on this information, the Joint Subcommittee on Aquaculture approved publication of the Resource Guide to Aquaculture Information. PRAISE participated in the creation of this publication.

PRAISE entered a cooperative agreement with PEACESAT, to improve information access for five Pacific Island sites. Under the agreement, residents of Guam, Saipan, Pohnpei, Belau, and Majuro can directly access the Aquatic Sciences and Fisheries Abstracts (ASFA) database through an Internet connection between the local PEACESAT station and the mainland vendor. This system cost $1,000 per year per locale.

A wealth of reports containing valuable, unique information are produced throughout the Pacific but never integrated into journals and conference proceedings. The inaccessibility of "gray literature" is a particularly serious problem in the Pacific, where libraries and other organizations that collect and disseminate information are few. Also, important work done in the region is not shared with the rest of the scientific community, which means regional work does not get the recognition it deserves. The Pacific Islands Gray Literature Project was established to address this impediment to information. To date, more than 100 Pacific Islands publications have been gathered for inclusion in the Aquatic Sciences and Fisheries Abstract (ASFA) database. In addition, "Pacific Islands Gray Literature Project: A Bibliography" was published.

PRAISE hosted the 20th Annual Conference of the International Association of Marine Science Libraries and Information Centers in Waikiki from October 9-13, 1994. Participants from more than 12 countries, including Iceland, Malaysia, and Russia, attended.

Year 8 progress and principal accomplishments

Objective: *Increase and ensure the continued usefulness of the PRAISE program through the use of CD-ROM database searching, telecommunications and new technologies as they develop, and disseminate information products as needed by the industry.*

Use of the workstations at both Hamilton Library and remote sites continued to increase. Since establishing the electronic network, the total number of system uses increased from about 400 per year to more than 8,000 per year. The 2,000 percent rise in use of the service was accomplished with no increase in staff.

During the eighth year of the program, the existing CD-ROM system was upgraded to effectively handle the tremendous increase in the use of the service. Hamilton Library adapted eight in-house workstations to Pentium computers. This was done in anticipation of allowing these machines to access both the Kapiolani Community College CD-ROM Local Area Network (LAN), which has better capability than the Hamilton

Library LAN and the Internet. This will provide access to the PRAISE WorldWide Web page as well as the aquaculture database. Test results of this system will allow progress toward other objectives.

The project instituted methods to allow the Pacific Islands to have cost-effective access to PRAISE, including making PRAISE Internet-compatible. A PRAISE home page on the WorldWide Web was established and is available to Guam, Saipan, and other Pacific Island sites with Internet connectivity.

Tests underway at the CTSA office and Hamilton Library will provide information that will eventually allow access to the aquaculture CD-ROM database either via direct telnet to Kapiolani Community College Library or to the PRAISE home page on the WorldWide Web.

In addition, the PRAISE program was positioned to take advantage of the upcoming national integration of aquaculture information, specifically by interacting with the U.S.D.A. Aquaculture Extension Service.

Objective: *Increase the efficiency of PRAISE through interaction with other information agencies.*

The Joint Subcommittee on Aquaculture (JSA) decided that legislative materials on the development and support of aquaculture should be included on AquaNIC, an Internet gateway to the world's electronic resources in aquaculture that is supported by the U.S. Department of Agriculture. David Coleman, a member of the JSA's Aquaculture Information and Technology Transfer Task Force, is attempting to gather relevant legislative materials from Hawaii and the Pacific Islands for AquaNIC. He met with AquaNIC coordinators in October 1995 to establish a procedure for downloading the information. Coleman gave a presentation on the cooperative project between PRAISE and PEACESAT and participated in discussions of Internet uses and procedures at the 1995 CYAMUS Marine Librarians Meeting.

A Pacific Aquaculture Legislation section has been established on the PRAISE home page on the WorldWide Web. Legislation information has been provided by the Hawaii Legislative Reference Bureau. Information from other Pacific islands is being sought.

Objective: *Increase the support base for the project through cooperative agreements with other agencies and information facilities.*

The cost to establish the remote workstations was shared by all the hosting institutions. The work on the "gray literature" bibliography received additional funding from the Pacific Island Network, USDA's National Agricultural Library, and the vendor for the ASFA database, into which the bibliography materials will be incorporated. The National Agricultural Library also provided co-funding to publish the bibliography. Additional base project funding was secured from the University of Hawaii, the Pacific Island Network, and the University of Hawaii Sea Grant Extension Service.

The Pacific Aquaculture Association purchased a photocopy machine for PRAISE use. The machine has significantly sped the process of providing documents for document delivery. It will also be used to provide clean copies for scanning of Pacific Islands documents that will then be indexed in the Aquatic Sciences and Fisheries Abstracts database.

The National Agriculture Library expressed interest in producing a bibliography of Pacific Islands "gray literature" based on the Information gathered under the auspices of the PRAISE "gray literature" project. The bibliography is available through PRAISE.

Work planned

The PRAISE server at Hamilton Library will be upgraded with new CD-ROM readers and equipment to allow improved access for users. The current system of searching from remote sites will continue, and project staff will continue to send documents to users upon request. The Pacific Islands "gray literature" bibliography will be updated, and new sources of funding will be sought to broaden the support base for PRAISE.

The vendor of the Aquatic Sciences and Fisheries Abstracts database and National Oceanic and Atmospheric Administration databases discussed the possibility of "gray literature" being electronically scanned and transmitted directly to the vendor. This would significantly speed incorporation of the data into the ASFA database. The scanning technology is also being investigated for the possibility of meeting additional document delivery objectives under consideration.

Impacts

This project has increased the accessibility of scientific information throughout the Pacific Region. In the last year, PRAISE users performed more than 8,000 searches, each of which lasted approximately 30 minutes. Commercial databases charge $102 per hour for connect time. Therefore, those searches would have cost approximately $408,000. The highest level of project funding from CTSA was $49,000 in year 8, which represents a savings of $359,000 in that year alone.

Support

This project received funding from the Center for Tropical and Subtropical Aquaculture (CTSA), the University of Hawaii (UH), Sea Grant Extension Service (SGES), the National Agricultural Library (NAL), the Center for Applied Aquaculture (CAA), the National Oceanic and Atmospheric Administration (NOAA) and jointly from the Pacific Island Network (PIN), and the Pacific Aquaculture Development Program (PADP).

		Other support							
Yr	CTSA	UH	SGES	PIN / PADP	NAL	CAA	NOAA	Total Other	Total Support
1	$7,000	$13,400	$1,500	$0	$0	$0	$0	$14,900	$21,900
2	$6,700	$12,600	$0	$0	$0	$0	$0	$12,600	$19,300
3	$6,000	$12,600	$3,300	$0	$0	$0	$0	$15,900	$21,900
4	$7,000	$14,100	$4,000	$0	$2,500	$0	$2,500	$23,100	$30,100
5	$20,000	$44,175	$3,500	$10,800	$0	$15,000	$0	$73,475	$93,475
6	$17,900	$24,000	$0	$5,800	$0	$0	$0	$29,800	$47,700
7	$28,000	$12,600	$0	$5,500	$0	$0	$0	$18,100	$46,100
8	$49,000	$11,400	$0	$5,500	$0	$0	$0	$16,900	$65,900
9	$25,000	$10,500	$0	$5,000	$7,500	$0	$0	$23,000	$48,000
Total	**$166,600**	**$155,375**	**$12,300**	**$32,600**	**$10,000**	**$15,000**	**$2,500**	**$227,775**	**$394,375**

Publications, manuscripts, or papers presented

Brown, C. L. and D. E. Coleman. 1991. Testing and Development of an Efficient, Remote CD-ROM System. CD-ROM Librarian. p. 13-18.

Coleman, D. E. 1990. Pacific Regional Aquaculture Information Service: Applied Technology and Development of a Long-Distance Information Service. In International Association of Marine Science Libraries and Information Centers Conference Series: Proceedings of the 15th Annual Conference.

Coleman, D. E. 1991. Remote CD-ROM Searching and Satellite Communications: From Pie in the Sky to Application. In International Association of Marine Science Libraries and Information Center Conference Series: Proceedings of the 16th Annual Conference.

Coleman, D. E. 1993. Gray Literature Project of the Pacific Regional Aquaculture Information Service. In Proceedings of the 19th Annual Conference of the International Association of Marine Science Libraries and Information Centers (IAMSLIC). Bethesda, MD.

Coleman, D. E. and R. L. Buettner. 1989. A Union List of Aquaculture Journals in Hawaii. Center for Tropical and Subtropical Aquaculture Publication #104. Waimanalo, Hawaii.

Coleman, D. E., D. Hanfman, and S. J. Tibbet, eds. 1991. Interactions of Aquaculture, Marine Coastal Ecosystems and Near Shore Waters: A Bibliography. Bibliography and Literature of Agriculture #105. National Agricultural Library. Beltsville, Maryland.

Guenther, K. and D. E. Coleman. 1994. Pacific Islands Gray Literature Project: A Bibliography. Center for Tropical and Subtropical Aquaculture Publication #115. Waimanalo, Hawaii.

Production of CTSA Educational Extension Materials

Dates of work, May 1995 through October 1996

Funding level

$31,217

Participants

Dr. Bruce Miller, Jill Ladwig, Richard Bailey, Sea Grant College Program, University of Hawaii
David Coleman, Hamilton Library, University of Hawaii

Project objectives

The goal of this project was to produce educational extension materials on the results of several past CTSA-funded projects. The materials, which are intended for a lay audience, consisted of manuals, fact sheets, and videos.

Progress and principal accomplishments

This project produced four extension fact sheets, one culture manual and a video. The first 2-page fact sheet, "Shrimp Diseases," contained sections titled: "Taura Syndrome Virus;" " HHNV and Runt Deformity Syndrome;" "Specific Pathogen-Free Broodstock;" "New Research;" and "Where to Turn for Help."

The second 2-page fact sheet discussed "Bacterial Diseases in Chinese Catfish," and included sections on "What Symptoms Should I Look For?;" "Treatments;" "New Research;" and "Where Should I Turn For Help?"

The third 2-page fact sheet, "Tilapia," included a discussion of the value of Hawaii's cultured tilapia crop, and sections titled: "The Illness — Hawaii Tilapia Rickettsia-Like Organism Disease;" "What Symptoms Should I Look For?;" "New Research;" and "Where Should I Turn for Help?"

The fourth 2-page fact sheet, "*Gracilaria* Gall Syndrome," discussed the background on *Gracilaria*, and includes sections on GGS — "The Symptoms;" "New Research;" and "Where Should I Turn for Help?"

The project also produced Starting a Successful Commercial Sponge Aquaculture Farm. The 20-page manual included the following sections:

- How to Use This Manual,
- Common Questions,
- Sponge Biology,
- Planning a Sponge Farm,
- Managing Your Sponge Farm,
- Harvesting, Cleaning, and Marketing Commercial Sponges,
- Local and Worldwide Markets,
- What If I Have Questions, and
- Glossary.

The video was titled "The Promise of Aquaculture." It will be used to educate legislators and other interested parties about the aquaculture regulatory process.

Impacts

This project met the CTSA goal of disseminating information by producing simple, easily understandable publications that farmers can use.

Support

This project was funded by the Center for Tropical and Subtropical Aquaculture and the University of Hawaii's Sea Grant College Program (SGCP).

		Other Support		
Year	**CTSA**	**SGCP**	**Total Other**	***Total Support***
One	$31,217	$8,000	$8,000	*$39,217*
Total	**$31,217**	**$8,000**	**$8,000**	***$39,217***

Publications, manuscripts, or papers presented

University of Hawaii Sea Grant College Program. 1996. The Promise of Aquaculture. Center for Tropical and Subtropical Aquaculture Video #V001. Waimanalo, Hawaii.

University of Hawaii Sea Grant College Program. 1996. Starting a Successful Commercial Sponge Aquaculture Farm. Center for Tropical and Subtropical Aquaculture Publication #120. Waimanalo, Hawaii.

University of Hawaii Sea Grant College Program. 1996. Shrimp Diseases. Center for Tropical and Subtropical Aquaculture Publication #121. Waimanalo, Hawaii.

University of Hawaii Sea Grant College Program. 1996. Chinese Catfish. Center for Tropical and Subtropical Aquaculture Publication #122. Waimanalo, Hawaii.

University of Hawaii Sea Grant College Program. 1996. Tilapia. Center for Tropical and Subtropical Aquaculture Publication #123. Waimanalo, Hawaii.

University of Hawaii Sea Grant College Program. 1996. *Gracilaria* Gall Syndrome. Center for Tropical and Subtropical Aquaculture Publication #124. Waimanalo, Hawaii.

University of Hawaii Sea Grant College Program. 1996. Video: The Promise of Aquaculture. Center for Tropical and Subtropical Aquaculture Video #V001. Waimanalo, Hawaii.

Aquaculture Extension and Training Support in the U.S.-Affiliated Pacific Islands

Dates of work, August 1989 through July 1996

Funding level

$657,470

Participants

Stephen Lindsay and Dr. Maria Haws, College of Micronesia, Pohnpei, Federated States of Micronesia.

Project objectives

The overall goal of this project is to provide extension and training support to private aquaculturists and to government fisheries and aquaculture staff to develop commercial and subsistence aquaculture crops within American Samoa, the Commonwealth of the Northern Mariana Islands (CNMI), the Federated States of Micronesia (FSM), the Republic of Belau, and the Republic of the Marshall Islands (RMI). Specific objectives related to that goal are to:

- conduct training courses in culture techniques and general biology of aquaculture species;
- provide extension support to private aquaculturists and government fisheries and aquaculture staff to develop commercial and subsistence aquaculture crops within the region;
- help develop and support hatcheries and growout farms for giant clams and other aquatic plant and animal species, including sponges, pearl oysters, seaweed, trochus, and green snails;
- assist in reef reseeding programs and surveys for giant clams, sponges, and other species as requested by local authorities; and
- continue to act as the scientific and aquaculture advisor to the FSM National Aquaculture Center in Kosrae.

Progress and principal accomplishments

This project began in 1988, when the Center for Tropical and Subtropical Aquaculture funded an aquaculture extension specialist for the region. Additional funding has been provided by Sea Grant Extension Service, the Pacific Island Network, the Pacific Aquaculture Development Program, the College of Micronesia, and the Federated States of Micronesia government.

Dr. Christine Crawford served as the extension specialist from 1989 to 1991, when Mr. Stephen Lindsay assumed the position. He resigned in February 1996; Dr. Maria Haws assumed the post from May through July 1996. The extension specialist provided technical advice and assistance to establish the FSM National Aquaculture Center in Kosrae and established a demonstration ocean growout farm on the reef outside the National Aquaculture Center. The specialist assisted with development of the major field site, including building and deploying enough off-bottom culture racks to hold all the clams. A training course in giant clam culture was conducted, after which trainees induced spawning in *T. gigas* and in 6 year-old *Hippopus hippopus*. A project was conducted to encourage the women of Kosrae to grow clams on the reef outside their homes. The specialist continues to act as the scientific and technical advisor to the facility and makes site visits to all the giant clam culture facilities in the region upon request.

Training courses in all aspects of giant clam spawning and culture were conducted in various locations throughout the region. Assistance was provided with giant clam reef reseeding programs, and surveys for giant clams, sponges, and other species were completed as requested by local authorities.

A series of lectures on aquaculture topics were presented to science classes at the American Samoa Community College and at the FSM Community College and to groups of Peace Corps volunteers in the FSM. In addition, lectures on pearl farming and giant clam culture were given to staff of the American Samoa Department of Marine and Wildlife Resources and to staff of the Marshall Islands Development Authority.

A giant clam demonstration farm was established in Pohnpei, FSM. Three sponge demonstration farms were established in various states of the FSM, and assistance was provided in marketing a colonial tunicate that grows on sponge farming lines. All giant clam culture facilities in the region received information on aquarium markets, local and international food markets, and reef reseeding. A private giant clam wholesaler in Saipan was assisted with obtaining the necessary permits and provided with information on clam availability and pricing from hatcheries in the region.

Literature and advice on aquaculture of various species were provided to private concerns and government agencies in the CTSA region. The species covered included mangrove crabs, pearl oysters, marine shrimp, freshwater prawns, marine sponges, live rock, soft coral, giant clams, mullet, grouper, trochus, eels, sea cucumbers, tilapia, and ornamental species. Field site evaluations were done in several cases. Approximately 3 percent of those who requested information and advice initiated aquaculture projects.

All locations were provided with information on the documentation required under the Convention on International Trade in Endangered Species (CITES) and by U.S. Fish and Wildlife Service to allow the export of giant clam products. The Republic of Belau, the Republic of the Marshall Islands, and the Federated States of Micronesia have completed the necessary steps and obtained permits to export giant clam products for non-food uses.

Year 7 progress and principal accomplishments

Objective: *Conduct training courses in culture techniques and general biology of aquaculture species*

Each year, the specialist conducts aquaculture training courses throughout the region. During the project's seventh year, the specialist conducted these training courses in all aspects of aquaculture.

- A training course in the culture of the marine gastropods trochus and turbot was conducted in the Marshall Islands.
- In June, an aquaculture workshop held in the Marshall Islands was attended by more than 40 people. The workshop included presentations on planning aquaculture projects, conducting feasibility studies, and pearl oyster culture.
- Presentations on various aquaculture topics were given for schools and the general public throughout the region. Presentations included "Aquaculture in the Pacific," "Reproduction in Tropical Bivalves and Gastropods," "Giant Clam Farming," "Marine Gastropod Farming," "Pearl Oyster Culture,""General Aquaculture," and "Aquaculture in the U.S.-Affiliated Pacific Islands."

Objective: *Provide extension support to private aquaculturists and government fisheries and aquaculture staff to develop commercial and subsistence aquaculture crops within the region.*

The agent continued to provide information on aquaculture of various species in response to requests from parties throughout the region. The information covered mangrove crab, marine and freshwater shrimp, sponges, soft coral, giant clams, pearl oysters, aquacultured live rock, freshwater aquarium fish, and marine ornamental fish.

Subscriptions to a relevant magazine were provided to operators of giant clam facilities, including the Marshall Islands Marine Resources Authority (MIMRA), Robert Reimers Enterprises (RRE), FSM National Aquaculture Center (NAC) in Kosrae, and the American Samoa Department of Marine and Wildlife Resources (DMWR).

Slide presentations and accompanying written materials on general aquaculture were developed. In addition, the project provided funding to produce a manual and accompanying video titled “Clams to Cash: How to Make and Sell Giant Clam Shell Products.” The manual and video provide details on how to produce value-added products from giant clam shells.

Objective: *Help develop and support hatcheries and growout farms for giant clams and other aquatic plant and animal species, including sponges, pearl oysters, seaweed, trochus, and green snails.*

Regional aquaculture businesses were assisted with developing markets for their products. Assistance with marketing and business matters remains a major concern of regional aquaculturists, most of whom have had marketing difficulties and have extensive questions regarding the topic.

Objective: *Assist in reef reseeding programs and surveys for giant clams, sponges, and other species as requested by local authorities.*

A black-lip pearl oyster stock assessment was conducted in Arno Atoll, RMI, and three people were taught survey techniques.

Work planned

A new extension agent will be hired by the end of 1996. The project will continue to provide extension support and training throughout the region. In addition, the manual and video on pearl oyster culture will be published and at least one workshop on soft coral culture will be held in the Marshall Islands.

Impacts

This project has provided vital technical assistance in all phases of aquaculture to farmers and government employees within the CTSA region. Both private and government aquaculture projects benefit from the expertise of an aquaculture extension specialist without incurring any costs for the assistance.

Support

This project received funding from the Center for Tropical and Subtropical Aquaculture (CTSA), the Federated States of Micronesia government (FSM), the Sea Grant Extension Service (SGES), the Pacific Island Network (PIN), the College of Micronesia (COM), the Pacific Aquaculture Development Program (PADP), and the United Nations Food and Agriculture Organization (FAO).

Extension and Training Support for the U.S.-Affiliated Pacific Islands

		Other				Support		Total	*Total*
Year	CTSA	FSM	COM	SGES	PIN	PADP	FAO	Other	*Support*
One	$100,000	$24,000	$7,000	$5,000	$4,000	$10,000	$0	$50,000	*$150,000*
Two	$85,870	$26,700	$7,000	$4,500	$4,500	$4,500	$2,500	$49,700	*$135,570*
Three	$83,600	$27,754	$7,000	$10,800	$10,800	$21,000	$6,000	$83,354	*$166,954*
Four	$70,000	$15,000	$2,000	$0	$6,000	$4,000	$0	$27,000	*$97,000*
Five	$75,000	$0	$3,000	$0	$2,000	$10,000	$0	$15,000	*$90,000*
Six	$98,000	$0	$3,000	$2,000	$2,000	$16,000	$0	$23,000	*$121,000*
Seven	$70,000	$0	$3,000	$2,000	$2,000	$12,000	$0	$19,000	*$89,000*
Eight	$75,000	$0	$3,000	$2,000	$2,000	$19,000	$0	$26,000	*$101,000*
TOTAL	**$657,470**	**$93,454**	**$35,000**	**$26,300**	**$33,300**	**$96,500**	**$8,500**	**$293,054**	***$950,524***

Publications, manuscripts, or papers presented

Crawford, Christine. 1991. Giant Clam Mariculture Information Sheet #2: CITES Requirements for the Export of Giant Clams. College of Micronesia. Kosrae, Federated States of Micronesia.

Crawford, Christine. 1991. Giant Clam Mariculture Information Sheet #3: U.S. Food and Drug Authority Ruling on Giant Clams. College of Micronesia. Kosrae, Federated States of Micronesia.

Crawford, Christine. 1992. A Review of U.S. Food and Drug Administration Requirements to Market Giant Clam Meat in the United States of America. CTSA Publication #109. Waimanalo, Hawaii.

Haws, Maria. 1996. Gems from the Sea: A Pearl Oyster Culture Manual. Center for Tropical and

Subtropical Aquaculture Publication #127. Waimanalo, Hawaii.
Heslinga, Gerald. 1996. Clams to Cash: How to Make and Sell Giant Clam Shell Products. Center for Tropical and Subtropical Aquaculture Publication #125. Waimanalo, Hawaii.
Heslinga, Gerald. 1996. Clams to Cash: How to Make and Sell Giant Clam Shell Products. Center for Tropical and Subtropical Aquaculture Video #V002. Waimanalo, Hawaii.
Lindsay, S. R 1991. Survival and Growth of Introduced Populations of the Giant Clam, *T. derasa*, on the Island of Yap Proper and the Outer Atoll of Woleai, FSM. Yap Marine Resources Division. Yap, FSM.

Gill Discoloration in *Penaeus stylirostris*

Dates of work, May 1994 through April 1996

Funding level

$17,300

Participants

Dr. Ilse Silva-Krott, College of Agriculture and Life Sciences, University of Guam
Dr. Jim Brock, State of Hawaii Aquaculture Development Program
Jeff Tellock, Guam Aquaculture Development and Training Center, Guam Department of Commerce

Project objectives

The objective of this project is to identify the primary and secondary causes of black gill discoloration in market-sized penaeid shrimp cultured in Guam.

Reason for termination

This project was terminated because all objectives were completed.

Progress and principal accomplishments

Objective: *Identify primary and secondary causes of gill discoloration in market-sized penaeid shrimp in Guam.*

This project entailed observation of shrimp growth and identification of lesions that occurred during growout of *Penaeus stylirostris* during dry and rainy seasons in earthen ponds. Samples of bottom soil taken from two shrimp ponds in July and December 1994 were analyzed to assess acid/alkaline balance, phosphorus, potassium, sodium, calcium, and magnesium. All were found to be within normal limits. Pond 2 was abandoned because of water leakage, and pond 2A was used instead. No soil samples were taken from pond 2A. Pond water samples were taken before stocking and biweekly thereafter and assessed for pH, oxygen saturation, salinity, ammonium contents, and temperature. Results showed fluctuations in salinity and elevated water temperatures.

Shrimp ponds were stocked at a density of 35 per square meter. Shrimp were sampled monthly, weighed, and examined for gross and histological abnormalities. The growth rate of shrimp in pond 1 averaged about 1.17 grams per week with an average weight of 18 grams at harvest after 125 days of growout. Shrimp in pond 2A grew an average of 0.7 gram per week and weighed an average of 19.6 grams at harvest after 185 days of growout. No significant mortalities occurred.

One hundred and four histological preparations of shrimp sampled from pond 1 were reviewed. Protozoa of *Zoothamium* spp. were found frequently and classified as incidental. Mild to moderate gill melanosis was seen in 43 preparations, and three cases showed stunted, thickened gills. Gill discoloration occurred during a dry period in October 1994. Water temperature rose as high at 36°C at the time, creating less than optimal conditions for the shrimp and possibly favoring the growth of disease-causing organisms. Three shrimp had hepatopancreatic granulomas with melanosis; it was, in one case, associated with an abdominal chronic granulomatous lesion. Hepatopancreas was normal with the exception of two cases in which it atrophied, suggesting reduced food intake. At harvest, the shrimp were collected by net, washed in fresh water, put on ice mixed with salt, and immediately transported to restaurants and stores. The manager did not report any problems with the sale of this harvest. Refrigeration of harvested shrimp over several days did not cause gill discoloration.

Animals sampled from pond 2A were found to have a high frequency of gill-fouling organisms present; histologic sections of shrimp samples taken in June 1995, showed numerous inflammatory gill lesions with occasional necrosis. Protozoa were present on 58 percent of all shrimp sampled; 23 percent of those had gills and mouth parts covered with protozoa with characteristics compatible with *Acineta* spp. rather than the *Zoothamium* spp. present earlier. These protozoa were found occasionally in high numbers; infection with them may be the cause of gill inflammation and necrosis. Infection of mouth parts may interfere with feeding. Forty-seven percent of shrimp collected in June had black gill disease concurrent with inflammatory gill lesions. Gross examination showed *Zoothamium* spp. protozoa on gills of 73 percent of shrimp harvested in September. Black gill lesions were not apparent. Refrigeration of harvested shrimp over several days did not cause gill discoloration. A total of 100 histological sections were reviewed. Other observations of shrimp culture conditions included highly crowded pond conditions, which were likely to further increase the spread of protozoa and other agents inducing inflammatory gill lesions.

Relatively long growout periods often increase the incidence of disease. The feeding technique consisted of a combination of hand feeding and timed automatic feeders. Lack of food probably slowed growth rates. A total of 1,200 pounds of shrimp were harvested from pond 2A. Harvesting was done as described for pond 1; no problems were reported with the sale of these shrimp.

Impacts

This project directly benefitted shrimp farmers in Guam by identifying causes of gill discoloration and producing an extension fact sheet that described ways to improve shrimp health. The project indirectly benefited shrimp farmers by providing basic data about shrimp growth rates, shrimp health, and water parameters in local ponds. In addition, the study resulted in recommendations to improve shrimp culture techniques being made to pond owners and managers. The manager of one shrimp farm improved the management techniques regarding pond preparation, feeding, and water quality. This resulted in reduced incidence of disease, improved growth rates, and shortened growout.

Support

This project, initiated under the Center for Tropical and Subtropical Aquaculture's (CTSA) Seventh Annual Plan of Work, received funding from CTSA, the University of Guam (UOG), the Hawaii State Aquaculture Development Program (ADP), and the Guam Department of Commerce (DOC).

		Other Support				
Year	**CTSA**	**UoG**	**DOC**	**ADP**	**Total Other**	**Total Support**
One	$17,300	$5,000	$3,000	$1,000	$9,000	$26,300
Total	**$17,300**	**$5,000**	**$3,000**	**$1,000**	**$9,000**	**$26,300**

Publications, manuscripts, or papers presented

College of Agriculture and Life Sciences, University of Guam. 1996. Aquafarmer Information Sheet for Prevention of Black Gill Disease in Marine Shrimp. Center for Tropical and Subtropical Aquaculture Publication #126.

Mangrove Crab as a Model for Development of a Quarantine System to Screen Species for Aquaculture in Guam

Dates of work, April 1994 through August 1996

Funding level

$50,234

Participants

Dr. Ilse Silva-Krott, Dr. John Brown, Dr. Robert Barber and David Crisostomo, College of Agriculture and Life Sciences, University of Guam
Dr. Jim Brock, Hawaii State Aquaculture Development

Project objectives

The overall goal of this project is to establish an aquatic animal quarantine area, which will be used to screen species for pathogens, at the University of Guam Marine Laboratory. Specific objectives related to that goal are to:

- construct an aquatic animal quarantine facility;
- capture local juvenile through adult mangrove crabs and transfer them into the quarantine area
- screen incoming and enhancement-reared crabs for biotic agents;
- identify a list of pathogens, diseases, and syndromes found in wild caught mangrove crabs;
- formulate pathogen and disease risk assessment for introduction of feral mangrove crab broodstock to the Guam Aquaculture Development and Training Center (GADTC);
- evaluate the economic feasibility of commercial scale mangrove crab production in Guam.

Reason for termination

This project was terminated because all the objectives were completed.

Progress and principal accomplishments

Objective: *Establish an aquatic animal quarantine area at the University of Guam Marine Laboratory.*

An area at the Marine Laboratory was identified for the quarantine facility. The area was fenced, and two 975-gallon tanks were installed. The tanks were connected to 5-micron and 1-micron cartridge water filters and a UV-sterilizer on both inflow and effluent water. An appropriate effluent water pump and a float valve were installed, and plywood covers were placed over the tanks to keep debris out. Holding baskets with plastic glass dividers were installed in the tanks. An official from the Guam Division of Aquatic and Wildlife Resources (DAWR) visited the facility and suggested several improvements that will be carried out. Project investigators are working closely with the DAWR in order to have the system approved within the next year.

Objective: *Capture local juvenile through adult mangrove crabs and transfer them into the quarantine area for pathogen and disease studies.*

A boat for setting crab traps was purchased. Nine crab traps were built and six more are being built. Four traps were deployed in the Ylig River, and two traps were deployed in Ylig Bay in early June. A total of 12 crabs were caught. Gross examination revealed no lesions or parasites. Two crabs were dissected for histopathology. The remaining crabs were placed in the holding baskets, which were abandoned after 1 week because the crabs destroyed them. The animals were transferred to individual buckets suspended under a drip water distribution system within the quarantine tank.

Objective: *Identify a list of pathogens and disease syndromes found in wild caught mangrove crabs.*

A pathology configuration microscope has been purchased and is being used for the histopathological studies on the mangrove crabs.

Objective: *Evaluate the economic feasibility of mangrove crab production in Guam as a prelude to further work in the area.*

The mangrove crab (*Scylla serrata*) reaches more than 20 centimeters in carapace width and more than 2 kilograms in weight. It naturally ranges from South Africa to Hawaii and from Japan to New Zealand and is indigenous to Guam. Due to a lack of habitat and strong local demand, Guam imports approximately 3,000 live crabs per month. The crabs retail for $8.25 to $8.75 per pound and have a ready market.

Investigators obtained mangrove crab production data from the Tungkang Marine Laboratories in Taiwan and the National Prawn Production Center in Malaysia and reviewed the literature on crab culture in the Philippines. They also visited crab farms, crab fattening operations, and crab sellers in Malaysia and Taiwan. Based on the information gathered, investigators project that mangrove crabs could be commercially cultured in Guam assuming the following three points:

- crabs could be cultured in ponds along with milkfish (This would necessitate displacing the milkfish on a pound-for-pound basis with crabs in polyculture.);
- the physical management of the pond would not need to be modified, so no additional costs would be incurred;
- commercial culture on Guam would use a maximum stocking density of one crab per square meter.

The investigators projected the following production parameters for Guam: 25-gram crabs would be stocked at a density of 1.0 crab per square meter, grown out for 180 days at an average rate of 1.75 grams per day to an average size of 340 grams. The projected survival rate is 75 percent and the project feed conversion ratio is 2.5. These estimates of the physical production parameters are derived from data from the other locations and have not been tested on Guam. However, given these points, investigators estimate that replacing 3,000 pounds of milkfish per acre of pond with mangrove crabs would yield a net gain of more than $9,000 per acre. Even using a stocking density of one crab per 2 square meters would result in a net gain of more than $4,500 per acre. In fact, the preliminary analysis indicates that the addition of mangrove crabs to milkfish ponds will always increase the profitability of the ponds.

Impacts

Aquaculture in Guam primarily produces tilapia, milkfish and shrimp. Diversification is necessary to develop the industry, but the Guam government has stringent regulations regarding importation of species. This project provided a sorely needed quarantine facility at which imported aquaculture species can be held in order to ensure their health status prior to introduction to farms or the Guam Aquaculture Development and Training Center's hatchery. Mangrove crabs were used as a test species for the quarantine facility because they could potentially be a valuable aquaculture crop in Guam.

		Other support			
Year	**CTSA**	**UOG**	**ADP**	**Total Other**	**Total Support**
One	$50,234	$18,000	$1,000	$19,000	$69,234
Total	**$50,234**	**$18,000**	**$1,000**	**$19,000**	**$69,234**

Support

This project was funded by the Center for Tropical and Subtropical Aquaculture (CTSA), the University of Guam (UOG), and the Hawaii State Aquaculture Development Program (ADP).

Publications, manuscripts, or papers presented

None

Disease Management for Hawaiian Aquaculture

Dates of work, April 1993 through October 1996

Funding level

$209,659

Participants

Dr. James Brock and Dee Montgomery, Hawaii State Aquaculture Development Program
Dr. Brad LeaMaster, Department of Animal Sciences, University of Hawaii
Dr. Rick Spencer, Hawaiian Marine Enterprises

Project objectives

The overall goal of this project is to develop management strategies to minimize losses from diseases at aquaculture farms in Hawaii. Specific objectives related to that goal are to:

- provide aquaculture health management extension support to commercial farms;
- identify contributing factors that may be important to the occurrence of bacterial disease during growout of Chinese catfish (*Clarias fuscus*);
- field test a preventive strategy to mitigate losses of cultured Chinese catfish (*Clarias fuscus*) during growout in Hawaii due to two bacterial diseases, *Aeromonas hydrophila* and *Edwardsiella tarda septicemia*;
- screen juvenile and adult cultured Chinese catfish and tilapia (*Oreochromis mossambicus*) for potential pathogenic fish viruses;
- assess the infectivity of IHHN virus in feces after passage through the digestive tract of a species of water bird;
- provide diagnostic and health management support to the CTSA-funded project titled "Ornamental Aquaculture Technology Transfer" and implement management practices and standard disease treatment strategies to improve fish survival and reduce the abundance of pathogenic parasites in imported groups of freshwater tropical aquarium fish;
- document the principal ectoparasites and assess their effects on cultured tilapia and mullet in a traditional Hawaiian fishpond;
- assess samples of *Gracilaria* spp. for the presence of *Gracilaria* Gall Syndrome (GGS), determine how the syndrome is transmitted, and identify potential chemical controls for it.

Anticipated benefits

This project focuses on problems that directly affect production of crops in Hawaii's aquaculture farms and provides disease screening for other CTSA-funded projects. Examples of assistance include developing procedures for control of bacterial diseases in cultured Chinese catfish, initial investigation of a serious new disease in cultured seaweed, a study to increase the understanding of transmission of selected viruses in cultured shrimp, documentation of ectoparasites on fish cultured in Hawaiian fishponds, and work focusing on disease control in ornamental fish culture in Hawaii. These efforts will improve production of Hawaii aquaculture facilities or procure new information that will eventually lead to improved health management strategies for improved aquaculture productivity in Hawaii.

Progress and principal accomplishments

Objective: *Provide aquaculture health management support to commercial farms.*

During year 1, investigators made 80 site visits to 10 farms to provide health management extension assistance under this project. During year 2, investigators made 240 site visits to 10 aquaculture farms on Oahu and Hawaii to provide health management extension assistance.

In May 1994, a serious disease outbreak occurred in farmed *Penaeus vannamei* in Kahuku, Hawaii. It caused mortality rates higher than 95 percent within 14 to 30 days of stocking. Studies initiated to determine the cause and a means of controlling the disease led to the discovery of a new shrimp virus in Hawaii. The virus was thought to be the Taura Syndrome agent, which was confirmed in subsequent studies by other laboratories. Additionally, this study found that

Hawaii farm that was affected by Taura Syndrome then began culturing *P. stylirostris* and achieved production levels equal to or greater than those achieved with *P. vannamei.*

Two bioassay studies were conducted to assess the effect of vaccination with a commercial bacterial lipopolysaccharide (LPS) preparation on acute, lethal exposure to TSV in juvenile SPF *Penaeus vannamei*. High mortality was observed after TSV exposure in both the vaccine and the control groups of shrimp.

Kahuku Shrimp Company's reproduction and hatchery operations have had limited production due to technical, feed, and disease problems. This project provided advisory and diagnostic assistance to the farm. Nauplii production improved, but hatchery output continued to be unstable.

Diagnostic testing as well as assessment of the population impact from IHHNV infection are being done at a commercial shrimp farm. Shrimp populations in all areas of the farm are being monitored. During year 3, evaluation of 116 specimens by dot-blot gene probe indicated that IHHNV infection was in the growout area of the farm, but not in the broodstock, reproduction, or hatchery facility. Because the farm was working with *P. stylirostris*, the initial strategy for control in growout has been to switch to *P. vannamei*. This strategy appears to be working; Taura Syndrome has not recurred on the farm.

Work continued to test hydrogen peroxide, which the Food and Drug Administration (FDA) has classified as a low regulatory priority compound for aquaculture, as a chemical control agent for treatment of common ectoparasite infestation of cultured freshwater and marine fish. During this reporting period, juvenile moi (*Polydactylus sexfilis*) with *Amyloodinium* spp. infection of the gills were treated with hydrogen peroxide to determine the fish's tolerance to hydrogen peroxide (e.g. concentration and duration) and to test the compound's efficacy as a chemical control of *Amyloodinium*.

Juvenile moi were found to tolerate exposure to hydrogen peroxide at 150 ppm for 30 to 60 minutes. Twenty-six fish were used in the study; 13 were hydrogen-peroxide treated and 13 were untreated control fish. Gill wet-mounts of treated juvenile moi showed significantly reduced numbers of *Amyloodinium* spp. This indicates that hydrogen peroxide may be an effective chemical for the treatment of ectoparasite disease caused by *Amyloodinium* species.

Objective: *Identify contributing factors that may be important to the occurrence of bacterial disease during growout of Chinese catfish (*Clarias fuscus*) and develop practical strategies for the control of bacterial diseases during their growout.*

During year 1, scientists in Thailand who have experience with disease management in freshwater aquaculture fishes in Asia were contacted for information. They forwarded a series of publications on the culture, environmental quality and disease problems for *Clarias* spp. The publications provided comparative information on diseases that have been a problem in Hawaii.

A study was initiated to determine the occurrence and severity of disease episodes in Chinese catfish reared in tanks. The study tracked the occurrence and severity of disease episodes in six tanks of catfish given the same feed. Fourteen dead fish were retrieved from the six tanks during the first sampling period. Those fish and, to a far lesser degree, the fish sampled for weighing showed physical changes that suggested internal bacterial infection, such as swelling of the abdomen over the anterior lobes of the kidney or small skin sores.

Investigators collected a set of water specimens from the six tanks and measured bacterial levels. The bacterium *A. hydrophila* was retrieved in samples from five of the tanks; the bacterium *E. tarda* was not identified from any of the samples.

Skin scrapings from a sample of five fish per tank were done to monitor the prevalence and the relative abundance of ectoparasites. The scrapings showed two types of ectoparasites, *Tricodina* spp. and *Gyrodactylus* spp., that are commonly associated with cultured Chinese catfish in Hawaii. The findings suggest that *Tricodina* spp. infestation had declined in the older groups of fish. *Gyrodactylus* spp. were found in fish from only one tank.

An initial database was developed on the physical and chemical water quality parameters in Chinese catfish culture tanks. Water samples were collected four times over approximately 24 hours. The samples were evaluated for temperature, dissolved oxygen, pH, carbon dioxide, hydrogen sulfide, secchi disc turbidity, hardness, alkalinity, chloride, total ammonia, nitrite, nitrate, and ortho-phosphate.

Water quality and commercial diet factors were evaluated in relation to the onset of disease episodes in Chinese catfish populations under farm conditions. The initial sample findings suggested that juvenile Chinese catfish can tolerate large diurnal variations in temperature, dissolved oxygen, carbon dioxide, and pH without the occurrence of high mortality episodes of bacterial or ectoparasitic disease. However, evaluation of the physical and chemical measurements during disease outbreaks suggested a positive correlation between occurrence of disease and elevated levels of ammonia or nitrate. Elevated levels of these compounds were associated with one of two factors: either the water supply is temporarily lost due to mechanical or electrical failure, or the phytoplankton/biological filtration community in the culture tank failed.

Bacterial pathogens were isolated from dead Chinese catfish during disease outbreaks. *A. hydrophila* accounted for an average of 80 percent of viable bacteria in the water samples. *E. tarda* rarely was isolated from catfish culture tank water. In addition, the bacterial pathogen *A. hydrophila* is the dominant flora in the water of these catfish culture tanks during periods of minimal losses to bacterial infection. This suggests that the animals are normally exposed to relatively stable numbers of *A. hydrophila* continuously throughout the culture period and that disease events involving this pathogen involve the contribution of more etiological variables than the bacteria and the host fish.

During year 3, vitamin C fortified feed was obtained for a trial to examine effect of high dietary vitamin C as a treatment to reduce bacterial disease losses in Chinese catfish farmed in Hawaii. Six cages were installed in the Hawaii Fish Company's reservoir in Mokuleia. Three hundred juvenile Chinese catfish will be stocked into each of the six cages for the trial, which will be conducted from September through December 1996.

Objective: *Field test a preventive strategy to mitigate losses of cultured Chinese catfish during growout in Hawaii due to two bacterial diseases,* Aeromonas hydrophila *and* Edwardsiella tarda septicemia*.*

During year 1, an isolate of *Aeromonas hydrophila* was propagated and used to produce an autogenous bacterin for a vaccination trial that was conducted in year 2. Fifty-four days following inoculation challenge with a known lethal dose of the same strain of *A. hydrophila*, survival of the bacterin-treated fish was 53 percent compared to 24 percent for the unvaccinated control group. No antibody titer to A. *hydrophila* was measured in the saline-vaccinated control fish tested in either the day 22 or day 54 samples. The results suggest that vaccination may be a practical tool to advert or reduce *A. hydrophila*-induced mortality of farmed Chinese catfish.

An autogenous formalin-killed bacterin was prepared from *Edwardsiella tarda* isolated from diseased Chinese catfish. In February and April when water temperatures were cooler, vaccination-plus-booster-plus-challenge trials were conducted with juvenile Chinese Catfish following a similar protocol to that applied in the *A. hydrophila* study. Following inoculation challenge with a known lethal dose of the same strain of *E. tarda*, survival was low in both the vaccinated and the control groups.

The two *E. tarda* vaccination trials gave different results than the trial with *A. hydrophila*: exposure of the *E. tarda* vaccinated fish did not elicit development of a serum antibody response, and no inoculation challenge was observed. Although further study is necessary before the reason is understood for the lack of an antibody response in the experimental fish, cooler water temperature is a plausible explanation. If this is the reason, then seasonal temperature changes will be an important criteria in the administration of bacterins to Chinese catfish.

Saprolegnia spp. and perhaps other aquatic saprophytic fungi, caused losses of eggs and post-hatched fry Chinese catfish. The Food and Drug Administration's Center for Veterinary Medicine (CVM) classified hydrogen peroxide as a low regulatory priority compound. Hydrogen peroxide was evaluated as a treatment for fungal infection of Chinese catfish eggs and fry at two farms. Chinese catfish hatching tanks dosed at 300 to 500 ppm hydrogen peroxide for 15 minutes as a single or multiple treatments had increased hatch of catfish fry and obviously lower presence of fungal mats than untreated control tank batches. These observations indicated that hydrogen peroxide is a useful chemical control for fungal infections of Chinese catfish eggs and fry.

Hydrogen peroxide was also tested as a treatment to control two Chinese catfish ectoparasites, *Gyrodactylus* spp. and *Trichodina* spp. Juvenile Chinese catfish were found to tolerate 30-minute treatment with 250 ppm hydrogen peroxide. Twenty-four hours after treatment, no *Gyrodactylus* spp. or *Trichodina* spp. were observed in wet-mounts of skin scrapings from the treated catfish, but an average of 18.4 *Gyrodactylus* spp. and 12.4 *Trichodina* spp. per fish appeared in wet mounts of skin scrapings of untreated control fish. This indicates that hydrogen peroxide may be useful as a chemical treatment for the control of ectoparasite infections in Chinese catfish.

During year 3, three preliminary experiments were conducted to test the hypothesis that elevated ammonia in Chinese catfish culture water results in the fish becoming more susceptible to lethal bacterial *septicemia* from *Aeromonas hydrophila*.

The first trial tested the feasibility of a static bath exposure to ammonia to find an ammonia level which, with moderate duration exposure (e.g., 5 to 10 days), was likely to be stressful but not lethal to juvenile Chinese catfish. Water for the trials had total hardness and alkalinity of 150 to 170 mg liter and 60 to 80 mg per liter, respectively. A 100-gallon treatment tank and a 100-gallon control tank were set up in the Anuenue Fisheries Research Center (AFRC) hatchery. Each tank was stocked with 10 juvenile Chinese catfish. One day after stocking, ammonium chloride was added to the treatment tank to achieve a total ammonia level of about 5 mg per liter. Daily thereafter for 6 days, total ammonia, pH, and temperature were measured in both tanks. Total ammonia, pH, and temperature varied from 4.0 to 5.2 mg per liter, 7.4 to 7.6, and 27 to 30°C, respectively, in the treatment tank and from 0.5 to 0.6 mg per liter, 7.2 to 7.6, and 27 to 30°C, respectively, in the control tank. The catfish in both groups fed actively and showed no difference in behavior or survival.

On day 7, additional ammonium chloride was added to the treatment tank to achieve a calculated total ammonia level of about 20 mg per liter. This ammonia level is at least twice as high as the highest level measured in local Chinese catfish culture systems. Total ammonia, pH, and temperature level varied from 22 to 24 mg per liter, 6.9 to 7.2, and 29.5 to 30°C, respectively, in the treatment tank and from 1.0 to 1.5 mg per liter, 6.9 to 7.2, and 28 to 29.5°C, respectively in the control tank. The fish were held for 4 days under these water conditions with no apparent difference between the control or the treatment groups.

Water in the two tanks was exchanged, and the temperature allowed to stabilize for 24 hours. Sodium carbonate was added to increase the pH in both the treatment and control tanks. The pH varied between 8.9 and 9.6 over a period of 2 days in the two tanks. Fish in both tanks displayed no adverse behavior and fed well. Ammonium chloride was added to the treatment tank to achieve an estimated 5 mg per liter of total ammonia. The fish remained active over the 4-day test period in both the control and treatment tanks. The pH level varied from 8.5 to 8.9 during this period, but total ammonia began to decline. Nitrite and nitrate were found to be elevated in the treatment tank water.

Both tanks were drained, cleaned, filled, and restocked with the juvenile catfish. After 1 day of acclimation, sodium carbonate was introduced to each tank, and, after several hours, ammonium chloride was added to achieve about 10 mg per liter total ammonia in the treatment tank water. The fish were held under these conditions for several days, and then the trial was terminated. Total ammonia and pH remained elevated in the treatment tank through the exposure period with no apparent adverse effect on the fish.

The results of the trials suggested that Chinese catfish could tolerate moderate duration exposure to total ammonia of about 10 mg per liter in water with pH of 8.5 to 9.0 and temperature of 28 to 30°C. This would result in the fish being held in water with an unionized ammonia concentration in the approximate range of 2 to 4 mg per liter. The level generally regarded as safe is 0.02 mg per liter for prolonged exposure to unionized ammonia in freshwater fish. This protocol subjects the experimental fish to about 100 times the safe level for a moderate duration, which should be sufficiently high to elicit a stress-induced effect on the immune function of Chinese catfish, if ammonia has this effect on this species of fish.

Preliminary studies to test the protocol for injection exposure of Chinese catfish to a predetermined concentration of the bacterium, *Aeromonas hydrophila*, demonstrated that an inoculum of about 105 viable bacteria per fish can be delivered for the bacterial challenge aspect of the study.

Objective: *Screen juvenile and adult cultured Chinese catfish and tilapia (*Oreochromis mossambicus*) for potential pathogenic fish viruses.*

During year 1, 60 tilapia of various ages and sizes were collected from five Oahu locations for a virus isolation study. Evaluation of various organ tissues revealed no viruses. These results support findings from previous tilapia disease cases on Oahu. Both wild and cultured tilapia (*Sarotherodon melanotheron* and *Oreochromis mossambicus*) populations have been afflicted by a previously unrecognized syndrome that causes high mortalities and has negatively affected production at several Oahu farms. Analysis of dying tilapia from various Oahu sites suggests that the cause is an intracellular rickettsia-like organism (RLO). Dead and dying Chinese catfish from an Oahu farm that has a history of chronic disease problems were evaluated by cell culture methods for viruses. Evaluation of various organ tissues showed no evidence of an infectious virus.

During year 2, about 300 juvenile to adult tilapia collected from five locations on Oahu were tested for pathogenic viruses. The results indicate that pathogenic viruses are not obviously present in cultured populations of tilapia on Oahu.

However, efforts under this objective were re-focused after the outbreak of a new disease observed in both wild and cultured tilapia populations on Oahu. *S. melanotheron* and *O. mossambicus* juveniles to adults were found susceptible to the disease, which has resulted in a high rate of mortality of fish populations. The disease has negatively affected production on the majority of commercial tilapia farms on Oahu.

Pathologically, tilapia affected by this syndrome have multiple, large pyo-granulomas systemically. An intracellular bacteria-like organism was identified by special histological stains and in transmission electron microscopy preparations of lesions from affected fish. Attempts to culture the organism on a variety of artificial bacterial media have been unsuccessful, as have attempts to grow the intracellular organism in cell culture.

Work on this new tilapia disease revealed the causative agent to be a species of rickettsia. Further work on the agent and the disease are ongoing. The Hawaiian tilapia RLO is the most important disease facing tilapia farmers on Oahu because of the disease's potential to cause high mortalities of fish.

Objective: *Field test an approach for IHHNV decontamination of shrimp ponds.*

During year 1, six ponds at the site of the former Amorient Aquafarm in Kahuku, Hawaii, were selected for the study. All six ponds were drained, and the soil's moisture content and pH balance were measured. The three control ponds were refilled with water within 3 days of draining. The surfaces of the three remaining ponds were spread with a layer of lime at a rate of 2,500 pounds per acre and were left undisturbed for 2 weeks.

Investigators sampled soil from the limed ponds weekly to determine the moisture content and pH level. The lime distribution was uneven, so the pH rose to 11 in some areas while remaining unchanged in other areas. After 2 weeks, the limed ponds were refilled and stocked at a per-acre rate of 60,000 *Penaeus vannamei* and 10,000 *P. stylirostris*; the latter are highly susceptible to IHHNV. Results were very disappointing. *P. stylirostris* survival and shrimp production levels were no better in the limed ponds than in the control ponds.

Objective: *Assess the infectivity of IHHN virus in feces after passage through the digestive tract of a species of water bird.*

During year 1, two cages were constructed to hold the two juvenile night herons obtained for the study. The birds adapted well to captivity and readily ate fish and fresh shrimp.

During year 2, three trials were conducted in which IHHNV-infected frozen shrimp carcasses (100 grams) were fed to an avian vector host and the feces from the bird were collected over 5 to 6 days following ingestion of the infected shrimp. The feces collected in the trial have been stored frozen and will be analyzed for IHHNV and infectivity to juvenile *P. stylirostris.* The studies to identify IHHNV in the feces from the bird vector have been postponed because of a backlog of work in our bioassay facility. The bird fecal samples for IHHNV evaluation by PCR-plus dot blot will be examined by the Aquaculture Pathology Group at the University of Arizona.

In year 3, six aquaria were each stocked with juvenile SPF *P. vannamei* from a population shown to be susceptible to TSV infection and disease. The resident avian vector was not fed for 1-day (day 0) because prior work showed that this bird clears the gut of contents within 12 to 18 hours. On day 1, the avian vector was fed 100 grams of previously frozen shrimp carcasses known to harbor infective TSV. The following morning, feces were collected from the plastic sheet on the bottom of the cage, and the majority of the feces were inoculated into the first shrimp tank in the bioassay room. The avian vector was not fed on day 1, and a small amount of droppings (0.5 gram) were collected and inoculated into the water of shrimp tank #2. On day 2, the bird was fed its normal ration of fish, and the plastic sheet was cleaned, disinfected, dried, and replaced on the bottom of the cage. On day 3, the bird feces were collected and inoculated into the water of shrimp tank #3. The process was repeated on days 4 and 5 so that five tank groups of indicator shrimp were exposed to feces of the bird. The sixth tank group of indicator shrimp served as the negative control for the trial.

The bioassay was run through day 21 and terminated. Shrimp survival in the six tank groups was 90 percent or higher, and no clinical indication of Taura Syndrome (TS) appeared in any animals in the study. Histologically, none of the 60 shrimp examined had tissue changes suggestive of TS. These results indicated the absence of transmission of the Taura Syndrome virus from avian feces to the susceptible juvenile *P. vannamei* indicator shrimp.

In addition, a trial was conducted to assess whether the tilapia RLO organism could be transmitted in bird feces to susceptible tilapia. Known RLO-infected dying tilapia were fed to the avian vector over 5 consecutive days. Each day the feces on the plastic sheet covering the bottom of the bird cage were collected and added to the water in a tank containing juvenile, RLO-free tilapia. Thirty days following addition of bird feces neither clinical, gross pathological or histological signs of RLO disease were found in the exposed or the unexposed control groups of tilapia. Survival was 95 to 100 percent in both groups of fish.

These findings indicate that the Hawaiian tilapia RLO does not appear to survive in an infectious form in feces after passage through an avian digestive tract.

Objective: *Test the susceptibility of the endemic caridean shrimp,* Halocaridina rubra*, to infection and disease by the penaeid shrimp viruses IHHN and the newly discovered Taura Syndrome virus (TSV).*

During year 3, work was conducted on two fronts. The first continued studies on birds as vectors of shrimp viruses. The second front tested *H. rubra* as a possible reservoir host for IHHN and TSV.

A colony of about 100 *H. rubra* was established in a 300-gallon holding tank in the isolation area at AFRC. The population of *H. rubra* were provided by Dr. Richard Brock, who collected the animals from a coastal pond in Kona, Hawaii. The virus challenge bioassay trials with *H. rubra* will begin in December 1996.

Objective: *Provide diagnostic and health management support to the CTSA-funded project titled "Ornamental Aquaculture Technology Transfer," and implement management practices and standard disease treatment strategies to improve fish survival and reduce the abundance of pathogenic parasites in imported groups of freshwater tropical aquarium fish.*

Eight groups of freshwater tropical fish, imported for the CTSA-funded project titled "Ornamental Aquaculture Technology Transfer," were evaluated and found to be free of diseases.

During year 1, diagnostic assistance was provided to three farmers who were losing fry and juvenile discus (*Symphysodon discus*) stock. Parasite and water quality problems were found. Parasite treatments were suggested and solutions to the water quality problems were recommended. Water quality monitoring data on temperature, dissolved oxygen, pH, carbon dioxide, alkalinity, hardness, chloride, total ammonia, nitrite, and nitrate are being gathered at one of the ornamental fish farms. The data will help in determining appropriate water quality parameters for tropical fish culture in Hawaii.

During year 2, 13 diagnostic case submissions were processed from the Ornamental Aquaculture Technology Transfer Project. Diagnostic assistance was provided for 26 case submissions of freshwater and marine aquarium fishes. Parasites and water quality problems were found, and recommendations were provided for treatment and improving the environmental conditions in the holding tanks or aquaria.

During year 3, project personnel made 108 trips to 15 farms. One hundred eighty-nine case submissions of samples were processed for laboratory diagnosis. Ten percent of the submissions comprised tropical ornamental fish. These included:

- seaweed held in a mixture of 80 percent seawater and 20 percent UV-sterilized freshwater and
- seaweed held in full strength seawater with no exposure to freshwater.

The effect of adding penicillin to seawater containing GGS-positive seaweed was tested. Preliminary observations suggest that penicillin reduces or eliminates GGS symptoms, which supports previous tests in flask cultures of GGS-positive *Gracilaria*. This finding implies that a bacterial agent may cause GGS. In year 2, further experiments at Hawaiian Marine Enterprises showed that the farm's fresh water supply was not a factor in GGS and that the trace nutrient solution used in seaweed culture was not a GGS contaminant. A series of trials were carried out to determine *Gracilaria* spp.'s tolerance level to each of seven fisheries therapeutants and the efficacy of the chemicals to control clinical GGS.

During the follow-up chemical treatment trials, the HME staff noted that GGS-afflicted seaweed in both chemically treated groups and untreated control groups in test aquaria recovered. Meanwhile, *Gracilaria* grown in the commercial culture tanks on the farm remained afflicted with GGS. This finding, confirmed repeatedly, led the investigators to think that some condition in the greenhouse had a curative effect on GGS. Further experiments led to the hypothesis that constant circulation in the aquaria seemed to have curative effect on GGS-afflicted seaweed.

The commercial seaweed culture tanks were aerated for 12 hours per day. As a result of the experiments, the air blowers in the commercial farm tanks have been run continuously with good results. This indicates that this physical factor can effectively control GGS on this farm. However, another land-based *Gracilaria* farm continues to have GGS outbreaks despite the use of constant aeration.

Impacts

This work had six principal impacts. The first was the demonstration that Chinese catfish respond to vaccination with a formalin-killed preparation of *Aeromonas hydrophila*. This opens the possibility of using vaccination as a means to mitigate disease from *A. hydrophila*.

The second impact was the demonstration of the usefulness of hydrogen peroxide as a means to control fungus infections of eggs and fry and as a chemical control agent for the ectoparasites *Trichodina* spp. and *Gyrodactylus* spp.

The third impact was the discovery that the new tilapia mortality syndrome is caused by a rickettsia-like organism (RLO). The identification of the etiological agent for the disease will help in developing practical control strategies.

The fourth impact was the discovery that Taura Syndrome is caused by a virus. Since the disease was first recognized in Ecuador in 1992, Taura Syndrome was believed to be caused by exposure to banana fungicides. This project, working in cooperation with Dr. Donald Lightner at the University of Arizona, was first to demonstrate that Taura Syndrome was caused by an infectious agent, which was a small virus. Work in several laboratories has confirmed this. This discovery has fundamentally changed the way many shrimp farmers in the Western Hemisphere view Taura Syndrome.

The fifth impact was the experimental determination and later field confirmation that *Penaeus stylirostris* is relatively resistant to disease impacts from Taura Syndrome. This finding led to the use of *P. stylirostris* at a shrimp farm in Kahuku, Hawaii, and the subsequent demonstration of the alternate species approach as a practical option for controlling Taura Syndrome.

The sixth impact was the discovery that continuous aeration reversed the progression of GGS in afflicted seaweed. This discovery led to the change to continuous aeration in the commercial culture tanks on the farm and, to date, an absence of reoccurrence of GGS on the HME farm site.

Work planned

During the next 6 months, investigators plan to:

- conduct further tests for IHHNV and to work with the commercial shrimp producer on containment of the infection to the growout area;
- conduct one or more trials to test the effect of elevated ammonia on susceptibility of juvenile Chinese catfish to injection challenge with a sub-lethal dose of *A. hydrophila*;
- build and stock experimental cages for a trial to assess the effect of low versus high stocking density on survival and production of Chinese catfish;
- conduct a trial to test the effect of high dietary vitamin C in reducing bacterial disease losses in Chinese catfish farmed in Hawaii;
- continue monitoring for IHHNV on shrimp farms in Kahuku. In addition, the temperature will be recorded in moist soil that has been covered with clear plastic and is exposed to direct sunlight;
- initiate IHHNV challenge trials with *H. rubra* and a second trial to assess TSV survival through the digestive tract of the avian vector;
- continue work to develop practical farm measures to mitigate the impact of Taura Syndrome on penaeid shrimp farming; and
- continue providing diagnostic and health management support to the CTSA-funded Ornamental Aquaculture Technology Transfer Project, including providing this assistance to ornamental fish producers and testing the feasibility of hydrogen peroxide treatment for use with ornamental species of fish.

Support

Support was provided by the Center for Tropical and Subtropical Aquaculture (CTSA), the University of Hawaii (UH), the Hawaii State Aquaculture Development Program (ADP), and Sea Grant Extension Service (SGES).

		Other support				
Year	CTSA	UH	ADP	SGES	Total Other	Total Support
One	$41,638	$15,988	$5,329	$0	$21,317	$62,955
Two	$68,116	$10,658	$5,329	$0	$15,987	$84,103
Three	$49,916	$13,323	$5,329	$0	$18,652	$68,568
Four	$49,989	$13,323	$7,600	$1,000	$21,923	$70,912
Total	**$209,659**	**$53,292**	**$23,587**	**$1,000**	**$77,879**	**$286,538**

Publications, manuscripts, or papers presented

Brock, J. A., R. Gose, D. V. Lightner, and K. Hasson. 1995. An Overview on Taura Syndrome, An Important Disease of Farmed *Penaeus vannamei*. In Swimming Through Troubled Waters, Proceedings of the Special Session on Shrimp Farming. Aquaculture ‘95. Browdy, C. L. and J. S. Hopkins (eds.). p. 84-94. World Aquaculture Society. Baton Rouge, Louisiana, U.S.A.

Brock, J. A. 1995. Update on Recent Research Findings on Taura Syndrome of Farmed *Penaeus vannamei*. In Proceedings of the 3rd Simposio Centroamericano Sobre Camaron Cultivado. April 3-5, 1995. Tegucigalpa, Honduras. In press.

Brock, J. A., D. V. Lightner, K. Hasson, and R. Gose. 1996. An Update on Taura Syndrome of Farmed Shrimp in the Americas. In Book of Abstracts, World Aquaculture Society ‘96. Bangkok, Thailand. p. 50.

Klien, M. R. G. 1996. An Epizootiological Study of Chinese Catfish, *Clarias fuscus:* Mortalities on Two Farms in Hawaii. Master of Science Thesis. In Animal Sciences. Submitted to the Graduate Division, University of Hawaii. 70 pp.

Development of Threadfin (*Polydactylus sexfilis*) Fry Production Technology

Dates of work, April 1993 through July 1996

Funding level

$204,500

Participants

Dr. Anthony Ostrowski and Dr. Kenneth Leber, The Oceanic Institute
Dr. Christopher L. Brown, Hawaii Institute of Marine Biology, University of Hawaii
Michael Fujimoto, Anuenue Fisheries Research Center

Project objectives

The overall goal of this 3-year project, initiated under the Center for Tropical and Subtropical Aquaculture's 6th Annual Plan of Work, was to develop the hatchery and nursery culture technology for Pacific threadfin (*Polydactylus sexfilis*). Specific objectives related to that goal are to:

- resolve bottlenecks in threadfin seedstock production, which will involve reducing mortality due to cannibalism by active and passive grading and by preventing cannibal development with optimal culture protocols during the larval rearing and stage 1 nursery phases;
- resolve bottlenecks in threadfin quality, including opercular deformities;
- demonstrate and document threadfin fingerling and fry mass production technology;
- exchange seedstock production technology with Hawaii's aquaculture industry by providing farmers with threadfin eggs and juveniles to conduct commercial hatchery and growout trials and estimating market potential; and
- develop a threadfin culture manual.

Reason for termination

This project was terminated because all the objectives were completed.

Progress and principal accomplishments

The first step in resolving the seedstock production technique was to secure a sufficient quantity of high-quality eggs from captive broodstock. In the first year of the project, investigators conducted a total of 148 spawns that produced 164,907,506 eggs with an average fertilization rate of 78.7 percent.

In an effort to overcome the high mortality rate of larvae during the first 15 days after hatching, investigators conducted 15 larval rearing (days 0 to 25) experiments in year 1. The first trial examined growth rate data from the rotifer feed stage (days 1 to 11). Cannibalism does not appear possible up to day 11. Seven trials compared survival and growth rates of larvae stocked at two different densities 10 eggs per liter and 30 eggs per liter. These trials were inconclusive, producing widely varying harvest densities and larval sizes.

Two trials compared the standard diet provided after day 15 with a diet composed of ground squid, smelt and krill. Five trials examined whether threadfin larvae would accept a pelleted feed diet between days 15 and 20. Efforts to wean these larvae from live feeds with four different types of pelleted feeds were unsuccessful. To date, weaning has taken place only after day 26.

A third goal under this objective was to develop a rearing technology with less than 1 percent mortality per day within the cannibalistic period (days 25 to 50). Investigators conducted a number of nursery stage 1 (days 26 to 40) and nursery stage 2 (days 41 to 60) experiments that examined the effects of grading, feeds and rearing systems on survival. These trials found that cannibalism is a phenomenon that occurs primarily during nursery stage 1 (days 26 to 40). Given optimum conditions of diet and rearing systems, cannibalism can be controlled, and survival rates of 90 percent or more can be achieved consistently during both nursery stages at stocking densities of at least 2 to 3 kilograms of fish per cubic meter.

Nursery stage 1 experiments examined the effects of size grading on survival to day 40. The results provided clear evidence that cannibalism can be significantly reduced by size grading. Ideally, the fish should be graded before day 26; however, grading even at day 26 significantly improved the survival rate during this stage.

Growth rates greater than 20 percent per day in nursery stage 1 and 10 percent in nursery stage 2 at feed conversion ratios approximating 1:1 can be obtained on pelleted, practical feeds. That means that for each kilogram of feed that a fish eats, the fish will grow 1 kilogram.

Another set of nursery stage 1 trials compared four diets, which were Rangen Nippai Brine shrimp flake feed (RNB); Rangen Salmon Elite (RAN); Oceanic Institute Mahimahi Nursery Diet (OIM); and Moore-Clarke Mahimahi diet (MCM), and three rearing systems, round tanks, oval raceways, and doughnut-shaped circular tanks.

Overall survival was highest for fish raised in oval raceways — 86 percent compared to 71 percent in doughnut tanks and 60 percent in round tanks. The combination of the OIM diet with the oval raceway resulted in a 63 percent improvement in fish survival compared to those raised in either round tanks and fed either the RNB or RAN diets.

Experiments conducted at Anuenue Fisheries Research Center (AFRC) examined the cause of the deformed operculum condition in juvenile threadfin. The experiment sought to:

- identify and document the types of deformities that occur when juvenile threadfin are fed different commercial diets;
- determine whether these deformities occur at a specific period of development; and
- determine whether a particular age is critical to normal opercular development.

Results of the experiments indicated that deformities may be caused by differences in the formulation of weaning diets. Although 23 to 26 day-old threadfin postlarvae readily accepted commercial semi-moist feeds formulated for salmon and trout (Bioproducts Biostarter and Biogrower Diets, respectively), these fish had more operculum deformities of various kinds by day 40 than fish that were fed a dry diet formulated for mahi mahi (Moore-Clarke Mahi Diet). Fish given the semi-moist diets exhibited not only flared and reduced operculum but also:

- lordosis,
- scoliosis,
- crooked lower jaw,
- dimple head, which is suspected to indicate an early stage of lordosis,
- cataracts, and
- pop eye.

The deformed fish were removed at day 40, but deformities continued to occur throughout the nursery stage 2 (day 41 through day 60). These results suggested either a lack of a nutrient or a reaction to the diet formulation or to the quality of an ingredient.

Another trial examined the effect of dietary vitamin C levels on the incidence of operculum deformities. Results were inconclusive because of problems attaining prescribed treatment levels in the commercial feeds tested. Other trials indicated that water quality also plays a role in the occurrence of operculum deformities in threadfin. Fish raised in oxygen concentrations above 80 percent saturation show a dramatic decrease in the incidence of flared operculum. Fish populations raised in water with less than 65 percent oxygen saturation have high incidences of flared operculum. Although mild operculum flaring is reversible, severe flaring is not. Fish with severely flared operculum also become more easily stressed during anesthetization. Therefore, it is recommended that juvenile threadfin be cultured under nearly oxygen-saturated conditions, that tanks be cleaned daily to remove feces and uneaten feed and that densities be reduced if oxygen levels begin to fall below 80 percent saturation.

During the first 2 years of the project, development of mass production techniques progressed rapidly. Based on a standard model developed at The Oceanic Institute, producing a 40 day-old juvenile threadfin costs an average of $0.58 per fish. A series of small tank trials were conducted at The Oceanic Institute to reduce juvenile production costs by reducing the cost of producing live feeds in the hatchery. Results of the trials, which used 30-liter and 1.5-cubic-meter tanks, indicated that the optimum live feed regimen in the hatchery employs rotifers until the threadfin larvae are 15 days old and then employs *Artemia* from that point. *Artemia* did not appear to substitute well for rotifers in larval rearing, and adding them early in the feeding regimen did not offer any advantage. This finding provides a substantial savings on the cost of these live food items.

Attempts to introduce pelleted feed on day 15 of the larval rearing cycle were inconclusive due to unusually high mortality in both the live-feed control groups and the treatment groups. The mortalities probably were caused by a combination of the small size of the tanks (30 liters) and an inability to control temperature.

Year 3 progress and principal accomplishments

Objective: *Resolve bottlenecks in threadfin production and quality by determining whether operculum deformities are inherited.*

During the third year of this project, investigators wanted to determine whether operculum deformities are inherited. The original trial design was modified when broodstock with deformed opercula failed to produce viable eggs after several attempts to induce spawning. Spawns from a single group of undeformed females were examined to determine whether the occurrence of shortened opercula was related to the individual spawn. In addition, anecdotal evidence obtained from red drum culturists in Gulf states suggested that opercular deformities might be related to how long eggs were exposed to air upon transfer from broodstock to rearing tanks.

An experiment was designed to determine the effect of egg handling technique and spawn number on overall incidence of gill deformity. Eggs from the second, third, and fourth consecutive spawns from a broodstock group were collected from the overflow drain catch of the broodstock tank and either netted and exposed to air for 5 seconds before being placed into rearing tanks or directly scooped with water in a 5-gallon bucket without direct exposure to air. Duplicate 1,500-liter tanks were used for each treatment combination, yielding four replicates for the main effect of spawn number and six replicates for the main effect of air exposure.

Results indicated that only spawn number significantly affected the extent of gill deformity in Pacific threadfin. Although the percentage of gill deformities noted on day 24 was high in all groups, affecting from 42 to 77 percent of fish, the percentage of deformed fish from spawn #4 was 25 percent lower than from either spawn #2 or spawn #3. No difference appeared in the rate of deformity of larvae reared from eggs that were either exposed to air or not exposed. Due to the high level of gill deformity noted, spawn number was not thought to be the primary reason for the occurrence of deformity in these fish. Special handling of eggs to minimize air exposure does not appear to be an important factor to reduce gill deformity, although waiting to take later spawns may be a contributing way to minimize the occurrence, like other factors shown to previously affect it.

Trials were conducted to determine the effects of fatty acids on gill deformities. Previous small-tank trials did not determine the incidence of gill deformity because the harvested fish were so small, so a second trial was conducted in larger, 1.5-ton units. Six tanks were stocked with an initial density of 30 larva per liter; the larvae were fed rotifers cultured on *Nannochloropsis occulata* until day 7. On day 7, two replicate tanks were fed rotifers enriched with an emulsified product containing either 30 percent docosahexanoic acid (DHA), 15 percent DHA, or an emulsified preparation made with flaxseed oil containing no DHA. Larvae were fed their respective rotifers and then *Artemia* enriched with the same preparations until day 28. The same high quality pelleted feed was offered to all larvae beginning on day 22.

Results indicated fish given live feed enriched with the high DHA product had a higher overall weight and a higher incidence of gill deformity compared to those given live feed enriched with flaxseed oil. Fish given live feed enriched with the moderate DHA product had an incidence of gill deformity similar to that of the latter group and lower than that of the former, but overall weight was not different than either group.

In stress tests conducted during harvest, fish fed rotifers enriched with flaxseed oil had survival rates of 62 percent the day after handling, whereas fish given the high DHA treatment had 99 percent survival and the fish given the moderate DHA treatment had 94 percent survival, which indicated better overall quality and health of fish in the latter groups. Overall results confirmed the importance of DHA enrichments on improving overall growth and survival of threadfin larvae. The reason for increased incidence of gill deformity due to high DHA treatment is unexplainable.

Results confirm those of the small-tank trial which showed that either moderate or high DHA treatments are required to maximize threadfin survival and produce healthy postlarvae.

Objective: *Resolve bottlenecks in threadfin production and quality by determining the effect of vitamin C on operculum deformities.*

Because previous trials examining the effect of dietary vitamin C on the incidence of gill deformity during nursery phase 2 proved inconclusive, an additional study and other dietary options were examined. Two experiments were conducted using feeds supplied by Moore-Clark, Inc. (Canada).

The first experiment compared two diets containing 45 percent protein that had varying levels of ascorbic acid polyphosphate (78 mg per kilogram and 671 mg per kilogram). In addition to the differences in vitamin C content, these diets were completely different formulations of different ingredients. The first was a trout diet, and the second was a modified diet designed specifically for mahi mahi. Each diet was fed to one tank of approximately 300 fish each from day 54 to day 78.

Results of the first trial indicated that both groups had similar survival rates. However, fish given the high vitamin C diet had a higher final mean weight of 15.9 grams and fork length of 98.0 mm than fish given the low vitamin C diet, which had a mean weight of 10.0 grams and fork length of 87.0 mm. The high vitamin C group also had only a 21.3 percent incidence of gill deformity, whereas the low vitamin C group had a 44.8 percent incidence of gill deformity. The water quality deteriorated as the amount of feed increased with the latter group of fish, which may have affected results. Results should be interpreted with caution because the diets were not the same formulations and only one tank was used per treatment.

The second experiment compared two diets with 45 percent protein. The first diet contained a fat level of 14 percent and a vitamin C level of 671 mg per kilogram. The second diet contained a fat level of 25 percent and a vitamin C level of 120 mg per kilogram. Diets were fed to two tanks of fish to day 67.

Preliminary results of the second trial indicate no difference in growth or survival between the two feeds. However, a higher incidence of deformity was observed in the group of fish given the low fat/high vitamin C diet (19%) than in the group of fish given the high fat/low vitamin C diet (14%). Further analysis is being done, but lack of replication also does not allow statistical interpretation of these results. No clear trend of diet on the incidence of gill deformity in threadfin is evident from these two trials.

Objective: *Exchange seedstock production technology with Hawaii's aquaculture industry by providing farmers with threadfin eggs and juveniles to conduct commercial hatchery and growout trials and estimating market potential.*

Approximately 61,00 juvenile threadfin were distributed to 10 cooperating commercial farms. Eggs were distributed on an availability basis. More than one million eggs were given to the state of Hawaii's Anuenue Fisheries Research Center. Eggs were also given to Bong Kim of the Hawaii Institute of Marine Biology, University of Hawaii, to conduct research.

A survey form was mailed to cooperating farmers to gather on-farm biological and cost information of threadfin growout. The results will be analyzed after the surveys are returned.

Two market surveys were conducted with fish raised at The Oceanic Institute. Fish were raised under contract agreement for sale by KTA Superstores, on the island of Hawaii, using fingerlings provided by the project.

A total 603 pounds of fish, with each fish weighing between 0.75 and 1.5 pounds, were air-freighted in two weekly batches to KTA. KTA, which provided market information, purchased the fish for $4.25 per pound and plus $0.35 per pound for air freight charges. Fish were sold whole to the general public beginning the weekend of February 7 through 13, 1996, at a retail price of $5.77 per pound.

Derek Kurisu, KTA Vice President, reported high consumer acceptance of the product. All of the more than 400 pounds shipped in the first week were sold within 2 days. Numerous repeat customers for the second shipment of fish remarked on the high quality and superior taste of the product. The 1.0- to 1.5-pound size was considered ideal for frying. A 2-pound product, which was not available in this test, was considered the best size for steaming, the traditional Chinese style of cooking threadfin.

Notably, blemished gill plates and fins and other gill deformities were not considered problematic. Customers and Kurisu personally did not feel that such factors adversely affected the appearance and presentation of fish.

In summary, KTA felt that it could easily sell 100 to 200 pounds of threadfin a week at a retail price of $6.79 to $8.99 per pound, depending upon the product supply. This would translate into a farm-gate price of $5.75 to $7.50 per pound. A 500-pound-per-week supply would yield a $5.99 per pound retail price and $5.25 per pound farm-gate price. Overall, cultured threadfin was considered a quality product with excellent shelf-life characteristics.

In addition, approximately 40 pounds of fish were supplied to the state of Hawaii's Department of Business and Economic Development and Tourism (DBEDT) for use in a vertical fish tasting event in Philadelphia, Pennsylvania, in February. The event is used as a marketing tool for seafood distributors, retailers, and restaurateurs to encourage the consumption of little-known types of fishes.

Pacific threadfin sent to Philadelphia were compared with seven East Coast fishes: Atlantic halibut, Atlantic salmon, flounder, mako shark, striped bass, swordfish, tilapia, and 10 West Coast fishes: bigeye tuna, sea bass, shortbill spearfish, striped marlin, ruby snapper, moonfish, crimson snapper, albacore tuna, gray snapper, and threadfin. Fish were ranked within various categories to describe their flavor, texture, taste, and aroma. Participants were also asked to rate which fish among the group they most preferred overall. Threadfin was compared within a category that included yellowtail flounder, hybrid striped bass, crimson snapper, and gray snapper.

Threadfin was well-received by all participants and ranked first preference within its group. An informal survey taken by DBEDT concluded that the threadfin was the most preferred of all fishes tested. DBEDT reported that threadfin would compete well on the mainland with hybrid striped bass, which normally sells for $3.00 per pound whole. Several chefs reported a willingness to pay up to $7.00 per pound for a comparable threadfin.

Objective: *Develop a threadfin culture manual*

A hatchery manual that explains methods of caring for and culturing broodstock, larvae, and fingerlings should be published by late Spring 1997.

Impacts

Diversification of aquaculture crops will provide a better economic base for the industry. Because Pacific threadfin are a high-value fish, perfection of technology to culture this species will offer farmers an opportunity to improve their overall profitability.

Results of the market research indicate that aquacultured Pacific threadfin are a very marketable product not only in Hawaii, but also in other areas of the United States as well. The fish was categorized as a superior product with potential to compete well with established aquacultured fishes at a premium price. This indicates a very strong export market to the mainland United States for farmers in Hawaii. This, together with potential export to Asian markets because of the ethnic acceptability of threadfin, bodes well for the future rise of a farm industry in Hawaii.

Resolution of the gill deformity issue in threadfin remains elusive, and a more in-depth approach is required to determine the primary cause. However, these gill deformities do not appear to be a major issue with the aquaculture potential of this species, in terms of its effects on survival or growth or on consumer appeal. Future research may be warranted, but it does not appear critical to advancement of the species into the marketplace in the near future.

Support

This project received funding from the Center for Tropical and Subtropical Aquaculture (CTSA), The Oceanic Institute (OI), the Hawaii Aquaculture Development Program (ADP), and the Anuenue Fisheries Research Center (AFRC).

Year	CTSA	Other Support				*Total Support*
		OI	ADP	AFRC	Total Other	
One	$42,000	$87,000	$15,000	$10,000	$112,000	*$154,000*
Two	$92,500	$50,000	$15,000	$10,000	$75,000	*$167,500*
Three	$70,000	$50,000	$15,000	$10,000	$75,000	*$145,000*
Total	**$204,500**	**$187,000**	**$45,000**	**$30,000**	**$262,000**	***$466,500***

Publications, manuscripts, or papers presented

Bass, P., E. Lee, and C. D. Kelley. 1995. The use of rotifers and *Artemia* nauplii as live feeds for Pacific threadfin. Aquaculture '95: Proceedings of the 1995 World Aquaculture Society meeting. February 1 - 4, 1995. San Diego, California.

Kelley, C. D., P. Bass, and K. Leber. 1995. The production of Pacific threadfin fry for stock enhancement and aquaculture. Aquaculture '95: Proceedings of the 1995 World Aquaculture Society meeting. February 1 - 4, 1995. San Diego, California.

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Leber, K. M., A. C. Ostrowski, R. Cantrell, and C. D. Kelley. 1996. Fingerling Production Technology for a High-Value Marine Finfish: Pacific Threadfin, *Polydactylus sexfilis*. Aquaculture America '96, Proceedings of the 1996 meeting of the U. S. Chapter, World Aquaculture Society. February 14 - 17, 1996. Arlington, Texas.

Ostrowski, A. C. 1995. Nursery and Growout Production Techniques for the Mahi Mahi (*Coryphaena hippurus*) and Pacific Threadfin (*Polydactylus sexfilis*). In Main, K. L., and C. Rosenfeld, eds. Culture of High-Value Marine Fishes in Asia and the United States: Proceedings of a Workshop. August 8 - 12, 1994. Honolulu, Hawaii.

Ostrowski, A. C., et al. (In review). Nursery production techniques for Pacific threadfin (*Polydactylus sexfilis*). Aquaculture. 1995.

Differential Growth Rate Studies in Cultured Commercial Sponges

Dates of work, April 1993 through April 1996

Funding level

$140,126

Participants

Richard Croft, College of Micronesia

Dr. Michelle Kelly-Borges, Natural History Museum, London, UK

Project objectives

The objectives of this 5-year project are to:

- improve the efficiency and production of commercial sponge farms by determining the factors responsible for variable growth rates in cultured sponges;
- obtain additional biological data; and
- maintain the existing demonstration nursery.

Anticipated benefits

Sponges grow very slowly, taking at least 2 years to reach market size. However, sponge growth rates vary widely; even sponges planted side by side grow at different rates. By determining the cause of growth rate variations, this project could improve the efficiency of sponge farms, thereby increasing the profitability to farmers.

Progress and principal accomplishments

Years 1 through 3

During the first year of the project, sponges were selected, cut, tagged, and planted for use in several investigations that were undertaken at the same time. The first study examined the cause of the large differences in growth rates of sponges growing under the same conditions.

The hypothesis was that certain areas of the parent sponge actively grow while other areas do not, which may explain why some sponges planted in the demonstration farm grew only 3 percent per month while others planted 3 feet away on the same line grew up to 15 percent per month. If this hypothesis proved true, the faster growing parts of the wild sponge would be used as cutting material.

Ball-shaped and bowl-shaped sponges were selected and cut into three layers—a top layer, a middle layer, and a bottom layer. The center of each layer was then cut out, resulting in a donut-shaped outside section and a disk-shaped inside section. The top layer from a bowl-shape sponge did not have an inside section. The six groups from the ball-shaped sponges and five groups from the bowl-shape sponges resulted in 1,100 cuttings, which were segregated by groups, tagged, and planted.

Within 4 to 5 weeks after planting the cut areas had healed, and cuttings were weighed for the first time. Each tagged section was weighed every 6 months. For some unknown reason, portions of some cuttings died and fell off just after planting. Thereafter, the cutting grew normally. If the lower growth rates were due to this "die back," growth rates would rise and the range of the average monthly growth rates would narrow. During the second and third years of the project, measurements showed this to be true.

As noted, the majority of the inside cuttings from most of the layers showed smaller overall increases in size. Cuttings from the center of each disk had none of the black, outer skin that covers parts of cuttings from the outside edge. This appeared to be the only physical difference between these two groups. The longer time needed for the inside cuttings to become totally covered again by the black skin may slow their overall growth rates.

All 11 groups of sponge cuttings showed a wide range of growth rates—from a low of 1 percent to a high of 17 percent—similar to those exhibited during the first growth rate study.

A second component to this experiment examined the differences in growth rates between cuttings taken from various sections of bowl-shaped and ball-shaped parent sponges. Ball-shaped and bowl-shaped parent stock were each cut into a top layer, a middle layer, and a bottom layer. The center of each layer was cut out, resulting in a donut-shaped outside section and a disk-shaped inside section (although the top layer of a bowl-shaped sponge does not have an inside section). The resulting 1,100 cuttings were then segregated into 11 groups, tagged, and planted together.

Tagged sponges from both the bowl-shaped and ball shaped parent stock grew an average of 4 to 7 percent monthly. The growth rates of all 11 groups of sponge cuttings range widely: from a low of -2 percent to a high of 18 percent. This may be attributed to the die back of portions of some of the cuttings. If so, the lower growth rates should climb during the next 12 months, and the range of growth rates should narrow.

At least twice as many cuttings from the inside section of the top, middle, and bottom sponge layers showed negative growth as cuttings from the outside layer. This may be because the inside cuttings do not have any of the black "skin" that covers the outside of sponges and probably protects the cuttings.

Another study examined the hypothesis that sponges exhibiting higher growth rates retain these high rates even after being cut into smaller pieces. If so, these should be the sponges used to replant or expand a farm, thus improving farm efficiency.

Only those sponges with a growth rate of 10 percent or more per month were selected for this experiment. High growth-rate sponges were divided into 100 cuttings, tagged, and planted.

None of the cuttings exhibited any "die-back." After only 12 months, many of these cuttings had reached the minimum commercial size of more than 600 grams, live, wet weight. After 24 months of growth, every sponge weighed at least 600 grams, and some weighed more than 1,200 grams. Larger sponges have greater commercial value. During the first growth study sponge cuttings took 24 months or more to reach commercial size, and 36 months to reach the size of the cuttings in this study. This clearly indicates that these sponges should be used to replant or expand a farm.

The third study examined whether cutting sponges with low growth rates stimulates their growth. The principal investigator selected 80 cultured sponges that weighed 400 grams or more and had exhibited growth rates of less than 5 percent per month. These sponges were divided into two or more cuttings of at least 200 grams each. The resulting 197 cuttings were tagged, weighed, and planted. These cuttings grew at monthly rates ranging from 3 - 9 percent and averaging 5 percent.

Some of these cuttings exhibited a partial "die back." Overall, the cuttings taken from slow-growing parent stock continued to grow slowly, with very few reaching commercial size after 24 months of growth. This indicated that slow-growing sponges in a commercial farm should be harvested and sold off as soon as they reach the minimum commercial size.

Objective: *Maintain the existing demonstration farm.*

Under the CTSA-funded Sponge Aquaculture Demonstration Project, five nursery areas were planted with more than 10,000 sponges. Two of these sites have been used for the growth experiments, and the other three sites provided cutting material to help local residents establish private sponge farms. Approximately 4,600 sponges from the nursery areas were provided to sponge farmers.

The nursery sites have also been used as training sites to train local people in sponge farming techniques. From late 1993 through 1996, 10 individuals started the training courses offered at these sites. Six of them started private sponge farms in Pohnpei's lagoon.

Although a variety of fouling organisms normally grow on sponge planting lines, in two areas of the nursery a species of colonial tunicate found growing on the surface of the cultured sponges caused the sponges to be misshapen. The nursery site was monitored monthly and the tunicates were cleaned off the sponges and lines.

A large number of the hanging lines used to suspend the sponges from the growing lines had broken and were replaced with heavier test lines. In addition, a number of the main growing lines had broken and were replaced.

Objective: *Obtain additional biological data.*

Dr. Michelle Kelly-Borges, a sponge systematist and ecologist who was hired as a project consultant, identified the Pohnpei sponge as *Coscinoderma mathewsi.* She designed several experiments for the project. These experiments will examine whether:

- the final morphology of cultured sponges is determined by the morphotype of the wild donor sponge, or by the environmental conditions during the culture period;
- the final morphology of cultured sponges is determined by the position in the donor sponge from which the explant was cut, or by the environmental conditions during the culture period; and
- the growth rate of cultured sponges is determined by the position in the donor sponge from which the explant was cut or by the environmental conditions during the culture period.

The wild donor sponges in the Pohnpei lagoon have three distinct morphologies: spherical or ball-shaped, vasiform or bowl-shaped, ring-shaped and digitate, which have small, finger-like extensions from the surface. Cuttings were taken six zones—the outside top, the inside top, the outside middle, the inside middle, the outside bottom, and the inside bottom of the sponge—within each sponge morphotype. Vasiform sponges have no inside top, and ring-shaped sponges have only the outside top and outside bottom. A minimum of three parent sponges of each morphotype and a minimum of 10 cuttings from each zone were required for statistical purposes.

Each of three parent sponges of the four morphotypes was selected, divided into the six zones, and cut. Approximately 900 cuttings were then tagged and planted by zone group. After 4 to 5 weeks, 10 or more healthy cuttings from each zone group was selected and placed into a basket. From this basket, cuttings were randomly selected and replanted for 12 to 18 months.

The survival rate of all tagged sponges tagged will be recorded. Growth data will be analyzed and compared within zones and morphotypes, within zones and between morphotypes, between zones and within morphotypes, and between zones and between morphotypes. A qualitative cluster analysis will be made of explant and donor morpohologies. The first two growth measurements were taken.

Work planned

Growth and morphological data will be collected, analyzed and compared during the fourth and fifth years of the project.

Impacts

This project has shown that sponges with high growth rates retain those high growth rates when cut into smaller pieces and replanted and should be used to replant and expand commercial farms to increase their efficiency and profitability. By the same token, slow-growing sponges retain their slow growth rates after being cut and replanted, so they should be harvested and sold as soon as they reach minimum size.

All 11 groups of sponge cuttings showed growth rates ranging from a low of 1 percent to a high of 17 percent. The lower rates appeared to result from portions of some of the cuttings dying back. The wide range in growth rates appeared not to depend upon the area of the parent sponge from which the cutting came and appeared to be the same for cuttings from both the ball-shaped and the bowl-shaped parent stock. This information will help farmers to improve their productivity.

Support

This project received funding from the Center for Tropical and Subtropical Aquaculture (CTSA), the College of Micronesia (COM), Pohnpei Marine Resources Division (PMRD), and Pohnpei Natural Products (PNP).

		Other support				
Year	**CTSA**	**COM**	**PMRD**	**PNP**	**Total Other**	**Total Support**
One	$30,380	$75	$ 625	$ 990	$1,690	$32,070
Two	$39,746	$75	$ 475	$7,535	$8,085	$47,831
Three	$30,000	$75	$ 550	$1,990	$2,615	$32,615
Four	$40,000	$50	$ 715	$7,150	$7,915	$47,915
Total	**$140,126**	**$275**	**$2,365**	**$17,665**	**$20,305**	**$160,431**

Publications, manuscripts, or papers presented

Kelly-Borges, M. 1996. Research report on aquaculture of the sponge *Coscinoderma mathewsi* in the Pohnpei7 Lagoon, Micronesia. Natural History Museum. London, United Kingdom.

Improvement of Tilapia Stocks in Hawaii Phase 1: Collection and Identification of Present Stocks

Dates of work, April 1994 through July 1996

Funding level

$49,180

Participants

Dr. Kevin Hopkins and Dr. Leon Hallacher, University of Hawaii at Hilo.

Project objectives

The objectives of this project are to:

- review the international, national, and Hawaii technical and regulatory status of tilapia stocks and strains related to improvement of the tilapia aquaculture industry in Hawaii;
- morphometrically and genetically assess the status of farmed stocks from 12 sites and wild tilapia stocks from 12 sites on six Hawaiian islands;
- determine growth characteristics of existing farmed tilapias and compare the data with the available scientific literature; and
- recommend measures to improve existing stocks with the available stocks or to propose importations of new tilapia strains or species based upon review of

the collected information.

Reason for termination

This project was terminated because all objectives were completed.

Progress and principal accomplishments

Objective: *Review the international, national, and Hawaii technical and regulatory status of tilapia stocks and strains related to improvement of the tilapia aquaculture industry in Hawaii.*

The scientific literature both on introductions of tilapia into Hawaii and on the impact of tilapia introductions worldwide was reviewed.

Objective: *Morphometrically and genetically assess the status of farmed stocks from 12 sites and wild tilapia stocks from 12 sites in Hawaii.*

All known tilapia farmers in Hawaii were interviewed to determine the history of their tilapia stocks. On the basis of these interviews, the sampling design was revised to minimize duplication and ensure that all possible stocks would be sampled. Tilapia were collected in two separate samples from the commercial and field sites shown in Table 1.

The sampled fish were photographed and measured. Eye, muscle, fin, and liver tissue from the collected fish were sampled and shipped to Genetic Analyses, Inc. in Smithville, Texas, for electrophoretic analyses. The collected fish were then fixed in formalin and transferred to alcohol for long-term storage. Meristic counts and taxonomic measurements were completed on all the preserved fish. Measurements taken included the number of gill rakers, the shape and size of the bone at the top of the gill arch, jaw length relative to body length, eye size, and fin size.

The first phase of the electrophoretic analyses examined the protein structure of sampled tissues to determine the species of tilapia. Based on results of this work, 15 isozymes were selected for closer analysis in the second phase. This work enabled investigators to determine the purity of local tilapia stocks and to provide fish breeders with recommendations regarding the suitability of using local fish for breeding stock.

Table 1. Sites From Which Tilapia Samples Were Taken

Island	Location	Tentative Field Identification
	UH-Hilo farm	*Oreochromis macrochir hybrid*
Hawaii	Jervis Fish Farm	Red *O. mossambicus*
	Okamura Fish Farm	*O. macrochir hybrid*
	Royal Hawaiian Sea Farms	Red *O. mossambicus*
		Sarotherodon melanotheron
Molokai	Kualapuu Reservoir	*O. mossambicus*
	Hanapepe River	*O. mossambicus*
	Nagao Farm	*Tilapia rendalli*
Kauai		*O. mossambicus x O. hornorum hybrid*
		S. melanotheron
	Amao Reservoir	*T. rendalli*
	Maui Plantation	*O. mossambicus*
Maui		*O. mossambicus*
	Kealia Fish Farm	*T. rendalli*
		O. macrochir
	UH Mariculture Research and	*O. aureus*
	Training Center	Red *O. mossambicus*
	Waianae Coast Community	Red *O. mossambicus*
	Development Authority	
	Nuuanu Reservoir #3	*T. rendalli*
Oahu		*T. zillii*
	McKinley High School	Red *O. mossambicus*
	Wahiawa Reservoir	*S. melanotheron*
	Anuenue Fisheries Research Center	Red *O. mossambicus*
	Honolulu Harbor	*S. melanotheron*
	Heeia Fish Pond	*S. melanotheron*
Philippines	Central Luzon State University,	
	Republic of the Philippines	*O. niloticus*

Objective: *Determine growth characteristics of existing farmed tilapias and compare the data with the available scientific literature.*

A computer database was created for entry of data collected during the project. The database, currently on a Macintosh® computer, uses a software program called Foxpro® that readily transfers across platforms, so it will be available for use on personal computers. Morphometric and meristic data were coded and transferred into a computerized database. Using this database, preliminary estimates of each characteristic (e.g., the number of scales in the lateral line, relative length of the head, etc.) were calculated and are now being compared to values published in scientific literature. Analyses of meristic and morphometric data were finished and the final report is being prepared.

Impacts

This project determined that crosses of *O. niloticus* are present in Hawaii. This information should assist farmers in getting approval to import pure strains of that species, which is considered the preferred species for food culture.

Support

This project was funded by the Center for Tropical and Subtropical Aquaculture (CTSA) and the University of Hawaii (UH).

Year	CTSA	Other Support UH	Other Support Total Other	Total Support
One	$49,180	$40,981	$40,981	$90,161
Total	**$49,180**	**$40,981**	**$40,981**	**$90,161**

Publications, manuscripts, or papers presented

None

Ornamental Aquaculture Technology Transfer

Dates of work, April 1993 through August 1996

Funding level

$250,200

Participants

Dr. Clyde Tamaru, Dr. Bruce Miller, Paul Olin, Richard Bailey and Brian Cole, Sea Grant Extension Service, University of Hawaii

Dr. Christopher Brown, Hawaii Institute of Marine Biology, University of Hawaii

Dr. Frank Chapman, University of Florida

Project objectives

The overall goal of this project is to establish consistent production and marketing of ornamental fish on existing Hawaii aquaculture farms. Specific objectives relating to that goal are to:

- obtain an assessment of the U.S. ornamental market;
- hire and retain an expert in commercial ornamental fish culture to support development of an ornamental production industry in Hawaii;
- import and evaluate the culture potential of selected ornamental fish species;
- evaluate the production economics of breeding and growout in selected species;
- establish commercial production of ornamental fishes on aquaculture farms in Hawaii;
- provide seedstock and technical support to farmers;
- conduct a business feasibility analysis in conjunction with the Pacific Business Center, University of Hawaii;
- develop breeding and production protocols for selected ornamental fishes; and
- produce a manual detailing practical ornamental fish culture methods for Hawaii farmers.

Anticipated benefits

The worldwide market for ornamental fish is about $1.4 billion per year. The United States alone imports approximately $40 million worth of tropical fish, and Florida's ornamental culture industry generates revenues of about $50 million annually. Hawaii's resources — clean, plentiful fresh and salt water, competitive air freight costs, and a warm climate with no freezes — could provide a competitive advantage in the ornamental fish culture market and further diversify the economic base of the aquaculture industry in the islands.

Progress and principal accomplishments

During the first year of this project, an assessment of the U.S. ornamental market was completed. It found that ornamental fish are kept principally by households in the U.S.A., Canada, Europe, and Japan. The U.S. market for ornamental fish is consistently growing, as are imports of ornamental fishes, which reached $45.2 million in 1993, and the net trade deficit. Most ornamental fishes sold through the pet trade are farm-raised freshwater species imported from Southeast Asia; wild-caught ornamentals from South America account for the second largest number of imports. Florida is the major domestic source of farm-raised ornamental freshwater fishes. The principal source of saltwater ornamental fishes is collection from the wild; the Philippines and Indonesia are the main exporters of these fishes. In the United States, saltwater ornamentals are collected from the coastal waters of Florida and Hawaii. Freshwater fishes account for 80 percent of the value of U.S. ornamental fish imports. Of the 1,539 species declared as ornamental fish, only 32 species — all freshwater animals — dominate the trade. The neon tetra and the guppy are the most popular ornamental fish in U.S. households, but a greater variety of species tends to penetrate the market as they become available. The average prices paid for imported ornamental freshwater fishes were 45 cents for egg-layers and 22 cents for live-bearers. Patterns of import and export trade activities indicate that the major ports for U.S. distribution of ornamental fishes are Los Angeles, which accounts for 39 percent of all trade activity, Miami, which accounts for 22 percent, New York, which accounts for 16 percent, and Tampa and Honolulu, which each account for 6 percent.

A business feasibility analysis was conducted for the project by the Pacific Business Center, University of Hawaii. The report was titled “A Business Analysis of the Ornamental Fish Market for Hawaii.” Investigators drew the following generalizations from the report.

- Profit margins for each species vary, but the ready markets and easy culture requirements of live-bearing species that fall in the middle of the profit spread make them good candidates for culture.
- Ornamental fish culture can be profitable in Hawaii by following patterns established in the successful Florida industry.
- Hawaii farmers will enjoy a distinct advantage by targeting Seattle for ornamental fish marketing because neither Florida nor Singapore can send farmed fish directly to Seattle, which serves growing markets in British Columbia.

In order to do the business analysis, the investigators developed an interactive software program to analyze the potential profitability of various mixes of ornamental fishes on Hawaii aquaculture farms. The software comprised a series of linked spreadsheets that evaluated the relative profit potential of various species, the best species mix for growout production, the best commitment of space and resources at a particular site, the best mainland markets for Hawaii farmers to target, production and shipping costs, landed prices, and overall profit potential. Reviewers recommended that the work group consider revising the software package to make it more user-friendly before distributing it to aquaculture farmers.

During the first year of the project, investigators hired Brian Cole, who has more than 10 years experience in ornamental fish production, including management of production systems, spawning induction, and disease diagnosis and treatment. Cole provided farmers interested in ornamental fish culture with technical assistance in the form of:

- public presentations on Oahu and the Big Island,
- site visits,
- hatchery design, and
- generating production data to be used in the development of business plans.

He developed and manages the demonstration ornamental fish culture facility at Windward Community College. The work included:

- installing a water treatment facility that impounds and aerates water to eliminate excess chlorine from the well water source;
- installing PVC liners in each of the ponds; and
- constructing an enclosed breeding facility equipped with 40, 10-gallon aquariums that are being used for production of high health, F_1 tiger barbs.

In addition, Rich Bailey of Sea Grant Extension Service commits 20 percent of his time to the project. Clyde Tamaru, the current project leader, responds to requests for technical assistance coming through the SGES office.

Demonstration growout trials in cages and tanks were completed at the Windward Community College facility for a number of ornamental species. Results indicated that increasing stocking densities inhibits growth or causes significant problems related to fin conformation or disease resistance. Subsequent trials evaluated higher densities.

Imported fish stocks were evaluated for pathogens using wet-mount and standard histological methods. The animals found to be free of specific pathogens were used to produce high health F_1 offspring, which served as founder broodstock for commercial producers. Dr. James Brock of the Hawaii State Aquaculture Development Program examined and certified the fish listed below as specific pathogen-free.

Common Name	Latin Name
jewel cichlids	*Hemichromis bimaculatus*
rosy barbs	*Barbus conchonius*
neon swordtails	*Xiphophorus helleri*
sunset swordtails	*Xiphophorus helleri*
red wag swordtails	*Xiphophorus helleri*
tinfoil barbs	*Barboides shwanenfeldi*
rainbow sharks	*Labeo erythrurus*
albino rainbow sharks	*Labeo erythrurus*

During the second year of the project, ornamental fish were stocked into ponds at two sites. Ponds at Hanohano Farms were stocked with sunset swordtails, neon swordtails, high-fin rosy barbs, pineapple swordtails, and jewel cichlids. Temperature, water flow, and water quality were monitored regularly. As of mid-October, excellent spawning and growth rates had been observed in all but one pond. Harvesting and marketing began in mid-October.

One pond at Fong's Plantation was stocked with rosy barbs, sunset swordtail broodstock, and catfish fry. Survival, growth, and reproduction have been good to excellent for both ornamental species. A second pond will be stocked with neon swordtail broodstock, F_1 tinfoil barbs, and F_1 tiger barbs. Rainbow sharks and tinfoil barbs will be separately stocked into two additional ponds. Four ponds at the Hawaii Aquaculture Group site in Kahuku were stocked with rosy barbs, swordtails, and albino rainbow sharks.

Fong's Plantation has produced neon swordtails, sunset swordtails, green swordtails, fancy goldfish, koi, rosy barbs, and feeder guppies at levels far higher than expected and has sold them in local and mainland markets. Current sales have been estimated at $20,000 to $25,000 per year and rising. Fong's is also growing broodstock tinfoil barbs, tiger barbs, and rainbow sharks for future production.

Hanohano Farms received assistance in establishing commercial production of three varieties of swordtails, rosy and tiger barbs, and two species that are discussed under the reverse osmosis objective (below). Additional funding from the Hawaii State Aquaculture Development Program provided fancy guppies, and project personnel have actively assisted in culture of that species.

Based on the findings of the business feasibility analysis, investigators began work on reproducing Amazon Basin fish using reverse osmosis technology during the second year of the project. This technology is most effective for production of discus and angelfish, two of the four most profitable species identified in the analysis. An environmentally-controlled, enclosed reverse osmosis production room was established at the Hawaii Institute of Marine Biology. Attempts to breed cardinal tetras have been unsuccessful but are continuing.

Hanohano Farms established an enclosed production room with environmental controls and a reverse osmosis water treatment system patterned after the facility at the Hawaii Institute of Marine Biology. Established breeding pairs of several varieties of angelfish and discus fish were introduced to the facility. The angelfish produced 3,000 marketable fry within four months after their introduction to the facility; the fry were sold for $0.40 each, for a total of $1,200.

During the third year of the project, another commercial farm on Oahu and a farm on Molokai joined the project as cooperating sites. The project also provided extension assistance in tropical fish culture to 22 entities, including other commercial farmers, various fish hobbyist clubs, and corporations on five Hawaiian islands.

Also during the third year of the project, investigators established a standardized production unit system for intensive culture of live-bearing, ornamental fish. The production units comprise two 12-foot, circular tanks outfitted with breeding cages and a series of growout cages in ponds. A breeding cage in one tank is stocked with 1,000 females and 200 male broodstock swordtails, which produce 7,500 fry over 15 days. Then the caged broodstock are moved to the second tank for another 15-day spawning cycle.

At that point, the 15 to 30 day-old fry from the first cycle were stocked into growout cages in ponds. Each production unit in the ponds has nine growout cages, from which the older fish are continually harvested and sorted for marketing. After an initial 3-month period, each production unit should be able to produce up to 15,000 fish per month.

Year 4 progress and principal accomplishments

Between January and May of 1996, project personnel responded to 19 requests for broodstock or fry. A total of 13,215 broodstock and fry were distributed to interested aquafarmers. Project personnel also responded to 200 requests for technical assistance in the form of site visits, verbal consultations, and workshops.

A series of six workshops titled "Warmwater Fish Disease Identification, Control, and Prevention" were conducted in August and September 1996 with Dr. James Brock of the State Aquaculture Development Program. Approximately 50 people participated in the workshops, which covered basic concepts of fish health, pertinent terminology, how to examine fish, interpretation and fish behavior and physical signs, water quality and husbandry concerns, laboratory tests and interpretation, the basics of parasitic, bacterial, fungal, and viral diseases, how to prepare and submit samples for laboratory tests, and traditional treatments.

Four intensive culture modules for commercial production of swordtails were established, and stock from three modules is being marketed. Data from all the modules are being obtained and summarized. Over a 6-month period, one commercial-scale module located at Hawaiian Marine Enterprises in Kahuku, Oahu, produced from 0.62 to 2.2 fry per female per day, which is equivalent to 20,000 to 78,000 fry per month. At the end of a 90-day growout period, fish ranged from 31.4 to 61.6 mm long and averaged 46.4 ± 6.7 mm long. A summary of this production data will be the basis for an oral presentation at the 1997 meeting of the World Aquaculture Society.

In addition, biological data, including size frequency distributions, length weight relationships, growth, and the effects of stocking density on growth and survival of swordtails, were summarized and/or obtained. The data were incorporated into the manual described under another objective. Biological data on growth of tiger barbs are also being incorporated into a manual. Data on the effects of stocking density on growth of swordtails also have been summarized and will be presented at the World Aquaculture Society conference in 1997.

Experiments testing the effects of commercial feeds on maturation, spawning, and growth were conducted using angelfish, *Pterophyllum scalars* as a test organism. The fatty acid profiles of two commercial flake feeds (#1 and #2) used during the experiment were obtained from the University of Hawaii Department of Environmental Biochemistry. Although the total amount of fatty acids did not differ significantly between the two diets, specific fatty acids were found to vary significantly between diets.

While 88 percent of the mating pairs of angelfish broodstock given commercial flake feed #1 spawned, only 40 percent of the mating pairs of angelfish broodstock given commercial flake feed #2 spawned. An experiment testing the feed being used for angelfish growout against two commercially available feeds designed for mahi mahi and trout was also conducted. The experiment was conducted using two strains of angelfish (marble and golden) fry from the same broods. Both the mahi mahi and trout diets resulted in significantly improved growth in both strains of angelfish over the course of the experiment.

The results clearly indicated that improved growth rate depends upon the type of commercial feed used. A lower feed conversion ratio is also evident and, when factoring into account the cost of the feeds used, a shortened growout period and reduced feed cost results in a substantial savings in growout costs. Similar results have also been obtained with goldfish. The data are being summarized in a manuscript to be submitted to the Journal of the World Aquaculture Society.

Objective: *Produce a manual detailing practical ornamental fish culture methods for Hawaii farmers.*

A series of manuals addressing the culture of ornamental fishes are being developed. "A Manual for the Commercial Production of the Swordtail, *Xiphophorus hellerii*" by Clyde Tamaru, Brian Cole, Rich Bailey, and Christopher Brown has been completed and should be published by early spring.

Several manuals are in draft form:
- "A Manual for the Commercial Production of the Tiger Barb" by Brian Cole, Clyde Tamaru, Rich Bailey, and Christopher Brown;
- "Enrichment of Artemia Nauplii for Hatchery Production of Ornamental Fish" by Clyde S. Tamaru, Harry Ako, and Restituto Paguirigan Jr.; and
- "Induction of Spawning of Fish: A Users Guide for Ornamental Species" by Clyde S. Tamaru, Brian Cole, Rich Bailey, and Christopher Brown.

A number of manuals are planned, and three are being drafted:
- "A Manual for the Commercial Production of the Gouramis" by Brian Cole, Clyde Tamaru, Rich Bailey, and Christopher Brown;
- "Freshwater Rotifer Culture for Use in Rearing of Freshwater Ornamental Fishes" by Clyde Tamaru, and Harry Ako; and
- "Disease Management of Freshwater Ornamental Fishes" by James Brock.

Work planned

Investigators plan to continue providing extension support to farmers interested in growing ornamental fishes and to produce an ornamental culture manual.

Impacts

This project will help to diversify the aquaculture industry in Hawaii and give farmers an inroad into the U.S. ornamental fish industry, which imported ornamental fish valued at $23.6 million in the first half of 1993. Most U.S. ornamental fish production is in Florida; few of those fish are shipped west of the Rocky Mountains, where demand is high. In addition, Japan and Canada are strong export markets for the U.S. ornamental industry. Hawaii will enjoy a distinct shipping advantage to the U.S. West Coast and Japan.

Support

This project received funding from the Center for Tropical and Subtropical Aquaculture (CTSA), Sea Grant Extension Service (SGES), the Hawaii state Aquaculture Development Program (ADP), and the Hawaii Institute of Marine Biology (HIMB).

		Other Support				*Total*
Year	CTSA	SGES	ADP	HIMB	Total Other	*Support*
One	$58,000	$2,050	$2,050	$0	$4,100	*$62,100*
Two	$122,200	$2,050	$2,050	$0	$4,100	*$126,300*
Three	$70,000	$7,022	$7,022	$6,082	$20,126	*$90,126*
Total	**$250,200**	**$11,122**	**$11,122**	**$6,082**	**$28,326**	***$278,526***

Publications, manuscripts, or papers presented

Bailey, R., C. S. Tamaru, B. Cole, C. Brown. 1996. Performance of an intensive culture module for commercial production of the swordtail, *Xiphophorus helleri*, in Hawaii. Abstract submitted to World Aquaculture '97.

Brown, C. L. 1995. Raising the Silver Arowana (*Osteoglossum bicirrhosum).* Center for Tropical and Subtropical Aquaculture Publication #117. Waimanalo, Hawaii.

Cole, B., C. S. Tamaru, R. Bailey, and C. Brown. Effects of stocking density on growth and survival of the swordtail, *Xiphophurus helleri*. Abstract submitted to World Aquaculture '97.

Tamaru, C. S., B. Cole, R. Bailey, and C. Brown. 1996. (In press). A Manual for the Commercial Production of the Swordtail, *Xiphophorus helleri.* Center for Tropical and Subtropical Aquaculture Publication #128. Waimanalo, Hawaii.

Semi-Intensive Milkfish Fry Production and Technology Transfer

Dates of work, May 1995 through April 1996

Investigators

Dr. Anthony C. Ostrowski, The Oceanic Institute
Michael Fujimoto, Anuenue Fisheries Research Center

Project objectives

The overall goal of this project are to produce milkfish fry and transfer the technology to industry. Specific objectives related to that goal are to:

- maintain and spawn broodstock to produce eggs;
- conduct larval rearing trials to produce fry;
- conduct growout trials at private farms;
- conduct trials to test milkfish as live bait;
- conduct a market and production cost analysis for milkfish fry; and
- develop recommendations for milkfish seedstock production.

Progress and principal accomplishments

Objective: *Maintain and spawn broodstock to produce eggs.*

The Oceanic Institute had a total of 94 milkfish broodstock at the beginning of this project. During June 1995, low oxygen levels in one pond resulted in the loss of 21 animals. Three more animals died in October 1995. By November 1995, another female died leaving 69 broodstock animals. Broodstock ponds were drained and dried to eliminate anemone infestation. Fish were transferred to 100-ton holding tanks for 2 weeks during the interim. At the time of transfer, the average weight of 43 females was 10.8 ± 1.6 kg, and of 27 males was 9.1 ± 1.4 kg. Fish are being fed a pelleted trout feed at an average 0.5 percent of body weight daily.

Objective: *Conduct larval rearing trials to produce fry*

Eggs were released during July and August 1995. A total of 689,000 eggs were collected. Fertilized eggs were reared in two batches. Each batch was stocked into three 4,000-liter tanks at an average density of 23 eggs per liter. Eggs were incubated and hatched at 32 ppt salinity. Freshwater was added gradually to tanks over the next week to obtain 15 ppt salinity, in which the fish were reared until day 22. Water exchange increased throughout the rearing from an initial rate of 10 percent of tank volume daily to 300 percent daily at day 40. Fish were fed rotifers (*Brachionus plicatilis*) at a maintained density of 20 per liter until day 22; the algae *Nannochloropsis occulata* was maintained at 500,000 cells per liter. On day 20, ground, powdered fish feed consisting of 45 percent protein and 12 percent lipid was introduced, and salinity was readjusted to 32 ppt. Results indicated that fingerling milkfish can be cultured using only rotifers and algae and switched easily to dry feed without a change in culture tanks.

Larval survival rates averaged 16.2±2.7 percent from the first rearing and 33.3±7.4 percent from the second rearing. Lower survival from the first rearing was attributed to dead eggs not being removed prior to stocking. Survival from hatched larvae was similar in the first and second rearing trials, averaging 75.6±15.9 percent and 62.6±13.9 percent respectively. Approximately 28,000 day 40 animals were produced from each of the six larval rearing tanks used. The two rearing trials produced a total of 166,000 day 40 animals that weighed from 0.9 to 1.0 grams and measured 19 to 22 mm standard length.

Gill deformities appeared in 25.0±0 percent of the animals from trial 1 and in 14.3±11.9 percent of the animals from trial 2; the higher incidence in trial 1 appeared to be related to differences in spawns from the broodstock. Trial 1 used a single spawn, whereas trial 2 used three consecutive spawns. During trial 2, gill deformities appeared in 28 percent of eggs used in the first spawn and in only 6 percent and 8 percent of subsequent spawns.

On day 40, fish were prepared for shipping to farmers on Oahu, Molokai, Kauai, and Hawaii. In a test packing, day 38 fish weighing 0.15 grams and measuring 2.0 to 2.5 cm were packed with oxygen in plastic shipping bags at densities of 150 grams and 100 grams. Survival averaged 99±1 percent over 7 hours for fish packed at both densities. That method was used to ship 44,000 fish to farmers on the outer islands; Oahu farmers picked up 109,000 additional fish.

Objective: *Conduct growout trials at private farms.*

Growout trials are underway at 10 cooperating farms on the islands of Oahu, Hawaii, Kauai, and Molokai. The fish were stocked into a variety of systems, including Hawaiian fishponds with and without cages, brackish water and freshwater ponds with and without cages, and circular saltwater tanks. Initial reports from farmers indicated high survival in all systems and no problems with feeding. As of October 1995, 90,000 fish remained on farm sites. Some fish were lost to predators and underfeeding. Farmers planned to continue to grow the fish to market size and sell them. A survey form was mailed to all participants to collect information regarding on-site biological and cost data. Investigators are awaiting return of the completed surveys.

Objective: *Conduct trials testing fry for use as baitfish.*

In February 1996, the project obtained cooperation from Pacific Ocean Producers (POP), a commercial longline fishing company, to conduct trials to gather preliminary information on the use of milkfish as bait on one of their longline vessels, the Marine Star.

Approximately 13,000, 6- to 7-inch-long milkfish were picked up by POP workers on February 27 and March 15, 1996. Fish were stocked into 1-ton transport tanks at a maximum density of 1,000 fish per tank, or one fish per liter, with supplemental oxygen, after water temperature was reduced with ice from 26°C to 22°C. Transport time from Oceanic Institute (OI) to dockside, and onto the Marine Star was approximately 1 hour. Fish were placed into two 6,000-gallon holding tanks onboard.

Three trials were conducted during three separate fishing trips. The first trip used live milkfish in alternating baskets (defined by the number of hooks between mainline floats) during each fishing set (defined by the length of one mainline that remains with baited hooks in the water for approximately 24 hours). Each basket contains approximately 20 to 25 hooks set 50 feet apart; hooks on remaining baskets would be baited with dead sanma. The second trip used live milkfish on alternating hooks regardless of basket. The third trip used a combination of the two previously described techniques.

Results of the cooperative longline trials were promising, but inconclusive. The first trip fished 1,600 live milkfish of 4,000 provided in 10 baskets compared to 70 baskets of frozen sanma (11,000 baits) over eight sets. Results showed that for every set, except one, live baits yielded 2.6 times higher percentage catch rate (1.69±0.28 percent) than sanma (0.65±0.06 percent). No correlation appeared between the size of fish caught or depth in which they were caught, although certain species of fish seemed to prefer one bait to another. Total survival of bait was high; in fact, approximately half the animals retrieved from a set were alive when brought back onto the vessel. Fish were not fed during the 4-week fishing trip.

The second trip reportedly fished 6,850 live baits (and an additional 4,000-plus holdovers from the first trip) out of l3,700 hooks in nine sets using alternating hooks. Catch rate between live bait (0.61±0.09 percent) and sanma (0.91±0.16 percent) was no different, although fish were reportedly lost on live bait due to broken lines because of the lighter tackle used. Jim Cook, co-owner of POP cited no confidence in the accuracy of reporting nor whether the prescribed fishing methods were actually used. He felt that for the data to be accurate, an objective, on-board observer should have been onboard to ensure that fishing sets are being conducted properly and results recorded accurately. As a result, POP did not agree to conduct the third fishing trial.

The remaining fish were distributed to a commercial bottom fisherman, Mr. Les Chatterton, owner and operator of the Ruthles. He agreed to test live milkfish versus dead squid, the typical bait used to catch targeted snapper species. Fishing trials are underway.

Objective: *Conduct a milkfish hatchery feasibility study.*

The two larval rearing trials were targeted to produced 100,000 fish each, operating the OI hatchery at full production. Egg production was estimated from the yearly cost of feeding 70 broodstock divided by the estimated number of eggs that could be produced during the normal spawning season.

OI hatchery production costs, including overhead, were estimated to be $0.26 per day 40 fingerling. Labor accounted for 48 percent of that cost; an average of 16 man-hours per day was dedicated to live feed culture. Table 1 shows the breakdown of costs for producing 100,000 day 40 milkfish and Table 2 shows the yearly cost of maintaining broodstock.

Table 1. Breakdown of costs for production of 100,000 Day 40 milkfish fry from the OI hatchery in three 4,000-liter tanks

Item	Amount	Total cost	Percentage of costs
Eggs	400,000	$ 600	2
Labor	672 hours / 42 days	$12,096	48
OI Overhead	@ 95% S & B	$11,491	45
Chemicals		$ 100	0
Dry feed	6.5 kg	$ 5	0
Seawater	480 tons	$ 10	0
Freshwater	5 tons	$ 2	0
Shipping boxes	59	$ 354	1
Air Freight	@ 16 / box	$ 944	4
Total	———	$25,602	——

Table 2. Yearly OI costs to maintain 70 10-kg broodstock milkfish (Estimated production is 3 million eggs monthly during July and August.)

Item	Amount	Total cost	Percentage of cost
Labor (salaries & benefits)	182 hours	$3,300	36
OI Overhead	@ 95% S&B	$3,135	34
Dry Feed	2% per day	$1,354	15
Supplies (nets, etc.)		$ 500	5
Water (@ $.150/gpm/month)	50 gpm	$ 900	10
Total	—————	$9,189	100

Objective: *Develop recommendations for milkfish seedstock production.*

Investigators are awaiting data from commercial cooperators.

Work planned

Additional trials will be conducted on the efficiency of milkfish as live bait. Data from the trials will be analyzed. Biological and cost data on milkfish growout will be collected from cooperating farmers and analyzed. Once all data is analyzed, investigators will prepare recommendations for commercial milkfish seedstock production.

Impacts

Approximately 13,000 fingerlings were distributed for the commercial fishing trials. Preliminary evidence suggests that milkfish are a promising live bait, but additional testing is necessary to confirm the fish's effectiveness.

Results of the commercial growout and fishing tests will be used to evaluate the potential market for fingerling production. This will be incorporated into a production cost analysis for hatchery production. The analysis will serve as a basis for economic decisions by potential investors or by state or local agencies to evaluate the feasibility of creating milkfish hatcheries in the state.

Support

This project received funding from the Center for Tropical and Subtropical Aquaculture (CTSA), The Oceanic Institute, the Hawaii State Aquaculture Development Program (ADP), and the Hawaii State Government (HSG).

Publications, manuscripts, or papers presented

No publications or presentations were produced during the reporting period.

		Other support				
Year	CTSA	OI	ADP	HSG	Total Other	Total Support
One	$92,500	$50,000	$15,000	$10,000	$75,000	$167,500
Total	**$92,500**	**$50,000**	**$15,000**	**$10,000**	**$75,000**	**$167,500**

Publications

Dates of work, March 1990 through October 1996

Funding level

$121,000

Participants

Dr. Kevan L. Main and Patti Killelea-Almonte, Center for Tropical and Subtropical Aquaculture, The Oceanic Institute

Project objectives

The overall goal of this project is to disseminate information on aquaculture. Specific objectives related to that goal are to:

- publish a quarterly newsletter to communicate information about the activities of the Center for Tropical and Subtropical Aquaculture and its funded projects and the latest information about aquaculture from the nation and the region;
- develop and publish a technical bulletin to communicate the status and progress of current activities to the CTSA Board of Directors, Industry Advisory Council and Technical Committee. The bulletin will also be sent to aquaculturists in the Pacific Region and upon request to other interested parties;
- produce and publish final reports of selected CTSA-funded projects. These publications will be distributed free of charge to commercial producers, aquaculture researchers, extension agents, and other interested parties throughout the Pacific Region, with limited distribution in the United States; and
- duplicate and distribute the other Regional Aquaculture Centers' videos and publications to information networks throughout the Pacific Region.

Anticipated benefits

In many locations in the Center for Tropical and Subtropical Aquaculture region, access to information is extremely limited, which handicaps the development of aquaculture. This project helps to overcome that obstacle by disseminating research results and other information that bears directly on commercial aquaculture production.

Progress and principal accomplishments

Objective: *Publish a quarterly newsletter to communicate information about the activities of the Center for Tropical and Subtropical Aquaculture and its funded projects and the latest information about aquaculture from the nation and the region.*

In August 1989, the Center developed and published the inaugural issue of its quarterly newsletter, CTSA Regional Notes. The staff handles all aspects of production for the Center's newsletter, including interviewing, researching, and writing articles, and shooting or obtaining photos. Regional Notes provides the latest information on Center activities and aquaculture throughout the Pacific Region. Published four times per year, it is distributed to approximately 1,000 individuals, organizations, and universities worldwide. In 1990, the newsletter was expanded by 1/3 and began carrying two regular columns:

- "PRAISE Pages" is a bibliography of journal articles; the column is prepared by David E. Coleman, coordinator of the CTSA-funded "Pacific Regional Aquaculture Information Service for Education." In each newsletter issue, Coleman compiles a bibliography on a specific topic of interest to Regional Notes readers;
- "Aquatips" provides recommendations and suggestions on specific aquaculture topics and problems from researchers and extension agents.

The newsletter also features news on CTSA-funded projects, government assistance programs for aquaculture, publications, and various information services that are available. In addition, it provides profiles of individuals and positions who provide services to aquaculturists, job openings in the region, and announcements about training courses.

Objective: *Develop and publish a technical bulletin to communicate the status and progress of current activities to the CTSA Board of Directors, Industry Advisory Council and Technical Committee. The bulletin will also be sent to aquaculturists in the Pacific Region and upon request to other interested parties.*

In February 1990, the Center staff developed and published its first set of Project Updates, technical bulletins that are distributed to the CTSA Board of Directors, Industry Advisory Council, and Technical Committee, and to extension agents and other interested parties upon request. Each set of Project Updates contains separate bulletins from one to six pages long on each active, funded project. Each bulletin provides details on the principal accomplishments for each objective and the principal investigators. In addition to writing and editing the bulletins, the staff does the artwork, layout and design, and works with printers to produce the final publication.

The Publications Project produced a 70-minute movie titled "CTSA Video Project Update." Center staff assisted with writing the script and shooting the background footage for the video. The staff worked closely with the Sea Grant Communications director on the editing and final production of the video. The "CTSA Video Project Update" was prepared to provide the CTSA Board of Directors, Industry Advisory Council and Technical Committee with the latest results from 12 Center-funded projects. The video was shown at the Industry Advisory Council meeting in March 1995 and at the Technical Committee meeting in April 1995 and was distributed throughout the region. A similar video, which featured different projects, was produced and shown in 1996.

Objective: *Produce and publish final reports of selected CTSA-funded projects. These publications will be distributed free of charge to commercial producers, aquaculture researchers, extension agents, and other interested parties throughout the Pacific Region, with limited distribution in the United States.*

The Center staff assists with publication of selected project final reports. Staff assistance includes editing the grammar and style of the reports, proofreading and designing them, and working with printers to produce the final documents.

During 1995, the Center staff assisted with publication of a bibliography developed under the 2-year, Center-funded project titled "Exploratory Study of Hawaii and Guam as High Health Aquaculture Stock Centers." The bibliography titled "A Bibliography of Specific Pathogen-Free Organisms," was published as CTSA Publication #116 in April 1995. The Center staff also assisted with publication of an extension fact sheet developed under the Center-funded project titled "Ornamental Aquaculture Technology Transfer." The fact sheet titled "Raising the Silver Arowana (*Osteoglossum bicirrhosum)*," was published as CTSA publication #117 in May 1995.

During 1996, the Center staff assisted with publication of a manual on making value-added products from giant clam shells. Titled "Clams to Cash: How to Make and Sell Giant Clam Shell Products," the manual was produced as part of the Center-funded project titled "Extension and Training Support in the U.S.-Affiliated Pacific Islands" and was published as CTSA publication #125 in August 1996.

Center staff also assisted in editing a sponge manual and four extension fact sheets that were produced under the Center-funded project titled "Production of CTSA Educational Extension Materials."

Objective: *Duplicate and distribute the other Regional Aquaculture Centers' videos and publications to information networks throughout the Pacific Region.*

The Center staff duplicated 11 videos produced by the other Regional Aquaculture Centers and distributed them to extension agents, libraries, and aquaculture concerns throughout the region. The Center staff also maintained a library of all videos produced by the Regional Aquaculture Centers and loaned them to interested parties upon request. In addition, the Center staff distributed publications produced by the other Regional Aquaculture Centers to extension agents and libraries throughout the region.

Work planned

The Center staff will continue to produce the quarterly newsletter, the technical bulletins, and selected project final reports.

Impacts

This project aids in disseminating aquaculture research results and information throughout the region in order to enhance viable and profitable U.S. aquaculture production that will benefit consumers, producers, service industries, and the American economy.

Support

This project was funded by the Center for Tropical and Subtropical Aquaculture (CTSA) through grants from the U.S. Department of Agriculture.

Year	CTSA	Total Support
One	$10,000	$10,000
Two	$10,000	$10,000
Three	$12,000	$12,000
Four	$15,000	$15,000
Five	$38,000	$38,000
Six	$18,000	$18,000
Seven	$18,000	$18,000
Total	**$121,000**	**$121,000**

Potential Drugs and Chemotherapeutants for Marine Shrimp Production: Selection, Evaluation, and Approval

Dates of work, March 1988 through August 1995

Participants

Dr. Donald V. Lightner, Thomas Bell, Rodney R. Williams, Department of Veterinary Science, University of Arizona

Dr. John Controulis, John Controulis, Ph.D., Inc.

Dr. Roger Fujioka, Water Resources Center, University of Hawaii

Dr. James Brock, Aquaculture Development Program, State of Hawaii

Reason for termination

This project was terminated because all objectives were completed.

Project objectives

The objective of this project was to identify and test compounds likely to be effective against shrimp diseases that have been identified as treatable.

Progress and principal accomplishments

Compounds will be selected based upon their reported efficacy, their potential to receive government approval under current federal regulations, and the manufacturer's interest in sponsoring studies.

To obtain Food and Drug Administration (FDA) approval for use of a compound to treat shrimp disease, a master file must be submitted that shows the compound is effective against disease, safe for the animals, and leaves no residue that could harm consumers. The master file data must come from a number of studies conducted *in vitro*, or in test tubes, and *in vivo*, or on live animals. Master file requirements comprise the following:

- Minimum Inhibitory Concentration (MICs) trials, which determine the lowest concentrations of a compound needed to completely inhibit the growth of selected bacteria isolated from diseased shrimp;
- animal safety studies, which determine whether the compound is safe for the animals;
- palatability studies, which determine the concentration of a compound in the feed at which the animals stop eating;
- and residue studies, which determine how rapidly residues of a compound are depleted from the animals' tissue. The FDA requires that withdrawal periods be long enough so that the animals' tissues show no detectable level of the compound.

Investigators completed work and submitted a Master File on formalin. As a result, the FDA approved the use of the compound in penaeid shrimp nurseries and growout. During the fifth year of the project, Western Chemical, which manufactures formalin, applied to the FDA for a label extension for use of the compound in shrimp culture. The FDA approved Western Chemical to market formalin for that use. This achievement was especially noteworthy because it marked the FDA's first approval of a compound for use in the culture of marine shrimp.

Media were evaluated and selected for use in recovering bacteria from shrimp hatchery water and larvae. Dominant bacteria were identified and isolated from samples collected at various U.S. shrimp culture facilities. In addition, bacterial pathogens from Ecuador were identified and, upon screening, a large number were found to be sensitive to Chloramphenicol.

An extensive database was compiled on the bacteria isolated during the project. The database will allow researchers to compare isolates from different areas to determine which are associated with disease. The database included information on each bacteria's structure and form, biochemical reactions, drug sensitivities, collection sources, host species, host species' health status, and other pertinent observations. The results of pharmacokinetic, bioavailability, and protein binding studies that were conducted on Mobay's enrofloxacin were analyzed. In addition, safety, palatability, and preliminary elimination studies were completed on:

- Arafin, the sarafloxacin manufactured by Abbott Laboratories. The studies indicated that neither palatability nor toxicity were problems even at 50 times the estimated treatment level of 200 mg per kilogram of feed. Elimination appears to be rapid, and a relatively short withdrawal period is indicated.
- Abbott's difloxacin with juvenile *P. vannamei*. The animals showed reduced palatability and survival when treated at levels two and four times higher than the estimated therapeutic level.
- Romet-30 with juvenile *P. vannamei*. Results indicated that up to 13,000 mg of the compound per kilogram of feed caused no ill effect to palatability and toxicity. However, because the FDA has expressed concern about all sulfa compounds in animal feeds, Hoffmann-LaRoche placed tissue analysis on very low priority, so this data was not available.

Large-scale field trials of oxytetracycline were conducted on Texas shrimp farms. The studies included pond and cage trials and laboratory trials for four different treatment levels for comparisons of the two treatment environments. Data from the studies indicated that oxytetracycline is effective in controlling Necrotizing Hepatopancreatis (NHP), which was formerly referred to as Texas Pond Mortality Syndrome, if the compound is administered before the disease is established in a majority of the shrimp and before most of the organ damage is done.

- In ponds, treatment at 3.0 grams OTC per kilogram of feed proved effective.
- In cage trials, treatment levels from 2.25 - 4.5 grams OTC per kilogram of feed were not significantly different in increasing the survival percentage. This indicates that the lower concentration may be just as effective as higher doses in decreasing mortality.

A second cage and laboratory study was conducted with animals from a pond previously treated with 3.0 grams OTC per kilogram of feed. The animals had been taken off the medicated diet, and the incidence of disease had increased. The retreatment data indicated that levels of OTC as low as 0.5 grams per kilogram of feed were as effective in maintaining survival as higher levels. Investigators initiated a study to determine the storage stability of oxytetracycline-medicated feed. The FDA requires such a study prior to approval of the compound in feeds for juvenile to adult size shrimp.

Objective: *With contractual and financial cooperation from one or more industry sponsors, obtain approval for the selected compound(s) from the appropriate federal government agency.*

Plans to conduct *in vitro* efficacy studies on dinitroaniline herbicides, the class of herbicides to which trifluralin belongs, were dropped after investigators learned that trifluralin can be used without approval from the Environmental Protection Agency (EPA). After discussions with officials from the National Pollution Discharge Elimination System (NPDES) permitting office in Washington, D.C. and with officials from EPA Region Six, investigators determined that the most economical way to regulate use of this compound would be through the NPDES permitting process, which is usually handled by state agencies. In Hawaii, that agency is the Department of Health; in other states, it may be the state environmental protection agency.

An NPDES permit for trifluralin would be necessary even if national registration of the compound were possible. Thus, each facility planning to use trifluralin must obtain a permit, which would eliminate potential problems with FDA inspectors. An application submitted to the NPDES permitting agency should include the following information on the compound:

- information on the current use practices in penaeid

culture available in published scientific literature;
- data indicating that the animals would carry no detectable residues to harvest. This has been submitted for publication by investigators at the University of Arizona and is available from them in draft form;
- a complete description of the proposed usage;
- worst case projections on the amount of trifluralin reaching the environment; and
- a copy of the letter stating that the EPA regulates the compound as a water quality agent.

Hawaii farmers who wish to pursue an NPDES permit for trifluralin should contact Dr. Leonard Young of the Hawaii State Aquaculture Development Program.

Impacts

Obtaining Food and Drug Administration approval for an agriculture drug has been estimated to cost $5 million to $10 million. The project funding, which resulted in approval of formalin for use in shrimp culture, represents a direct cost benefit of $4 million to $8 million. Safe, effective therapeutic compounds will help U.S. shrimp farmers to achieve higher production levels and to compete better against unregulated foreign producers.

Recommended follow-up activities

Aquaculture industry members are urged to support the National Biological Service's Chemical Drug Registration Program and the National Coordinator for New Animal Drug Applications, Rosalie Schnick, who will coordinate Investigational New Animal Drug applications for the U.S. aquaculture industry.

Support

This project received funding from the Center for Tropical and Subtropical Aquaculture, the University of Arizona (UA), the University of Hawaii (UH), and the Hawaii State Aquaculture Development Program (ADP).

		Other Support			Total	**Total**
Year	CTSA	UA	UH	ADP	Other	**Support**
One	$192,145	$12,086	$5,679	$5,329	$23,094	$215,239
Two	$165,704	$0	$0	$5,329	$5,329	$171,033
Three	$193,000	$0	$0	$5,329	$5,329	$198,329
Four	$165,000	$6,019	$0	$3,730	$9,749	$174,749
Five	$100,788	$2,216	$1,136	$1,066	$4,418	$105,206
Six	$155,667	$2,223	$1,136	$1,066	$4,425	$160,092
Total	**$972,304**	**$22,544**	**$7,951**	**$21,850**	**$52,345**	**$1,024,649**

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Park, E. D., R. R. Williams, J. Stamm, and D. V. Lightner. (submitted). Palatability and safety of Romet-30® to penaeid shrimp *Penaeus vannamei*. Journal of the World Aquaculture Society.

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Williams, R. R., T. A. Bell, and D. V. Lightner. (in revision). Trifluralin residues in juvenile *Penaeus stylirostris* exposed as larvae in water treatments for the control of *Lagenidium* and *Sirolpidium* zoospores. Aquaculture.

Pacific Communications Survey Project: Needs and Resources

Dates of work, April 1988 through June 1989

Participants

Mr. Michael Hamnett and Dr. Barbara Moir, Social Science Research Institute, University of Hawaii

Reason for termination

This project was terminated because all the objectives were completed.

Project objectives

The objectives of this project were to:

- define the communications needs of regional organizations, federal and state agencies, and University of Hawaii programs that deal with the Pacific Islands; and
- document the experience of those agencies and organizations in establishing communications links using a range of technologies with programs and agencies in the Pacific Islands.

Principal accomplishments

Objective: *Define the communications needs of regional organizations, federal and state agencies, and University of Hawaii programs that deal with the Pacific Islands, document their experience in establishing communications links using a range of technologies with programs and agencies in the Pacific Islands.*

Many of Hawaii's educational, research, and business outreach programs communicate regularly with Pacific Island countries and territories. The communications infrastructure is generally fragile and flawed. With the broad aim of mapping information conduits from Hawaii's educational organizations to their Pacific colleagues, the project surveyed 36 local organizations to learn their telecommunications needs and resources. The survey participants were:

1. U.S. International Trade Administration,
2. Agricultural Development in the American Pacific, University of Hawaii (UH),
3. Pacific International Center for High Technology Research,
4. School of Travel Industry Management (UH),
5. U.S. Economic Development Administration,
6. Hawaii Institute of Geophysics (UH),
7. National Marine Fisheries Service,
8. Water Resources Research Center (UH),
9. Hawaii English Language Program (UH),
10. Waikiki Aquarium (UH),
11. Pacific Business Center Program (UH),
12. Pacific Research Institute for Information Systems and Management (UH),
13. Pacific Fisheries Development Foundation,
14. South Pacific Regional Agricultural Development Program (UH),
15. Joint Institute for Marine and Atmospheric Research (UH),
16. Hawaii Institute of Marine Biology (UH),
17. Sea Grant Program (UH),
18. Center for Tropical and Subtropical Aquaculture,
19. Curriculum Research and Development Group (UH),
20. Law of the Sea Institute (UH),
21. U.S. Army Corps of Engineers,
22. Pacific Basin Development Council,
23. Hawaii Natural Energy Institute (UH),
24. Hawaii Undersea Research Laboratory (UH),
25. Cooperative Fisheries Research Unit (UH),
26. Pan-Pacific Education and Communication Experiment by Satellite (PEACESAT)
27. Pacific and Asian Management Institute (UH),
28. Institute for Astronomy (UH),
29. School of Public Health (UH),
30. International Center for Health Promotion and Disease Prevention (UH),
31. Western Pacific Fisheries Management Council,
32. University Library (UH),
33. Pacific Regional Educational Program,
34. Pacific Telecommunications Council,
35. U.S. Soil Conservation Service, and
36. School of Library Sciences (UH).

The survey revealed that most organizations:

- communicated primarily by letters, memos, and voice;
- communicated via U.S. mail, facsimile machines, or voice telephones;
- communicated via computer communications only when their activities were largely hard science

research;
- communicated via single-side band and PEACESAT radio services minimally, if at all, and had significantly decreased their use of telex;
- emphasized reliability over cost in their communications needs;
- ranked timeliness higher than cost;
- communicated most frequently with the U.S.-Affiliated states in the Pacific Basin; and
- differed widely in budgeted communications costs, from PEACESAT's $379 per month to the International Center for Health Promotion and Disease Prevention's $7,000 per month.

The resulting recommendations rested on two key assumptions that underlay participants' comments.

A. While concern for cost existed, each organization's staff stressed the need to cut transit time for all messages and staff time in learning and using various communications media.

B. The chief obstacle to using advanced telecommunications and computer technology to communicate with the Pacific was a technological culture gap between those fluent in hardware and software and others who grappled with mechanical glitches and poor documentation.

To address these needs, project recommendations advised:
- sorting messages into high and low priority documents by deadlines, need for revisions, need for legal signatures, point-to-point versus point-to-multipoint;
- enhancing budgeting and planning by recording Pacific telecommunications costs for at least 2 consecutive months, implementing depreciation timelines for equipment and factoring salary and time for personnel to learn software into project plans;
- increasing use of facsimile machines in areas where technical support for computer services is limited, budgeting for a full-time computer specialist either on staff or as a consultant, and sharing costs with other programs if possible;
- organizing a users' network of Pacific research telecommunications to educate university and state administrators and to negotiate package discounts with commercial services; and
- and using free PEACESAT services for low priority documents while saving research funds for expensive commercial communications services to send critical documents with high reliability requirements.

Impacts

This project provided the Center for Tropical and Subtropical Aquaculture (CTSA) and other entities within the region with a clear picture of the communications needs and resources available at Hawaii-based education, training, and technical assistance programs serving the countries and territories of the CTSA region. This allowed CTSA and other entities to determine how best to serve and communicate with entities in its region.

Recommended follow-up activities

None

Support

See table at bottom of page.

This project received support from the Center for Tropical and Subtropical Aquaculture (CTSA), the International Center for Health Promotion and Disease Prevention (ICHPDP), the Pacific Basin Development Council (PBDC), the Pacific International Center for High Technology Research (PICHTR), the Social Science Research Institute (SSRI), and the South Pacific Regional Agricultural Development Project (SPRADP).

Publications, manuscripts, or papers presented

University of Hawaii Social Science Research Institute. 1989. Hamnett, M., ed. Pacific Telecommunications. Needs and Resources: An Assessment Survey of Hawaii-Based Research, Training and Extension Organizations. Center for Tropical and Subtropical Aquaculture Publication #103. Waimanalo, Hawaii.

		Other Support						Total
Year	CTSA	ICHPDP	PBDC	PICHTR	SSRI	SPRADP	Total Other	Support
One	$2,000	$1,890	$1,890	$1,890	$1,890	$1,890	$9,450	$11,450
Total	**$2,000**	**$1,890**	**$1,890**	**$1,890**	**$1,890**	**$1,890**	**$9,450**	**$11,450**

Production of Freshwater Prawns and Marine Shrimp for Commercial Farms in the Western Pacific Region

Dates of work, April 1988 through April 1992

Participants

Mr. William FitzGerald, Guam Department of Commerce
Dr. Steve Nelson and Mr. David Crisostomo, University of Guam
Dr. James Brock, Aquaculture Development Program, Hawaii Department of Land and Natural Resources

Reason for termination

This project was terminated because all the objectives were completed.

Project objectives

The expansion of the aquaculture industry was a priority for Guam, and other U.S.-Affiliated Pacific Island nations expressed strong interest in similar commercial development. The overall goal of this 5-year project was to aid development of the industry by producing seedstock to supply the aquaculture farms on Guam and in the Western Pacific Region and diversifying the aquaculture products available to the domestic markets within the region. Specific objectives related to those overall goals were to:

- bring the Guam Aquaculture Development and Training Center into full operation,
- train staff and interested farmers and local residents in aquaculture hatchery and growout techniques, and
- produce seedstock of key species in support of the development of commercial aquaculture on Guam and the Western Pacific Islands.

Principal accomplishments

Objective: *Bring the Guam Aquaculture Development and Training Center into full operation.*

The Guam Aquaculture Development and Training Center (GADTC) was an abandoned facility taken over by the government for back taxes. With the assistance of this project, the facility was repaired and refurbished to fully operational condition. Thereafter, GADTC staff began post-larval production cycles of *Penaeus stylirostris,* which is also known as blue shrimp and was obtained from Hawaii, and established broodstock populations of freshwater prawns (*Macrobrachium rosenbergii*), marine shrimp (*P. stylirostris*), and rabbitfish (*Siganus fuscescens*) at GADTC. In addition, standard operating procedures were established for routine mass production of phytoplankton and rotifers, and production was begun.

In addition, a computerized bibliographic database of locally available technical literature was developed, a technical note series was established as a means of disseminating information gained through GADTC program, and an expanded library of aquaculture books, extension publications, and research reports was organized at GADTC.

Objective: *Train staff and interested farmers and local residents in aquaculture hatchery and growout techniques.*

Hatchery technicians were trained in production of prawn and shrimp postlarvae, phytoplankton, and rotifers. In addition, a technical note series was established as a vehicle for disseminating technical information gained through the programs at GADTC. More than 12 University of Guam (UOG) undergraduate and graduate students were trained in aquaculture techniques at GADTC. Other agencies provided funding to train more than 20 GADTC technicians in Tahiti at France Aquaculture, in Taiwan at Tungkang Marine Laboratory, and in Hawaii at The Oceanic Institute.

All aquaculture farms on Guam were surveyed to identify needs and desired technical assistance. Thereafter, farmers were contacted weekly to ensure open communication and solve emerging problems. Farmers were introduced to new products, improved feeds and feeding methods, oxygen monitoring strategies and equipment, the use of pH monitoring equipment, new harvesting and handling techniques, and live animal transport methods. A demonstration *P. stylirostris* project conducted at a cooperating commercial farm resulted in two additional farms undertaking shrimp culture. An instructional and informational marine shrimp culture booklet was produced and distributed to farmers.

In addition, a television program featuring the commercial culture of blue shrimp was produced and aired, and a brochure describing blue shrimp culture methods used in Guam was published. Articles about GADTC and Guam aquaculture were published in *World Aquaculture* magazine and *INFOFISH International*, and a public relations program to promote the commercial aquaculture industry and increase the visibility of GADTC was launched. Tours of the GADTC facility and local farms were provided to more than 1,100 individuals including potential farmers, school groups, investors, and visiting dignitaries. A directory of government agencies offering financial assistance to aquaculture farmers recovering from typhoon damage was complied, and a brochure describing the commercial and government loan and financial assistance programs available to commercial aquaculture farmers in Guam was published.

Objective: *Produce seedstock of key species in support of the development of commercial aquaculture on Guam and the Western Pacific Islands.*

Techniques to rear the early larval stages of rabbitfish were established, *M. rosenbergii* production levels reached 1 million per year and up to 10 million specific pathogen-free (SPF) *P. stylirostris* post-larvae were produced year-round. GADTC was the only hatchery in both the Pacific Region and the United States that maintained SPF *P. stylirostris.* As a result, GADTC's *P. stylirostris* production served not only Guam and the region but also commercial farms in Hawaii and research facilities in Florida, South Carolina, Arizona, and Hawaii. Approximately 250,000 broodstock were maintained in reserve. Samples were taken periodically and analyzed by Dr. James Brock of the Hawaii State Aquaculture Development Program and by Dr. Donald Lightner of the University of Arizona to ensure their continued SPF status.

GADTC staff conducted market surveys to identify local demand and price structures for shrimp. Retail outlets were surveyed to identify the sizes, product forms, and prices of shrimp available to consumers. Shrimp raised at GADTC were introduced to retail markets to determine the demand and pricing for fresh, locally raised shrimp. These studies gave farmers information they needed to optimally position their product in the retail market, and encouraged farmers to devote more ponds to shrimp production. GADTC staff provided technical support during all phases of production and marketing for each new pond that was stocked. Ultimately, all four commercial aquaculture farms undertook shrimp production.

GADTC staff then focused on the possibility of selling fresh shrimp to local hotels and restaurants. Chefs and buyers were surveyed to identify the shrimp products being used and interest in locally farmed shrimp. Samples of fresh shrimp were delivered to a sizable cross-section of restaurants for evaluation. The resulting information was provided to farmers. A shrimp tasting was held at an upscale local restaurant to highlight the availability of Guam-raised blue shrimp and to bring together farmers and chefs to facilitate the ordering process. Mass media advertising and table tents promoting locally grown aquaculture products were developed and distributed.

A series of five typhoons during the period from December 1990 through November 1992 and an earthquake of 8.2 on the Richter scale severely damaged *M. rosenbergii* production facilities at the four farms that were culturing the freshwater prawn. Only one farm was able to resume production during the life of this project.

Impacts

This project addressed the major constraint to development of commercial aquaculture, which was a lack of seedstock production to support the local industry. This was resolved with the development of the Guam Aquaculture Development and Training Center as a focal point for aquaculture production, research, and training activities. GADTC benefited not only from the Department of Commerce's management and operation of the facility but also from advice from researchers and students at the University of Guam Marine Laboratory and the UOG Marine Advisory Program. In addition, GADTC benefited from regional input from the Center for Tropical and Subtropical Aquaculture (CTSA), The Oceanic Institute, France Aquaculture, Institut Francais de Recherche et Exploration de Mer (IFREMER), the South Pacific Commission, the University of Hawaii, and the Hawaii

State Aquaculture Development Program (ADP). Finally, as a result of this project, four aquaculture farms undertook culture of freshwater prawns and marine shrimp.

Recommended follow-up activities

It was recommended that the GADTC obtain funding from other sources, particularly the Guam Department of Commerce, to maintain the specific pathogen-free status of shrimp and prawn broodstock and the production of seedstock for interested farmers. In addition, it was recommended that GADTC focus on further diversification of food and aquarium species available for commercial growout and marketing.

Support

This project received support from the Center for Tropical and Subtropical Aquaculture (CTSA), the U.S. Department of the Interior (DOI), the Pacific Aquaculture Association (PAA), and the Guam Department of Commerce (DOC).

Publications, manuscripts, or papers presented

Nelson, S. G., S. A. Lock, and L. A. Collins. 1992. Growth of the rabbitfish, *Siganus randalli* woodland, in relation to the feasibility of its culture on Guam. University of Guam. Mangilao, Guam.

Nelson, S. G. and S. DeC. Wilkins. 1994. Growth and respiration of embryos and larvae of the rabbitfish *Siganus randalli* (Pisces, Siganidae). Journal of Fish Biology. 44: 513-525.

		Other Support				Total
Year	CTSA	DOI	PAA	DOC	Total Other	Support
One	$120,000	$173,000	$0	$60,000	$233,000	$353,000
Two	$119,959	$127,000	$5,000	$59,000	$191,000	$310,959
Three	$74,200	$0	$5,000	$37,000	$42,000	$116,200
Four	$110,000	$0	$5,000	$55,000	$60,000	$170,000
Five	$40,000	$0	$5,000	$60,000	$65,000	$105,000
Total	**$464,159**	**$300,000**	**$20,000**	**$271,000**	**$591,000**	**$1,055,159**

An Aquaculture Resource Manual for Hawaii and the U.S.-Affiliated Pacific Islands

Dates of work, March 1989 through May 1991

Participants

Mr. Mark Brooks, Mr. Allan T. Tom, Mr. Paul Olin, and Ms. Linda Koch, Sea Grant Extension Service, University of Hawaii

Mr. David Crisostomo, Cooperative Extension Service, University of Guam

Reason for termination

This project was terminated because all the objectives were completed.

Project objectives

This project produced a regional catalog and directory for aquaculture supplies, services, and information that:

- enhanced communications between producers, buyers, information specialists, and other professionals in the aquaculture industry within the widely diverse and isolated island states of CTSA's region;
- met practical information requirements of start-up and working aquaculturists in a consolidated, consistent, and useful format consistent with the time demands on most farmers;
- served regional development planners as a supply side or commercial infrastructure assessment of each island state;
- served growth of private industry as an educational catalog in itself and as a stimulus to production ideas, business opportunities, and creative

entrepreneurship; and
- served as a tool for extension personnel to more efficiently serve their operating sector clients, saving the time and effort currently involved in providing routine information to entry-level questions.

Principal accomplishments

This project produced Aqua Pages: An Aquaculture Resource Manual for Hawaii and the American-Affiliated Pacific Islands. The resource manual contained two sections. In a format similar to the telephone directory's "yellow pages," the first section uses particular goods as headings, below which are listed each of the companies that carry that item. For example, 14 companies, their addresses, and phone numbers are listed under the heading "Anchors and Boat Supplies." Area codes and phone numbers are listed for U.S. companies; country codes and phone numbers are given for companies outside the United States.

The second section provides information specific to each island group within the CTSA region. The comprehensive list of topics includes:

- Producers of Aquaculture Products;
- Community and Educational Projects;
- Laboratory Services;
- Researchers;
- Funding Agencies;
- Public Advisory Services;
- Information Services;
- Marketing;
- Shipping;
- Product or Equipment Suppliers; and
- Getting Started.

Impacts

This publication provided aquaculturists within the region with an easy reference guide to suppliers and the help available to them in their area. More than 850 copies of Aqua Pages: An Aquaculture Resource Manual for Hawaii and the American Affiliated Pacific Islands were distributed throughout the United States and the U.S.-Affiliated Pacific Islands.

Recommended follow-up activities

None

Support

This project was funded by the Center for Tropical and Subtropical Aquaculture (CTSA), the Hawaii State Aquaculture Development Program (ADP), and the University of Hawaii Sea Grant Extension Service (SGES).

		Other Support			
Year	CTSA	ADP	SGES	Total other	Total support
One	$34,855	$15,487	$3,872	$19,359	$54,214
Total	**$34,855**	**$15,487**	**$3,872**	**$19,359**	**$54,214**

Publications, manuscripts, or papers presented

University of Hawaii Sea Grant Extension Service. 1991. Olin, P., ed. Aqua Pages: An Aquaculture Resource Manual for Hawaii and the American-Affiliated Pacific Islands. Center for Tropical and Subtropical Aquaculture Publication #102. Waimanalo, Hawaii.

Introduction of Aquaculture to Secondary Schools in Hawaii and the American-Affiliated Pacific Islands

Dates of work, March 1989 through March 1990

Participants

Mr. Steven A. McKay, Anderson Valley Agriculture Institute
Dr. Jim Leising, University of California at Davis

Reason for termination

This project was terminated because all the objectives were completed.

Project objectives

The objectives of this project were to:
- assemble aquaculture curriculum resources into a readily usable form for secondary school teachers;
- work with secondary teachers to help them determine the optimum way to integrate aquaculture into their schools' curriculum and implement a program through which secondary school students could work with elementary school students in aquaculture;
- link instruments with specialists and producers and establish a framework and working relationship so that they can work together to identify aquaculture resources on their own islands;
- help teachers and students establish aquaculture production and marketing programs on their own islands, in their own schools, or in cooperation with an existing producer; and
- establish a communication network between programs on different islands.

Principal accomplishments

Objective: *Assemble aquaculture curriculum resources into a readily usable form for secondary school teachers.*

A national literature search was conducted to identify secondary level aquaculture curriculum resources and instructional materials to assist teachers in improving these programs. Curriculum resource packages identified from the literature search and example aquaculture course outlines were distributed during the phase 1 workshops.

Objective: *Work with secondary teachers to help them determine the optimum way to integrate aquaculture into their schools' curriculum and implement a program for secondary school students to work with elementary school students in aquaculture.*

Thirteen workshops and seminars were conducted for agricultural teachers and administrators in Hawaii and the U.S.-Affiliated Pacific Islands comprising American Samoa, the Commonwealth of the Northern Mariana Islands (CNMI), the Federated States of Micronesia (FSM), Guam, the Republic of Belau, and the Republic of the Marshall Islands. The first phase comprised a series of six workshops that assisted teachers in learning how to plan curriculum and organize a program of aquacultural education. The second phase comprised a series of seven workshops that focused on the implementation of program plans in curriculum, supervised occupational experience, and leadership development. Investigators distributed an aquaculture course curriculum that covered the following topics:
- Introduction to Aquaculture,
- Species Selection,
- Site and Type of Aquaculture Farm,
- Construction of Aquatic Environments,
- Culture Methods, and
- Management.

Programs were developed to implement aquacultural literacy programs in elementary schools in the islands, and some schools developed courses in marine biology and aquaculture.

Objective: *Link instruments with specialists and producers and establish a framework and working relationship so that they can work together to identify aquaculture resources on their own islands.*

During the workshops, resource personnel and aquaculture specialists were identified at each site to provide support for aquacultural education programs on the islands. Aquacultural program advisory committees were developed on each island. The committees included marine biologists, aquaculture specialists, commercial producers, parents, school administrators, and other interested community members. The committees were seen as fundamental to continued development of aquaculture education in the islands.

Objective: *Help teachers and students establish aquaculture production and marketing programs on their own islands, in their own schools, or in cooperation with an existing producer.*

Consultations were conducted with teachers and students to initiate and expand aquaculture demonstration projects at school sites. The Saipan Marine Biology Club and the agriculture program on Maui implemented aquaculture demonstration projects. Chapters of the Future Farmers of America (FFA) were established at high schools in the CNMI, the FSM, and Guam. Some participating FFA students spent their junior year of high school in an exchange aquaculture vocational education program at Anderson Valley. During their senior year back at home, they assisted in aquaculture programs at their high schools and at elementary schools.

Objective: *Establish a communication network between programs on different islands.*

A communication network was explored during the first phase of workshops, and PEACESAT and ADAP computer networks were identified as possible sources. During the second phase of workshops, Anderson Valley Agriculture Institute established an ADAP computer link with Guam, the CNMI, American Samoa, Belau, the FSM, and the Marshall Islands. ADAP was used to disseminate curriculum and instructional materials for teachers, and conferences and professional development seminars that originated at Anderson Valley Agriculture Institute and Lahainaluna High School were broadcast to participating teachers and administrators via PEACESAT. Some aquaculture teachers and administrators participated in professional development opportunities, such as the Summer Science Institute at the University of California at Davis.

Impacts

Education is vital to furthering development of aquaculture in the Pacific Islands. As a result of this project, teachers were trained in teaching methods, an aquaculture curriculum was introduced, courses were implemented, and aquaculture demonstration projects were undertaken. In addition, several chapters of Future Farmers of America were established in the Pacific Islands, and students were able to participate in a student exchange program.

Recommended follow-up activities

It was recommended that the investigators continue their outreach work with funding from other sources.

Support

This project was supported by the Center for Tropical and Subtropical Aquaculture, and Other Institutions.

Year	CTSA	Other Institutions [1]	Total Support
One	$11,000	$46,500	$57,500
Total	**$11,000**	**$46,500**	**$57,500**

[1] The Other Institutions were the University of Hawaii, the College of the Pacific Islands, Pacific Basin Development Council, the U.S. Department of Commerce, and the U.S. Department of Interior.

Publications, manuscripts, or papers presented

None

Economic Feasibility of Aquaculture in the Commonwealth of the Northern Mariana Islands

Dates of work, March 1989 through February 1991

Participants

Mr. Patrick Bryan, Department of Land and Natural Resources, Commonwealth of the Northern Mariana Islands
Mr. William FitzGerald, Department of Commerce, Government of Guam
Dr. Steve Nelson, University of Guam

Reason for termination

This project was terminated because all the project objectives were completed.

Project objectives

The objectives of this project were to:

- identify species appropriate for aquaculture in Saipan, Tinian, and Rota;
- determine the development and operational costs of the four most likely aquaculture candidates;
- determine the development and operational costs and revenues for a small-scale giant clam hatchery facility and growout facility in the adjacent lagoon at Twekesbury Park, Rota;
- identify suitable sites for aquaculture on the islands of Saipan, Tinian, and Rota;
- determine opportunities and constraints for aquaculture development on Saipan, Tinian, and Rota;
- identify local and export market opportunities for the identified species; and
- assess local interest in development of aquaculture as a means of economic sustenance.

Principal accomplishments

The final report, published and distributed in early 1991, found that the Commonwealth of the Northern Mariana Islands' low labor costs and low corporate tax rate offer two distinct advantages to the development of aquaculture. However, the limited land area and high land prices form major constraints to aquaculture development, as does a limited domestic market.

In light of that, the CNMI appears to hold potential for developing a small aquaculture industry to help diversify the economy, generate government revenue, and reduce imports. Investigators evaluated the possibility of culturing 12 species and selected four as most appropriate. They included detailed analyses of a number of biological, physical, and economic factors for those species. To aid development of the industry, the report recommended actions in 13 key areas.

1. Natural resources:
   - lease suitable government lands for aquaculture development;
   - identify and employ growout methods that optimize land use;
   - protect coastal water quality so that commercial aquaculture can be developed;
   - ensure that indigenous aquatic species will not be adversely affected by species introduced for aquaculture;
2. Government services:
   - establish a lead agency to advocate and coordinate research, development, planning, and economic aspects of the aquaculture industry;
3. Regulatory and legal considerations:
   - determine the acceptability of using public land for aquaculture and the potential application of current regulatory and legal requirements needed to implement effective and efficient government policies on aquaculture development;
   - expand support services within the CNMI in proportion to development of the aquaculture industry and limit subsidized support services to smaller, resident commercial developments;
4. Capital:
   - encourage foreign investment in aquaculture — specifically those investments that ease access to export markets;
5. Manpower:
   - periodically evaluate the aquaculture industry's manpower needs in relation to the local labor force;
6. Training:
   - promote aquaculture as a career and develop a technical training program of appropriate scale if development of resident manpower is more desirable than importing foreign labor;

• coordinate government training and private entities' employment of residents;
• incorporate an aquaculture demonstration operation into the College of the Northern Mariana's proposed model farm;
• include in the college curriculum workshops and short courses that are integrated with the establishment of a demonstration farm and the support of the government's lead aquaculture agency;

7. Integration of resources:
• identify opportunities to integrate aquaculture activities with those of other sectors;

8. Market:
• improve information on seafood products entering the commercial market; analyze and monitor various aspects of the market supply and demand as industry develops;
• stabilize production of consistently high quality products to fill local market demands;
• publicize aquaculture products and disseminate information on their nutritional value;
• evaluate potential export markets for high-value products; identify and develop high-value product market niches in which the CNMI may participate;
• explore the development of a fish-processing facility;

9. Species evaluation:
• explore the commercial culture of marine shrimp, milkfish, tilapia and rabbitfish, winged oysters for blister pearls in Garapan Lagoon, giant clams for sashimi for the tourist trade, edible seaweeds such as Gracilaria or Caulerpa for use as fresh vegetables;

10. Development and operational costs for four selected species:
• evaluate organisms and select those most appropriate for initial aquaculture development in the CNMI;
• four organisms — marine shrimp, rabbitfish, milkfish, and tilapia — were selected;
• evaluation of the four included sections on taxonomy and morphology, natural history, environmental tolerances, growth and production, feeding and food conversions, reproduction, stocking, previous and current culture practices, market information, proposed culture methods for the CNMI, and a detailed discussion of development and operation costs, including biological, physical, and economic considerations;

11. Production costs:
• phase farm development to meet the market demand for the product; design farms for maximum efficiency but with enough flexibility to switch to another species as market opportunities arise;
• monitor markets to determine price elasticity to ensure that prices don't fall below an acceptable level;

12. Giant clam hatchery:
• establish the objectives of giant clam culture and determine whether seedstock or growout stock could be better provided by the region's existing facilities;
• explore in detail the markets for 1 year-old clams;

13. Systems:
• explore various culture systems, from semi-intensive to intensive;
• defer pursuit of ocean thermal energy conversion for aquaculture;
• monitor Ocean Thermal Energy Conversion (OTEC) development in other areas and, if commercial viability is demonstrated, solicit private investors, possibly through a joint venture.

Impacts

This report provided a basis for CNMI government agencies and private investors to evaluate the potential for aquaculture from an informed perspective.

Recommended follow-up activities

None

Support

This project was funded by the Center for Tropical and Subtropical Aquaculture.

Year	Center for Tropical and Subtropical Aquaculture	Total support
One	$30,500	$30,500
Total	**$30,500**	**$30,500**

Publications, manuscripts, or papers presented

Nelson, S. G. and W. J. FitzGerald, Jr. 1990. Development of aquaculture in the commonwealth of the Northern Mariana Islands: A feasibility study. Center for Tropical and Subtropical Aquaculture Publication #105. Waimanalo, Hawaii.

Giant Clam Training and Demonstration in the Outer Marshall Island Atoll Communities

Dates of work, March 1989 through February 1993

Participants

Mr. Aliksa Andrike, Republic of the Marshall Islands

Reason for termination

This project was terminated because all the objectives were completed.

Project objectives

The objectives of this project were to:

- continue lagoon nursery operations for 1 to 3 year-old juvenile giant clams begun by the Marshall's Community Action Agency in Majuro lagoon;
- begin outer island nurseries in the outer-lying atolls to growout 3 year-old juveniles to harvest size;
- train and educate outer island communities about modern aquaculture techniques through the establishment of nurseries; and
- develop a giant clam culture training manual.

Principal accomplishments

This project established giant clam nurseries on Jaluit, Ailinglapalap, and Namarik atolls. After discussion with atoll councils, the investigators surveyed potential sites and recommended sites for the nurseries to the councils, and the sites were selected. One person from each atoll, who served as the atoll's "giant clam specialist," attended a 4-week nursery training program at the Community Action Agency's Kalalin nursery in Majuro. That individual then received an initial shipment of Tridacna derasa for growout. He in turn trained other local residents to grow out the clams. The project also produced a giant clam culture training manual in English and Marshallese.

Impacts

This project introduced aquaculture to the outer atolls of the Marshall Islands and helped to establish giant clam growout sites on three separate atolls.

Recommended follow-up activities

None

Support

This project received funding from the Center for Tropical and Subtropical Aquaculture (CTSA) and the government of the Republic of the Marshall Islands (RMI).

		Other Support		
Year	CTSA	RMI	Total other	Total support
One	$15,621	$17,106	$17,106	$32,727
Two	$18,700	$24,076	$24,076	$42,776
Total	**$34,321**	**$41,182**	**$41,182**	**$75,503**

Publications, manuscripts, or papers presented

Marshall Islands Job Training Program Agency/Private Industry Council. 1992. Kobaia, H., ed. Giant Clam Project Training Manual: Experience in nursery management and clam farming techniques. Majuro, Republic of the Marshall Islands.

Giant Clam Aquaculture Sociological Survey

Dates of work, March 1989 through February 1992

Participants

Mr. Flinn Curren, Pohnpei Marine Resources Division, FSM
Dr. Kevin Foster, University of Rhode Island, Kingston, RI
Dr. Barbara Moir, Northern Marianas College, CNMI

Reason for termination

This project was terminated because all the objectives were completed.

Project objectives

The goal of this project was to conduct sociological studies of natives of Pohnpei, Chuuk, and Rota regarding giant clam aquaculture.

Principal accomplishments

The survey found that tridacnid clam consumption patterns were similar throughout all three survey locations. In all locations, the majority of respondents said giant clams were eaten anytime. In all locations, the preferred preparation method was clam meat eaten raw with lime juice, salt, or other seasonings. Clam distribution was primarily for personal consumption and for family and friends.

Responses indicated no areas on reefs where clam taking was traditionally prohibited. Judging from surveyed wholesale and retails prices for 200-mm clams, local market prices appeared too low to sustain development of commercial clam aquaculture without subsidies for clam seed and ocean nursery cage costs. Future development of a commercial clam aquaculture industry would need to rely on tourist and restaurant markets, value-added clam products, and export markets. Development of subsistence clam gardening could be achieved provided individuals and government are willing to bear establishment and labor costs.

Impacts

Giant clam species had a high potential for commercial success as well as for substitution for imported foods and for relieving pressure on other threatened food species in the Pacific Islands. Numerous aquaculture planning meetings indicated giant clams had the highest priority for mariculture development in the U.S.-Affiliated Pacific Islands. However, funding was needed for manpower, travel, training, clam stock, surveys, feasibility studies, communications, and a small amount of equipment and supplies to establish and support demonstration farming and to provide extension for the technology.

This was the first sociological survey conducted to assess the attitudes of Pacific Islanders concerning giant clam aquaculture. It aided managers and planners in assessing potential sociological problems of clam aquaculture and helped identify individuals interested in such work. It also helped address development and management issues as well as the facilitation of data collection. This project provided those interested in commercial clam culture in Micronesia with some of the information needed to make an informed decision about starting such a venture.

Recommended follow-up activities

None

Support

This project received funding from the Center for Tropical and Subtropical Aquaculture and the Pohnpei Marine Resources Division.

Year	CTSA	Other Support		Total Support
		Marine Resources	Total Other	
One	$21,764	$3,000	$3,000	$24,764
Total	**$21,764**	**$3,000**	**$24,764**	**$24,764**

Publications, manuscripts, or papers presented

Curren, F., K. Foster, and B. Moir. 1991. A Survey of Potential Clam Growers in Rota, Commonwealth of the Northern Mariana Islands, and Chuuk and Pohnpei States, Federated States of Micronesia. College of Micronesia Land Grant Program. Pohnpei, FSM.

A Market Study of Pacific Giant Clam Products

Dates of work, March 1989 through December 1994

Participants

Dr. Yung C. Shang and Dr. PingSun Leung, University of Hawaii
Dr. Clem Tisdell, University of Queensland
Dr. Paul Callaghan and Dr. John Brown, University of Guam

Reason for termination

This project was terminated because all the objectives were completed.

Project objectives

The overall goal of this 3-year project was to obtain information about the market potential for giant clam products and to assess the economic feasibility of culturing the animals commercially. Specific objectives related to that goal were to:

- characterize the existing markets for major giant clam products in four key countries — the United States, Japan, Taiwan, and Australia;
- test market giant clams as food items in Honolulu and Guam and as aquarium specimens on the U.S. mainland and in Australia; and
- evaluate the economic feasibility of producing different giant clam products by:
    - collection of production cost data;
    - economic evaluation of different giant clam products; and
    - a comparative economic study.

Principal accomplishments

During the first year of the project, information on markets for giant clam products in Japan, Taiwan, and Australia was collected. Giant clams were found to be popular as sushi and sashimi and in pickled form in Okinawa. The animals were little known as food items on the Japan mainland; however, a limited market existed there for giant clams as aquarium specimens and for their shells. Taiwan had a market for giant clam adductor muscle in either fresh or frozen form. Australia had a thriving market for giant clam shells. The results of the first year were published in 1991 under the title "Report on a Market Survey of Giant Clam Products in Selected Countries."

During the second year of the project, test marketing was conducted for giant clams as food at 13 restaurants in Honolulu. Preliminary data showed that 68 percent of patrons who tried giant clam dishes would be willing to order them again. Unfortunately, although all the permits necessary to import giant clams had been obtained, the Hawaii state health department embargoed the second shipment and refused to release it for the marketing test. In subsequent tests conducted in Saipan, consumers gave giant clams an average 3.5 rating on a scale of 1 to 5. A survey of chefs in the participating restaurants showed that most preferred 3 year-old clams over 2 year-old clams because the older clams had a thicker, firmer meat texture.

Test marketing of giant clams as aquarium specimens was conducted at several cooperating mainland aquarium supply stores. Customers indicated a willingness to pay \$10 to \$15 for a 5- to 10-cm clam and \$15 to \$30 for a 10- to 15-cm clam. The results of the second year of the project were published in July 1993 under the title "Test Marketing of Giant Clams as Seafood and as Aquarium Specimens in Selected Markets."

During the third year of the project, production cost analyses were completed on giant clam culture systems being used in the Republic of the Marshall Islands, the Federated States of Micronesia, the Republic of Belau, and American Samoa. The results of these analyses were published in December 1994 under the title "Production Economics of Giant Clam (*Tridacna* spp.) Culture Systems in the U.S.-Affiliated Pacific Islands."

Two distinct culture systems were analyzed in the Marshall Islands. One system used raceways in the hatchery phase, and the other system used floating tanks. Both systems use floating platforms outfitted with plastic trays during the nursery phase and growout the clams on shallow fringing reefs. The raceway system produced an 8 month-old *Tridacna gigas* for $0.41, and the floating tank system produced the same age *T. gigas* for $0.23. Raising 3 year-old clams in the raceway system cost $5.08 each and in the floating tank system cost $4.83 each.

The Micronesian Mariculture Demonstration Center (MMDC) in Belau used a land-based hatchery and combined land-based raceways and ocean-cage nurseries to produce 2 year-old *T. derasa*. Producing a 1 year-old clam, which was done entirely in the land-based system cost $0.82. Producing a 2 year-old clam, which used ocean cages for the last 12 months, cost $1.41.

The facilities in American Samoa and Kosrae, FSM, were similar to the MMDC but had lower production levels. Producing a 2 year-old *T. derasa* cost $1.23 in Kosrae and $3.20 in American Samoa.

The optimal harvest time to maximize economic returns for *T. derasa* varied depending on the product derived. Optimal harvest age ranged from:

- no production to 13.2 years if only the shells were assumed to be salable;
- no production to 9.2 years if only the adductor muscles were assumed to be salable;
- no production to 10.5 years if only the other meat was assumed to be salable;
- no production to 9.0 years if all meat was assumed to be salable; and
- no production to 10.7 years if all products were assumed to be salable.

Year	CTSA	Other support UH	UOG	UQ	Total other	Total support
One	$53,200	$29,234	$0	$12,984	$42,218	$95,418
Two	$79,600	$30,404	$9,480	$16,218	$56,102	$135,702
Three	$30,000	$42,847	$0	$16,866	$59,713	$89,713
Total	**$162,800**	**$102,485**	**$9,480**	**$46,068**	**$158,033**	**$320,833**

Impacts

This 3-year project provided giant clam farmers in the region with information regarding potential markets and constraints to culturing and marketing giant clams that was vital to help them to make informed business decisions.

Recommended follow-up activities

The economic feasibility study should be updated to reflect significant changes that occurred during 1993 and 1994. At the time this study was initiated, farmers were primarily marketing their giant clams as food products. However, during 1993 and 1994, farmers began selling their giant clams at higher prices to the aquarium market.

Support

This project received funding from the Center for Tropical and Subtropical Aquaculture (CTSA), the University of Hawaii (UH), the University of Guam (UOG), and the University of Queensland (UQ).

Publications, manuscripts, or papers presented

Leung, P. S., Y. C. Shang, K. Wanitpraphа, and X. Tian. 1993. Production economics of giant clam (*Tridacna* species) culture systems in the U.S.-Affiliated Pacific Islands. Center for Tropical and Subtropical Aquaculture #114. Waimanalo, Hawaii.

Shang, Y. C., P. S. Leung, and C. Price. 1990. Marketing of giant clam products. Presented at the International Conference on Fisheries Economics and Trade. December 1990. Santiago, Chile.

Shang, Y. C., C. Tisdell, and P. S. Leung. 1991. Report on a market survey of giant clam products in selected countries. Center for Tropical and Subtropical

Aquaculture Publication #107. Waimanalo, Hawaii.
Shang, Y. C., P. S. Leung, J. Brown, and C. Tisdell. 1992. Test marketing of giant clams as seafood and as aquarium specimens in selected markets. Center for Tropical and Subtropical Aquaculture Publication #110. Waimanalo, Hawaii.
Tisdell, C. 1989. Market for giant clam shells: Report on a survey of retailers and wholesalers in Southeast Queensland, Australia. Research Reports and Papers in Economics of Giant Clam Mariculture, #1. Queensland, Australia.
Tisdell, C. 1989. Market for giant clams as aquarium specimens: Report on a survey of retailers of supplies for saltwater aquariums, Southeast Queensland, Australia. Research Reports and Papers in Economics of Giant Clam Mariculture, #3. Queensland, Australia.
Tisdell, C. 1990. Exploring the demand for farmed giant clams and their components: Approaches and Problems. Research Reports and Papers in Economics of Giant Clam Mariculture, #7. Queensland, Australia.
Tisdell, C. 1991. Report on possible demand for giant clam meat by Tongan descendants in Australia: Inferences from interviews conducted in the Brisbane area. Research Reports and Papers in Economics of Giant Clam Mariculture, #8. Queensland, Australia.
Tisdell, C. 1991. A report on the test marketing of giant clams as aquarium specimens in Brisbane, Australia. Research Reports and Papers in Economics of Giant Clam Mariculture, #26. Queensland, Australia.
Tisdell, C. and T. Vinnicombe. 1992. The market for giant clams as aquarium specimens in Sydney and Melbourne: Results of a telephone survey of retail outlets. Research Reports and Papers in Economics of Giant Clam Mariculture, #28. Queensland, Australia.
Tisdell, C. 1992. Interest of Asian restaurants in Queensland in using giant clam meat in their cuisine and their knowledge of it. Research Reports and Papers in Economics of Giant Clam Mariculture, #35. Queensland, Australia.
Tisdell, C. and C. H. Chen. 1992. Notes on the use of giant clam meat for food in Taiwan. Research Reports and Papers in Economics of Giant Clam Mariculture, #36. Queensland, Australia.
Tisdell, C. and Y. Kuronuma. 1992. Interest of Japanese restaurants in Brisbane in using giant clam meat in their cuisine and their knowledge of it. Research Reports and Papers in Economics of Giant Clam Mariculture, #37. Queensland, Australia.
Tisdell, C., J. Barker, and B. Stevens. 1993. Business strategies for the growing of giant clams: What paths have been or are being followed by enterprises? Research Reports and Papers in Economics of Giant Clam Mariculture, #38. Queensland, Australia.
Tisdell, C. and Y. Kuronuma. 1993. Giant clam in Japanese cuisine — Brisbane trials and the use of giant clam meat in the Ryukus. Research Reports and Papers in Economics of Giant Clam Mariculture, #39. Queensland, Australia.

Sponge Aquaculture Demonstration Project

Dates of work, March 1989 through March 1994

Participants

Mr. Richard Croft, College of Micronesia
Mr. Flinn Curren, Pohnpei Marine Resources Division, FSM

Reason for termination

This project was terminated because all the objectives were completed.

Project objectives

The objectives of this project were to:

- establish a small-scale demonstration sponge nursery farm to provide preliminary data on growth of Pohnpei “wool” bath sponges after cutting and planting;
- maintain and improve the demonstration farm’s stock of cultured sponges;

- obtain growth data on sponges planted on the demonstration farm;
- develop a cost-effective method for cleaning large quantities of sponges;
- finalize the economic feasibility of culturing sponges;
- prepare a long-range plan for developing the sponge aquaculture industry in the FSM;
- develop a training outline to be used for future extension work; and
- train local residents in sponge culture techniques and assist them with starting their own private farms.

Principal accomplishments

During the first year of this project, stocks of wild sponges were located, and a farm site was surveyed, selected, and planted. First, 150-pound-test nylon fishing line was strung between two sets of coral heads. Lighter test line, which was used for planting lines, was strung from the heavy lines in parallel rows 2 feet apart. The upper 2/3 of the wild sponge parent stock were cut away, and while submerged, divided into cuttings that weighed at least 200 grams each.

The resulting cuttings were then threaded with loops of tarred, 35-pound-test nylon fishing line, and the loops suspended from the planting lines spaced 14 to 16 inches apart. The demonstration farm was planted with more than 5,000 cuttings. One hundred sponges planted in five sectors of the farm were tagged; these were measured every 6 months. Sponges in the five sectors grew at average rates ranging between 8 and 17 percent per month during the first year. Growth data gathered in subsequent years showed average growth rates of 7.5 percent per month for sponges in Farm Sector A, 6.9 percent in Farm Sector AA, 7.2 percent in Farm Sector B, and 7.0 percent in Farm Sector C. The overall average growth rate was 7.15 percent per month. Within each sector, growth rates varied greatly, ranging from 4 to 9 percent in Sector A, 4 to 12 percent in Sector AA, 3 to 15 percent in Sector B, and 3 to 13 percent in Sector C.

A cost-effective method for cleaning the sponges after harvest was developed. Preliminary market and economic research was done. First, samples of 2 year-old sponges were sent to potential buyers on the U.S. mainland. Two buyers responded very favorably, indicating that they would pay $1 to $1.25 for a 4- to 5-inch sponge. A West Coast buyer indicated a willingness to buy 200,000 sponges per year. Based on that information, an economic evaluation was done by a researcher at the University of New South Wales. It showed that commercial sponge farming in Micronesia has strong potential for success.

Then, 120 sponges from a private farm were marketed at a Guam/Micronesian Island Fair. During the 3-day event, 107 sponges were sold for an average price of $6. Beginning in 1991, sponges from the private farm were sold in the Pohnpei tourist market. Those sponges, priced at $6 each, sold briskly. Although marketing the sponges this way involves a packaging cost, the overall profit to farmers is considerably more than what was offered by the mainland buyers.
The coasts of other islands were surveyed for wild sponge stocks. Sponges of a type similar to Pohnpei sponges were found in the waters off Chuuk State, FSM. Other varieties of sponges that may have commercial value were found in the waters off the Republic of the Marshall Islands.

A draft sponge culture training manual was developed. Five Pohnpei residents were trained in sponge culture techniques and assisted with starting their own private sponge farms. Each individual was given 2,500 sponge cuttings and assisted with planting them. The project investigator visited each farm monthly to inspect it and answer farmers' questions. During the monthly visit, the investigator brought each farmer 100 new sponge cuttings and planting supplies to expand his farm. This maintained the farmers' enthusiasm during the lengthy sponge growout period and allowed them to start harvesting and marketing some of their large sponges sooner rather than using them to expand the farms. The five farmers began to harvest and sell limited numbers of sponges during early 1995. At that point, other Pohnpei residents expressed interest in learning culture techniques and starting farms.

Impacts

This project not only offers Micronesian residents the opportunity to start their own aquaculture businesses but also helps to diversify narrowly based economies of the U.S.-Affiliated Pacific Island nations.

Recommended follow-up activities

It was recommended that a project be undertaken to investigate the cause of the wide variation in sponge growth rates. That project, titled “Differential Growth

Rate Studies in Cultured Commercial Sponges," was begun under CTSA's 6th Annual Plan of Work and is ongoing.

Support

This project received funding from the Center for Tropical and Subtropical Aquaculture (CTSA), the College of Micronesia (COM), the Pohnpei Marine Resources Division (MRD), and the principal investigator (PI).

Publications, manuscripts, or papers presented

Brown, J. and R. A. Croft. 1994. Marketing Commercial Sponges to the Tourist Trade. Presentation at the World Aquaculture Society Meeting. January 14-18, 1994. New Orleans, Louisiana.

Croft, R. A. 1991. Recommendations for Establishing a Sponge Industry Within the Region. Center for Tropical and Subtropical Aquaculture Publication #106. Waimanalo, Hawaii.

		Other support				
Year	CTSA	COM	MRD	PI	Total other	Total support
One	$10,500	$0	$4,220	$0	$4,220	$14,720
Two	$10,900	$0	$4,970	$0	$4,970	$15,870
Three	$16,124	$0	$1,700	$0	$1,700	$17,824
Four	$38,550	$8,100	$0	$0	$8,100	$46,650
Five	$30,380	$75	$625	$7,315	$8,015	$38,395
Total	**$106,454**	**$8,175**	**$11,515**	**$7,315**	**$27,005**	**$133,459**

Croft, R. A. 1994. An overview of Commercial Sponge Farming Activities in Micronesia. Presentation at the World Aquaculture Society Meeting. January 14 - 18, 1994. New Orleans, Louisiana.

Introduction of New Aquaculture Species for Biological Culture Assessment

Dates of work, March 1991 through August 1994

Participants

Dr. Christopher Brown and Dr. Kirk Hahn, Hawaii Institute of Marine Biology, University of Hawaii

Dr. Bruce J. Miller, Mr. Paul Olin, Mr. Richard Bailey and Mr. Brian Cole, Sea Grant Extension Service, University of Hawaii

Dr. David Leighton, San Diego State University

Reason for termination

This project was terminated because all the objectives were completed.

Project objectives

The objectives of this project, initiated under the Center for Tropical and Subtropical Aquaculture's 4th Annual Plan of Work, were to:

- document the review process for import permit applications and prepare and publish specific guidelines for preparation and submission of permit applications; and
- import the warm-water abalone species, *Haliotis diversicolor supertexta* and *H. fulgens*, for growout trials at two commercial facilities, and publish a brochure to disseminate results of the growout trials and recommendations for abalone culture in Hawaii.

Principal accomplishments

Objective: *Document the review process for import permit applications and prepare specific guidelines for preparation and submission of permit applications. Publish the guidelines as an extension fact sheet.*

Investigators completed the process to obtain a species import permit for the warm-water abalone species, *Haliotis diversicolor supertexta* and *H. fulgens*. They then prepared and published a fact sheet explaining the process, which documented the following key points:

Importation of all living organisms to Hawaii is regulated by the Plant Quarantine Branch of the state Department of Agriculture, which maintains lists of approved, restricted, and prohibited species. Organisms on the prohibited list cannot be imported to Hawaii. Both importation and possession of species on the restricted list require permits. An import permit is required for species on the conditionally approved list; such animals are not monitored after their arrival in Hawaii.

To import a living organism to Hawaii, an individual must submit an import permit application to the Plant Quarantine Branch Subject Matter Specialist in Honolulu. The Branch employs specialists in invertebrate and aquatic biota, microorganisms, insects, land vertebrates, and plants. The application form must be completed with the species, number, import dates, supplier, and responsible person. A vital component of the import permit application is a species profile, which must include:
• the complete species name,
• potential local prey,
• potential competitors,
• potential local predators,
• hybridization potential,
• feeding strategy and diet,
• reported diseases and treatments,
• species origin,
• specific goals and objectives of the importation,
• quarantine procedures and facilities,
• mode of reproduction,
• whether the species is protected or endangered,
• potential impact of introduction,
• requirements for reproduction,
• potential benefits of introduction,
• control or eradication measures,
• a description of holding or culture facilities,
• collection methods and permits,
• requirements of adults, eggs, and larvae, and
• migration and dispersal mechanisms.

The completed permit application is submitted to the plant quarantine branch of the health department, where it begins a 20-step review process. The review may result in issuance of a permit with conditions for import of the selected species.

Objective: *Import the warm-water abalone species* Haliotis diversicolor supertexta *and* H. fulgens *and conduct growout trials at two commercial facilities. Publish a brochure to disseminate the results of the growout trials and recommendations for abalone culture in Hawaii.*

Growout trials of the green abalone, *H. fulgens*, and the Taiwanese abalone, *H. diversicolor supertexta*, were conducted for 12 months at two cooperating commercial farms, Hawaiian Marine Enterprises on Oahu and Royal Hawaiian Seafarms on the island of Hawaii. The trials demonstrated that both species potentially could be profitable as supplemental crops on commercial seaweed culture facilities in Hawaii if surplus and off-grade seaweed were used as abalone feed. The results of the growout trials were published in two extension fact sheets that provide information on:
• import and quarantine requirements and procedures,
• sources of abalone seed,
• shipping procedures,
• background on the development of abalone culture,
• environmental requirements for abalone culture,
• the results of the project growout trials, and
• opportunities for abalone culture in Hawaii.

Impacts

Commercial aquafarmers often find the process to import new species to Hawaii to be a lengthy and confusing process. This project clearly defined the steps involved, and more importantly, the information required by regulators of the process. This eliminated some of the confusion for farmers who want to import new aquaculture species.

Recommended follow-up activities

None

Support

This project received funding from the Center for Tropical and Subtropical Aquaculture (CTSA), the Sea Grant Extension Service (SGES), the Hawaii State Aquaculture Development Program (ADP), Hawaiian Marine Enterprises (HME), and Royal Hawaiian Seafarms (RHS).

Year	CTSA	Other support SGES	ADP	HME	RHS	Total other	Total support
One	$21,300	$3,300	$3,325	$6,100	$6,100	$18,825	$40,125
Total	**$21,300**	**$3,300**	**$3,325**	**$6,100**	**$6,100**	**$18,825**	**$40,125**

Publications, manuscripts, or papers presented

Olin, P. 1993. Importing live organisms to Hawaii: procedures and permitting. Fact Sheet #1. Center for Tropical and Subtropical Aquaculture. Waimanalo, Hawaii.

Olin, P. 1994. Abalone Culture in Hawaii: Importation, quarantine and seed suppliers. Fact Sheet #2. Center for Tropical and Subtropical Aquaculture. Waimanalo, Hawaii.

Olin, P. 1994. Abalone culture in Hawaii: *Haliotis fulgens* and *Haliotis diversicolor supertexta*. Fact Sheet #3. Center for Tropical and Subtropical Aquaculture. Waimanalo, Hawaii.

Expert System Disease Module for Hawaiian Aquaculture

Dates of work, March 1991 through October 1995

Participants

Dr. Stephen Itoga, Department of Information and Computer Sciences, University of Hawaii

Dr. Jim Brock, Aquaculture Development Program, State of Hawaii

David E. Coleman, Hamilton Library, University of Hawaii

Reason for termination

This project was terminated because all the objectives were completed.

Project objectives

The objectives of this project are to:

- develop an expert system disease module, a computer software program that will help aquafarmers and extension personnel diagnose and manage diseases of tilapia cultured in Hawaii;
- redesign the major modules of the expert system to take advantage of the graphical user interface of Microsoft Windows® and implement the new design using that environment; and
- implement the new design using the Macintosh development environment.

Progress and principal accomplishments

The goal for the first 2 years of work, initiated under the CTSA 4th Annual Plan of Work, was to develop an expert system disease module, a computer software program that could help aquafarmers and extension personnel diagnose and manage diseases of tilapia cultured in Hawaii.

To start, investigators surveyed 94 groups involved in aquaculture, asking them to rank species for inclusion in the expert system. Based on the results of the survey, investigators selected tilapia for the program.

A list of diseases and syndromes of tilapia cultured in Hawaii was compiled, and published information on tilapia diseases was gathered and indexed. In addition, information on local disease problems was drawn from the files of Dr. Brock, a veterinarian and state aquaculture disease specialist who has worked with cultured tilapia for more than 10 years.

A computer software program, PDC Prolog®, was tested and found suitable to be the basis of the expert system. Slides of animals showing various disease symptoms were digitized and incorporated into the software.

The expert system disease module was completed as Center for Tropical and Subtropical Aquaculture Publication #111. Titled "Hawaii Aquaculture Module Expert System," the program runs in a Microsoft DOS® environment. It has five components:

- "Review information," which is divided into six sections:
    1. An Overview of HAMES,
    2. Reading Topics, Glossary,
    3. Laboratory Methods,
    4. Bibliography,
    5. Extension Assistance, and
    6. Vendors;
- "Volume determination," a utility designed to compute the volume of a wide variety of containers;
- "Solve a problem," which uses observation and selected water parameter tests to help the tilapia farmer understand diseases that may affect tilapia in Hawaii. It is divided into four sections:
    1. Field Observations,
    2. Microscopy,
    3. Water Analysis, and
    4. Ammonia;
- "Treatment," which contains seven sections:
    1. List of Chemicals,
    2. Ectoparasites,
    3. Anesthesia,
    4. Bacteria,
    5. Disinfection,
    6. Fungi, and
    7. Algae Control;
- "Control this session," which allows users to view data and information from the last session, save the current session, or print all the data and information in the current session.

The expert system comes complete with an instruction manual and is available on either 3.5" or 5.25" floppy diskettes. A $10 fee to cover the cost of the diskettes is charged for distribution outside the CTSA region. As of October 1995, more than 100 copies of the expert system had been requested and distributed.

Objective: *Redesign the major modules of the expert system to take advantage of graphical user interface of Microsoft Windows and implement the new design using that environment.*

Investigators have completed redesigning the program to operate in an Microsoft Windows® environment. The implementation is currently being refined and tested. The new program will contain enhanced graphics and a more "user-friendly" interface. The Windows program will be available by the end of 1995.

Objective: *Implement the new design using the Macintosh development environment.*

Investigators have implemented the design to operate on Macintosh computers. They are currently testing the implementation of the program. The Macintosh version of the software will be available by the end of 1995.

Impacts

The cost of developing a computer software program can be several hundred thousand dollars. The project funding therefore represents a cost benefit of at least $10 for each dollar spent.

Recommended follow-up activities

After the program has been in use by farmers, its programming and technical information may need revisions and updating.

Support

This project received funding from the Center for Tropical and Subtropical Aquaculture, the Hawaii State Aquaculture Development Program (ADP), and the University of Hawaii (UH).

		Other support			
Year	CTSA	ADP	UH	Total other	Total support
One	$16,709	$2,208	$5,000	$7,208	$23,917
Two	$27,849	$1,325	$5,000	$6,325	$34,174
Three	$30,000	$4,800	$5,000	$9,800	$39,800
Total	**$74,558**	**$8,333**	**$15,000**	**$23,333**	**$97,891**

Publications, manuscripts, or papers presented

Brock, J. A., S. Itoga, Y. Liu, H. Fujii, and D. E. Coleman. 1992. Hawaii Aquaculture Module Expert System. Center for Tropical and Subtropical Aquaculture Publication #111. Waimanalo, Hawaii.

Aquaculture Marketing Assistance Program for Guam

Dates of work, April 1992 through June 1994

Participants

David P. Crisostomo, Dr. John W. Brown, and Dr. Jeff Barcinas, University of Guam

Reason for termination

This project was terminated because all the objectives were completed.

Project objectives

The objectives of the project were to:

- educate consumers about Asian catfish in order to expand the market for the product on Guam;
- conduct preliminary market research, including surveys of consumers at cooking demonstrations to learn their acceptance of catfish and to estimate the size of the potential market; and
- investigate partial mechanization for processing Asian catfish.

Principal accomplishments

Objective: *Educate consumers about Asian catfish in order to expand the market for the product on Guam.*

Investigators completed two educational publications. The first was a pamphlet describing how to prepare Asian catfish for cooking. The second publication was a set of six cards bearing recipes developed by home economists at the College of Agriculture and Life Sciences at the University of Guam. Five hundred of the 1,000 copies printed of each were distributed through the Guam Cooperative Extension Service; the remainder are being distributed upon request from farmers.

Objective: *Conduct preliminary market research, including surveys of consumers, at cooking demonstrations to learn their acceptance of catfish and to estimate the size of the potential market.*

Investigators held a series of cooking demonstrations and taste tests at the four largest supermarkets on the island. Those who tasted the fish samples were asked to complete a short questionnaire that asked:

- whether respondents were the primary food shoppers for their households;
- the number of individuals in their households;
- the number of times per week their households ate seafood;
- the ethnic extraction of their households;
- their rating of the catfish samples on a scale from "not good" to "excellent;"
- whether they would buy Asian catfish in the future; and
- what a reasonable price would be in their opinions.

Ninety-seven percent of the 120 self-selected respondents indicated their willingness to purchase Asian catfish in the future, and on average, rated a price of US $5.68 per kilogram as reasonable. Filipinos ate seafood most frequently, followed by "others," Chamorros, mixed households, and U.S. mainlanders. Respondents rated the smoked Asian catfish highest, followed by the gingered Asian catfish, fried Asian catfish, and fried channel catfish.

Objective: *Investigate partial mechanization of processing Asian catfish.*

Investigators sent Asian catfish samples to a small plant in Mississippi for processing. Plant workers were able to process 50 to 60 kilograms of Asian catfish per man-hour with a yield of 59 percent per headed, skinned and dressed fish. However, a sensitivity analysis showed that processed Asian catfish would be viable in the local market only at the lowest fresh fish price, which is $3.30 per kilogram. Farmers sold live milkfish and tilapia for $6.60 per kilogram; both animals could be raised in the same ponds used for catfish. Thus, raising lower value catfish would have been less profitable than raising milkfish or tilapia.

Impacts

Several educational products on Asian catfish were developed to expand potential markets, and a consumer taste test and survey informed farmers about consumer preferences. This project determined that consumers were willing to buy Asian catfish if fillets were available in markets. However, the price of the processed product was too low to make the additional processing economically feasible.

Recommended follow-up activities

None

Support

This project received funding from the Center for Tropical and Subtropical Aquaculture (CTSA) and the University of Guam (UOG).

Publications, manuscripts, or papers presented

Crisostomo, D. P., J. W. Brown, and J. Barcinas. 1992. Guam catfish: A delicious, nutritious aquaculture product. Guam Cooperative Extension Service, College of Agriculture and Life Sciences, University of Guam. Mangilao, Guam.

Guam Cooperative Extension Service. 1993. Yellow Gingered Catfish; Baked Garlic Catfish; Breaded Fried Catfish; Oriental Catfish; Sweet & Sour Catfish; Escabeche. College of Agriculture and Life Sciences, University of Guam. Mangilao, Guam.

		Other support		Total
Year	CTSA	UOG	Total other	support
One	$16,907	$3,960	$3,960	$20,867
Total	**$16,907**	**$3,960**	**$3,960**	**$20,867**

Exploratory Study of Hawaii and Guam as High Health Aquaculture Stock Centers

Dates of work, March 1991 through September 1994

Participants

Dr. Christopher L. Brown and Dr. Kirk Hahn, Hawaii Institute of Marine Biology, University of Hawaii

Dr. James Brock, Hawaii State Aquaculture Development Program

William FitzGerald, Guam Department of Commerce

Dr. Gary Pruder, The Oceanic Institute

Reason for termination

This project was terminated because all the objectives were completed.

Project objectives

The objectives of this project, were to:

- construct an overview of established agricultural methods for producing, maintaining, and distributing specific pathogen-free (SPF) livestock in the United States;
- construct an overview of established aquacultural methods for developing, maintaining, and distributing SPF livestock in U.S. catfish, trout and salmon, Norwegian salmon, Japanese flounder, and the international ornamental fish trades;
- conduct a preliminary survey of the demand for SPF marine shrimp in Hawaii and Guam;
- estimate the approximate world market size and market value of at least three species of SPF marine shrimp, *Penaeus vannamei*, *P. monodon*, and *P. chinensis*; and
- develop a business opportunity report, which includes a preliminary business plan, system design and operating protocol for a modular facility for the housing and distribution of SPF broodstock, and seed marine shrimp and other aquaculture species. The plan will include potential operating and capital costs, levels of production, and rates of return for the selected species.

Principal accomplishments

Objective: *Construct an overview of established agricultural methods for producing, maintaining, and distributing specific pathogen-free (SPF) livestock in the United States; construct an overview of established aquacultural methods for developing, maintaining, and distributing SPF livestock in U.S. catfish, trout and salmon, Norwegian salmon, Japanese flounder, and the international ornamental fish trades*

Investigators attended an SPF marine shrimp breeding workshop sponsored by The Oceanic Institute's Marine Shrimp Program in July 1991. The workshop focused on the genetics of breeding animals within closed populations and in specialized facilities. At the workshop, a recommendation was made that a second SPF facility be established "to secure continued production of SPF stock and to reduce the risk of losing SPF progress to contamination of the single facility."

An extensive database was developed on SPF culture techniques used to produce a wide variety of organisms. Those techniques were reviewed for their applicability to the culture of penaeid shrimp, the species deemed most likely to be the first choice for large-scale commercial SPF culture in Hawaii.

The majority of SPF culture research was done on pigs and laboratory animals such as mice and rats. Two concepts from pig breeding could be adapted to shrimp hatchery production.

- U.S. and Canadian pig research has emphasized breeding programs. It demonstrated that genetic variability can be maintained in broodstock even when the initial population is small.
- The hatchery owner and commercial farmers develop an interdependent relationship. Farmers must depend on the integrity of the hatchery to deliver certified high health animals. The hatchery owner must build trust by:
    - maintaining a close relationship with the farmers;
    - educating them to new techniques; and
    - guaranteeing that, should the hatchery experience a problem, no animals will be sold until it is resolved.

Workers at SPF facilities are the most probable sources of pathogen contamination. Designing a facility to ensure its cleanliness, as well as educating workers to be aware of their personal habits at all times, is vital.

Techniques using SPF broodstock to produce juveniles for resale to commercial aquaculture producers are being used in the Norwegian salmon industry. Although the basic techniques are the same as those used in rearing terrestrial animals, the use of water adds new aspects. The cost of moving and sterilizing water is much higher and more difficult to accomplish. Most culture facilities use flow-through systems, which could be disastrous for aquatic species. In-flowing water presents a constant potential source of pathogen contamination. Freshwater species are somewhat easier because deep wells that are free of pathogens can be used. However, water for marine species is typically drawn from near-shore subtidal zones. Semi-open, recirculating systems can reduce the cost of sterilization and filtration while maintaining the high quality water necessary for an SPF facility.

By definition, SPF production is intended to prevent the contamination of broodstock by specified pathogens that have a deleterious effect on the animals' growth and reproduction. Thus a thorough knowledge of these pathogens — including their mode of transmission, life cycle, hosts, resistance to antibiotics, and effects on the animals — is necessary before techniques can be developed to prevent their introduction to stocks. Work on SPF Norwegian salmon helped to define the aspects of shrimp culture that need to be analyzed. Having extensive records on the growth and reproduction of the broodstock, and the survival and growth of the offspring is essential for a successful breeding program and continued production of healthy, genetically variable offspring.

Objective: *Conduct a preliminary survey of the demand for SPF marine shrimp in Hawaii and Guam; estimate the approximate world market size and market value of at least three species of SPF marine shrimp,* Penaeus vannamei, P. monodon, *and* P. chinensis.

The market study of Ecuador, the Philippines ,Thailand and Indonesia, was completed and published in June 1994. Some interesting points came from the market study.

- In 1991, shrimp aquaculture from these four countries totaled approximately 350,000 metric tons and accounted for more than half the world total of shrimp produced from aquaculture.
- All four countries made a major commitment to the production of postlarvae from hatcheries, and all four have experienced production problems caused by either animal quality or disease.
- All four countries had well developed, fully integrated shrimp farming sectors with the technological capability to incorporate a program of specific pathogen-free culture readily into their existing culture systems.

- Indications were that Ecuadorian farmers would readily accept specific pathogen-free *P. vannamei* because the species has been shown to be resistant to runt deformity syndrome, which is caused by the presence of the IHHN virus.
- Speculation on the market for SPF stocks in Southeast Asia proved more difficult. The region primarily produced *P. monodon*, which had not been studied under production conditions, so the impact of both *monodon* baculovirus and IHHN was unclear. However, shrimp farmers in Southeast Asia have shown in interest in development of an SPF *P. monodon* stock.

Objective: *Develop a business opportunity plan for a high health shrimp facility in Hawaii and Guam.*

The business plan, completed and published in July 1994, raised a number of interesting points.

- Worldwide production of marine shrimp grew from 84,022 metric tons in 1982 to 663,800 metric tons in 1991, an increase of 790 percent. In 1991, Asian countries produced 522,840 metric tons of shrimp, and Western Hemisphere countries produced 140,960 metric tons of shrimp.
- The increasing frequency of viral diseases seriously threatens future production of farmed marine shrimp and forms, in the opinion of some shrimp experts, the biggest obstacle facing worldwide shrimp production. For example, during the mid-1980s, Ecuadorian shrimp farmers lost about 15 percent of their *P. vannamei* production to a virus. In Taiwan, *P. monodon* production fell from 70,000 tons in 1986 to 20,000 tons in 1989 due to a virus.
- Markets for SPF shrimp are potentially large in both the Western Hemisphere and Asia. Annually, Western farmers, mainly in Ecuador, utilize 30 billion nauplii, 21 billion post-larvae, and 120,000 broodstock of *P. vannamei* worth approximately $85 million. Each year Asian farmers use 41 billion nauplii, 16 billion post-larvae, and 36,000 broodstock with a combined value of approximately $90 million.

Impacts

This project provided interested aquaculturists in Hawaii and Guam with information they would need before establishing facilities to produce high health shrimp for sale to producers throughout the world.

Recommended follow-up activities

None

Support

This project received funding from the Center for Tropical and Subtropical Aquaculture (CTSA), the University of Hawaii (UH), and the Hawaii State Aquaculture Development Program (ADP).

		Other support			
Year	CTSA	UH	ADP	Total other	Total support
One	$39,791	$6,082	$2,208	$8,290	$48,081
Two	$61,531	$6,082	$2,208	$8,290	$69,821
Total	**$101,322**	**$12,164**	**$4,416**	**$16,580**	**$117,902**

Publications, manuscripts, or papers presented

Brown, C. L., K. O. Hahn, and D. E. Coleman. 1995. A bibliography of specific pathogen-free organisms. Center for Tropical and Subtropical Aquaculture Publication #116. Waimanalo, Hawaii.

Hahn, K. O., C. L. Brown, and G. D. Pruder. 1994. Standard agricultural practices for the culture of specific pathogen-free organisms, and their applications to aquaculture. Reviews in Fisheries Science. 2(4): 315-330.

Iversen, R. T. B. and C. L. Brown. 1993. A preliminary business opportunity plan for specific pathogen-free shrimp culture facilities in Hawaii and Guam. Center for Tropical and Subtropical Aquaculture Publication #113. Waimanalo, Hawaii.

Kuljis, A. M. and C. L. Brown. 1992. A market study of specific pathogen-free shrimp. Center for Tropical and Subtropical Aquaculture Publication #112. Waimanalo, Hawaii.

Pruder, G. D., C. L. Brown, J. N. Sweeney, and W. H. Carr. 1995. High health shrimp systems: Seed supply — theory and practice. In Browdy, C. L. and J. S. Hopkins, eds. Swimming through troubled water: Proceedings of the Special Session on Shrimp Farming, Aquaculture '95. World Aquaculture Society. Baton Rouge, Louisiana.

Investigation of Mullet Net-Pen Stocking Densities in Coastal Fishponds for a Stock Enhancement Nursery Program

Dates of work, April 1992 through October 1995

Funding level

$88,700

Participants

Dr. Kenneth Leber, The Oceanic Institute
Dr. Robert Nishimoto, Department of Aquatic Resources, Hawaii State Department of Land and Natural Resources
Mary Brooks, Hawaiian Island Sea Farms

Project objectives

The overall goal of this project, which received funding under the Center for Tropical and Subtropical Aquaculture's 5th and 7th Annual Plans of Work, was to determine whether traditional Hawaiian fishponds and other in-ocean ponds could play a role in improving inshore fisheries by serving as nursery facilities for stock enhancement efforts. Specific objectives related to that goal are to:

- synthesize available literature and data on Hawaii fishponds in reference to socio-economic feasibility issues related to their use as a stock enhancement resource;
- develop transport methods to transfer mullet from the hatchery to the coastal fishponds;
- determine the optimal stocking density for juvenile mullet in net pens;
- determine the optimal net-pen design for mullet nursery culture in coastal fishponds;
- compare growth and survival of juvenile mullet in net-pens with land-based tank culture; and
- establish preliminary economic data for use in analysis of the cost of net-pen operations and production.

Reason for termination

This project was terminated because all the objectives were completed.

Principal accomplishments

Objective: *Synthesize available literature and data on Hawaii fishponds in reference to socio-economic feasibility issues related to their use as a stock enhancement resource.*

A report on general conditions in coastal Hawaiian fishponds similar to Heeia Fishpond, which is the site of this project, was compiled. Fishponds played an important historical role in Hawaii, but in modern times, a long period of neglect, disuse, and lack of appreciation for their potential reduced their number from 360 to approximately 70. In the past 10 years, state, federal, and private efforts began fishpond restoration and operation projects.

Hawaiians built several types of fishponds, which are typically shallow, with a muddy, sandy or rocky substrate, and soft bottom overlying the fringing coral reef. Larger ponds are often found within embayment areas that provide wave protection. Heeia Fishpond is a type of fishpond known as "loko kuapa," which are from 300 to 600 years old. These fishponds are marine enclosures in which a section of fringing reef is fully surrounded by a wall built of rock and coral. The walls usually extend to the edge of the reef and are adjacent to deeper marine waters and currents. Water and fish pass from ocean to pond through gates called "makahas." Tides and currents frequently create water exchange rates of 100 percent per day. Loko kuapa were used to raise primary herbivores, such as mullet and milkfish, and to hold excess fish from abundant ocean catches.

Objective: *Determine effective and efficient net-pen designs for mullet nursery systems in shallow coastal fishponds.*

A survey of fish containment systems was completed. A special pen, which combined the best features and eliminated the disadvantages of fixed and free-floating systems, was developed. Eighteen of the 2-cubic-meter cages, built of hard plastic, quarter-inch mesh with a unique mooring system that allowed them to float at high tide and be supported off the bottom in extremely shallow water at low tide, were deployed and stocked. The cages remained stable during 40-mile-per-hour wind gusts. However, the small-gauge mesh necessary to contain the juvenile mullet proved to be an ideal surface for rapid, abundant growth of filamentous algae. The algae restricted water flow, was difficult to remove, and seemed to be a factor in chronic, low-grade mortality across all stocking densities within the trial population. Because of these problems, different containment systems were designed and tested.

A 200-square-meter, rectangular, escape-proof fenced pen was built. It was initially built with a fence of 1/8-inch mesh surrounded by a 1-inch mesh fence; both were attached to the pond bottom. To control algal fouling, the inner fence can be removed and replaced with clean fencing of a slightly larger mesh size. The prototype was stocked with 15,000 juvenile mullet in two shipments from The Oceanic Institute. Fish from one of the stocking shipments were stressed during transport because dissolved oxygen concentrations fell to extremely low levels. Thus, the second year of the project developed transport methods that ensured the healthy condition of fish transported from the hatchery to the fishpond.

Observations of mullet behavior in the test pen showed that the fish schooled and fed regularly, grew rapidly, had easy access to the bottom substrate, and appeared to resist parasitic outbreaks, which were very prevalent in the first cage design. The higher water volume in the new pen appeared to reduce rapid deterioration of water quality and resulted in a more stable culture environment. However, the rectangular shape and nearshore location presented distinct disadvantages. First, the fish tended to group in the corners of the rectangle, making fish maintenance and sampling more difficult. Second, the nearshore location prevented adequate water circulation. For these reasons, investigators modified the pen design to an octagonal-shape and placed it farther from shore.

A large-scale, octagonal pen, 40 feet in diameter, underwent testing to determine if any design refinements were needed before three pens were stocked for the year 2 growout trial. In August 1994, the octagonal pen was stocked with 20,000, 25- to 35-millimeter mullet post-larvae for growout trials. The pen was located farther from shore to ensure a high water turnover rate and prevent fouling. Trial results showed that this size and style pen is conducive to high survival and growth. The pen style was also easy to maintain, operate, and harvest and prevented predator entry and crop escape. This design was economical and efficient to construct, could be used for other species and locations, and could be easily enlarged.

Objective: *Determine the optimal stocking density for juvenile mullet in net-pens.*

In the trials using nine cages of the original design, fish were stocked at three densities: 1 fish per liter, 1.5 fish per liter, and 2 fish per liter. Results indicated that growth and survival were independent of the three stocking density treatments. Survival rates ranged from a low of 3 percent in the high density cage to a high of 34 percent in the low density cage. Although the final fish count from each of the nine cages varied considerably, overall survival and growth rates were independent of stocking density.

Objective: *Develop transport methods to transfer mullet from the hatchery to the coastal fishponds.*

A key objective of this project was to develop fish transport methods that would assure the healthy condition of striped mullet postl-arvae on arrival at coastal ponds after transport from the hatchery. If post-larvae are healthy when stocked in the pond, they can more easily resist stress-mediated disease and early mortality. Transport techniques for shipping stage 1 fingerlings are being adapted to shipping post-larvae both intra-island by truck and inter-island by barge or air freight.

Post-larvae ranging from 25 mm to 35 mm were packed in aerated plastic boxes within boxes and transported from The Oceanic Institute to Heeia Fishpond by truck. The plastic bags were then hand-carried directly to experimental net-pens and stocked.

Researchers also set up a tank system for conducting experiments designed to establish an interisland barge transport capability. A 1,476-liter live fish transport tank was fitted with a battery-powered oxygen system consisting of an oxygen tank, regulators, dissolved oxygen meter, and diffusers. The tank was also fitted with a circulation and aeration system with bioactive filters, agitators, and carbon dioxide to ensure water quality. The entire system was on a portable platform. The refinement of the system produced a viable, cost-effective method to transport fish interisland by barge.

Objective: *Compare growth and survival of juvenile mullet in net-pens with land-based tank culture.*

Growth and survival rates in the original cages were far poorer than those obtained in the land-based tanks at The Oceanic Institute. However, the newly designed net-pens appeared promising as cost-effective systems for growing out juvenile fish in fishponds for aquaculture or stock enhancement purposes. Results from the final harvest in mid-July showed variable survival and slow growth. The investigators concluded that using this system for mullet aquaculture was not economically viable.

Objective: *Establish preliminary economic data for use in analysis of the cost of net-pen operations and production.*

Because problems with the year 1 cage design resulted in poor fish survival and growth, an economic analysis of operations and production is not viable based on year 1 economic data. However, cost data collected during the growout trials indicated that the unit and operations costs per fish produced related directly to the scale of operation. Generally, costs are lower per fish with larger cage units.

Results from the first year of the project showed that cage cleaning methods had the greatest impact on operating expenses. The redesigned pen used in the second year of the project employed a mesh removal and replacement system to control algal fouling rather than the manual cleaning methods that were necessary with the year 1 cage design. Thus, cleaning expenses were minimized. However, the poor growth rates and survival of the mullet in the growth trials showed that this system is not economically viable for aquaculture of mullet. It may be viable for other species.

Impacts

This project developed a unique net-pen system for use in traditional Hawaiian coastal fishponds. The associate investigator plans to use private funding to test the net-pen with other species, including Pacific threadfin (*Polydactylus sexfilis*), which is a highly valued local food fish.

Support

This project was funded by the Center for Tropical and Subtropical Aquaculture (CTSA).

Year	CTSA	Total support
One	$28,700	$28,700
Two	$60,000	$60,000
Total	**$88,700**	**$88,700**

Publications, manuscripts, or papers presented

None

Commercial Feasibility of Giant Clam Mariculture in American Samoa

Dates of work, March 1989 to October 1995

Participants

Henry Sesepasara, Ray Tulafono, Bonnie Ponwith, Lui Bell, Pio Gaisoa, Dominique Gebauer, John McConnaughey, Fa‘asega Kuresa, Ioelu Seve, and Fa‘atauvaa Lam Kitiona, American Samoa Department of Marine and Wildlife Resources (DMWR)

Reason for termination

This project was terminated because all objectives were completed.

Project objectives

The overall goal of this 5-year project, initiated under the Center for Tropical and Subtropical Aquaculture’s 2nd Annual Plan of Work, is to establish a giant clam culture demonstration station to be used for training, extension, and economic feasibility studies. Specific objectives for the fifth and final year of the project related to that goal are to:

- produce giant clam juveniles for distribution to local farmers;
- develop and establish participation of the private sector in giant clam farming on the intertidal and near intertidal zones;
- investigate and develop local markets in American Samoa for giant clams;
- conduct a business feasibility study of giant clam hatchery and lagoon farming in American Samoa;
- develop an aquaculture education and training extension program that will target farmers, villages, and government leaders and complement existing DMWR education program.

Principal accomplishments

During the first year of the project, a project investigator obtained training in giant clam spawning and culture techniques from the Micronesian Mariculture Demonstration Center (MMDC) in the Republic of Belau. Investigators negotiated a lease for a hatchery site. In July 1990, Lui Bell, senior fisheries biologist for the government of Western Samoa, was hired and assumed management of the project. By October 1990, enough of the hatchery facility was built so that spawning inductions could be conducted on *Tridacna derasa* imported from the MMDC. *T. derasa* was chosen as the target species for the project because broodstock and seed were readily available from the MMDC.

Investigators also continued to monitor growth, development, and survival of clams in the Nuuuli lagoon growout nursery, which were planted in 1987 under a DMWR project. Predation by *Cymatium* snails was the biggest cause of mortalities. In June 1991, investigators constructed PVC racks fitted with cages to hold trays of clams for growout. This hanging method was found to reduce mortalities from *Cymatium* snail predation.

The first successful spawning inductions were conducted in January 1991. Through trial and error, investigators determined that the intensive method of larval culture was best suited to conditions at the facility. In addition, providing shade to reduce algal blooms during the juvenile stage was necessary. Investigators established a total of eight ocean growout nurseries, three of which were managed by private citizens who had been trained in nursery management techniques at the DMWR hatchery and nursery. According to DMWR statistics, giant clams were unavailable in the market place in American Samoa after 1988, probably because wild clam stocks were so depleted. However, DMWR records showed that clam meat had nearly tripled in price from 1982 to 1988.

School classes from grade one through college took field trips to the hatchery and nursery sites. During these visits, investigators lectured on clam taxonomy, general biology, the status of local giant clam species, and giant clam culture methods and constraints. These educational efforts were intended both to make the students more environmentally aware and interest them in aquaculture.

Objective: *Produce giant clam juveniles for distribution to local farmers.*

Investigators built a new pump house and saltwater intake line and installed a new water pump. Tests showed the pump could achieve flows of 150 gallons per minute, more than double the rate required to fill all the raceways twice daily. The saltwater intake line was extended to deeper water further from shore because of poor water quality conditions near shore.

In October 1994, the new hatchery manager, John McConnaughey, received training in spawning and larval rearing techniques from the regional aquaculture extension specialist. During the project's fifth and final year, the hatchery shifted its emphasis from production of *T. derasa* to *Hippopus hippopus*, a species better-suited to conditions in American Samoa. Although *T. derasa* grows slightly faster, *H. hippopus* is more resistant to the predator snail *Cymatium*, which is the major cause of clam mortalities in American Samoa.

Investigators attempted to induce spawning in approximately 120 broodstock *H. hippopus* and *T. derasa* over 7 consecutive days in late October. However, significant quantities of viable eggs and sperm were not released, and the broodstock that were sacrificed for gonads had very regressed gonads. Whether this was due to seasonal factors or the broodstock being spawned too many times is unclear.

In December 1994, *T. derasa* broodstock spawned spontaneously. On the following day, approximately 3 million eggs were filtered, concentrated, moved to indoor settling tanks, and reared according to protocol described in the giant clam manual by Heslinga et al. (1990). Zooxanthelle extracts were added to all tanks. The clams exhibited good survival rates and normal growth rates. In March, samples from two raceways showed the average clam had reached 3.0 mm in one raceway and 5.4 mm in another. Several hundred clams were transferred to the Nuuuli nursery site and to two private farms and continue to grow well.

T. maxima broodstock collected from the wild were induced to spawn on two occasions in 1995, and the resulting larvae are being grown out. *T. derasa* and *H. hippopus* broodstock spawned spontaneously every week from July through September 1995, when brought to the adult holding tank from the ocean nursery. Adult nudibranches introduced to raceways with 100 day-old clams appeared to be effective at controlling algae, which had been a problem at the facility. However, as of June 1995, algae growth slowed considerably at the same time that a small, bright red annelid worm appeared in the tanks. Clam larval survival has improved as a result.

Security has been a major concern for the project, with numerous clams having been stolen. The situation improved with the installation of security gates in the access road.

Objective: *Develop and establish participation of the private sector in giant clam farming on the intertidal and near intertidal zones.*

Four individuals in three villages expressed interest in starting giant clam farms, and three of the areas were surveyed. Two sites in Nuuli are expected to provide a habitat similar to the project's nearby nursery site. The third site, located in Maicata, was determined to be less than ideal because the reef is only about 200 meters wide, has no barrier, and is exposed to high wave conditions.

Table 1. Results of Preliminary Survey of Restaurant and Fish Market Owners

Date Clams Were Spawned	April 1991	June 1992
Age of Clams	3.8 years old	2.6 years old
Average Size of Clam	204 mm	133 mm
Acceptable Price Range for Whole Clams	$2 to $4/clam	$1.75 to $2.50/clam

Announcements were made on local television and in newspapers to recruit potential clam farmers. Sixteen individuals responded and received training in clam nursery culture. They were then assisted with starting their own small, private ocean farms and each given 25, 3.5 year-old clams. The farmers are responsible for routine care of the clams. Project personnel visit each farm monthly to collect growth and survival data. Four of the 16 farms were destroyed by high waves in July.

Objective: *Develop an aquaculture education and training extension program that will target farmers, villages, and government leaders and complement existing DMWR education program.*

An experimental nursery is being established at the American Samoa Community College's new marine science laboratory. The nursery will be stocked with 25 - 50 *T. derasa*. In addition, a number of how-to pamphlets are being developed for private farmers in Samoa.

Objective: *Investigate and develop local markets for giant clams in American Samoa.*

In February 1995, project personnel took *T. derasa* to several fish markets and restaurants to evaluate market acceptance. Market and restaurant owners said they would be interested in purchasing the clams and suggested prices they would be willing to pay (Table 1). Two taste tests and a clam marketing study designed by the University of Hawaii's Pacific Business Center Program were conducted. Data analysis and reports are being prepared and should be available by the end of the year. In addition, project personnel conducted another market study through which farmers have the opportunity to market clams and determine the prices they are likely to obtain. The farmers purchase clams for $1 per pound and then sell them at the times, locations, and prices of their choosing.

Impacts

This project has started three giant clam growout nurseries that are run by private citizens and 12 additional ocean growout nurseries, thus providing diversification to the narrowly based economy and protecting the remaining wild stocks of clams. In addition, educational efforts help to increase young people's awareness of the status of American Samoa's wild giant clam stocks and interest in mariculture.

Recommended follow-up activities

None

Support

This project received funding from the Center for Tropical and Subtropical Aquaculture (CTSA), the Pacific Aquaculture Development Program (PADP), and the American Samoa Department of Marine and Wildlife Resources (DMWR).

		Other support			Total
Year	CTSA	PAA/PADP	DMWR	Total other	support
One	$17,300	$0	$17,900	$17,900	$35,200
Two	$15,000	$0	$23,500	$23,500	$38,500
Three	$20,000	$11,200	$22,410	$33,610	$53,610
Four	$29,000	$23,000	$28,100	$51,100	$80,100
Five	$29,000	$15,000	$47,900	$62,900	$91,900
Total	**$110,300**	**$49,200**	**$139,810**	**$189,010**	**$299,310**

Publications, manuscripts, or papers presented

None

Administrative Support 1996

Dates of work, January through December 1996

Participants

Dr. Kevan L. Main, Director; Patti Killelea-Almonte, Research Assistant; Alcian Clegg, Secretary, Center for Tropical and Subtropical Aquaculture, The Oceanic Institute

Project objectives

The objectives of this project are to:

- establish and operate a regional center that will conduct aquaculture research, development, and demonstration for the enhancement of a viable and profitable commercial aquaculture industry in the United States;
- develop and maintain a national program of cooperative and collaborative research, extension, and development activities among public and private institutions; and
- provide support for the development and implementation of projects of the Center for Tropical and Subtropical Aquaculture.

The Center for Tropical and Subtropical Aquaculture (CTSA) is jointly administered by the University of Hawaii and The Oceanic Institute. The Center's administrative activities are overseen by the Executive Committee of the CTSA Board of Directors. The Executive Committee includes the designated representative of the University of Hawaii and The Oceanic Institute. In 1987, The Oceanic Institute was designated as the site of the CTSA Administrative Center. In this capacity, The Oceanic Institute will disburse funds to other participating institutions and serve as a legal and physical agent in the receipt and disbursement of funds.

Anticipated benefits

The mission of the Center for Tropical and Subtropical Aquaculture is to support aquaculture research, demonstration, and extension education to enhance viable and profitable U.S. aquaculture production that will benefit consumers, producers, service industries, and the American economy.

Progress and principal accomplishments

The Center for Tropical and Subtropical Aquaculture Administrative Center staff provided a variety of support services to the Board of Directors, the Industry Advisory Council, the Technical Committee, various project review panels and delegations, and project work groups during 1996. The Administrative Center staff has processed nine Annual Plans of Work and is currently processing the tenth. The support services provided in 1996 are described below.

The Administrative Center staff provided support for the completion of the 10 projects funded under the 6th Annual Plan of Work, for 14 projects funded under the 7th Annual Plan of Work, for 11 projects funded under the 8th Annual Plan of Work, and for 11 projects funded under the 9th Annual Plan of Work. That support included monitoring project status and progress, preparing subcontracts, tracking budget expenditures, reviewing progress reports, and assisting principal investigators with problems.

In March 1996, CTSA underwent a program review by CSREES. Center staff prepared slide and overhead presentations and various written materials for the review team, in addition to arranging lodging for the team and meetings for team members with members of CTSA's Board of Directors, Industry Advisory Council, and Technical Committee. The review evaluated CTSA's priority setting process, its overall implementation of research and extension educational program, and performance review process. The review team reported that it was "pleased with the overall management and operation of the Center." The team felt that the IAC was, "committed to the Center," and that industry representatives were, "pleased with the overall performance of the Center in meeting the diverse needs of the industry in the region. The Center continually has identified opportunities and taken action to enhance the overall management and effectiveness of the program."

In support of the 10th Annual Plan of Work, the Administrative Center Staff assisted Industry Advisory Council Members in developing their priority list, assisted Technical Committee Groups in preparing problem statements, and prepared the Preliminary Plan of Work. The staff then solicited proposals and assisted work groups in preparing the documents. The Center Staff also solicited external and CTSA panel reviews of the proposals and drafted the Tenth Annual Plan of Work.

The Administrative Center staff functions as the work group for the project titled "Publications." Work under that project included producing a quarterly newsletter, annual technical bulletins, project final reports, a video, and providing editing assistance to a series of publications produced under the CTSA-funded project titled "Production of CTSA Educational Extension Materials." Details on the work completed under the Publications project are provided in the chapter titled "Publications."

In addition, the Center staff continued to reproduce and distribute a computer software program and accompanying instruction manual that was developed under the Center-funded project titled "Expert System Disease Module for Hawaiian Aquaculture." The DOS version of the software program, titled "Hawaii Aquaculture Module Expert System," was published as CTSA Publication #111 in March 1994. The Windows and Macintosh versions of the software program were published as CTSA Publication #118 and 119, respectively, in 1995. The Center staff also distributed other publications by CTSA and the other Regional Aquaculture Centers upon request.

Work planned

During 1997, the CTSA Administrative Center Staff will continue to provide all these forms of support for the Center's regional aquaculture projects. Further, the Center staff will:

- coordinate the review of annual reports;
- prepare the USDA grant package for fiscal year 1997;
- organize and provide documentation and assistance for CTSA meetings;
- visit Pacific Island project sites to review the status of currently funded projects and assist in the development of proposals for the 11th year program;
- complete the 10th Annual Plan of Work and submit it to USDA;
- participate in the National Coordinating Council; and
- work with other fisheries and aquaculture agencies throughout the region.

Finally, the Administrative Center staff will prepare the 11th Annual Plan of Work for submission to the U.S. Department of Agriculture.

North Central Regional Aquaculture Center Compendium Report
for the Period May 1, 1989 to August 31, 1996

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Introduction

The U.S. aquaculture industry continues to be one of the fastest growing sectors within U.S. agriculture, although at a lesser rate than what occurred during the 1980s. Production in 1994 reached 666 million pounds and generated approximately $751 million for producers. The impact of U.S. aquaculture is substantial accounting for approximately 181,000 jobs and generating an estimated $5.6 billion annually. Yet, anticipated growth in the industry, both in magnitude and in species diversity, continues to fall short of expectations.

Much of what is known about aquaculture science is a result of institutional attention given to our traditional capture of wild fisheries with the goal of releasing cultured fishes into public waters for enhancement of declining public stocks. Despite extensive efforts to manage wild populations for a sustained yield, as a nation we consume substantially greater amounts than we produce. Much of the United States' demand for seafood has been met by imports. The U.S. imports over 40% of its fish and shellfish and, after Japan, is the world's second largest importer of seafood. Fisheries imports are the largest contributor to the U.S. trade deficit among agricultural products, and the second largest after petroleum, among all natural resources products. The value of imported fisheries products more than doubled during the 1980s and has continued to increase in the 1990s. In fact, the $12.5 billion value for 1995 was a record. In 1995, the trade deficit was $4.2 billion for all fisheries products, $3.5 billion of which was for edible fish and shellfish.

Landings for most commercial capture fisheries species and recreational fisheries of the United States have been relatively stable during the last decade, with many fish stocks being overexploited. In this situation, aquaculture provides an opportunity to reduce the trade deficit and meet the rising U.S. demand for fish products. A strong domestic aquaculture industry is needed to increase U.S. production of fish and shellfish. This can be achieved by a partnership among the Federal Government, state and local public institutions, and the private sector with expertise in aquaculture development.

Congress recognized the opportunity for making significant progress in aquaculture development in 1980 by passage of the National Aquaculture Act (P. L. 96-362). Congress amended the National Agricultural Research, Extension, and Teaching Policy Act of 1977 (P. L. 95-113) in Title XIV of the Agriculture and Food Act of 1981 (P. L. 97-98) by granting authority to establish aquaculture research, development, and demonstration centers in the United States in association with colleges and universities, State Departments of Agriculture, federal facilities, and non-profit private research institutions. Five such centers have been established: one in each of the Northeastern, North Central, Southern, Western, and tropical/sub-tropical Pacific regions of the country. The 1996 Federal Agriculture Improvement and Reform Act (FAIR) (P. L. 104-127) otherwise know as the Farm Bill, has reauthorized the Regional Aquaculture Center program at $7.5 million per annum. As used here, a center refers to an administrative center. Centers do not provide monies for brick-and-mortar development. Centers encourage cooperative and collaborative aquaculture research and extension educational programs that have regional or national application. Center programs complement and strengthen other existing research and extension educational programs provided by the U.S. Department of Agriculture (USDA) and other public institutions. As a matter of policy, centers implement their programs by using institutional mechanisms and linkages that are in place in the public and private sector.

The mission of the Regional Aquaculture Centers (RACs) is to support aquaculture research, development, demonstration, and extension education to enhance viable and profitable U.S. aquaculture production which will benefit consumers, producers, service industries, and the American economy.

The North Central Regional Aquaculture Center (NCRAC) was established in February 1988. It serves as a focal point to assess needs, establish priorities, and implement research and extension educational programs in the 12 state agricultural heartland of the United States which includes: Illinois, Indiana, Iowa, Kansas, Michigan, Missouri, Minnesota, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin. NCRAC also provides coordination of interregional and national programs through the National Coordinating Council for Aquaculture (NCC). The council is composed of the RAC directors and USDA aquaculture personnel.

Organizational structure

Michigan State University (MSU) and Iowa State University (ISU) work together to develop and administer programs of NCRAC through a memorandum of understanding. MSU is the prime contractor for the Center and has administrative responsibilities for its operation. The Director of NCRAC is located at MSU. ISU shares in leadership of the Center through an office of the Associate Director who is responsible for all aspects of the Center's publications, technology transfer, and outreach activities.

At the present time the staff of NCRAC at MSU includes Ted R. Batterson, Director and Liz Bartels, Executive Secretary. The Center Director has the following responsibilities:

- serving as executive secretary to the Board of Directors, responsible for preparing agenda and minutes of Board meetings;
- serving as an ex-officio (non-voting) member of the Technical Committee and Industry Advisory Council;
- coordinating the development of research and extension plans, budgets, and proposals;
- coordinating and facilitating interactions among the Administrative Center, Board of Directors, Industry Advisory Council, and Technical Committee;
- monitoring research and extension activities;
- arranging for review of proposals for technical and scientific merit, feasibility, and applicability to priority problems and preparing summary budgets and reports as required;
- recruiting other Administrative Center staff as authorized by the Board of Directors;
- with assistance of the Economics and Marketing Work Group, Technical Committee, or others preparing a summary of regional aquaculture, including production statistics and sales, and identifying technical, financial, and institutional constraints to expanding production. The summary shall include sections addressing established industries, development industries, and opportunities for new product development, and recommended research needs;
- maintaining liaison with other RACs; and
- serving on the NCC.

At the present time the staff of NCRAC's Office for Publications and Extension Administration at ISU includes Joseph E. Morris, Associate Director and Glenda Dike, Secretary. The Associate Director has the following responsibilities:

- serving as head of Publications for NCRAC, including editor of the Center's newsletter;
- serving as the NCRAC liaison with national aquaculture extension programs, including in particular, extension programs of the other four USDA RACs; and
- serving as a member of NCRAC's Extension Executive Committee.

The Board of Directors (BOD) is the primary policy-making body of the NCRAC. The BOD has established an Industry Advisory Council (IAC) and Technical Committee (TC). Membership of the BOD consists of two persons from the IAC (the chair and an at-large member), a representative from the region's State Agricultural Experiment Stations and Cooperative Extension Services, a member from a non-land grant university and representatives from the two universities responsible for the center: Michigan State and Iowa State. The IAC is composed of representatives from each state's aquaculture association and six at-large members appointed by the BOD who represent various sectors of the aquaculture industry and the region as a whole. The TC is composed of a sub-committee for Extension (TC/E) and a subcommittee for Research (TC/R). Directors of the Cooperative Extension Service within the North Central Region appoint representatives to the TC/E. The TC/R has broad regional make-up and is composed of scientists from universities and state agencies with varied aquacultural expertise who are appointed by the BOD. Each sub-committee of the TC has a chairperson who serves as an ex-officio member of the BOD.

NCRAC functions in accordance with its *Operations Manual* which is periodically amended and updated with BOD approval. It is an evolving document that has changed as the Center's history lengthens. It is used for the development of the cooperative regional aquaculture and extension projects that NCRAC funds.

Administrative operations

Since inception of NCRAC February 1, 1988, the role of the Administrative Center has been to provide all necessary support services to the BOD, IAC, TC, and project work groups for the North Central Region as well as representing the region on the NCC. As the scope of the NCRAC programs expand, this has entailed a greater work load and continued need for effective communication among all components of the Center and the aquaculture community.

The Center functions in the following manner.

- After BOD approval of Administrative Center costs, the Center submits a grant to USDA/CSREES/Grants Management Branch for approval. To date the Center has received nine grants from USDA for FY88 (Grant #88-38500-3885), FY89 (Grant #89-38500-4319), FY90 (Grant #90-38500-5008), FY91 (Grant #91-38500-5900), FY92 (Grant #92-38500-6916), FY93 (Grant #93-38500-8392), FY94 (Grant #94-38500-0048), FY95 (Grant #95-38500-1410), and FY96 (Grant #96-38500-2631) with monies totaling $6,440,981. Currently, five grants are active (FY92-96); the first four grants (FY88-91) have terminated.
- The Center annually coordinates a program planning meeting which sets priorities for the next funding cycle and calls for regional workshops to develop project outlines to address priority problem areas.
- Work Groups, which are formed at the workshops, submit project outlines to the Center. The projects are peer reviewed by experts from both within and outside the region.
- The BOD, using reviewers' responses, decides which projects are to be approved and funding levels. The Center conveys BOD decisions to all Project Work Groups. Those that are approved for funding are asked to submit revised project outlines incorporating BOD and reviewers' comments.
- The Center then submits the revised project outlines as a Plan of Work (POW) to USDA for approval.
- Once a POW is approved by USDA, the Center then prepares subcontracts for each participating institution. The Center receives all invoices for subcontractual agreements and prepares payment objective(s) are then included in a workshop announcement that is broadly distributed throughout the North Central Region. The workshops are 1-day events to establish a work group that will develop a project outline over the summer months. Work group members will be those who have demonstrated that they have the expertise and facilities for undertaking the proposed work in regard to a particular objective or objectives. The proposed work cannot deviate from the objective or objectives included in the workshop announcement. The work group elects a chair and secretary. The chair is responsible for submitting the project outline to the NCRAC Director; the secretary is responsible for preparing minutes from the workshop that are distributed to all attendees. All project outlines are peer reviewed. The reviewers' comments are used by the BOD in making the final selection of projects and level of funding at the following year's annual Program Planning meeting. All work group members are apprised of the BOD decisions. Revisions of projects approved by the BOD are submitted by the work group chair to the NCRAC Director. The revised project outlines are then included in a POW that is submitted to USDA. Upon approval by USDA, the Center issues subcontracts to the funded work group members.

Time frame

- Program Planning meeting: early winter.
- Workshops: late-spring, early summer.
- Project outlines developed over the summer by work group members who participated in the workshops. These project outlines are then submitted to the Center in the fall and peer reviewed.
- The Board of Directors at the following year's Program Planning Meeting selects the projects to be funded.
- Project outline revised and submitted to the Center by May.
- Revised projects are then submitted in June as a POW (or an amendment to a POW) to USDA for approval. Once approved by USDA subcontracts are let by the Center with a start date of September 1.

By following this procedure, it takes approximately 18 months from the time of identifying a priority area until inception of a project to address the issue in question.

Workshops
The purpose of the workshops is to bring together those who are best qualified to work on project objectives by virtue of a demonstrated record of expertise and access to facilities required in the project. These people form a work group for the purpose of writing a project outline to address the problem in question. The following criteria typically apply to those projects that are funded by NCRAC.

- Involves participation by two or more states in the North Central Region;
- requires more scientific manpower, equipment, and facilities than generally available at one location;
- approach is adaptable and particularly suitable for inter-institutional cooperation resulting in better use of limited resources and a saving of funds;
- will complement and enhance ongoing extension and research activities by participants, as well as offer potential for expanding these programs;
- is likely to attract additional support for the work which is not likely to occur through other programs and mechanisms;
- is sufficiently specific to promise significant accomplishments in a reasonable period of time (usually up to 2 years);
- can provide the solution to a problem of fundamental importance or fill an information gap;
- can be organized and conducted on a regional level, assuring coordinated and complementary contributions by all participants.

The NCRAC program pays no overhead to participating institutions nor tuition remission, has no brick-and-mortar money, and relies on in-place salaried personnel, equipment, and facilities to carry out the projects. Due to the collaborative and cooperative nature of these regional projects, no one individual or institution receives a significant portion of the total project funds.

Project reporting

As indicated in Table 1, NCRAC has funded a number of projects for many of the project areas it has selected for research and extension activities. For example, there have been five separately funded projects in regard to Extension and six for walleye. Project outlines have been written for each separate project within an area, or the project area itself if only one project. These project outlines have been submitted in Plans of Work (POWs) or amendments to POWs for the grants as indicated in Table 1. Many times, the projects within a particular area are merely continuations of previously funded activities; while at other times they are addressing new objectives. Presented below are Progress or Termination Reports for all projects that were underway or completed during the period May 1, 1989, to August 31, 1996. May 1, 1989, marked the beginning of the first projects funded by NCRAC.

All publications, manuscripts, or papers presented for all funded NCRAC project areas are listed in the Appendix.

Table 1. North Central Regional Aquaculture Center funded projects.

Project area	Project number	Proposed duration period	Funding level	Grant number
Extension	1	5/1/89-4/30/91	$39,221	88-38500-3885
			$37,089	89-38500-4319
	2	3/17/90-8/31/91	$31,300	89-38500-4319
	3	9/1/91-8/31/93	$94,109	91-38500-5900
	4	9/1/93-8/31/95	$110,129	91-38500-5900
	5	9/1/95-8/31/97	$10,875	92-38500-6916
			$25,725	95-38500-1410
			$348,448	
Economics and marketing	1	5/1/89-12/31/91	$127,338	88-38500-3885
			$34,350	89-38500-4319
	2	9/1/91-8/31/92	$53,300	91-38500-5900
	3	9/1/93-8/31/95	$40,000	93-38500-8392
			$254,988	
Yellow Perch	1	5/1/89-8/31/91	$76,957	88-38500-3885
			$85,723	89-38500-4319
	2	6/1/90-8/31/92	$92,108	90-38500-5008
	3	9/1/91-8/31/93	$99,997	91-38500-5900
	4	9/1/93-8/31/95	$150,000	93-38500-8392
	5	9/1/95-8/31/97	$200,000	95-38500-1410
			$704,785	
Hybrid Striped Bass	1	5/1/89-8/31/91	$68,296	88-38500-3885
			$68,114	89-38500-4319
	2	6/1/90-8/31/92	$101,000	90-38500-5008
	3	9/1/91-8/31/93	$96,550	91-38500-5900
	4	9/1/93-8/31/95	$168,000	93-38500-8392
	5	9/1/95-8/31/97	$160,000	95-38500-1410
			$661,960	
Walleye	1	5/1/89-8/31/91	$177,517	89-38500-4319
	2	6/1/90-8/31/92	$111,657	90-38500-5008
	3	9/1/91-8/31/92	$109,223	91-38500-5900
	4	9/1/92-8/31/93	$75,000	89-38500-4319
	5	9/1/93-8/31/95	$150,000	93-38500-8392
	6	9/1/95-8/31/97	$117,897	94-38500-0048
			$57,103	95-38500-1410
			$798,397	
Sunfish	1	6/1/90-8/31/92	$130,758	90-38500-5008
	2	9/1/92-8/31/94	$149,799	92-38500-6916
	3	9/1/94-8/31/96	$174,999	94-38500-0048
			$455,556	
Salmonids	1	6/1/90-8/31/92	$9,000	89-38500-4319
			$120,799	90-38500-5008
	2	9/1/92-8/31/94	$149,997	92-38500-6916
	3	9/1/94-8/31/96	$200,000	94-38500-0048
			$479,796	
NCR Aquaculture Conference	1	6/1/90-3/31/91	$7,000	90-38500-5008
National Aqua. Extension Workshop	1	10/1/91-9/30/92	$3,005	89-38500-4319
Crayfish	1	9/1/92-8/31/94	$49,677	92-38500-6916
Baitfish	1	9/1/92-8/31/94	$61,973	92-38500-6916
Wastes/Effluents	1	9/1/92-8/31/94	$153,300	92-38500-6916
National Aquaculture INAD/NADA Coordinator	1	9/1/93-8/31/94	$2,000	89-38500-4319
		5/15/95-5/14/96	$5,000	94-38500-0048
		5/15/96-5/14/97	$6,669	92-38500-6916
			$3,331	
			$17,000	

Project Termination or Progress Reports

Extension

Progress Report for the Period May 1, 1989 to August 31, 1996

NCRAC funding level

$328,923 (May 1, 1989 to August 31, 1996)

Participants

Fred P. Binkowski, University of Wisconsin-Milwaukee, Wisconsin
James E. Ebeling, Ohio State University, Ohio
Donald L. Garling, Michigan State University, Michigan
Jeffrey L. Gunderson, University of Minnesota, Minnesota
F. Robert Henderson, Kansas State University, Kansas
John Hochheimer, Ohio State University, Ohio
Anne R. Kapuscinski, University of Minnesota, Minnesota
Terrence B. Kayes, University of Nebraska-Lincoln, Nebraska
Ronald E. Kinnunen, Michigan State University, Michigan
Christopher C. Kohler, Southern Illinois University-Carbondale, Illinois
David J. Landkamer, University of Minnesota, Minnesota
Charles Lee, Kansas State University, Kansas
Joseph E. Morris, Iowa State University, Iowa
Kenneth E. Neils, Kansas State University, Kansas
Robert A. Pierce II, University of Missouri, Missouri
Daniel A. Selock, Southern Illinois University-Carbondale, Illinois
LaDon Swann, Purdue University Indiana/Illinois
Administrative Advisor:
David C. Petritz, Purdue University, Indiana

Project objectives

1. Strengthen linkages between North Central Regional Aquaculture Center (NCRAC) research and extension work groups.
2. Enhance the North Central Region (NCR) extension network for aquaculture information transfer.
3. Provide in-service training for Cooperative Extension Service, Sea Grant Advisory Service, and other landowner assistance personnel.
4. Develop and implement aquaculture educational programs for the NCR.
5. Develop aquaculture materials for the NCR including extension fact sheets, bulletins, manuals/guides, and instructional video tapes.

Anticipated benefits

The NCRAC Extension Work Group will promote and advance commercial aquaculture in a responsible fashion through an organized education/training outreach program. The primary benefits will be:

- increased public awareness through publications, short courses, and conferences regarding the potential of aquaculture as a viable agricultural enterprise in the NCR;
- technology transfer to enhance current and future production methodologies for selected species, e.g., walleye, hybrid striped bass, yellow perch, salmonids, and sunfish, through hands-on workshops and field demonstration projects;
- improved lines of communication between interstate aquaculture extension specialists and associated industry contacts; and
- an enhanced legal and socioeconomic atmosphere for aquaculture in the NCR.

Progress and principal accomplishments

Objective 1

Due to the efforts of aquaculture extension personnel in the NCR, NCRAC's Board of Directors formally adopted guidelines for extension's involvement in all NCRAC-funded projects. These guidelines integrate research and extension activities so that extension service personnel can better serve their clientele groups. In addition, aquaculture Extension Work Group members have:

- served as extension liaisons, if not active researchers, for every funded NCRAC project;
- assisted in writing and developing the NCRAC Walleye Culture Manual that was edited by Bob Summerfelt of Iowa State University;
- assisted with the planning, promotion, and implementation of the hybrid striped bass, walleye and yellow perch workshops held throughout the region;
- helped conduct a survey of crayfish producers in the NCR and completed a report on *Orconectes immunis* for inclusion in the Crayfish Work Group report;
- provided the NCRAC Economics and Marketing Work Group with information relevant to that group's efforts to develop cost of production budgets and expected revenues for the commercial production of food-sized hybrid striped bass, walleye, and yellow perch in the NCR;
- participated as Steering Committee members for a regional public forum regarding the National Aquaculture Development Plan of 1996;
- assisted NCRAC in obtaining information on the 1995 status of aquaculture in the NCR. The information will be used to develop NCRAC's regional aquaculture situation and outlook (S&O) report. Extension specialists often coordinated the effort to develop a cover letter, prepare a mailing list and send the survey out, and to follow up to assure a high response rate;
- conducted educational programs for the Wisconsin Aquaculture Association on non-indigenous aquatic nuisance species and implications for aquaculture as well as participating in the annual meeting of the Great Lakes Fish Health Committee providing input as it relates to aquaculture.

Objective 2

The demand for aquaculture extension education programs cannot be met by the few specialists in the NCR (4.0 FTE). Networking of specialists and Cooperative Extension Service (CES) designated contacts has maximized efficiency of education programs and minimized duplication. The NCRAC Extension Project is designed to assess and meet the information needs of the various clientele groups through cooperative and coordinated regional educational programming. In fact, individual state extension contacts often respond to 10-15 calls per month from outside their respective state as well as interacting with colleagues with mutual concerns related to developing aquaculture activities.

Prior to mid-1994, little coordination of international aquaculture information sharing existed. National and international agencies producing information could only be obtained by contacting the respective sources of this information. Also, individual CES personnel relied heavily on information produced by individual states or through regional cooperative projects. As Internet access extended beyond educational institutions and governmental agencies, a clear need developed to utilize the Internet to reach a much broader audience. In the age of an "information overload" the need for a centralized gateway to the ever increasing number of aquaculture resources in electronic format was apparent.

The development of the Aquaculture Information Network Center (AquaNIC) has been instrumental in reaching the public with valuable and timely information. It has been funded, in part, by NCRAC and has currently over 4,000 contacts per month from more than 50 countries to this web site. AquaNIC receives direction from a national steering committee from public and private sector aquaculture. AquaNIC began on a Gopher Server in July 1994 and moved to a World Wide Web server in January 1996. AquaNIC (ag.ansc.purdue.edu/aquanic/) houses more than 1,650 extension publications, governmental documents, image files, comprehensive e-mail lists, newsletters, calendars, job announcements, and résumés. In addition, AquaNIC has 190 pointers to other aquaculture and fisheries related web sites. Ongoing promotional campaigns through mouse pads and access information cards has increased the level of awareness of this new resource available to the world aquaculture industry. It is the gateway to the world's electronic resources in aquaculture including the Regional Aquaculture Centers.

AquaNIC also serves as the home of NCRAC's web site (ag.ansc.purdue.edu/aquanic/ncrac) which was developed in conjunction with NCRAC administrative staff and the Illinois-Indiana Sea Grant Program. The web site provides electronic versions of NCRAC extension publications, directories, operations manuals, and newsletters.

Aquaculture handbooks have been developed and distributed to each NCRAC designated aquaculture extension specialist and selected CES and Sea Grant field staff member.

As with any organization, there have been changes in NCRAC extension personnel since the inception of the project. Landkamer was the primary aquaculture extension contact for Minnesota. However, he left the university and Kapuscinski became the primary contact person until 1992 when Gunderson assumed that responsibility. In 1994 there were two changes: in Kansas, Neils replaced Henderson and in Illinois, Kohler replaced Selock. There continues to be changes in NCRAC extension personnel since the inception of the project; Hochheimer has replaced Ebeling in Ohio while Lee replaced Neils in Kansas in 1996.

Objective 3
In-service training for CES and Sea Grant personnel and other landowner assistance personnel have been held in most of the states in the region. Training has been in the areas of basic aquaculture and safe seafood handling including HACCP (Hazard Analysis Critical Control Point).

Objective 4
A number of workshops, conferences, videos, field-site visits, hands-on training sessions, and other educational programs have been developed and implemented.

There have been workshops on general aquaculture, fish diseases, commercial recirculation systems, aquaculture business planning, crayfish culture, pond management, yellow perch and hybrid striped bass culture, rainbow trout production, in-service training for high school vocational-agricultural teachers, and polyploid induction in sunfish held in the region.

Two North Central Region Aquaculture Conferences have been held. The first in Kalamazoo, Michigan, was held in March 1991. The second was held in February 1995 in Minneapolis, Minnesota. These regional meetings were attended by hundreds of individuals including persons from Canada. The next conference is scheduled for February 1997 in Indianapolis, Indiana.

On April 10, 1993, over 700 viewers from 35 states and Canada watched the first national interactive teleconference on aquaculture, "Investing in Freshwater Aquaculture," that was broadcast from Purdue University. It was a televised satellite broadcast for potential fish farmers. The program consisted of 10, 5- to 7-minute video tape segments which addressed production aspects of channel catfish, crayfish, rainbow trout, hybrid striped bass, tilapia, yellow perch, baitfish, and sportfish. A set of course materials was available prior to the program. Three times during the program, a question and answer period was available to the audience through a toll free telephone number. Questions not answered during the program were answered by mail afterwards. The entire teleconference is available as a videotape from NCRAC's Publications Office, as well as two other videotapes by the University of Nebraska-Lincoln that are reprises of the broadcast.

Objective 5
Numerous fact sheets, technical bulletins, and videos have been written or produced by various participants of the Extension Work Group. These are listed in the Appendix.

Work planned

Efforts will continue in regard to strengthening linkages between research and extension work groups as well as enhancing the network for aquaculture information transfer. Participants will also continue to provide in-service training for CES, Sea Grant, and other land-owner assistance personnel. Educational programs and materials will be developed and implemented. This includes development of a sunfish culture guide, yellow perch culture guide and videos, hybrid striped bass culture guide, a publication on fee-fishing (sunfish), tilapia culture information packet, and a publication on yellow perch culture in flowing water systems.

Additional workshops developed and hosted by state extension contacts will be advertised in surrounding states to take advantage of the NCRAC Extension Network and the individual expertise of Extension Work Group participants.

Several additional NCRAC fact sheets, technical bulletins, and videos will be developed by various Work Group members.

Impacts

- In-service training for CES and Sea Grant personnel has enabled those professionals to respond to initial, routine aquaculture questions from the general public.
- Development of aquaculture education programs for the NCR has provided "hands-on" opportunities for prospective and experienced producers. Approximately 5,000 individuals have attended workshops or conferences organized and delivered by the NCRAC Extension Work Group. Clientele attending regional workshops learned of aquaculture development strategies in other areas of the country and acquired information which was of direct use to their own enterprises. Education programs also created situations where problems encountered by producers were expressed to extension personnel who later relayed them to researchers at NCRAC work group meetings for possible solutions through the research effort.
- Fact sheets, technical bulletins, and videos have served to inform a variety of clients about numerous aquaculture practices for the NCR. For instance, "Making Plans for Commercial Aquaculture in the North Central Region" is often used to provide clients with initial information about aquaculture, while species specific publications on walleye, trout, and catfish have been used in numerous regional meetings and have been requested by clients from throughout the United States. Publications on organizational structure for aquaculture businesses, transportation of fish in bags, and others are beneficial to both new and established aquaculturists. In a 1994 survey, NCRAC extension contacts estimated that NCRAC publications were used to address approximately 15,000 client questions annually.
- NCRAC extension outreach activities have helped to foster a better understanding and awareness for the future development of aquaculture in the region.
- In the brief time since AquaNIC began more than 25,000 people from 49 countries have chosen to use AquaNIC as an alternative to or in conjunction with traditional means of obtaining information. Primary users by countries are: U.S. (40%), Canada (5%), Australia (3%), and the United Kingdom (2%). As a gateway to electronic resources in aquaculture, AquaNIC has increased the timeliness and variety of information available to outreach educators, governmental agencies, and individual users while more effectively utilizing existing personnel resources. AquaNIC can be accessed anytime and, therefore, alleviates the challenges associated with office hours, time zones or weekends. Several groups have recognized the benefits AquaNIC provides to the world aquaculture industry and have established long-term partnerships with AquaNIC to assist them in distribution of their resources. Key groups using AquaNIC to house their web sites include: the World Aquaculture Society, NCRAC, Indiana Aquaculture Association, and the Illinois Aquaculture Industry Association.

Publications, manuscripts, workshops, or conferences

See the Appendix for a cumulative output for all NCRAC-funded Extension activities.

Support

Years	NCRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1989-91	$107,610	$237,107				$237,107	$344,717
1991-93	$94,109	$152,952				$152,952	$247,061
1993-95	$110,129	$198,099		$250,000	$55,000	$503,099	$613,228
1995-96	$17,075	$70,968				$70,968	$88,043
Total	**$328,923**	**$659,126**		**$250,000**	**$55,000**	**$964,126**	**$1,293,049**

Economics And Marketing

Project Termination Report for the Period May 1, 1989 to August 31, 1993

NCRAC funding level

$214,988 (May 1, 1989 to August 31, 1993)

Participants

Susan B. Kohler, Southern Illinois University-Carbondale, Illinois
Marshall A. Martin, Purdue University, Indiana
Patrick D. O'Rourke, Illinois State University, Illinois
Jean R. Riepe, Purdue University, Indiana
Extension liaisons:
Donald L. Garling, Michigan State University, Michigan
Terrence B. Kayes, University of Nebraska-Lincoln, Nebraska
LaDon Swann, Purdue University, Indiana

Reason for termination

The objectives for this work were completed.

Project objectives

1. Identify existing and needed economic data; develop statistical reporting methods; design an information management system and prototype annual situation/outlook report on the North Central Region (NCR) aquaculture industry; begin collecting and compiling a regional database; and prepare a situation/outlook report.
2. Develop and implement an extension program designed to educate current and potential aquaculture producers on the need to provide accurate economic information on their operations.
3. Investigate economic production and marketing feasibility for selected species currently produced in the NCR and other species which offer commercial potential.
4. Identify existing policy impediments and incentives for expanded aquaculture development in each participating state within the NCR.

Principal accomplishments

The first Situation and Outlook (S&O) Report for the 12-state NCR was published in August 1993. The report was developed to compile preliminary statistics and information about aquaculture in the NCR. Data and information contained in the report was obtained from a variety of sources including an extensive mail survey conducted in 1991 to gain information about the aquaculture industry in the NCR during 1990. Accurate information from respondents to that and other surveys was the result of the extension educational program of the project. Numerous contacts were established with state agencies interested in commercial aquaculture (e.g., natural resources, environmental licensing/permitting, and agriculture agencies), state aquaculture associations, state extension services, Sea Grant programs, and economic development groups in each of the states within the NCR seeking input and support for information gathering components of the project. Key individuals respected by the aquaculture industry were chosen from those various groups to facilitate legitimation of the data and to explain the value of accurate economic data reporting.

Two surveys of retail, wholesale, and other firms that comprise the traditional marketing channels for fish and seafood products within the NCR were completed in 1990 and 1991. The results of these surveys determined that channel catfish, trout, salmon (salt- and freshwater), freshwater shrimp, and tilapia were the five cultured freshwater species that were most frequently sold in the NCR. The species that were judged to have the most marketing potential were walleye, yellow perch, bluegill (sunfish), largemouth bass, and frogs. The surveys also indicated that the general perception of farm-raised products was positive within seafood distribution channels. Compared to wild-caught species, farm-raised aquaculture products were perceived as being fresher, of higher quality, and had greater price stability.

Trout and catfish cost of production budgets were developed. Based on the 1990 survey of producers in the NCR, these two species are the largest revenue generators for this region's growers.

There were 65 trout producers who grew trout and sold in excess of $1,000 during 1990. Twenty-nine of these producers were chosen for the cost of production study to reflect differences in sizes of operations. Nineteen of the facilities were visited in person; the remaining 10 were sent a questionnaire and then interviewed by telephone. The initial 19 were also contacted by telephone, as needed, to clarify initial responses when questions arose. Of the 10 participants who were not visited, five did not respond to the telephone survey. Three questionnaires were not used because the data provided was too incomplete to develop budgets. The remaining 21 producer surveys provided the data base for the trout study. The level of cooperation received from trout growers in completing a very lengthy and difficult questionnaire was a very pleasant surprise.

Data were collected for the calendar year 1991. Producers were asked to provided information about variable and fixed operating costs of raising trout, the prices of variable and fixed inputs for which producers would have price data, type(s) of fish stocked, stocking size, market size, food conversion rate, and physical relationships such as water temperature and flow rates.

The 21 producers were divided into three groups. The small group contained nine producers with gross sales of $1,250 to $45,000, with average sales of $20,039. These producers sold an average of 8,845 kg (19,500 lb) live weight. The medium group contained seven producers with sales ranging from $92,178 to $130,000, and averaging $108,220. Output averaged 278,671 kg (61,435 lb) live weight. The large group contained five producers who averaged $324,184 in sales and 72,746 kg (160,375 lb) live weight in output. The smallest of this group sold $225,000 during 1991.

Variable plus fixed operating costs were $21,140 for the average small producer resulting in negative operating revenues of $1,101; so there was a negative balance before any allocation to the operator's labor, management and investment. The medium and large groups had variable plus fixed costs of $95,927 and $239,510, respectively, leaving returns of $12,293 and $84,674, respectively, available for operator's labor, management, and investment.

The cost and revenue data were of questionable validity at best. Most of the trout producers interviewed have very weak cost accounting skills and, therefore, have limited ability to evaluate whether their trout operations are profitable or not. In addition, trout operations as a group are very complex when compared to other agricultural production enterprises because of the number of growing ranges for the fish (hatching eggs, selling or buying fingerlings, selling or buying stockers, and selling food size fish). Also, there are a large variety of production facilities, i.e., ponds, raceways, cages, etc. and all possible combinations of these facilities, plus variations in the costs of obtaining water. Experience suggests that very basic educational programs in management, cost accounting, and budgeting would be highly beneficial to these producers in NCR.

It was determined that states within the NCR are in various stages of aquaculture policy development. Minnesota, Illinois, and Indiana have enacted legislation promoting the development of the aquaculture industry. At the other end of the spectrum, North and South Dakota have so few producers that private fish culture is not likely to reach the public agenda for quite some time. Most of the states in the region fall somewhere in between, with aquaculture interests working toward the development of a state aquaculture development plan or the implementation of legislation supporting the industry.

Five major resource policy issues for aquaculture in the region were identified: (1) regulatory jurisdiction, (2) predator control, (3) water quality, (4) regulation of game and non-native species, and (5) environmental contamination. While producers have generally favored the classification of aquaculture as agriculture, in hope of avoiding environmental regulation, the research indicated that there is little reason to believe that such a reclassification will resolve the underlying substantive issues.

Most policies affecting the growth and marketing of fish in the NCR are found in state natural resources agencies. They are found in these agencies because they were developed to regulate open access fisheries, and now are the only policies that apply to cultured settings. However, for aquaculture to become more feasible, reconsideration of this regulatory policy framework is necessary. A cautionary and studied approach is suggested for any attempt to revise the regulatory structure that affects fish farmers. Producer issues in many cases have similarities to those for other livestock enterprises, but often in increased intensity because of application in water instead of on land.

The investigators found that one of the barriers to the growth and development of the aquaculture industry in the NCR was a lack of comprehensive information on the state laws and regulations that affect the industry. One of the outputs of the project was the publication of a digest that includes the laws related to the marketing and production of aquacultural products for the 12-state NCR. The publication entitled "Aquaculture Law in the North Central States: A Digest of State Statutes Pertaining to the Production and Marketing of Aquacultural Products" was published in May 1992 as the first publication in NCRAC's Technical Bulletin Series.

Impacts

The S&O Report provided a widely distributed report of the state of aquaculture in the NCR. It indicated that farm-raised aquaculture products have become more important in fulfilling seafood markets in the United States.

The marketing study showed that the primary species being cultured in the NCR are highly marketable and are well-accepted in commercial seafood marketing channels.

The benefits of the cost of production budgets are two. First, for the first time, budgets using North Central trout and catfish producer data are available for use by regional producers and NCRAC Extension agents in assisting producers and others to assess the profitability of existing and proposed fish enterprises. These budgets are also useful in helping producers assess the feasibility of growing other species in the region. Second, these budgets provide an educational tool in the hands of Extension agents to teach fish growers how to improve cost accounting and budgeting procedures on their operations. Improved cost accounting will make producers better managers and assist regional researchers in assessing the feasibility of growing particular species under varying conditions.

Incorporation of cost of production budget parameters into budget software will give current or potential fish producers, financial institutions, and policy makers regional results about the feasibility of producing trout and catfish in various locations of the NCR. In addition, the data on some costs such as water and facilities will be transferable to other species of interest.

Recommended follow-up activities

A new Economics and Marketing Work Group will begin to develop cost of production budgets and expected revenues for the raising of food-sized walleye, yellow perch, and hybrid striped bass on farms in the NCR.

Publications, manuscripts, or papers presented

See the Appendix for a cumulative output for all NCRAC-funded Economics and Marketing activities.

Support

Years	NCRAC USDA funding	Other support					Total support
		University	Industry	Other federal	Other	Total	
1989-91	$161,688	$59,683				$59,683	$221,371
1991-92	$53,300	$66,457				$66,457	$119,757
Total	**$214,988**	**$126,140**				**$126,140**	**$341,128**

Economics And Marketing

Progress Report for the Period September 1, 1993 to August 31, 1996

NCRAC funding level

$40,000 (September 1, 1993 to August 31, 1996)

Participants

Susan B. Kohler, Southern Illinois University-Carbondale, Illinois
Marshall A. Martin, Purdue University, Indiana
Patrick D. O'Rourke, Illinois State University, Illinois
Jean R. Riepe, Purdue University, Indiana
Extension liaisons:
Donald L. Garling, Michigan State University, Michigan
Terrence B. Kayes, University of Nebraska-Lincoln, Nebraska
LaDon Swann, Purdue University, Indiana

Project objective

Develop cost of production budgets and expected revenues for the raising of yellow perch, walleye, and hybrid striped bass on farms in the North Central Region (NCR).

Anticipated benefits

The overall goal of this collaborative project is to enhance walleye, yellow perch, and hybrid striped bass production by developing enterprise budgets for various production systems for these species in the NCR. This supports the mission of the North Central Regional Aquaculture Center (NCRAC), especially by conducting research "for the enhancement of viable and profitable commercial aquacultural production in the United States for the benefit of producers, consumers, and the American economy."

The cost of production and budgeting components of this project offer the potential to help in identifying production systems for walleye, yellow perch, and hybrid striped bass most likely to be commercially viable in the NCR. Information on production costs is quite limited for these species, especially walleye and yellow perch. Enterprise budgets for real and prototype systems will enable producers or potential producers to assess the expected costs for their own operation, for a new operation, or for increased production in their present operation in an objective and comprehensive manner.

This project will benefit the aquaculture industry in the NCR in several ways, even though there are some limitations in using these budgets given the "emerging" status of the industry and the small number of commercial producers in these three species.

- First, objectively developed cost information is typically more accurate than subjectively developed cost information or no information on costs at all. These budgets will give producers an idea of how enterprise budgets should be organized, what types of data need to be collected, and why good record keeping is essential. The production values and relationships upon which the cost structure are based, while not standardized in the industry, should serve as a rough rule-of-thumb by which aquacultural producers can gauge their management skills.
- Second, enterprise budgets are an excellent management tool and are the cornerstone for financial analysis of aquaculture operations for producers and investors. These budgets may stimulate potential and current aquacultural producers to put together budgets and analysis for their own unique enterprises.
- Third, enterprise budgets are also the cornerstone for sensitivity analysis (yet another management tool). Undertaking sensitivity analysis will enable economists, producers, and potential producers to better understand the relative importance of cost and production items in the budget and the impact on profitability.
- Finally, realizing that the budgets produced under the auspices of this project will not be the final, definitive budgets for production of these species in the NCR, they will serve as a solid starting base from which to better understand the potential profitability of alternative species, production systems, life stages, etc.

In a more indirect way, the enterprise budgets will accomplish two other important things.

- One, the budgets may help guide research and extension decisions concerning hybrid striped bass, walleye, and yellow perch by NCRAC work group participants, the Industry Advisory Council (IAC), the Board of Directors, and the supporting committees.
- Second, the budgets will provide an opportunity for the economists and other personnel developing the budgets to interact with aquaculture producers, researchers, and extension personnel in the NCR. This type of interdisciplinary interaction is vital for the improved understanding and communication of all vital aspects of aquaculture in the NCR.

Economic feasibility analysis will help producers evaluate technical advances in fish production. This contribution is critical as a guide to future research funding in the various species and production systems suitable for commercial production. The distribution of research results from this project will provide a structured and objective framework for profitability and financial analysis of hybrid striped bass, walleye, and yellow perch aquaculture systems for producers, financial institutions, and others.

Progress and principal accomplishments

Hybrid striped bass

Kohler has compiled a review of the literature on hybrid striped bass production and production costs. The literature reviewed is summarized in an annotated bibliography. This bibliography will be available to anyone needing the information.

Kohler has developed hybrid striped bass cost of production estimates based on six recent published reports on hybrid striped bass production. These estimated production costs were presented at the NCRAC Hybrid Striped Bass Workshop in November 1995.

Walleye

O'Rourke and Illinois State University graduate students continued an extensive walleye production and culture literature review. The primary focus of the literature review was to evaluate research findings that might be useful in ascertaining the cost of production for walleye fingerlings and food-sized fish under intensive and extensive culture regimes. Very little economic research was found and even less was found that was documented well enough to be useful.

Work has advanced on identifying and analyzing the cost of production for food-sized walleye in intensive culture systems. The second M.S. thesis on walleye to come from this project was officially finished in December 1995. It reported on an economic feasibility analysis of a tank based intensive food-sized walleye system.

Yellow perch

Riepe's analysis of yellow perch production in ponds and cages is reported in NCRAC Extension Fact Sheet #111 and NCRAC Extension Technical Bulletin #111, both ready for release. While developing cost estimates for yellow perch aquaculture, Riepe investigated feed and fingerling prices and procurement with various suppliers. A fact sheet on managing feed costs was developed and is in final review by Riepe as a NCRAC Extension Fact Sheet.

Work planned

The distribution of research results from this project is proceeding, primarily for the walleye species. The research on cost of production in tank culture systems for fingerlings and food sized walleye will be organized in fact sheet or technical bulletin format for release to producers, financial institutions, and others.

Riepe will complete the review of the fact sheet on managing feed costs. This is expected to be published as a NCRAC Extension Fact Sheet.

Impacts

Kohler and O'Rourke presented the review of hybrid striped bass production costs as well as profitability and volume-cost business analysis tools at the NCRAC Hybrid Striped Bass Workshop in November 1995. The information developed and presented is anticipated to be directly useful to the attendees (producers and potential producers) as they consider their own operations and intentions in light of the cost data and analytical tools presented.

This project has already benefited the aquaculture industry in the NCR through the workshop presentations. As a result of this NCR project, economists have been able to develop and deliver presentations on economic issues in aquaculture production to current and potential aquacultural producers. These presentations and the publications which follow may reduce the impacts of uninformed investment decisions by current and potential aquaculture entrepreneurs.

Publications, manuscripts, and papers presented

See the Appendix for a cumulative output for all NCRAC-funded Economics and Marketing activities.

Support

Years	NCRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1993-95	$40,000	$59,683				$59,683	$99,683
Total	**$40,000**	**$59,683**				**$59,683**	**$99,683**

Yellow Perch

Project Termination Report for the Period May 1, 1989 to August 31, 1993

Total funding level

$354,785 (May 1, 1989 to August 31, 1993)

Participants:

Fred P. Binkowski, University of Wisconsin-Milwaukee, Wisconsin
Paul B. Brown, Purdue University, Illinois
David A. Culver, Ohio State University, Ohio
Konrad Dabrowski, Ohio State University, Ohio
Donald L. Garling, Michigan State University, Michigan
Terrence B. Kayes, University of Nebraska-Lincoln, Nebraska
Jeffrey A. Malison, University of Wisconsin-Madison, Wisconsin
Robert J. Sheehan, Southern Illinois University-Carbondale, Illinois

Extension liaison:

Donald L. Garling, Michigan State University, Michigan

Non-funded collaborators:

Forrest Williams, Bay Port Aquaculture, Inc., West Olive, Michigan
David Northey, Coolwater Farms, Dousman, Wisconsin

Reason for termination

The objectives for the first three NCRAC-funded projects on Yellow Perch were completed.

Project objectives

1. Compare the survival, growth, feed conversion, and proximate composition of offspring from selected Northern, Southern, and Great Plains stocks of yellow perch, at different life history stages and at different temperatures.
2. Evaluate the survival, growth, and feed conversion of yellow perch raised at various loadings or rearing densities in selected flow-through and pond culture systems.
3. Evaluate and improve the efficiency of various methods of inducing triploidy in yellow perch, and compare the survival and growth to market size of the triploids produced with that of normal diploids.
4. Compare pond and intensive culture methods for the production of yellow perch fingerlings.

Principal accomplishments

The principal goal of this work was to develop practical strategies for commercial yellow perch aquaculture under the diverse environmental conditions that exist in the NCR. Much of this goal was realized. During its 4-year history, the two projects examined: (1) the suitability of selected wild perch brood stocks obtained from different geographic locales as candidates for potential brood stock development; (2) the applicability of selected conventional production technologies to perch aquaculture; (3) the potential of using chromosomal triploidy induction to enhance growth; and (4) the relative merits of pond versus intensive culture methods for the production of perch fingerlings, and the nutrient composition of live-food organisms versus perch fry raised to different sizes (stages of development) under different culture conditions (different pond sites and laboratories, pond versus intensive culture).

With respect to objective 1, studies at the University of Wisconsin-Milwaukee (UW-Milwaukee) found variations in percentage of survival and swim bladder inflation between perch fry from different stocks, and research at Purdue University (Purdue) identified significant differences in the growth of perch fingerlings from these same stocks at various rearing temperatures. In overview, these variations and differences appeared to be primarily reflective of the geographic locales from which the brood fish and fertilized eggs of the different stocks were selected, which is a factor that should be considered when selecting brood stock for the production of perch. Thus, producers in the northern and southern parts of the NCR should probably use brood stock from their own respective parts of the region, and not expend undue time and resources seeking "super" perch from stocks with presumed superior performance traits that have not been documented by properly controlled experimental procedures.

As part of objective 2, Michigan State University (MSU) and University of Wisconsin-Madison (UW-Madison) researchers unequivocally demonstrated that perch can be raised to market size using conventional aquaculture production technologies in a time frame similar to that of such important commercially cultured species as channel catfish. The demonstration of this fact is perhaps one of the project's most important practical benefits, because it underscores the importance of matching species selection for aquaculture development with climatic conditions and available resources. This research effort was also important because it helps refute the notion that, except for salmon and trout, finfish aquaculture in the North, owing to the shorter growing season, cannot be competitive with aquaculture production at southern or tropical latitudes. The temperature requirements of perch for successful reproduction and optimum growth make the commercial culture of this species in the principal catfish producing states of the South highly unlikely.

Regarding objective 2, MSU investigators, working with Bay Port Aquaculture Inc. of West Olive, Michigan, have demonstrated that perch can be raised on a commercial scale at high densities in flow-through tanks, using research-based procedures for estimating carrying capacity based on the dissolved oxygen requirements and ammonia tolerance limits of perch. Such an approach to perch aquaculture, employing intensive procedures similar to those used in commercial trout production, should be particularly applicable to situations where an inexpensive, abundant source of high-quality temperate (i.e., 18-24°C; 64.4-75.2°F) water is or can be made available for "grow out" - from natural springs, wells, and/or the utilization of dependable waste heat or clean cooling water from such providers as electric power generating stations.

Using a different approach, UW-Madison researchers, working with Coolwater Farms of Dousman, Wisconsin, have demonstrated that the commercial-scale culture of perch in ponds can be feasible, if sufficient quantities of inexpensive groundwater (which ranges between about 8 and 14°C across the NCR; 46.4 and 57.2°F) is available to moderate pond water temperature highs during the summer and ice formation during the winter. Such groundwater addition also helps maintain elevated dissolved oxygen concentrations, and facilitates ice control during the winter to provide access for management and feeding and to prevent equipment damage. The benefit of this approach is that it provides a ready means of producing commercial quantities of perch in those parts of the region where pond construction is feasible and groundwater is abundant and available at a reasonable cost.

Studies by researchers at the UW-Madison and Southern Illinois University-Carbondale (SIUC) on objective 3, have shown that while direct triploidy induction in fertilized eggs produces perch that exhibit retarded gonadal development and somewhat higher fillet yields than is observed in normal diploid fish, direct triploidy induction does not significantly enhance growth. Investigators at the UW-Madison have developed effective procedures to induce triploidy in perch either by heat or hydrostatic pressure shocks, but have shown that such shocks exert a negative influence on growth independent of ploidy change. Accordingly, unless perch can be marketed on the basis of fillet yield or lack of reproductive competence, instead of total body weight, it is difficult to envision how direct triploidy induction can benefit commercial perch aquaculture. Researchers at the UW-Madison have also developed procedures for producing tetraploid brood perch, which presumably can be backcrossed with diploid fish to produce triploid eggs via natural fertilization, rather than by using physical or chemical shock treatments. Triploid perch produced by crossing tetraploid and diploids may grow faster than diploids, but this potential benefit has not yet been tested either experimentally or in practice.

Researchers at the UW-Madison and UW-Milwaukee, working collaboratively on objective 4, have clearly demonstrated that with recently developed "best available" techniques, fry and early-fingerlings perch raised in ponds exhibit better survival and growth and far fewer problems with swim bladder inflation and spinal deformities than perch reared intensively in tanks since hatching. Furthermore, after habituation to formulated feed and intensive culture conditions, pond-reared perch often continue to out-grow fish reared entirely by intensive methods. Over the years, UW-Madison investigators have continued to develop improved procedures for incubating and hatching perch eggs, rearing and harvesting ever-increasing numbers of perch fingerlings from ponds (up to 1,000,000 per surface ha; 2,470,097/acre), and habituating early-fingerlings (16-18 mm total length; 0.63-0.70 in) to formulated feed and intensive culture conditions using internal tank lighting. The development of these procedures represents another one of the project's most important practical benefits, because they provide fish farmers with a ready means of producing large numbers of perch fingerlings that are habituated to formulated feed and ready for "grow out" to market size.

After 3 years of research on objective 4, UW-Madison and UW-Milwaukee investigators have found that despite significant improvements in procedures and fry survival, problems with swim bladder inflation and cannibalism continue to be serious impediments to the large-scale intensive production of perch fingerlings. UW-Milwaukee researchers demonstrated that problems with early development and habituation of fry to intensive culture conditions were not as serious with perch originating from the Prquimans River in North Carolina, as with perch fry originating from other locales. Ohio State University (OSU) investigators discovered no significant differences in the amino acid compositions of young perch from Wisconsin and Ohio, suggesting similar nutritional needs across perch stocks. Researchers from OSU also found suggestive evidence, but no causal or clear-cut functional linkages, that dietary ascorbic acid deficiencies may be responsible for the high incidence of spinal deformities often observed in perch larvae reared under intensive culture conditions, and that certain long-chain fatty acids may be important in the diets of young perch.

Impacts

The principal impacts of the completed NCRAC yellow perch project have been the development and/or expansion of two of the NCR's leading commercial perch aquaculture operations, the actual or planned start up of several new commercial perch aquaculture ventures, the utilization of the project's newly developed knowledge and production procedures by a number of fish farmers, and the training of numerous graduate and undergraduate students at the participating institutions.

Specific examples

The research done by MSU on the intensive flow-through culture of perch in tanks (objective 2) played a key role in the development and expansion of Bay Port Aquaculture Inc. of West Olive, Michigan. Similarly, all of the perch net-pen and pond production research (objective 2) and many of the perch fingerling production studies (objective 4) reported by the UW-Madison were done at Coolwater Farms of Dousman, Wisconsin, and directly involved Coolwater Farms' personnel in these investigations. As a consequence, Coolwater Farms has greatly expanded both the scope and efficiency of its operations.

Private producers that are actually known to have recently started culturing perch largely as a consequence of the project, or who have made significant investments to soon start, include one in Iowa, three in Indiana, one in Michigan, two in Nebraska, two in Ohio, and two in Wisconsin. Other perch aquaculture ventures in the region may have recently started or may soon become operational, but this cannot be presently documented.

The UW-Madison has reported that several "aquaculture endeavors in Iowa, Indiana, and Michigan have based their business plans on the pond culture of perch,"; that one in Iowa "is evaluating the use of net-pens,"; that "at least three commercial fish farms (one each in Wisconsin, Michigan, and Ohio) have begun to rear all-female perch,"; and that "at least three commercial perch aquaculture operations in the upper Midwest" have or are implementing the "egg hatching and fingerling production and training methods developed" during the project. The UW-Milwaukee has reported training fish farmers in its procedures for producing perch fingerlings intensively in tanks.

Purdue has indicated the partial training of two graduate students and three undergraduates, as part of the project; MSU reported training two graduate and several undergraduate students. In overview, all of the principal investigators and technical staff of the various laboratories and institutional programs participating in the project gained tremendous insights and new knowledge about the culture and biology of the yellow perch, and a better appreciation of the benefits of regional and inter-institutional collaboration.

Recommended follow-up activities

Building on the results of these projects, NCRAC provided funding for another Yellow Perch project which began on September 1, 1993, and will run for 2 years. The objectives of this new project are to: (1) determine the commercial scale feasibility and improve on the best intensive tank and pond culture practices for the production of yellow perch fingerlings and (2) determine the commercial scale feasibility of raising food-size yellow perch in flow-through raceways or tanks, open ponds, and large net-pens, comparing the best available formulated diets. A number of commercial fish farmers in the NCR have been named as major participants in this new project; many aspects of which will not be possible without their full cooperation and support.

The importance of UW-Milwaukee and OSU investigations on objective 4 is that they demonstrate that considerable additional research will probably be required to develop the procedures and diets necessary to successfully culture perch larvae intensively in tanks on a large scale. Based on experience with other species with small larvae, a long-term investment in selective breeding or in research on perch larval diet development might make such intensive culture technically feasible. Whether or not it would be commercially competitive with the improved methods developed in recent years for culturing young perch in ponds is unclear, particularly considering that continued improvements in the latter approach are likely.

Publications, manuscripts, or papers presented

See the Appendix for a cumulative output for all NCRAC-funded Yellow Perch activities.

Support

Years	NCRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1989-91	$162,680	$213,435				$213,435	$376,115
1990-92	$92,108	$400,543				$400,543	$492,651
1991-93	$99,997	$165,394				$165,394	$265,391
Total	**$354,785**	**$779,372**				**$779,372**	**$1,134,157**

Yellow Perch

Progress Report for the Period September 1, 1993 to August 31, 1996

NCRAC funding level

$257,086 (September 1, 1993 to August 31, 1996)

Participants:

Fred P. Binkowski, University of Wisconsin-Milwaukee, Wisconsin
Paul B. Brown, Purdue University, Illinois
Konrad Dabrowski, Ohio State University, Ohio
Donald L. Garling, Michigan State University, Michigan
Terrence B. Kayes, University of Nebraska-Lincoln, Nebraska
Jeffrey A. Malison, University of Wisconsin-Madison, Wisconsin
Extension liaison:
Donald L. Garling, Michigan State University, Michigan
Non-funded collaborators:
Harlan Bradt, etc., Coolwater Farms, LLC, Cambridge, Wisconsin
William Hahle, Pleasant Valley Fish Farm, McCook, Nebraska
John Hyink and John Wolf, Alpine Farms/Glacier Springs Trout Hatchery, Wisconsin
Dave Smith, Freshwater Farms of Ohio, Inc., Urbana, Ohio
Michael Wyatt, Sandhills Aquafarm, Keystone, Nebraska
Nebraska Game & Parks Commission, Calamus State Fish Hatchery, Burwell, Nebraska
Forrest Williams, Bay Port Aquaculture, Inc., West Olive, Michigan

Project objectives

1. Continue to improve larval rearing techniques by developing and evaluating different starter diets in relation to size at transfer to formulated feeds under selected environmental conditions.
2. Continue to improve pond fingerling production through examination of in-pond feeding techniques using physical/chemical attractants and improved harvesting strategies for different sizes of fingerlings from various types and sizes of ponds.
3. Continue development of extension materials and workshops emphasizing practical techniques coinciding with production events to meet the needs of established and potential yellow perch culturists through on-site presentations at two or more locations in different parts of the region.

Anticipated benefits

This project addresses priority needs identified by the North Central Regional Aquaculture Center (NCRAC) Industry Advisory Council (IAC) for advancing yellow perch aquaculture in the North Central Region (NCR). The IAC has indicated that one major constraint that presently limits perch aquaculture is the lack of reliable methods of producing perch fingerlings habituated to formulated feeds. In addition, there is a continuing need to provide producer training on key aspects of perch aquaculture, and to transfer advances in perch culture technology to the public sector.

The information generated by these projects will greatly assist perch producers in their efforts to reliably raise the large number of perch fingerlings needed by the industry. Improvements in pond fingerling techniques will immediately increase the availability of fingerlings to the industry because almost all fingerlings currently available are produced in ponds. Research on the effect of spawner size on larval size and on starter diet formulation for yellow perch will improve intensive fry rearing techniques and decrease the dependence on live feeds. Laying the foundation for use of one of the more potent and proven legal flavor additives for fish requires quantifying two critical nutritional requirements for yellow perch; the total sulfur amino acid and choline requirements. These values alone are beneficial in terms of developing a diet for yellow perch and provide the foundation for evaluation of betaine as a flavor additive in diets.

Extension activities will continue to promote and advance yellow perch culture through expanded outreach, education, and training programs. Additional extension materials (bulletins, fact sheets, and audiovisual materials) developed by the NCRAC Yellow Perch and Extension Work Groups and a series of hands-on workshops and field demonstrations will transfer current technology to established and potential fish farmers, and increase public awareness of the potential of yellow perch aquaculture as a viable agricultural enterprise in the NCR. In addition, this project will develop improved technologies for certain key facets of yellow perch aquaculture. Finally, the results of experiments incorporated into this proposal will immediately help fish farmers improve the production efficiency of yellow perch.

Progress and principal accomplishments

As an integral component of this project, private producers have cooperated by providing facilities, fish, feed, day-to-day husbandry, and routine data collection. At its inception, this project included the participation of eight different private fish farms in various parts of the NCR. Participating university researchers provided project oversight on experimental design, advice or direct assistance with the technical set-up of any specialized experimental systems, supervision and assistance on critical end-point data collection, and analyses of results.

In year 1 of the project (September 1, 1993 to August 31, 1994), significant progress was made at certain sites at testing selected research-based production technologies. Accordingly, from an extension perspective, the project is successfully building and/or expanding working relationships between NCRAC researchers and certain regional fish farmers, testing various research-based technologies under practical production conditions, transferring knowledge from academia to the private sector, and identifying private producers who are both capable and willing to sustain a collaborative technology evaluation and demonstration effort. Several of the original private-sector collaborators have either met or have worked hard to meet their project commitments.

Objective 1

Researchers at Michigan State University (MSU) directed their efforts in 1996 towards studying the effects of female spawner size on the size of eggs and fry. Spawning stock were collected from the outer Saginaw Bay, Lake Huron and transported to Bay Port Aquaculture, West Olive, Michigan. Bay Port workers held the fish until they could be manually spawned. Eggs were sampled from females divided into six size classes in 25 mm (1 in) increments from 200 to 350 mm (7.8 to 13.8 in).

Subsamples of eggs were collected from the ends and center of each ribbon. Approximately 1 gram of eggs from each subsample was weighed and fixed in Stockard's solution for subsequent measurements. The ribbon segments were fertilized and placed into specially designed incubator trays and incubated in well water at 11.5°C (52.7°F). Nine days after fertilization, measurements of larval mouth gape (height and width) and total length were taken using a dissecting microscope in conjunction with the Optimas imaging system, BioScan™. The data is currently being analyzed. Preserved egg samples were used to determine the number of eggs/g and 25 eggs were measured along the long axis of the egg outer diameter and the yolk membrane. Preliminary evaluation of egg size indicates a positive relationship with the length of the maternal parent.

A sulfur amino acid requirement study is underway at Purdue University (Purdue) and should be completed by December 1996. Through 4 weeks, fish fed 1.0% methionine are growing better than fish fed lower concentrations in the diet.

Studies at Ohio State University (OSU) have been designed to evaluate the use of pancreatic enzymes and a digestive tract neurohormone, bombesin, in the diets offered to 0.6 g (0.02 oz) yellow perch. Perch fry were raised initially in ponds (Ohio Valley Fisheries, Inc.) and were transferred to an indoor facility and accustomed to an artificial commercial diet (Ziegler). Studies on three experimental diets and one commercial diet fed to triplicate groups of yellow perch are being conducted using 40 L (10.6 gal) glass tanks at OSU. Experimental diet 1 is supplemented with either pancreatic digestive enzymes (PD), diet 2 with bombesin and PD, and diet 3 with nothing. Results indicated no significant differences between treatments. However, all experimental diets resulted in better growth of yellow perch than the commercial salmonid starter.

An accompanying study using the same batch of fish, the same commercial diet, and three different experimental diets was conducted at the Piketon Research and Extension Center. Four groups per treatment were used and a semi-purified, casein-gelatin diet (#l) was tested along with diets based on krill and squid meals (#2) or fish meals (#3). In a trial in Piketon, 4 weeks of feeding resulted in significantly lower growth rate of perch fed a semi-purified diet (gain 70+/-8%) than both experimental diets (105+/-11% - 115+/-15%) or a commercial diet (104+/-7%).

Objective 2

An experiment was conducted by University of Wisconsin-Madison (UW-Madison) researchers at Coolwater Farms, LLC, to determine key parameters for producing yellow perch fingerlings habituated to formulated feeds and reared in ponds for an entire growing season, and to compare the performance of two types of pond lighting and feeding systems. Ponds are currently being harvested and production data are being collected. Observations made by Coolwater Farms culturists indicate that improvements in pond lighting and feeder design markedly reduced the labor needed for husbandry and system maintenance.

In the late spring and early summer of 1996, University of Nebraska-Lincoln (UNL) investigators compared the utility of different lighting systems, combined with a specially designed trap-net, to harvest photopositive young-of-the-year (YOY) yellow perch on a large scale from ponds. Previous research using similar capture gear had demonstrated that up to 38,000 young yellow perch could be captured per 30-min effort from heavily stocked, shallow (<1.25 m; 4.1 ft) earthen ponds of 0.4 ha (1 acre) surface area or less.

The 1996 trials compared the utility of different configurations of lights arrayed on rafts that could be easily pulled from an opposing pond shoreline to the trap-net. Trials were conducted at the Calamus State Fish Hatchery (near Burwell, Nebraska), in two plastic-lined 0.2 ha (0.5 acre) ponds that when full have an average depth of well over 1.25 m (4.1 ft). Both ponds were stocked with about 225,000 yellow perch fry, and managed by standard procedures used by the Nebraska Game and Parks Commission. Harvesting trials were initiated when the fish in each pond reached 19 mm (0.7 in) total length.

Two light-raft systems were tested. The lights on both could be turned on or off by remote control. One raft was equipped to broadcast a total of 250 W of omnidirectional light below water. The second was equipped to broadcast a total of 910 W of omnidirectional-submerged, directional-submerged, and directional-above-surface lighting. The directional lighting on the latter system was broadcast forward of the raft as it was pulled through the water.

The trap-net was fitted with a string of five, 75 W submerged lights that were turned off sequentially to draw fish into an open-top harvest pot, designed to facilitate the low-stress crowding and capture of small fish. The design of this trap-net has been proven effective at capturing large numbers of photo-positive young fish when used in combination with a variety of lighting systems in shallow earthen ponds.

The results of the 1996 UNL trials were that the 910 W light raft effected the capture of significantly greater numbers of yellow perch (about 5,000 fish per 30-min capture effort) than the 260 W light raft (about 3,800 fish per capture effort). The number of capture efforts made with each system were 14 and 17, respectively. One particularly noteworthy observation was that the numbers of yellow perch captured per unit effort in 1996 was significantly down from previous years (typically 10,000-20,000 fish per capture effort). This was attributed primarily to the fact that the Nebraska Game and Parks Commission added AquaShade® to the ponds to prevent excessive algae growth, and possibly to the greater depth of the ponds used in 1996. AquaShade® is a commercially available product that reduces light transmittance in water.

Extremely poor weather conditions, combined with budgetary shortfalls, precluded UNL testing of this or similar harvesting equipment at sites other than the Calamus State Fish Hatchery. Three years of research by UNL investigators on the use of light to harvest YOY yellow perch indicate that it is a very useful tool but can yield highly variable results, depending on a number of factors, e.g., pond depth and area, plankton concentrations, presence of aquatic vegetation, size, and age of fish.

Objective 3

During 1996, two "Intensive Aquaculture of Yellow Perch in Conjunction with Recirculating Aquaculture

Systems" workshops were sponsored by the University of Wisconsin Sea Grant Institute, which included NCRAC Extension and Yellow Perch Work Group members. Alpine Farms (Sheboygan Falls, Wisconsin) personnel participated as aquaculture industry cooperators to provide their practical experience with, and knowledge of, yellow perch rearing in their recirculating aquaculture system (RAS) technology.

The program for the first workshop included a morning session with lecture presentations and an afternoon poster session during which small groups of attendees had the opportunity for direct contact with the presenters, having their specific questions answered and problems solved. In order to maximize personal contact with the presenters, the number of attendees at this workshop was limited to 75.

ln the weeks following this workshop, small groups of workshop attendees were given the opportunity for additional direct hands-on advisory service concerning the technology for intensive rearing of yellow perch. These on-site activities were conducted at the University of Wisconsin System Aquaculture Institute in Milwaukee, and at Alpine Farms where they observed demonstrations on the intensive aquaculture of yellow perch in conjunction with a RAS.

A second 1-day workshop on the intensive culture of yellow perch with RAS was held in June 1996. The agenda for this workshop included lecture presentations on RAS operation and technology, water quality management in RAS, relevant aspects of yellow perch biology under intensive rearing, and the economic and business aspects of yellow perch culture. The format of this workshop was designed to focus on the most important topics and maximize the interaction between workshop attendees and aquaculture experts during an extended question/answer session. Eighty-five people attended this workshop.

Kayes of UNL conducted a workshop in Nebraska, part of which covered methods of harvesting yellow perch in ponds. In addition, progress was made on producing a videotape on the small-scale processing of yellow perch, in cooperation with videographers at Kansas State University.

Work planned

Objective 1

Preliminary studies were conducted at MSU to develop larval rearing tank designs similar to those that have been used successfully in raising larval walleye and mahi mahi. The initial design will be improved in 1996-97 and used in feed acceptance studies. Also in 1996-97, MSU researchers will use their findings from 1995-96 to select spawners from size classes that produce favorable hatchability and mouth size traits in their fry. The fry will be used for nutritional studies comparing live and formulated dry diets.

After completion of the methionine requirement at Purdue, the dietary choline requirement will be quantified, then the ability of betaine to supply part or all of the choline requirement will be determined. Work at OSU will continue to evaluate the use of pancreatic enzymes and a digestive tract neurohormone, bombesin, in the diets offered to young yellow perch.

Objective 2

A second experiment on pond fingerling production will be conducted by UW-Madison researchers at Coolwater Farms, LLC. This experiment will evaluate strategies to maximize fingerling survival and crop uniformity in perch cultured throughout a growing season.

Nearly all the NCRAC funds allotted to UNL for research on objective 2 were exhausted in 1996. In 1996-97, UNL investigators will evaluate and compare the data collected over the past 3 years on harvesting YOY fish using light in preparation for submitting the findings to a peer-reviewed journal for publication, and as part of a NCRAC project termination report.

Objective 3

A workshop demonstrating key facets of fingerling production and grow-out is being planned by UW-Madison researchers for June 1997.

The "Intensive Aquaculture of Yellow Perch in Conjunction with RAS Technology" workshops presented by University of Wisconsin-Milwaukee in 1996 provided the framework for the presentation of a hands-on workshop to be organized and presented in

1997. They intend to install a demonstration RAS at the University of Wisconsin System Aquaculture Institute in Milwaukee that can be directly used for hands-on participation and training of workshop attendees.

A NCRAC-sponsored conference and two workshops on yellow perch aquaculture will be held in Nebraska in 1996-97. Also, the videotape on the small-scale processing of yellow perch, which was proposed by Kayes of UNL, should be completed.

Impacts

Defining critical nutritional requirements for targeted species reduces feed costs and overall cost of production. These data will be important pieces of information for manufacturers of feed. This research provides strong evidence that commercial diets for salmonids need to be modified to meet nutritional requirements of yellow perch. These new diet formulations may significantly improve growth rate of yellow perch fry. Further, definite use of legal flavor additives may alleviate the problems of poor feed acceptance by larval and growout perch.

Studies on pond fingerling production by UW-Madison researchers have shown that research based production strategies can be used on a commercial scale to produce large numbers of yellow perch fingerlings at a relatively low cost. Lights and automatic feeders used to habituate fingerlings to formulated feeds while they remain in ponds can be used throughout the first growing season, eliminating the need for a separate feed-training phase of production. Improvements in feeder design may increase reliability and decrease capital and operational costs.

The field trials conducted by UNL investigators have demonstrated both the utility and the limitations of using light to harvest YOY yellow perch. Present indications are that light is being used by increasing numbers of fish farmers to harvest young yellow perch (as well as other species) in several states including Ohio, Minnesota, and Wisconsin.

Workshops done on yellow perch aquaculture in the NCR have stimulated increased interest in this species among established fish farmers, potential fish farmers, and the general public. In the past year, requests for information on yellow perch aquaculture have increased significantly; for example, requests for yellow perch culture information from Kayes at UNL have increased by about 500%.

Publications, manuscripts, or papers presented

See the Appendix for a cumulative output for all NCRAC-funded Yellow Perch activities.

Support

Years	NCRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1993-94	$75,000	$87,240	$30,000	$10,000[a]		$127,240	$202,240
1994-95	$75,000	$81,587	$30,000	$81,000[abc]		$192,587	$267,587
1995-96	$107,086	$145,814	$20,000	$134,000[ac]		$299,814	$406,900
Total	**$257,086**	**$314,641**	**$80,000**	**$225,000**		**$619,641**	**$876,727**

[a] Sea Grant/USDC/NOAA
[b] USDI, Bureau of Indian Affairs
[c] EPA

Hybrid Striped Bass

Project Component Termination Report for the Period May 1, 1989 to August 31, 1993

NCRAC funding level

$232,960 (May 1, 1989 to August 31, 1993)

Participants:

Terrence B. Kayes, University of Nebraska-Lincoln, Nebraska
Christopher C. Kohler, Southern Illinois University-Carbondale, Illinois
Jeffrey A. Malison, University of Wisconsin, Wisconsin
Robert J. Sheehan, Southern Illinois University-Carbondale, Illinois
Extension liaison:
Joseph E. Morris, Iowa State University, Iowa

Reason for termination

The objectives for this work on hybrid striped bass were completed.

Project objectives

1. Obtain and maintain (in captivity) populations of spawning size white bass.
2. Define reproductive development in wild and captive white bass by characterizing seasonal changes in hormone titers and gonadal histology.
3. Evaluate the effects of selected photoperiod/temperature and hormonal manipulations on gonadal development and spawning in white bass brood stock.

Principal accomplishments

Southern Illinois University-Carbondale (SIUC) researchers have successfully captured adult white bass, acclimated them to tank culture conditions, and trained them to accept formulated feed. Some fish have been held in captivity for over 3 years. This level of domestication is not known to have been achieved with white bass in any other laboratory or commercial enterprise.

Considerable numbers of white bass spawns have been accomplished using various hormonal/temperature/photoperiod manipulations over the course of this project. Fish have been accelerated to spawn as early as January, and have had their spawning delayed to as late as October. Accordingly, techniques have been developed that allow successful spawning of white bass any season of the year. Moreover, female white bass that successfully spawned in October 1992 were successfully induced to spawn again in April 1993. Thus, it was demonstrated that white bass can be successfully spawned twice in a 7-month period. It was also shown that male white bass held at or above spawning temperatures (15°C; 59°F) produced viable sperm for at least 2 months. Average hatching rates have also been improved from 25% to 50%. These findings represent major steps toward the development of domesticated white bass brood stocks to be used for hatchery production of hybrid striped bass.

Injection levels of a synthetic luteinizing hormone-releasing hormone analogue (LhRha) and human chorionic gonadotropin (hCG) have been identified that greatly improve upon previous results at SIUC, and elsewhere, with respect to controlled spawning of white bass. Data indicate that hCG dosages considerably less than that traditionally used to induce final egg maturation are more useful in white bass. In addition to providing guidance for improved spawning performance, these data have positive implications toward eventual regulatory approval of hCG by FDA for spawning *Morone* species.

Annual rhythms of serum levels of estradiol-17 and testosterone, as well as gonadal growth and histology of the wild and the three captive populations of white bass were documented and correlated with actual spawning events.

Impacts

Domestication

The development of a protocol to habituate adult white bass to captivity, including training to dry formulated feeds, allows for developing domesticated brood stock. Domesticated brood stock is clearly advantageous by:

- obviating need to collect brood stock from wild,
- resolving numerous regulatory issues regarding collection and hauling of wild brood stock,
- allowing for brood stock selection programs, and
- ensuring availability of brood stock when needed.

Out-of-season spawning
The development of efficacious procedures to manipulate sexual maturation and induce out-of-season spawning is important for optimal management of brood stock. It leads to:
- greater predictability of gamete production,
- reduced incidence of failed spawnings,
- reduced incidences of brood stock losses due to toxemia, and
- production of fertilized eggs and fry at predetermined times throughout the year.

Hatching rates
Improvements in hatching rates allows for increased hatchery production or reduction in brood stock needs.

Hcg dosages
Determination of the most efficacious hCG dosages not only improves spawning performance, but these data have positive implications toward eventual regulatory approval of hCG by the FDA for spawning *Morone* species. As a direct consequence of this work:
- standard dosages of hCG are being tested for efficacy in a multi-state Investigational New Animal Drug (INAD) application being administered by Auburn University through sponsorship of Intervet Inc.;
- hCG will be tested for animal safety by SIUC under sponsorship of Intervet Inc.; and
- these projects will collectively provide FDA with necessary information to make a determination for approval of hCG for brood fish.

Recommended follow-up activities
NCRAC funded a follow-up study that is focused on developing procedures to intensively rear white bass larvae to a stage when they will consume formulated feed (see next Project Component Termination Report). A proposed study for the next NCRAC funding cycle will, among other topics, compare three strains of white bass in yield trials. Collectively, the results from these studies should pave the way to undertake a white bass brood stock selection program.

Publications, manuscripts, or papers presented

See the Appendix for a cumulative output for all NCRAC-funded Hybrid Striped Bass activities.

Support

Years	NCRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1989-91	$136,410	$93,436				$93,436	$229,846
1991-93	$96,550	$54,317				$54,317	$150,867
Total	**$232,960**	**$147,753**				**$147,753**	**$380,713**

Hybrid Striped Bass

Project Component Termination Report for the Period June 1, 1990 to August 31, 1996

NCRAC funding level

$269,000 (June 1, 1990 to February 29, 1996)

Participants

Fred P. Binkowski, University of Wisconsin-Milwaukee, Wisconsin
George G. Brown, Iowa State University, Iowa
Paul B. Brown, Purdue University, Indiana
Konrad Dabrowski, Ohio State University, Ohio
James E. Ebeling, Ohio State University, Ohio
Christopher C. Kohler, Southern Illinois University-Carbondale, Illinois
Jeffrey A. Malison, University of Wisconsin, Wisconsin
Robert J. Sheehan, Southern Illinois University-Carbondale, Illinois
Bruce L. Tetzlaff, Southern Illinois University-Carbondale, Illinois
M. Randall White, Purdue University, Indiana
Extension liaison:
Joseph E. Morris, Iowa State University, Iowa

Reason for termination

The objectives for this work on Hybrid Striped Bass were completed.

Project objectives

1. Develop larval diets and economically feasible techniques to convert hybrid striped bass young from zooplankton to prepared diets.
2. Develop intensive hatchery production techniques for white bass and to "domesticate" white bass by producing brood stock originating from induced spawns.
3. Improve methods for storage and transport of striped bass and white bass gametes.

Principal accomplishments

In a comparative study conducted at Southern Illinois University-Carbondale (SIUC), hatching rates for embryos incubated in Heath trays (28.2%) were equivalent to tannic acid-treated (150 mg/L water) embryos incubated in Heath trays (22.9%) or McDonald jars (22.4%).

Facilities to intensively rear larval white bass were established at Ohio State University (OSU), SIUC, and the University of Wisconsin-Milwaukee (UW-Milwaukee). White bass larvae from three separate spawning trials were shipped by overnight freight to OSU and UW-Milwaukee. Attempts to rear larval white bass were minimally successful. Less than 1% survival rates were obtained by day 122 at UW-Milwaukee, day 45 at OSU, and day 24 at SIUC.

A group of white bass sac-fry shipped from SIUC to UW-Milwaukee was introduced evenly by volume into 12, 60 L (15.9 gal) flow-through aquaria. Each aquarium contained approximately 300 sac-fry. These fish were offered "green tank" water and the three experimental diets that were provided by Purdue University (Purdue). The length of the cylindrical food particles ranged from approximately 0.5 to 1.7 mm (0.02 to 0.07 in) and the diameter was 420-595 mm. White bass sac-fry are approximately 3.5 mm (0.14 in) in total length. The cross sectional diameter of the feed approximated the width of the entire head (550-630 mm) of white bass sac-fry, and was outside of the range of the width of the mouth. UW-Milwaukee researchers ground portions of the diets in a mortar and pestle and sieved it through a 150 mm mesh to obtain more suitable-size particles. From May 26-31, 1995, each of the three ground and sieved diets was offered to fry in triplicate aquaria along with "green tank" water. The controls received only "green tank" water. No feeding activity or interest by the fry in the formulated diets was observed. Mortality of the sac fry was heavy in all the tanks and by May 31 (within 6 days), less than a dozen fry were observed in any of the aquaria and more than half of them had only one or no living fry. At this point the trial was terminated.

Researchers at SIUC found that both hybrid striped bass crosses at a 2-5 g (0.07-0.18 oz) size range readily convert from zooplankton to formulated feed. Over 90% of the fish converted to formulated feed within 2 days as compared to 70-85% after 7 days for large-mouth bass which were trained in a "side-by-side" study. Preliminary results indicate that white bass and reciprocal-cross hybrids are equivalent in this regard and can make the switch between day 21 and 28 after hatch. Original cross hybrids can generally be switched at day 7 after hatch.

A problem facing hybrid striped bass aquaculturists is that hybrid fry are not always available. Gametes must be obtained from two species that may not be spawning simultaneously or are located in different geographical areas. Therefore to facilitate hybrid production, viable *Morone* semina need to be readily available when ripe eggs are available.

To aid in the solution of this problem, procedures for reliable short-term (refrigerated) and long-term (cryopreservation) storage of striped bass (*Morone saxatilis)* semina were developed. Initially, the characteristics of high quality spermatozoa were examined to determine methods for assessing sperm quality and developing effective sperm handling techniques. This led to the formulation of extenders for short-term (less than 21 days) refrigerated (1°C; 33.8°F) storage. The quality of stored seminal samples was tested by determining sperm motility percentages and developing a sperm quality index (SQI). Refrigerated extended seminal samples were routinely stored for 14 days with 50% sperm motility.

Cryopreservation procedures were developed and sperm quality of cryopreserved seminal samples of striped bass were assessed. Fertility tests with these samples were performed with white bass (*M. chrysops*) eggs and results were compared to those results when using (fresh) white bass semen.

Ten media containing dimethylsulfoxide (DMSO) were used to cryopreserve striped bass spermatozoa. Although all media successfully cryopreserved spermatozoa, the best motility (SQI 2.3: about 50%) was obtained with samples cryopreserved in the five media containing 4% DMSO. Using the criteria for high quality semen, the samples cryopreserved in media containing 4% DMSO with or without trehalose and bovine serum albumin gave the best motility results and were used in fertility tests with white bass eggs. Straws of the cryopreserved samples were transported from Florida to SIUC packed in dry ice. These were then stored in liquid nitrogen until used in fertility tests. Striped bass spermatozoa were cryopreserved with relatively simple methods. This may partially be because of the small size of the sperm, causing damage by the freezing process to be minimal since the cryogenic medium penetrates the whole cell very rapidly and the actual freezing may be rapid enough to prevent damaging ice crystal formation.

In the hybrid cross, the study was pursued until the hatch of normal larvae. Although success with cryopreserved spermatozoa has previously been reported for striped bass results were determined on the basis of cleavage, which does not necessarily indicate the normal development of diploid embryos. Fertility was tested using striped bass semen cryopreserved in cryogenic media and white bass eggs. The percent fertilization based on the number of hatched, normal larvae was 6.2% for the cryopreserved semen and 2.5% for the eggs fertilized with fresh control white bass semen (dead and abnormal larvae were excluded). This represented a 251.2% hatch from cryopreserved semen related to control semen. No development was found in control eggs (unfertilized eggs) tested for parthenogenesis.

The motility intensity of thawed and activated cryopreserved spermatozoa was roughly equivalent to that of seminal samples activated after 14-21 days of refrigerated storage, indicating that cryopreservation of striped bass semen may be the best option when storage time exceeds 21 days.

Emphasis was also focused on developing refrigerated and frozen storage methods for white bass spermatozoa. Evaluations of sperm motility and nuclear magnetic resonance (NMR) were used as measures of success in developing methodologies. NMR was used to measure the availability of high energy phosphorus compounds to power flagellar movements in spermatozoa.

Sperm quality was best when seminal samples were extended prior to shipping and when they were transported in tissue-culture flasks which provided a larger air space than the microcentrifuge tubes which were also tested as shipping containers. Extenders with simple formulations, including one that was essentially only a saline solution, were as good or better than a more complex extender solution for maintaining sperm quality during refrigerated storage at 1°C (33.8°F). The simple saline solution extender maintained good sperm quality for up to 1 month of refrigerated storage.

Declines in high-energy phosphorus compounds and increases in their breakdown product, as measured via NMR, corresponded with declines in sperm motility over time during refrigerated storage of semen. However, NMR detected differences in stored energy in spermatozoa among seminal samples when no such differences in sperm motility were detected, indicating that NMR may be a more sensitive measure of sperm quality.

It was found that a cryogenic solution consisting of a simple extender and DMSO as the cryoprotectant performed as well as more complex cryogenic media in sperm motility tests. Fertility was somewhat reduced using cryopreserved semen, as compared to semen which had been extended and stored at 1°C (33.8°F) for about 1 week. Cryopreservation reduced white bass sperm motility to 5 to 25% of motility in fresh semen samples, a reduction similar to that found in seminal samples which are extended and stored under refrigeration for about 4 weeks. It is recommended that refrigerated storage be used for white bass semen if storage times of 1 month or less are anticipated. Cryopreservation is the better option, if sperm storage is to exceed 1 month.

Impacts

Studies by the Hybrid Striped Bass Work Group demonstrate that:

- improvements in hatching rates allows for increased hatchery production or reduction in brood stock needs;
- improvements in larval rearing techniques of white bass will allow “true” domestication;
- improvements in switching hybrid striped bass fingerlings from zooplankton to formulated feeds will increase production efficiency;
- *Morone* semen which is to be stored should be kept cold at all times subsequent to stripping;
- white bass injected with hCG once per month and held at 15°C (59.0°F) produced 2 to 3 times as many spermatozoa as compared to those either given hCG once per week or no hCG but otherwise treated similarly—using this approach allowed semen to be obtained from each fish once per week for several months;
- semen should be diluted with an extender prior to shipping and transported on ice;
- relatively simple extender solutions (saline solutions) are effective for refrigerated storage of *Morone* semen;
- tissue culture flasks proved to be better than microcentrifuge tubes for shipping white bass semen—this difference was attributed to the oxygen in the larger air space of the former;
- *Morone* semen can be extended and stored at 1°C (33.8°F) and good motility can be retained for 3 to 4 weeks;
- initial evaluations indicated that changes in NMR spectra of seminal samples are consistent with changes in sperm motility; however, NMR may provide a more sensitive measure of semen quality;
- cryopreservation reduced sperm motility by about 50%, as compared to extended semen;
- a relatively simple cryogenic medium (4% DMSO in a simple extender solution) was effective for storing *Morone* semen;
- excellent fertility in white bass eggs was obtained using cryopreserved striped bass semen, and good fertility was obtained using cryopreserved white bass semen;
- based on reductions in sperm motility, cryopreservation is the better option for *Morone* semen if it is to be stored for more than 3 to 4 weeks, whereas refrigerated storage is better for shorter storage times.

Recommended follow-up activities

NCRAC is currently funding studies aimed at comparing different geographical strains of hybrid striped bass and white bass in ponds. These studies are incorporating spawning, sperm storage, and hatchery procedures developed in this project. The sperm storage protocols are also being tested in industry settings. Collectively, the results from past and current studies should pave the way to economically undertake hybrid striped bass culture in the NCR. Continued demonstration of the technologies developed need to be undertaken with industry partners.

Publications, manuscripts, or papers presented

See the Appendix for a cumulative output for all NCRAC-funded Hybrid Striped Bass activities.

Support

Years	NCRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1990-93	$101,000	$94,000				$94,000	$195,000
1993-96	$168,000	$119,440				$119,440	$287,440
Total	**$269,000**	**$213,440**				**$213,440**	**$482,440**

Hybrid Striped Bass

Progress Report for the Period September 1, 1995 to August 31, 1996

NCRAC funding level

$90,270 (September 1, 1995 to August 31, 1996)

Participants

Fred P. Binkowski, University of Wisconsin-Milwaukee, Wisconsin
Michael L. Brown, South Dakota State University, South Dakota
Paul B. Brown, Purdue University, Indiana
Terrence B. Kayes, University of Nebraska-Lincoln, Nebraska
Christopher C. Kohler, Southern Illinois University-Carbondale, Illinois
Jeffrey A. Malison, University of Wisconsin, Wisconsin
Joseph E. Morris, Iowa State University, Iowa
Robert J. Sheehan, Southern Illinois University-Carbondale, Illinois

Extension liaison

Joseph E. Morris, Iowa State University, Iowa

Non-funded collaborators

Robert Lyons, Lyons Enterprises, Morocco, Indiana
Gary Shirley, Shirley's Fish Farm, Lafayette, Indiana
Mike Freeze, Keo Fish Farm, Inc., Keo, Arkansas
Scott Lindell, AquaFuture, Turners Fall, Massachusetts

Project objectives

1. Examine fry (phase 1) to fingerling (phase 2) production of three strains of white bass and three strains of hybrid striped bass (sunshine bass) in ponds with and without lights and vibrating feeders.
2. Conduct field testing of fingerling (phase 2) to advanced fingerling (phase 3) production of three strains of hybrid striped bass (sunshine bass) in various culture systems.
3. Extension component:

a. Coordinate selection of various culture systems and implement field testing (fingerling to advanced fingerling to food size).
b. Write an initial culture manual using the information generated by all the hybrid striped bass research sponsored by North Central Regional Aquaculture Center (NCRAC).
c. Produce associated fact sheets, bulletins, and videos for hybrid striped bass research in the North Central Region (NCR).

Anticipated benefits

The overall goal of this collaborative project is to enhance hybrid striped bass aquaculture in the NCR. Hybrid striped bass are consistently identified as a high priority species within the NCR and consistently ranked as a preferred species to eat (unpublished data from Purdue University). Out-of-season spawning of white bass has been achieved in an ongoing NCRAC-sponsored project. The development of intensive larval culture techniques for this species will allow for its full domestication. The development of techniques for semen storage (cryopreservation and extended) preclude the need for maintaining large numbers of male striped bass brood stock. The logical next step is to conduct field trials of several strains of white bass and hybrid striped bass in various culture systems.

Existing producers need to improve the economics of hybrid striped bass production by increasing stocking densities and improving feeds. The break-even production cost of hybrids grown in cages is reported to be $2.29 to $3.45/kg ($1.04 to $1.56/lb). Those values were based on a stocking density of 100/m^3 (2.8/ft^3) and feed costs of $0.55/kg ($0.25/lb). As production of hybrids increases on a regional and national scale, market price will likely decrease. Thus, this research will help maintain current profit margins as production increases. The knowledge gained from this study should be of immediate use by the aquaculture industry. The extension component of the study will assure that research information gets to the industry in a user-friendly form. Although the proposed project is not directly interregional with respect to physical performance, lines of communications have and will continue to be maintained with the Hybrid Striped Bass Grower's Association and other researchers, specifically: Harrell, Woods, and Zohar at the University of Maryland; Smith and Jenkins at the South Carolina Department of Natural Resources; and Hodson and Sullivan at North Carolina State University.

Progress and principal accomplishments

Objectives 1 and 2

Southern Illinois University-Carbondale (SIUC)

Brood stock acquisition

Adult white bass were acquired by SIUC researchers from three regions representing the extremes of white bass' native range: Arkansas, South Dakota, and Lake Erie. Arkansas white bass were collected by trap netting in the Arkansas River. The South Dakota stock of white bass was collected by South Dakota State University (SDSU) by angling in Lake Kampeska, South Dakota. Lake Erie white bass were collected by commercial fishermen in Sandusky Bay of Lake Erie. Brood fish were held at SIUC in recirculating systems in winter conditions (8°C [46.4°F] and 10 hours daylight) until all three stocks of fish were collected.

Spawning of Brood Stock and Incubation of Larvae

Once all three stocks of fish were acquired, temperature and number of daylight hours were gradually increased until 16°C (60.8°F) and 14 hours, respectively, were reached. During this warm-up phase brood fish were fed minnows on a daily basis. When spawning temperature and number of daylight hours were obtained, female white bass were injected with hCG at a rate of 150 IU/kg (68.4 IU/lb) to induce ovulation. Males were injected at a rate of 100 IU/kg (45.5 IU/kg) to enhance semen production. Extended striped bass semen was obtained from Keo Fish Farm, Arkansas, so that sunshine bass could be produced.

At least 10 females of each stock ovulated, at which point the eggs were manually stripped and divided into two allotments. One allotment of the eggs was fertilized with white bass neat semen to produce pure white bass, while the other allotment of eggs was fertilized with extended striped bass semen to produce sunshine bass. Both allotments of eggs were treated with tannic acid to reduce the adhesiveness of the eggs. Eggs were then incubated in MacDonald jars until hatch. Hatch was complete at about 48 hours postfertilization.

Enumeration and Stocking of Larvae

At 4 days of age, the larvae were enumerated and subsequently stocked into ponds. In order to enumerate the larvae, ten samples of 100 mL (3.4 oz) were randomly drawn from each holding tank. The number of larvae in each sample was counted. From the 10 samples an average number of larvae per volume was calculated. This average value was used to extrapolate to the total volume of the holding tank. This procedure was repeated for all six stocks of fish.

Ponds used in this study are approximately 0.04 ha (0.10 acres); however, each pond's length and width were measured to determine its individual surface area. Larvae were transported from the holding tanks to the Touch of Nature pond facility in bags containing approximately one-third fish and water and two-thirds pure oxygen. Stocking of larvae began at dusk and continued after dark. Larvae were stocked at a rate of 500,000/ha (202,350/acre). Each stock of fish was stocked in quadruplicate.

Pond Filling and Fertilization

Ponds were filled 5-10 days prior to stocking and incoming water was filtered using a nylon "sock" with a mesh size of 500 mm. Ponds were fertilized using both cottonseed meal and 8-32-16 inorganic fertilizer. A single application of cottonseed meal was administered at 350 kg/ha (312.3 lb/acre) 4-5 days prior to stocking. The inorganic fertilizer was applied at 25 kg/ha (22.3 lb/acre) twice weekly for 5 weeks. An additional application of cottonseed meal was administered once weekly at 25 kg/ha 22.3 lb/acre) starting in week 4.

Feeding of Phase 1 Fingerlings

Training the fish to accept prepared feed began 21 days poststocking. Fish were offered frymeal twice a day at 5-10 kg/ha/day (4.5-8.9 lb/acre/day). Once fish were observed accepting prepared feed, pellet size was increased as necessary and fish were fed to satiation. Feed amounts were recorded twice daily.

Harvesting Phase 1 Fingerlings

At 36-41 days of age phase 1 fingerlings were harvested by seining. Survival rate varied from pond to pond, but was generally poor. The highest survival rate for any pond was 21.3%, while the lowest survival rate was 0.0%. Survival rates were markedly higher for hybrid striped bass ponds versus white bass ponds averaging 12.8% and 2.6%, respectively. Average weight of an individual fish in any particular pond was inversely related to its survival rate; that is, if a pond had a high survival rate, then the average weight of an individual within that pond tended to be low. This trend is reflected in the relatively low average weights of hybrid striped bass (1.5 g; 0.05 oz) and the relatively high average weights of white bass (2.2 g; 0.08 oz). Average weights were calculated by weighing 120 individuals from each pond.

Phase 2 Production

Phase 1 fingerlings which were harvested were re-stocked for phase 2 production. Due to a lack of fish, all three white bass stocks were eliminated from this segment of the experiment. Both Arkansas and South Dakota hybrid striped bass stocks were restocked in triplicate, while Lake Erie hybrid striped bass were only restocked in duplicate. The stocking rate used for phase 2 production was 25,000 fish/ha (10,117.5/acre). Fish were offered feed two times per day. Fish were fed to satiation and feed amounts were recorded twice daily. One grass carp was stocked per pond to serve as a control on aquatic vegetation.

Harvesting Phase 2 Fingerlings

At the end of the growing season, phase 2 fingerlings were harvested by seining. Survival rates ranged from a low of 49.2% to a high of 85.8%. Survival rates for both Arkansas and Lake Erie hybrid striped bass were about 72%, while the survival rate for South Dakota hybrid striped bass was only 56.6%. The average weight of individual fish also varied from stock to stock. The highest average weight was 90.2 g (3.2 oz) for Lake Erie hybrid striped bass, while South Dakota and Arkansas hybrid striped bass had average weights of 69.0 g (2.4 oz) and 58.4 g (2.1 oz), respectively. Average weights were calculated by weighing 50 individuals from each pond.

South Dakota State University (SDSU)

Two groups of hybrid striped bass fingerlings were transported from SIUC to SDSU to conduct strain comparison and density experiments. The two groups of offspring were produced either with female white bass collected from the Arkansas River, Arkansas, or Lake Kampeska, South Dakota, and stored striped bass spermatozoa maintained at SIUC, originating from Keo Fish Farm, Arkansas. These experiments, which began on August 16, 1996, will continue for approximately another 110 days.

The culture system for both experiments consists of 110 L (29.1 gal) glass aquaria connected as a closed freshwater recirculating system with a delivery rate of approximately 1 L/min (0.26 gal/min). Ammonia, nitrite, nitrate, pH, hardness, alkalinity, and carbon dioxide are measured every 2 days. Water temperature is maintained at 22°C (71.6°F) and dissolved oxygen is maintained near saturation by continuous aeration; both are monitored several times weekly. A light/dark cycle of 12 h light/12 h dark is maintained using incandescent lighting controlled by an automatic electric timer.

Initial mean weights were 3.6 g (0.13 oz) and 2.9 g (0.10 oz) for Arkansas and South Dakota hybrids, respectively. The diet (38% protein, 8% crude fat) used in both experiments was obtained from Southern States Cooperative, Inc. (Richmond, Virginia). All fish were conditioned for a 2-week period by feeding a #4 crumble *ad libitum* two times per day. Randomly selected fish from each strain group were then stocked in individual aquaria to provide four replicates. The feed was supplied to fish initially at a rate of 10% of body weight per day equally divided into four feeding periods. All feeding is done with belt feeders. The feeding rate will be progressively reduced to 3% of body weight during the experiment to minimize overfeeding while maintaining a level approaching satiation. Also, pellet sizes fed are periodically increased with graded changes in body size. Waste material is siphoned from each aquaria every other day.

Group and individual measurements are made at weekly intervals. Feed allotments are adjusted weekly. The same general protocol is being applied to the density experiment. Four replicates each of 5 (45/m^3; 1.3/ft^3), 15 (136/m^3; 3.9/ft^3), or 30 (273/m^3; 7.7/ft^3) South Dakota hybrids per 110 L (29.1 gal) aquaria are being maintained at present. Performance characteristics (e.g., growth, conversion, condition, survival) are monitored in both experiments.

Purdue University (Purdue)
In the first year of the Purdue project, a private producer was going to provide fingerlings for the first evaluation, but none of the fish survived overwinter at the producer's site. A secondary supplier was identified and fish were brought to Indiana. However, most of those fish died due to the stress of a 15-hour haul. Cages were stocked at both field sites and will be harvested in November 1996. The tank loading study was initiated in late-summer 1996 and will be completed by December 1996. In a series of studies, soy products have been evaluated as a replacement for fish meal in diets. Maximum incorporation of raw soybeans was less than 20%, while roasted soybeans could be incorporated up to 20%. Solvent-extracted soybean meal could be incorporated up to 40% of the diet if sufficient mineral supplementation was provided.

Objective 3
Iowa State University and SIUC
Kohler and Morris served as co-chairs for the NCRAC Hybrid Striped Bass Workshop that was held in November 1995, in Champaign, Illinois. The topics for the workshop included larval culture, cage culture, brood stock management, and an industry perspective; the 35 attendees were from Illinois, Iowa, Indiana, and Missouri. NCRAC-funded speakers included Chris Kohler, Sue Kohler, and Bob Sheehan (SIUC), George Brown and Joe Morris (Iowa State University), and LaDon Swann (Purdue). A hybrid striped bass fact sheet developed by Morris and Kohler is in press.

Work planned

Objectives 1 and 2
SIUC
Phase 2 fingerlings were redistributed at a rate of 4,940 fish/ha (2,000/acre) for phase 3 growout. There are five replicates for each stock of hybrid striped bass. Feeding will resume as early in spring 1997 as possible and will occur twice a day until the end of the 1997 growing season.

Studies in aquaria are being conducted to compare the three strains of hybrid striped bass and the three strains of white bass. This study is being conducted as a "control" for the pond studies.

University of Wisconsin-Madison and University of Nebraska-Lincoln
Pond studies comparing survival of larval hybrid striped bass and white bass with and without lights and vibrating feeders will be carried out in 1997.

SDSU
During the summer of 1997, plans are to evaluate performance characteristics of three strains of hybrid striped bass (Arkansas, South Dakota, and Lake Erie female white bass sources) under flowthrough conditions. Phase 2 fish, supplied by SIUC, will be transported to SDSU and stocked in an indoor flow-through system comprised of 1,100 L (290.6 gal) rectangular tanks. Each strain will be stocked into a minimum of three replicate flow-through tanks at similar densities.

Commercial feed will be dispensed by belt feeders. General environmental conditions will be similar to those maintained during fall 1996. Trials will continue until each strain reaches a marketable size. Dressed and fillet proportions, and composition will be determined following harvest.

Purdue
Fish for the second year of the project have been acquired and will be reared at the Purdue University Aquaculture Research Facility. Thus, problems in acquisition and transport have been eliminated. Two commercial aquaculture facilities (Lyons Enterprises and Shirley's Fish Farm) will be stocked in April 1997 and fish grown until November of 1997. Stocking densities will be 100, 150, and 200 fish/m^3 (2.8, 4.2, and 5.7 fish/ft^3). Tank stocking densities will range from 50 to 300 fish/m^3 (1.4 to 8.5 fish/ft^3), with flow rates held constant at 1.0 L/min (0.26 gal/min).

Objective 3
The culture manual will be written in 1997. One or more videos will also be developed.

Impacts

Much of the technology developed over the course of NCRAC-sponsored hybrid striped bass research was incorporated in the current project. For example, wild white bass brood stock were obtained from three distinct geographic locations and transported to SIUC where they were habituated to captivity and induced to spawn using hormones. Stored striped bass semen obtained from Keo Fish Farms, Arkansas, was then used to produce hybrid striped bass. Eggs were incubated using the jar technique and fry were stocked into newly fertilized ponds. Fingerlings were switched to formulated feed in the ponds and phase 2 production was carried out. Feed-trained fingerlings were also sent to SDSU for additional studies in aquaria and raceways. Identification of the maximum density of fish in cages and tanks will allow maximum use of production space and resources; thus, increasing profitability of culture. Use of soybean products in diets decreases the cost of feed, while not sacrificing weight gain or health of fish. Further, these new formulations can be manufactured in the NCR. These studies, taken collectively, will not only meet the stated objectives of the project, but also will serve as a demonstration of the bulk of the technology developed by the NCRAC Hybrid Striped Bass Work Group since its inception.

Publications, manuscripts, or papers presented

See the Appendix for a cumulative output for all NCRAC-funded Hybrid Striped Bass activities.

Support

Years	NCRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1995-96	$90,270	$85,117	$55,019			$140,136	$230,406
Total	**$90,270**	**$85,117**				**$140,136**	**$230,406**

NCRAC has funded five Hybrid Striped Bass projects. This progress report is for the fifth project which is chaired by Christopher C. Kohler. The project continues and expands upon the first four projects. It began on September 1, 1995, and will conclude on August 31, 1997.

Walleye

Project Component Termination Report for the Period, September 1, 1989 to August 31, 1993

NCRAC Funding level

$321,740 (May 1, 1989 to August 31, 1993)

Participants

Thomas G. Bell, Michigan State University, Michigan
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Anne R. Kapuscinski, University of Minnesota, Minnesota
Terrence B. Kayes, University of Nebraska-Lincoln, Nebraska
Jeffrey A. Malison, University of Wisconsin-Madison, Wisconsin
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Extension liaison
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Non-funded collaborators
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Iowa DNR, Rathbun State Fish Hatchery, Iowa
Iowa DNR, Spirit Lake State Fish Hatchery, Iowa
Minnesota DNR, Devil's Track Hatchery, Minnesota
Nebraska Game & Parks Commission, Calamus State Fish Hatchery, Burwell, Nebraska
Ohio DNR, London State Fish Hatchery, Ohio
U.S. Fish & Wildlife Service, Garrison Dam National Fish Hatchery, North Dakota
U.S. Fish & Wildlife Service, Valley City National Fish Hatchery, North Dakota
U.S. Fish & Wildlife Service, Gavins Point National Fish Hatchery, South Dakota
U.S. Fish & Wildlife Service, Genoa National Fish Hatchery, Wisconsin

Reason for termination

The objectives for this work on Walleye were completed.

Project objectives

1. Develop baseline information on the mechanisms regulating the natural reproductive cycle of wild and pond-held walleye by characterizing seasonal changes in hormone titers and gonadal histology.
2. Develop methods for manipulating the annual reproductive cycle of walleye to induce out-of-season spawning.
3. Evaluate zooplankton seeding for pond culture of fingerling walleye.
4. Evaluate strategies for control of clam shrimp in ponds used for culture of fingerling walleye.
5. Determine the etiology of noninflation of the gas bladder of intensively cultured walleye fry.

Principal accomplishments

Objective 1

The endocrine and gonadal changes during the annual reproductive cycle of walleye were described for the first time. No differences were observed between the developmental patterns of wild walleye (captured primarily from the Mississippi River near Minneapolis, Minnesota) and walleye held in ponds in Carbondale, Illinois. Gonadal growth in wild male walleye began in August/September, and testes contained mature spermatozoa by late fall (October/November). Mature spermatozoa could be expressed from males collected from January through April. Testosterone levels were low (<0.5 ng/mL) throughout the summer, rose in October to a plateau of >1.0 ng/mL from November through January, and peaked in March at 2.8 ng/mL. Serum levels of 11-ketotestosterone were <10 ng/mL from late April through January, and rose in March and early April to >35 ng/mL.

In wild female walleyes, oocytes increased in diameter from 184.0 $\pm$ 18.6 mm in October to 998.7 $\pm$ 39.8 mm in November. This increase occurred coincident with a marked rise in gonadosomatic indices (GSIs) and circulating levels of serum estradiol-17 (from 0.2 ng/mL to 3.7 ng/mL), the steroid responsible for stimulating hepatic vitellogenin synthesis in teleosts. Just prior to spawning in March, oocytes were approximately 1,500 mm in diameter. Following spawning, average GSIs in females declined from 15.3% to 1.5%. Data from *in vitro* cultures of walleye oocytes conducted at the University of Wisconsin-Madison (UW-Madison) suggested that 17,20-dihydroxy-4-pregnen-3-one (17,20-P) may be the steroid responsible for inducing final oocyte maturation in walleye. *In vivo* studies showed that 17,20-P levels rose very transiently to approximately 2 ng/mL coincident with final oocyte maturation. Taken together, these results suggest that vitellogenesis and spermatogenesis are at or near completion as early as mid-January, and that simple environmental and/or hormonal manipulations could be used to induce spawning from mid-January to late March.

Objective 2

University of Nebraska-Lincoln (UNL) and UW-Madison investigators developed methods to manipulate the annual reproductive cycle and induce out-of-season spawning of walleye. Wild adult walleye were captured in autumn by the Nebraska Game and Parks Commission from Lake McConaughy, Elwood Reservoir, and Merritt Reservoir in Nebraska, and by the Iowa Department of Natural Resources (DNR) from the Mississippi River. The Nebraska fish were transported to the Calamus State Fish Hatchery near Burwell, Nebraska, where they were maintained in a lined pond. In early December, these fish were transferred to earthen ponds at the Gavins Point National Fish Hatchery near Yankton, South Dakota. Walleye captured from the Mississippi River were transported directly to the Gavins Point Hatchery. All fish were fin-clipped to identify their origin, and transported by UNL personnel. At the Gavins Point hatchery, the walleye were separated by sex and overwintered in two 0.07 ha (0.17 acre) ponds stocked with forage fish.

In January, February, and March, 16-20 females and 4-5 males were recaptured from the Gavins Point hatchery ponds and transferred to the Calamus State Fish Hatchery, where they were placed in indoor tanks. There, the water temperature was gradually increased over a 10-d period to 10°C (50°F) and the photoperiod was set at 12- h light/12-h dark. Females were subject to one of four injection regimes: (1) saline on days 0 and 2; (2) human chorionic gonadotropin (hCG) on day 0 and day 2; (3) a synthetic luteinizing hormone-releasing hormone analogue (LHRHa) on day 0 and day 2; or (4) hCG on day 0 and 17,20-P on day 2. No difference in response patterns to these treatments was observed in walleye of different origins.

At each month, all hormone injection regimes successfully induced GVBD (germinal vesicle breakdown) and ovulation in at least some of the females, whereas none of the saline-injected fish underwent GVBD or spawned. In January, hCG was the most effective treatment for inducing ovulation. In February and March, egg survival was the highest in hCG-treated fish. At all times, the 17,20-P treatment resulted in very low egg survival and small egg size. The results demonstrate that appropriate hormone and environmental treatments can be successfully used to induce spawning in walleye from late January through March. The most effective hormone treatment in this regard was hCG. Regardless of when it was used, hCG at 150 and 500 IU/kg (days 0 and 2, respectively) (68 IU/lb and 227 IU/lb) induced spawning 6-8 days after the last injection.

Objective 3

Investigators at Iowa State University (ISU) conducted experiments in 1989 and 1990 to evaluate zooplankton seeding (inoculation) for pond culture of fingerling walleye at the Valley City National Fish Hatchery (VCNFH), Valley City, North Dakota, and Garrison Dam National Fish Hatchery (GDNFH), Riverdale, North Dakota. Zooplankton for the inoculation experiments were collected with an air-lift pump and cage system or by filtering inflowing water to raceways. In both cases, the zooplankton that were used for seeding the ponds were those large enough to be retained by a 0.5-mm (0.02-in) screen. All of the inocula consisted of cladocera (*Bosmina* and *Daphnia*) as well as cyclopoid and calanoid copepods. The zooplankton inoculation was done during the first week after fry stocking. The zooplankton inocula ranged from 0.4 to 28.8 kg/ha, or 70 to 990 organisms per m^3 based on pond volume. In 1989, 95 to 100% of the inoculum was *Daphnia pulex*; in 1990, 77% of the inoculum used at Valley City was *Daphnia pulex*, but at Garrison Dam, only 13.5% were *Daphnia* and 67% were copepods. Over 2 years, 22 ponds were inoculated with zooplankton, and 22 ponds served as the controls (i.e., without zooplankton inoculation).

In each year and at both hatcheries, the average yield (kg/ha, and number/ha) of walleye fingerlings from ponds that had received the zooplankton inoculation was lower than the control ponds. The yield of walleye fingerlings (number per acre) from ponds receiving a zooplankton inoculation at the VCNFH in 1989 was 21.8% less than the control ponds, and 22.4% less in 1990; at GDNFH, the yield from ponds receiving the zooplankton inoculation was 50.9% less in 1989 and 66.9% in 1990 than the control ponds. Also, data combined over both years and both hatcheries showed that smaller biomass yield of fingerlings (42.7 kg/ha; 38.1 lb/acre) was obtained from ponds that were seeded with zooplankton than the control ponds that were not inoculated (54.9 kg/ha; 49.0 lb/acre).

Overall, the findings indicate that zooplankton inoculation of culture ponds during the week fry are stocked had reduced fish production. These findings indicate that zooplankton inoculation as a pond management strategy must be undertaken with caution. Ponds should not be inoculated with large cladocera such as *Daphnia pulex* shortly after stocking walleye fry because the larger zooplankton in the inoculum have a competitive advantage over the smaller zooplankton *copepoda* nauplii and other smaller zooplankton that serve as important prey for first feeding larval walleye. This does not mean that an inoculation with smaller zooplankton or use of larger zooplankton will not be desirable, however, the findings demonstrate that the timing of such methods for biomanipulation need to be carefully evaluated. At these hatcheries, the normal inflowing water carried an abundance of zooplankton, but if zooplankton populations are not abundant in the water used to fill the ponds (i.e., when ground water is used) or if zooplankton numbers decline during the culture interval, inoculation may be used to initiate or to sustain zooplankton populations. However, prior research on the effects and benefits of zooplankton inoculation is limited, and it has not been systematically studied in walleye fingerling culture.

Objective 4

ISU investigators carried out studies on the ecology of clam shrimp at the GDNFH. In 1992, the studies were carried out on 23, 0.64 ha (1.58 acre) ponds during the culture season for northern pike, and 19 of the same ponds during the season for walleye. Ponds at the GDNFH were first used to raise northern pike. They were drained after 3 to 4 weeks to harvest the pike, then refilled to raise walleye. Adult clam shrimp were observed in 12 of the 23 ponds during northern pike culture, and 10 of the 19 ponds during walleye culture. Northern pike were cultured up to 29 days in ponds with clam shrimp, while pike were cultured a maximum of 22 days in ponds without clam shrimp. Survival and yield (number/ha and number/pond) of northern pike was significantly lower in ponds with clam shrimp compared to ponds without clam shrimp. Similar differences, although not significant, were seen in walleye culture ponds. Northern pike and walleye were cultured longer in ponds with clam shrimp, implying that fish growth is reduced in culture ponds with clam shrimp. The majority of large clam shrimp found during the walleye culture season were most likely hatched during northern pike culture. When ponds are used in tandem to raise northern pike and walleye, to prevent development of clam shrimp during the walleye culture the ponds should be thoroughly dried between culture periods.

Objective 5

Studies on the etiology (cause for) of noninflation of the gas bladder (NGB) were carried out as a collaborative effort among ISU, Michigan State University (MSU), and University of California-Davis (UCD). The study objectives included development of methods for intensive rearing of larval walleye on formulated feeds (ISU), identification of pathological lesions that will indicate the etiology of non-inflation of the gas bladder (MSU), and a description of developmental histology of the gas bladder, pneumatic duct, and other tissues and glands (UCD).

Each year, researchers at ISU obtained 1- to 5-day posthatch walleye fry or eyed-eggs from at least three cooperating state and federal fisheries agencies. ISU personnel reared walleye fry in an intensive culture environment and fed the fish a formulated feed, "fry feed Kyowa" (BioKyowa, Inc.), sizes B-400 through B-700, for 21-30 days. Each lot of fish obtained each year was used to evaluate different intensive culture treatments. Culture conditions involved different tank design (cylindrical and square), single-pass and recycle water systems, and pH. These different culture systems have aspects of them that may influence feed particle density (i.e., feeding success affects survival) and water quality (i.e., surface films or pH) which, in turn, may affect gas bladder inflation.

The fry samples provided a progression in age and size of fish, some collected before and others after feeding, with and without the yolk sac, and fish with and without gas bladder inflation, from a variety of experiments in which environmental variables differed substantially. MSU investigators found degenerative changes in the gas bladders (i.e., hyperplasia and abundance of macrophages) which were indicative of an inflammatory disease, and preliminary evidence to suggest a microbial infection as a specific initiating process. The observation of bacteria in the macrophages suggested a bacterial infection, at least as a secondary invader.

UCD investigators found that inflation of the swimbladder began on the sixth day posthatch, coinciding with the time of yolk sac depletion and initiation of feeding. In larvae with noninflated swimbladders, the pneumatic duct was obvious and its diameter remained fairly constant (25-45 mm) through the nineteenth day posthatch, but the pneumatic duct atrophied in larvae with inflated swimbladders. During the interval of swimbladder inflation, from the sixth to the 12th day posthatch, the common bile duct and pneumatic duct both opened to undifferentiated foregut where surfactant-like secretions from the common bile duct could affect fragmentation of large ingested air bubbles for transfer into the relative small-diameter pneumatic duct. After the 12th day posthatch, however, the pyloric sphincter developed and separated the common bile duct in the intestine from the pneumatic duct in the dorsal wall of the stomach. Thus, this finding indicates that differentiation of the foregut prevents inflation of larvae after 12th day posthatch.

The day for these events, however, will vary depending on water temperature.

Impacts

Objectives 1 and 2

These studies initially generated the basic knowledge of the reproductive cycle of walleye that was needed to begin efforts at controlling reproduction in walleye. This information was subsequently used to develop methods for inducing out-of-season spawning in walleye from late January through March.

The investigators also detailed techniques useful for synchronizing spawning in walleye, resulting in greater predictability of gamete production, and reduced incidence of failed spawning, gamete resorption and subsequent brood fish losses. These techniques can be used to increase hatchery efficiency and reliability.

Recently, UW-Madison personnel successfully led an effort to gain FDA-INAD approval to use hCG to induce spawning in walleye and yellow perch. This approval involves three regional private sector producers, and was done with the help of and in conjunction with the Iowa DNR.

Walleye producers (including the Iowa DNR) have used the technologies developed from these studies to produce walleye fry 9-12 weeks prior to the normal spawning season, and thereby greatly extended the period of time during which larval walleye can be reared intensively. This, in turn, has greatly increased the efficiency of existing intensive fry culture systems, facilitated research on the intensive culture of walleye fry, and aided hatcheries in their efforts to produce larger walleyes for stocking.

Objective 3

Research on the use of zooplankton inoculation for pond culture of fish has not been systematically studied in walleye fingerling culture. In the present study, zooplankton inoculation of walleye culture ponds during the week walleye fry were first stocked reduced survival and yield. It was surprising to find lower survival and production in ponds supplemented with zooplankton because the method is believed to increase forage for fingerlings. However, basic studies by aquatic ecologists has long demonstrated the difficulties of precise prediction of zooplankton dynamics in ponds and lakes. Moreover, the use of zooplankton inoculation is an unproven method for biomanipulation of aquatic ecosystems, even as small as fish culture ponds. Zooplankton inoculation may be beneficial in ponds filled with water which is devoid of planktonic life (e.g., well water), but at hatcheries which fill ponds with surface water there may be no benefit of adding zooplankton and it may actually be detrimental to production.

These findings indicate that zooplankton inoculation as a pond management strategy must be undertaken with caution. It seems, however, that ponds should not be inoculated with large cladocera shortly after stocking walleye fry because seeding ponds with zooplankton that are too large to be eaten by first feeding walleye may encourage a competitive advantage for the larger zooplankton over the smaller cladocera and copepods that are essential prey for first feeding larval walleye. In this study, the organisms used for seeding were generally larger than 2 mm (0.08 in) which is larger than first feeding walleye (about 9 mm; 0.35 in) can consume. It has been reported by others that the mean length of zooplankters in gut contents of first feeding walleye was 1.1 mm (0.04 in) at one study site and 0.8 mm (0.03 in) at another study site.

Objective 4

Basic studies on the ecology of clam shrimp in culture ponds demonstrate that strategies for control of clam shrimp in culture ponds need to consider both the life history characteristics of clam shrimp and fish cultural practices. Clam shrimp life history information provided insight into pond management strategies to reduce the impacts of clam shrimp on fish production. The typical habitat of most North American clam shrimp species is small, ephemeral ponds. The key to clam shrimp survival in this habitat is their ability to produce eggs that are highly resistant to drying, mechanical injury, and freezing. Clam shrimp problems in fish culture ponds are persistent because the resting eggs are resistant to mechanical injury, sunlight, and desiccation. Clam shrimp resting eggs can survive long periods of direct sunlight and wind, which they encounter when culture ponds are drained for harvest. Control measures for clam shrimp include interruption of the wet-dry cycle in fish culture ponds, a fill-drain-and-fill strategy, biological control, and chemical control. A fill-drain-and-fill strategy would involve partial pond filling in the spring long enough for clam shrimp eggs to hatch, then drained to flush out the newly hatched clam shrimp nauplii. The current tandem culture system at the GDNFH is a type of fill-and-drain strategy. At GDNFH, the northern pike culture season seems to end before clam shrimp reach sexual maturity, and many juvenile clam shrimp are flushed out before they were able to produce either summer or resting eggs. Also, many, but not all juveniles stranded on the pond bottom die before the ponds are refilled. These practices reduced the abundance of clam shrimp during the walleye culture season because clam shrimp that are hatched during the first week of northern pike season were unable to reproduce before they were washed out when the ponds were drained to harvest the northern pike. Although many of the clam shrimp were washed out, as observed in the catch basin when the ponds were drained, some clam shrimp are carried-over to the walleye culture season by surviving in the kettle and on the wet, pond bottom. Although these clam shrimp would be killed with a longer drying period, it is not possible to delay refilling (mean of 1.6 days in 1992) because hatching of these walleye has already been delayed to facilitate the double-cropping strategy. Biological control of clam shrimp with a predaceous fish does not seem to be effective because neither northern pike nor walleye culture feed on clam shrimp. Chemical control may be possible. Quicklime (calcium oxide, CaO) or slaked lime (calcium hydroxide, $Ca(OH)_2$) is generally recognized as safe as a pond sterilant by FDA and can be applied at the rate of 1,500 kg/ha (1,338 lb/acre) as quick lime or 2,000 kg/ha (1,784 lb/acre) as slaked lime. Lime is often used as a pond disinfectant to kill infectious organisms and parasites, including fish, tadpoles, and insects. The toxicity of lime to clam shrimp resting eggs has not been evaluated, but it is a potential treatment for killing clam shrimp eggs if the lime is applied to the moist, pond bottom after it is drained at the end of each production season. In a hatchery such as the GDNFH, the best time to make a lime application would follow the walleye harvest. Previously, trichlorfon (commercially sold as Masoten™ or Dylox™) was widely used for control of clam shrimp, but is not registered for use in fish culture ponds. Other studies show that trichlorfon treatment may be detrimental to zooplankton and invertebrates.

Objective 5

Culture tanks equipped with a surface spray, using about 10% of the inflow directed through a 90% nozzle to the water surface, removed the oil film, and feed and bacterial growth from the tank surface, thereby greatly enhancing gas bladder inflation. Gas bladder inflation, which was 20-40% without a surface spray, was 80 to 100% with a spray. Circular tanks (cylindrical tanks) with black-painted side walls were found to be more effective culture vessels than cuboidal tanks or tanks with blue-colored side walls. A near neutral pH is a healthier environment for larval fish than one supplied with water with high pH (> 8.5).

Development of a successful intensive culture system is essential for use of out-of-season spawning of walleye when ponds are not available for stocking. The successful development of techniques for out-of-season spawning and intensive culture system for rearing larval walleye represent a major breakthrough in walleye culture, opening new opportunities of research and commercial culture.

Recommended follow-up activities

Objectives 1 and 2

Further efforts should be directed at developing techniques to induce out-of-season spawning in walleye throughout the year.

Objective 3

The findings of negative effects from zooplankton inoculation suggest the need for further research to provide further explanation, and the need to define how and when (i.e., the timing) zooplankton inoculation may be used in pond culture of walleye. Distinction needs to be made between ponds receiving zooplankton from the water supply and those filled with well water and devoid of zooplankton. Likewise, little attention has been given to measuring the quantity and impact of zooplankton inoculation from the water supply of fish hatcheries using surface water sources.

Objective 4

Strategies for control of clam shrimp in culture ponds with quicklime (calcium oxide, CaO) or slaked lime (calcium hydroxide, $Ca(OH)_2$) need evaluation because these chemicals are approved for use in aquaculture.

Objective 5

Non-inflation of the gas bladder has been a major constraint to successful mass culture of walleye. The development of tank design and a spray-system to remove surface contaminants was a major breakthrough, however, survival is still typically less than 50% and further research would be beneficial to improve commercial feasibility. Research is especially needed on use of turbid water, optimizing light intensity, and feeding strategies for enhancing survival and growth of larval walleye.

Publications, manuscripts, or papers presented

See the Appendix for a cumulative output for all NCRAC-funded walleye activities.

Support

Years	NCRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal[a]	Other[b]	Total	
1989-91	$177,517	$127,535		$17,511		$145,046	$322,563
1991-92	$109,223	$73,242		$8,935		$82,177	$191,400
1992-93	$35,000	$26,475		$9,424	$40,990	$76,889	$111,889
Total	**$321,740**	**$227,252**		**$35,870**	**$40,990**	**$304,112**	**$625,852**

[a]University of Wisconsin Sea Grant Program/USDC/NOAA

[b]Nebraska Game and Parks Commission

NCRAC has funded six walleye projects. This project component termination report covers work undertaken for the first, third, and fourth projects. Robert Summerfelt chaired the first and third projects and Jeffrey A. Malison chaired the fourth project. The third project continued the first project for an additional year whereas the fourth project expanded upon earlier projects.

Walleye

Progress Report for the Period September 1, 1995 to August 31, 1996

NCRAC funding level

$117,897 (September 1, 1995 to August 31, 1996)

Participants

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Extension liaison

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Non-funded Collaborators

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Nebraska Game & Parks Commission, Calamus State Fish Hatchery, Burwell, Nebraska

Project objectives

1. Evaluate growth, feed efficiency, and stress responses as functions of density, loading, temperature, and feeding regimes (feeding rate and frequency) under tank and open-pond rearing conditions for raising juvenile walleye to food size.

2. Characterize the economics and institutional aspects of the domestic market for walleye as food fish, fingerlings, and other intermediate products.
3. Offer several workshops in the North Central Region (NCR), using extension materials (fact sheets, videos, etc.) and other information that has or will be developed necessary to demonstrate the technology of culturing walleye and its hybrids.
4. Complete performance evaluations of walleye (sauger hybrids) to finalize research initiated during the 2-year project period of the June 1993 proposal - including studies on fillet yield, proximate analysis, and organoleptic properties.

Anticipated benefits

This project is addressing priority needs identified by the North Central Regional Aquaculture Center (NCRAC) Industry Advisory Council, as well as specific objectives adopted by the NCRAC Board of Directors, to advance the development of commercial walleye aquaculture in the NCR. Two major lines of research are being pursued: first, to determine whether this species can be cultured to food size under practical conditions, at rearing densities and in a time frame conducive to commercialization; and second, to evaluate the nature and scope of the domestic market for walleye.

In addition, research is being completed to determine if one or more combinations of walleye and sauger genetic stocks can be used to produce hybrids that exhibit superior growth and performance characteristics, compared to purebred walleye. Collectively the data generated by research on both purebred and hybrid walleye will provide critical information to facilitate economic analyses of production costs, and provide extension professionals and private fish producers with new knowledge and training materials on key aspects of walleye aquaculture.

The research being done as part of this project on the production of walleye to food size is: (1) evaluating survival, growth, feed efficiency, and stress responses under various culture conditions; (2) examining methods of estimating growth and feeding rates under conditions that span the optimum temperature range for juveniles, with the goal of developing feeding tables for walleye; (3) adapting a bioenergetics model for use in projecting walleye growth and making feeding recommendations under various culture conditions; and (4) completing studies comparing the growth, performance, and other characteristics of walleye hybrids and purebreds up to food size.

The research being done to investigate the domestic walleye market is documenting critical information about the historic and potential imports of Canadian walleye, and the potential negative price impacts such imports could have on a fledgling domestic walleye aquaculture industry. A clearer understanding of wild-caught supplies, market pricing systems, marketing channels, and institutional structures will assist walleye producers to position themselves better to achieve profitability; help plan production, financing and marketing strategies; and provide insights on the potential effect farm-raised walleye products will have on the domestic market for this species.

Progress and principal accomplishments

Objective 1

Research in year 1 of the project on this objective was conducted by investigators from Iowa State University (ISU), the Illinois Natural History Survey (ILNHS), University of Nebraska-Lincoln (UNL), and the University of Wisconsin-Madison (UW-Madison). Much of the work done was preparatory to definitive research that will be completed in year 2.

Studies by ISU investigators were done using walleye hatched and raised to fingerling size at ISU in 1995. Final weight, percentage weight gain, and specific growth rate (percent weight gain per day) was greater for walleye cultured at 25°C (77.0°F) than at 20°C (68.0°F), but the difference was not statistically significant ($P \leq 0.05$). At 25°C (77.0°F), growth (total percentage weight gain) and specific growth rate in fish of 250-300 mm (9.8-11.8 in) total length (TL), at feeding rates of 1.5 and 2.0 (percent body weight per day) were not significantly different.

Work by ILNHS investigators has thus far focused on modifying an existing bioenergetics model for walleye using more recently developed metabolic parameters and better measures of specific inputs. Determinations of caloric levels contained in individual whole walleye and formulated feeds (by Parr adiabatic bomb calorimetry) and feces (by microbomb calorimetry) are nearing completion. Preparations have been made to enter data into the model from tank experiments using water temperature and food consumption as input variables.

The main focus of UNL investigators in year 1 of the project was to raise a large number of age 0 juvenile walleye for use in year 2 production trials aimed at culturing fish to market size under practical conditions. On June 6, 1996, UNL researchers harvested about 43,200 walleye of 28.5 mm (1.1 in) mean TL and 0.7 g (0.02 oz) mean body weight from 0.4 ha (1.0 acre) production ponds at the Calamus State Fish Hatchery near Burwell, Nebraska. Equal numbers of these fish (about 2,700) were assigned to 16 840 L (222 gal) cylindrical tanks, enclosed in a darkened Aquashelter® (Tuttle Industries, Friend, Nebraska).

All 16 tanks were equipped with in-tank lighting and 24-hour belt feeders (Zeigler Bros., Gardners, Pennsylvania), and supplied with Calamus Reservoir water run through packed columns for aeration. A feeding trial was conducted comparing a diet developed for juvenile walleye by Rick Barrows of the U.S. Fish and Wildlife Service (Bozeman, Montana) and “Silver Cup” salmon starter-series diet (Murrey Elevators, Murrey, Utah). Far more walleye were habituated to the Barrow’s diet than the Silver Cup diet. However, overall survival from the beginning to the end of the trial was extremely poor.

On July 12, 1996, less than 3,000 of the original 43,200 walleye remained alive, despite every effort to maximize survival. This poor survival was attributed primarily to facilities problems, though cannibalism was also a contributing factor. Significant disease problems were not observed. By October 9, 1996, only 973 walleye remained alive, though they were healthy and in excellent condition. Their mean total lengths and body weights were 161 mm (6.3 in) and 33.3 g (1.17 oz), respectively. On that date, the remaining walleye were placed in tanks supplied with 13.3°C (55.9°F) well water for overwintering.

Investigators at UW-Madison conducted experiments to measure changes in blood serum concentrations of cortisol, glucose, and chloride, following acute stress challenge tests of walleye acclimated to different water temperatures. Preliminary findings suggest that the stress-induced cortisol rise in walleye is far quicker and returns to baseline values faster than in rainbow trout, but peak values in the two species are comparable. After being stressed, walleye held at 15°C (59.0°F) had lower peak cortisol levels, which took longer to return to baseline levels, than walleye held at 21°C (69.8°F). Holding walleye above their thermal optimum (25°C; 77.0°F) prior to being stressed accelerated the initial cortisol rise to peak levels and delayed the return to baseline - suggesting a stronger, more prolonged stress response.

Objective 2

Investigators at Illinois State University completed a walleye market survey and an in-depth walleye fingerling culture and fish market literature review. The primary focus of the literature review was to identify any past research that might prove useful in describing the market for walleye fingerlings. Little of use was found. Information on the fingerling markets in Canada, the U.S., and the NCR was collected from research and extension experts, and from public and private suppliers and producers of walleye fingerlings using telephone interviews and mailed survey instruments.

Purdue University researchers conducted an in-depth literature review, which included the trade literature for food wholesalers, supermarkets, and restaurants; and developed a mailing list for those types of firms for the 12 states in the NCR. Supermarket and restaurant surveys were initiated in 2 phases. Phase 1 surveys differed for restaurants and supermarkets, and were limited to asking for general information on purchases and sales of fishery products. These surveys identified those restaurants and supermarkets where walleye was sold in 1996. The initial mailing was completed in the last week of August and the first week of September 1996.

North Dakota State University (NDSU) investigators have recently begun to collect published and secondary data on walleye exports from Canada to the U.S. This work got underway in August 1996.

Objective 3

Two NCRAC-sponsored workshops on walleye aquaculture were held in 1996. The first, “Intensive Culture of Walleye: From Fry to Fingerlings on Formulated Feed,” was held on May 7, 1996, at the Max McGraw Wildlife Foundation, Dundee, Illinois. Robert Summerfelt and Richard Clayton of ISU were the principal speakers, and Tom Harder of the McGraw

Foundation provided a tour and detailed description of the McGraw fry culture facilities. The workshop covered nearly all aspects of walleye fry culture - including design of a large-scale culture system, fish husbandry techniques, and feeding. Terry Kayes of UNL, videotaped the workshop with the assistance of Ron Kinnunen of Michigan State University. Nineteen people attended the workshop; five from Illinois, three from Iowa, two from Minnesota, four from Michigan, one from Nebraska, and four from Pennsylvania.

The second NCRAC workshop, "Production of Advanced Fingerling Walleye: Growth of Minnows in Ponds and Intensive Culture of Formulated Feed," was held on June 18, 1996, at Spirit Lake, Iowa. This workshop was co-sponsored by the Iowa Department of Natural Resources. Techniques for the production of advanced (127-203 mm; 5.0-8.0 in) fingerling walleye were presented. Participants observed a partial harvest of Welch Lake, a 23 ha (56.8 acre) undrainable pond, with a large seine. A site visit was made to the Spirit Lake State Fish Hatchery to observe procedures for training walleye fingerlings to formulated feed. Fifteen people attended this workshop; four from Iowa, two from Michigan, four from Pennsylvania, and five from Wisconsin.

Objective 4

Studies by UW-Madison investigators comparing hybrid and purebred walleye produced from several geographically different stocks of walleye and sauger were recently completed, but the data has not been fully analyzed. To date, hybrid walleye have exhibited superior growth to purebreds at all sizes up to food size. Food size Spirit Lake walleye (Mississippi River sauger gained 1.23 g/day (0.043 oz/day) compared to 0.45 g/ day (0.016 oz/day) for purebred walleye. Significant differences in the growth and performance of walleye purebreds and hybrids from different geographic stocks were observed.

Organoleptic trials and proximate analyses of carcass composition revealed little or no difference between purebred and hybrid walleye. Taste panels expressed a high degree of consumer preference for these fish, describing them as firm, flaky, and tender, with an absence of any off-flavors. Proximate analyses indicated that fillets were very low in fat (1.1-1.7%).

Work planned

Objective 1

Investigators at ISU were unable to use a bioenergetics model to calculate feeding rate in year 1 of the project, because ILNHS collaborators were unable to provide measurements of energy values for walleye and formulated feeds until August 1996. Therefore, in year 2 a retrospective analysis will be undertaken using energetics data, combined with actual feeding and growth data, to estimate how the bioenergetics model can be used to determine feeding rates. Additional experiments with "feeding the gain" will be completed, and that procedure will be compared with the bioenergetics method, to estimate feeding rates for the production of food-size walleye.

In year 2, ILNHS researchers will complete their calorimetric studies and modification of the walleye bioenergetics model. Simulations with the model will evaluate potential growth and feeding rates of walleye of various sizes under different aquaculture conditions.

Studies by UNL investigators will focus on evaluating the effects of rearing density on culturing juvenile walleye to food size in tanks, as described in the original proposal. The overwinter survival and growth of juvenile walleye maintained in tanks on well water at 13.3°C (55.9°F) will also be examined. Because of the poor survival of fish in year 1 and the resulting small numbers of advanced fingerling walleye available, no studies on the production of food-size fish in ponds will be possible in Nebraska in year 2 of the project. A shortfall in funding also precludes this possibility.

Investigators at UW-Madison, however, will conduct a study to characterize the growth and performance of walleye cultured to food size in ponds in year 2 of the project. Also, a second experiment to study the spacial requirements of near-food-size walleye in tank culture systems will be conducted. Details on these investigations are outlined in the original project proposal.

Objective 2

Investigators at Illinois State University will develop research and extension-oriented publications on the U.S. walleye fingerling market, based on data collected from public and private producers and suppliers, the research findings of NDSU, and other sources.

Researchers at NDSU will collect and evaluate secondary data on the export of walleye products from Canada to the U.S., as well as develop a report on the institutional components of the wild-capture fishery in Canada.

Purdue investigators will send phase 2 surveys to all those firms identified by phase 1 surveys as restaurants and supermarkets where walleye products were sold in 1996. The phase 2 surveys will ask targeted questions about walleye purchases and sales. Different surveys have been developed for restaurants and supermarkets. All other types of firms (e.g., fishery products whole-salers, brokers, and food service distributors) will be surveyed with the same, single survey instrument.

Objective 3

Two workshops on walleye aquaculture are planned for 1997. Both will be organized by Robert Summerfelt of ISU. The first workshop will be held in Ames, Iowa on February 25, 1997, and will cover the intensive culture of walleye fry. The second workshop will be held at Spirit Lake, Iowa on April 16-17, 1997, and will provide demonstrations of walleye brood stock collection, spawning methods, and egg incubation. In 1997, Kayes of UNL will edit and produce an introductory videotape on the intensive culture of walleye fry.

Objective 4

Investigators at UW-Madison will complete the analysis of all data collected under this objective in year 2 and will submit two manuscripts for publication, comparing the growth, performance, proximate analyses, and organoleptic qualities of hybrid and purebred walleye.

Impacts

Objective 1

The ongoing project will provide information that can be used to prepare guidelines and tables for predicting growth and determining appropriate feeding rates of juvenile to food-size walleye under different culture conditions, determine whether walleye can be raised to food size under practical production conditions, and help determine which culture techniques can be used to rear this species in a time frame and manner conducive to commercialization.

Objective 2

Research to date on this objective has generated no measurable economic impacts. But the research findings on this objective should produce valuable insights on the domestic markets for walleye as food fish, fingerlings, and other intermediate products.

Objective 3

The workshops on walleye aquaculture have provided the participants with conceptual information as well as demonstrations of important methods. This experience should enhance the ability of participants to learn from reading and doing, as well as undertake more advanced culture technologies.

Objective 4

The identification of hybrid walleye (sauger crosses) that have superior growth, performance, and other characteristics “put to use” should significantly reduce the time and costs required to produce food-size walleye.

Publications, manuscripts, or papers presented

See the Appendix for a cumulative output for all NCRAC-funded walleye activities.

Support

Years	NCRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1995-96	$117,897	$143,355				$143,355	$261,252
Total	**$117,897**	**$143,355**				**$143,355**	**$261,252**

NCRAC has funded six walleye projects. This progress report is for the sixth project, which is chaired by Terrence B. Kayes. The project continues and builds upon the first five projects. It began on September 1, 1995, and will conclude on August 31, 1997.

Sunfish

Project Component Termination Report for the Period, June 1, 1990 to August 31, 1995

NCRAC funding level

$280,577 (June 1, 1990 to August 31, 1995)

Participants

Donald L. Garling, Michigan State University, Michigan
Robert J. Sheehan, Southern Illinois University-Carbondale, Illinois
Bruce L. Tetzlaff, Southern Illinois University-Carbondale, Illinois
Extension liaison
Joseph E. Morris, Iowa State University, Iowa

Reason for termination

The objectives for this work on sunfish were completed.

Project objectives

1. Determine the mechanisms of sex control in sunfish and to produce and evaluate polyploid sunfish and hybrids.
2. Determine optimal stocking densities and relationships between temperature and growth for *Lepomis*, *Lepomis* hybrids, and triploid *Lepomis*.

Principal accomplishments

An evaluation of both cold and pressure shocks of varying magnitudes, initiation times (time after mixing egg and sperm), and durations to determine the optimum treatments to produce tetraploid (organisms with twice the number of normal chromosomes) bluegill (*Lepomis macrochirus*) has been completed at Michigan State University (MSU). Tetraploidy was induced in five of the 16 cold shock treatments tested. Maximum induction rates of 40% are comparable to those achieved in other species. Of the 10 pressure treatments examined, none were successful in producing tetraploids. Relative survival ranged from <1 to 34% for bluegill exposed to cold shock treatments or pressure shock treatments, respectively.

Twenty-seven combinations of pressure (41,369, 48,264, or 55,158 kPa), shock durations (2, 3, or 4 min), and post-fertilization shock initiation times (2, 3, or 4 min) were tested at Southern Illinois University-Carbondale (SIUC) to identify treatments which would most efficiently induce triploidy in green sunfish (*L. cyanellus*) male (bluegill female F_1 hybrids). Several of the shock treatments produced 100% triploids with at least 90% survival relative to controls. The two shock treatments which appeared to be most effective were: (1) 48,265 kPa for 4 min begun 2 min postfertilization and (2) 41,369 kPa for 2 min begun 3 min postfertilization. A paper based on this work appeared in the "Journal of the World Aquaculture Society;" it is the first publication on shock-induced triploidy in *Lepomis*.

Using starch gel electrophoresis, a diagnostic genetic technique, SIUC investigators found that they could distinguish among three species of sunfish, bluegill, green sunfish, and pumpkinseed (*L. gibbosus*). Furthermore, use of this technique made it possible to identify hybrids of these species; however, it did not allow for the identification of triploids.

Bluegill had a lower mean weight and poorer food conversion after 121 days of growth in a trial comparing bluegill, green sunfish, and male bluegill x female green hybrids. No significant differences were found between green sunfish and hybrids for final weight, specific growth rate, percent weight gain, or food conversion. Growth occurred over the entire range of temperatures tested, 8- 28°C (46-82°F) at 5°C intervals; 23°C (73°F) was optimum.

Male bluegill x green sunfish female triploid and diploid F_1 hybrid growth performance was compared in a 230-day trial at 23°C (73°F). Diploids showed larger final weight and better specific growth rate, percent weight gain, and food conversion. In a third growth trial at SIUC, diploid male bluegill/female green sunfish F_1 hybrids and green sunfish were compared to triploid male green sunfish/female bluegill F_1 hybrids over 235 days. Diploid taxa were selected on the basis of the results of the 121-day growth trial. No significant differences in weight, specific growth rate, percent weight gain, or food conversion were found. Green sunfish had lower dress-out weights than either hybrid. Gonadal somatic index was higher in the green sunfish than in the diploid and triploid hybrids. The vast majority of the green sunfish became sexually mature and were producing gametes over the range of tested temperatures, 8-28°C (46-82°F). Growth occurred at all temperatures; 18°C (64°F) was optimum. Lower growth rates and reduced optimum temperature were attributed in this trial to the use of fish larger than the ones used in the all-diploid growth trial.

Given the presumption of sterility and other potential advantages, triploids are a viable alternative for intensive food fish culture; they will not reproduce in culture units and will not cause genetic contamination of wild stocks. Male green sunfish/female bluegill F_1 hybrid triploids and male bluegill/female green sunfish diploid F_1 hybrids performed similarly in growth trials at SIUC and appeared to be the best candidates for food fish production.

The pressure-induced triploidy and allozyme species identification techniques derived at SIUC were used to produce gynogens (an organism with only maternal chromosomes) in a study to investigate the genetic sex determination system in bluegill. Heterologous (green sunfish) spermatozoa were irradiated, 15-360 sec, with 1500 uW/cm^2 of 254 nm wavelength UV light to deactivate the DNA. The irradiated spermatozoa were then used to activate bluegill eggs.

Control eggs which were not shocked but activated with irradiated sperm were all (N = 37) haploid; controls which were fertilized with normal spermatozoa and not shocked were all diploid (N = 21). Sperm irradiation times of 120, 150, or 180 sec plus the hydrostatic shock produced 48 diploids (gynogens) and no individuals with other ploidy levels or green sunfish loci, indicating 100% gynogen production efficiency.

Supposed gynogen larvae (N = 150) were then produced and stocked into a pond. Seven sexually mature gynogens were recovered from the pond. All seven were pure bluegill, based on allozyme analysis, and female. The probability of obtaining seven females from a 1:1 sex ratio population is only 0.008. This is strong evidence that the female is the homogametic sex and that an XX/XY genetic sex determination system occurs in bluegill.

This is the first study reporting induced gynogenesis and gynogen sex ratios in bluegills; this provides the foundation necessary for developing a technique for all-female production in bluegill. Sex reversal of gynogens would yield phenotypic males that would produce all-female progeny when crossed with normal females. This strategy could be used to eliminate reproduction in bluegill culture units, developing techniques for eliminating reproduction is one of the more important goals of the North Central Regional Aquaculture Center (NCRAC) Sunfish Research Effort.

Stocking densities for sunfish in cages and ponds were also evaluated at SIUC. Hybrid sunfish (bluegill male x green sunfish female) grew better at densities of 100 (3 fish/ft^3) and 200 fish/m^3 (6 fish/ft^3) than at 400 fish/m^3 (11 fish/ft^3) in cages. Food conversion was best at the lowest density and it became worse as density was increased.

Growth of hybrid sunfish was directly related to stocking density in ponds at the tested densities of 7,410, 4,940, and 2,470 fish/ha (3,000, 2,000, and 1,000 fish/acre). Food conversion also improved as density increased in ponds. Food conversion (weight of food fed/weight gain) ranged from 2.5 to 5.3 in the highest and lowest density ponds, respectively.

Impacts

Control of sunfish reproduction

The development of protocols for reducing reproduction in these fishes allows for the potential of increased growth of these fish in aquaculture systems as opposed to unrestrained reproduction. The two shock treatments which appeared to be most effective were: (1) 48,265 kPa for 4 min begun 2 min post-fertilization; and (2) 41,369 kPa for 2 min begun 3 min post-fertilization.

Optimal stocking densities

Information garnered from this work indicates the desirabilities of these fish for aquaculture in the NCR. The sunfish species and diploid and triploid hybrids which were evaluated showed optimum growth at temperatures of 18 to 23°C (64-73°F); all groups grew across a temperature range of 8 to 28°C (46-82°F).

Even at 8°C (46°F), the sunfish species, diploid and triploid hybrids, that were evaluated showed 150 to 200% weight gains over 121 days, and growth rates generally increased with increasing temperature. In light of results from this research project, the male bluegill x female green sunfish diploid F_1 hybrid and the male green sunfish x bluegill sunfish triploid F_1 hybrid appeared to be the best candidates for aquacultural production.

A stocking rate of 200 fish/m^3 (6 fish/ft^3) is the recommended stocking density for sunfish in cages. In open pond culture, the recommended stocking density for sunfish is 12,355 to 17,297 fingerlings/ha (5,000 to 7,000 fingerlings/acre).

Recommended follow-up activities

The male green sunfish x female bluegill triploid hybrid grew as well, or better than, the other four *Lepomis* taxa (green sunfish, bluegill, male bluegill male x female green sunfish hybrid, and male bluegill x female green sunfish triploid hybrid) evaluated. There is a need to evaluate growth in this triploid to a larger size, because triploids, theoretically, have their greatest growth advantage over diploids after the diploids reach sexual maturity.

One of the major impediments to open pond culture of sunfish is uncontrolled reproduction. Monosex sunfish production would eliminate this problem. An important first step has been taken in the development of monosex stock production in sunfish. Induced gynogenesis in bluegill has been accomplished at SIUC. The mating of sex-reversed XX gynogens to normal XX females would produce all-female progeny. Although females did not grow as well as males in SIUC's studies, their growth performance could be considerably enhanced in the absence of males. There is a need to explore monosex stock production in sunfish to determine if this is a viable means for controlling reproduction in culture units.

Publications, manuscripts, or papers presented

See the Appendix for a cumulative output for all NCRAC-funded sunfish activities.

Total support for the first two projects

Years	NCRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1990-92	$130,758	$96,710				$96,710	$227,468
1992-94	$149,799	$343,160	$3,200[a]		$29,830[b]	$376,190	$525,989
Total	**$280,557**	**$439,870**	**$3,200**		**$29,830**	**$472,900**	**$753,457**

[a]American Fishing Tackle Manufacturing Association
[b]Illinois Natural History Survey

NCRAC has funded three sunfish projects. This project component termination report covers work undertaken for the first and second projects. Bruce L. Tetzlaff chaired the first project and Robert J. Sheehan chaired the second project.

Sunfish

Project Component Termination Report for the Period, June 1, 1990 to August 31, 1996

NCRAC funding level

$280,557 (June 1, 1990 to August 31, 1996)

Participants

Michael L. Hooe, Illinois Natural History Survey, Illinois
Robert J. Sheehan, Southern Illinois University-Carbondale, Illinois
Bruce L. Tetzlaff, Southern Illinois University-Carbondale, Illinois
James R. Triplett, Pittsburg State University, Kansas
David H. Wahl, Illinois Natural History Survey, Illinois

Extension liaison

Joseph E. Morris, Iowa State University, Iowa

Reason for termination

The objective for this work on sunfish was completed.

Project objective

Determine optimum stocking densities and relationships between temperature and growth for crappie, crappie hybrids, and triploid crappie.

Principal accomplishments

Hybrid and pure stock crappies were produced at the Sam Parr Biological Station during spring 1993 and 1994 (no black crappie were produced in 1994) by Illinois Natural History Survey (INHS) personnel with assistance from Southern Illinois University-Carbondale (SIUC) researchers. Diploid F_1 hybrid and triploid F_1 hybrid crappies were produced by crossing white crappie females with black crappie males. In spring 1994, ponds were drained and 1,300 - 1,500 fish of each stock (85-100 mm [3.3-3.9 in] total length) were provided for Pittsburg State University (PSU) and 400-500 of each stock were provided for SIUC. In early summer 1994, additional pure stock black and hybrid crappie were provided for SIUC (300-400 of each stock, 100-150 mm [3.9-5.9 inch] total length). Starch-gel electrophoresis for all brood fish confirmed genetic integrity of the fry.

Observations derived from PSU research

- Capture and post-transport mortalities were very high, but a small percent of the wild caught white crappie (2%) from summer 1993 survived and showed significant growth. These fish were moved indoors for further feeding trials in a recirculating system.
- Optimum stocking densities were not adequately determined for white crappie. In all cases (1.8 to 5.0 kg/m^3; 0.1-0.3 lb/ft^3) overall survivability in cages was poor. However, survival was high and growth was acceptable in indoor trials at densities of 4-5 kg/m^3 (0.2-0.3 lb/ft^3).
- The high mortalities (57-98%) related to capture and transport of wild caught white crappie during 1993 were reduced to 0% in 1994. Approximately 4,200 fish were transported from the Sam Parr Biological Station in Illinois to the PSU Research Reserve in Kansas in two hauls of 8 hours (702 km; 436 mi) each, without any mortalities. The fish were handled and transported at night with temperatures less than 20°C (68°F) at 4-6 mg/L dissolved oxygen using oxygen diffusers and water treatments of 0.5% salt, PolyAqua™ (0.175-0.375 mL/L), and AmQuel™ (0.125 mL/L). Prior to handling for measurements, fish were anesthetized in Finquel™.
- White crappie, which were wild caught and fed in cages through the summer of 1993, were moved indoors in November and kept in two tanks in a recirculating system at a density of 4-5 kg/m^3 (0.2-0.3 lb/ft^3) for nearly 18 months. During the six feeding trials, only 17 of the 71 fish died; 16 of these were killed accidently by a single high chlorine event.
- Black crappie out-performed both white crappie and hybrid crappie in the second year of the cage culture trials. Black crappies showed the greatest growth rates, feed acceptance, uniformity, and survivability, with white crappies intermediate, and hybrid crappie showing poorest overall performance.
- Fish consumed and grew on 2.5 mm (0.1 in) Biodiet™ pellets in both cage trials and recirculating system trials. Examination of the abdominal cavity in all

cases revealed fatty livers and the cavity packed with fat.
• Observations of feeding activity in recirculating tanks suggested the formation of feeding hierarchies. Separate feeding experiments in aquaria during the summer of 1994 as part of a National Science Foundation (NSF) research training academy confirmed the presence of a dominance hierarchy.

A growth trial was conducted at SIUC using black, white, and hybrid crappie. White crappie used in the trial had been subjected to a pressure shock; about 66% of them were triploids. Ten 550 L (145.3 gal) circular tanks, each equipped with biofiltration, aeration, and heating and cooling systems, were used in the growth trial. The circular tanks were partitioned into three compartments, with each compartment receiving equal amounts of the inflow water. All three taxa were evaluated in each tank, one taxon per compartment constituted a replicate, 20 fish per replicate. Despite a protracted training period, feed acceptance was poor during the growth trial and none of the taxa grew well at any of the test temperatures. In most cases, test fish actually lost weight during the trial.

A second growth trial was designed so that growth of black and hybrid crappie would be evaluated against hybrid *Lepomis* sunfish (female green sunfish x male bluegill), a sunfish taxa known to be a good performer in recirculating systems under a variety of water temperatures. In this trial, a more protracted period of time was used to attempt to habituate black and hybrid crappies to prepared diets. The initial mean weight of the hybrid sunfish (60.1 g; 2.1 oz) was considerably greater than the black crappie (26.5 g; 0.9 oz) and hybrid crappie (30.4 g; 1.1 oz), but this was largely due to differences in body conformation and condition; there were only small differences in mean initial total length among the hybrid sunfish (14.7 cm; 5.8 in), black crappie (12.5 cm; 4.9 in), and hybrid crappie (13.0 cm; 5.1 in).

The growth trial was terminated at the end of 56 days when it became evident that hybrid crappie were not growing at some of the test temperatures. The extended training period appeared to be successful for black crappie in this trial. Black crappie grew at all test temperatures and had weight gains ranging from about 20 to 45%; hybrid sunfish had weight gains of 48 to 75% at 10 to 18°C (50.0 to 64.4°F). At test temperatures of 10 and 14°C (50.0 and 57.2°F), the hybrid crappie lost weight and showed the poorest growth in comparison to either the black crappie or hybrid sunfish at the other test temperatures. The best growth during the trial was shown by the hybrid sunfish at 18°C (64.4°F). Percent weight gains for black crappie were the highest among the three taxa at 22 and 26°C (71.6 and 78.8°F). However, instantaneous growth rate for black crappie was not better than that for hybrid sunfish at the two highest tested temperatures. Mean survival rate was high for all three taxa with all of the hybrid sunfish and 97% of the other two taxa surviving the trial.

Hybrid sunfish showed their best growth at temperatures of 18°C (64.4°F) or less whereas black and hybrid crappie showed their best growth at temperatures of 18°C (64.4°F) or more. This may be significant, since farmers in our region would have more of an advantage over southern producers with culture animals that grow better at lower temperatures.

Although effective procedures for inducing triploidy in *Lepomis* are available (see the 1994-95 Annual Progress Report), methods developed for crappie have not proved to be as successful. Prior to this study, the best triploid induction rate obtained at SIUC with crappie, using pressure shocks similar to those effective in *Lepomis,* was 66%.

A study conducted at SIUC was designed to develop more effective methods for inducing triploidy in crappie and to test the hypothesis that the temperature at which fertilized eggs are incubated may influence the effectiveness of shocks. The approach used by SIUC researchers was to hold the magnitude (6,000 psi) and duration (3 min) of the pressure shock constant while varying post-fertilization shock initiation time (2 to 7 min, tested at 1 min intervals) and the incubation temperature (17, 20, and 23°C; 62.6, 68.0, and 73.4°F) of the developing embryos prior to and during the shock treatment.

Incubation temperature did not affect triploid induction rate but better triploid induction rates were obtained as post-fertilization shock initiation times were increased. The most effective shocks for producing triploids in *Lepomis* were initiated at 2 to 3 min post-fertilization. Based on frequencies of deformed larvae and triploidy induction rate, the longer post-fertilization times were more successful with white crappie eggs. The highest triploidy induction rate SIUC researchers obtained (about 95%) occurred at a post-fertilization time of 7 minutes and at an incubation temperature of 20°C (68.0°F). This suggests that longer post-fertilization shock initiation times need to be investigated to optimize triploid induction procedures for white crappie.

Impacts

- Findings from PSU indicate survivability in cages is a major problem for cage culture of crappie, but this may be a function of cage design. Consideration of capture and transport methods is vital to minimizing initial mortality losses. PSU researchers determined that black crappie were the most suitable species for cage culture.
- PSU researchers have developed capture, transport and handling techniques that can markedly reduce mortality problems associated with crappie in aquaculture settings.
- Hybrid sunfish had their best growth at temperatures of 18°C (64.4°F) or less whereas black and hybrid crappie had their best growth at temperatures of 18°C (64.4°F) or more. This may be significant, since farmers in this region would have more of an advantage over southern producers with culture animals that grow better at lower temperatures.
- Pressure shock procedures for inducing triploidy in white crappie were developed which yielded more than 90% triploids; it appears that pressure shocks for inducing triploidy in the white crappie need to be applied at a much later time after fertilization, as compared to findings for *Lepomis*.

Recommended follow-up activities

- Cage design needs to be modified and evaluated for crappie culture.
- Continue to evaluate black crappie in recirculating systems.
- Further study needed on transport and stress in crappie.
- Evaluate importance of acclimation in reducing stress.
- Develop feeding strategies that reduce the impact of feeding hierarchies and fat accumulation on growth.
- Re-evaluate density effects associated with stress conditions.
- Determine optimal temperatures for growth and feeding.
- *Lepomis* taxa have not required extended training periods to habituate them to prepared diets. Black, white, and hybrid crappie have been much more difficult to habituate to prepared diets, and they do not feed as aggressively, especially at lower temperatures. This is largely responsible for the poorer overall performance of crappies, as compared to *Lepomis* taxa, under tank culture conditions. There is a need to explore avenues to enhance the response of crappies to prepared diets.

Publications, manuscripts, or papers presented

See the Appendix for a cumulative output for all NCRAC-funded sunfish activities.

Total support for the first two projects

Years	NCRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1990-92	$130,758	$96,710				$96,710	$227,468
1992-94	$149,799	$343,160	$500[a]	$10,000[b]	$4,200[c]	$357,860	$507,659
Total	**$280,557**	**$439,870**	**$500**	**$10,000**	**$4,200**	**$454,570**	**$735,127**

[a] KOCH Industries - Koch Flexrings

[b] National Science Foundation - STARS Research

[c] $3,000 from Kansas Department of Wildlife & Parks - white crappie and hauling tanks and $1,400 from the City of Pittsburg Water Department - anthracite coal

NCRAC has funded three sunfish projects. This project component termination report covers work undertaken for the first and second projects. Bruce L. Tetzlaff chaired the first project and Robert J. Sheehan chaired the second project.

Sunfish

Progress Report for the Period September 1, 1994 to August 31, 1996

NCRAC funding level

$174,999 (September 1, 1994 to August 31, 1996)

Participants

Fred P. Binkowski, University of Wisconsin-Milwaukee, Wisconsin
Paul B. Brown, Purdue University, Indiana
Donald L. Garling, Michigan State University, Michigan
Robert S. Hayward, University of Missouri-Columbia, Missouri
Terrence B. Kayes, University of Nebraska-Lincoln, Nebraska
Christopher C. Kohler, Southern Illinois University-Carbondale, Illinois
Joseph E. Morris, Iowa State University, Iowa
Douglas B. Noltie, University of Missouri-Columbia, Missouri

Extension liaison

Joseph E. Morris, Iowa State University, Iowa

Non-funded collaborators

Denzil Hughes, Farmland Industries, Inc., Kansas
Fountain Bluff Fish Farms, Illinois
Illinois Department of Conservation, Little Grassy Fish Hatchery, Carbondale, Illinois
Jim Frey, Jim Frey Fish Hatchery, West Union, Iowa
Ron Johnson, Spruce Creek Fish Farm, Minnesota
Myron Kloubec, Kloubec Fish Farms, Amana, Iowa
Missouri Department of Conservation, Missouri
Tribal Council, Red Lake Band Chippewa, Wisconsin
National Biological Service, Midwest Science Center (formerly USFWS National Fisheries Contaminant Research Laboratory), Missouri

Project objectives

1. Produce a production manual, accompanying videos and other information as necessary to demonstrate the technology for culturing centrarchids.
2. Determine the major nutritional requirements for centrarchids and to compare their growth and performance using available commercial feeds in laboratory and field settings.
3. Determine the best feeding management strategies for culturing centrarchids in laboratory and field settings.

Anticipated benefits

At the 1993 Program Planning Meeting held in Madison, Wisconsin, the North Central Regional Aquaculture Center (NCRAC) Industry Advisory Council specifically requested the development of extension educational materials in the form of a production manual and accompanying videotapes, as a high priority need for demonstrating the commercial feasibility of centrarchid sunfish aquaculture in the region. Such information is needed to enable this industry to enlarge.

Defining the critical nutritional requirements for targeted sunfish will enable development of diets that meet, but not exceed, their requirements. Feed costs are typically the largest annual variable cost; thus, minimizing nutrient concentrations decreases costs without impairing weight gain or health of individuals. Protein requirements of sunfishes are poorly understood, which hinders their economic potential in food fish culture. Accurate estimates of protein requirements for hybrid sunfish that have sex ratios skewed towards males may prove useful in promoting maximal growth rates as well as minimizing feed costs.

Significant progress has been made with regard to sunfish brood stock development (bluegill and black crappie), spawning, acceptance of prepared diets, and good growth response. Most of the research and commercial production of sunfish has focused on utilizing pond systems (extensive aquaculture). However, to a lesser extent this same effort has been directed at intensive aquaculture. With a better understanding of the early life stage feeding strategies the aquaculture industry will be able to broaden the scope of sunfish aquaculture to include rearing these fish under intensive conditions.

Progress and principal accomplishments

During the 1994-96 period University of Nebraska-Lincoln (UNL) researchers were to produce two 10-20-minute educational video tapes on selected topics covered in the new sunfish production guide. However, due to time constraints at UNL these videotapes are postponed until 1997. Michigan State University (MSU) and ISU personnel have completed drafts of the new sunfish culture guide. The individual chapters will be reviewed during winter 1996; the guide is scheduled for completion by summer 1997.

There have been numerous sunfish hybrids produced by both researchers and private aquaculturists; these hybrids have varying percentages of male offspring and growth rates. The hybrid sunfish used by NCRAC researchers is the F_1 offspring resulting from crossing a female green sunfish (*Lepomis cyanellus*) with a male bluegill (*L. macrochirus*).

At Southern Illinois University-Carbondale (SIUC), researchers used practical diets containing crude protein levels of 32, 36, 40, and 44% and compared their ability to promote growth of hybrid sunfish in two culture systems: recirculating culture system and culture ponds.

Recirculating culture system

Year 1 adult hybrid sunfishes (source: Fountain Bluff Fish Farms, Illinois; mean initial weight = 37.1 g; 1.3 oz) were stocked at a density of 28 fish per 300 L (79.3 gal) circular tank (three replicates per treatment). Flow rates were 30 L/min (7.9 gal/min) and water temperature was maintained at approximately 24°C (75.2°F). Feeding rates were 2%/day divided into two feedings during the 98-day growth trial. Survival ranged from 93 to 100% and did not differ significantly between treatments ($P \leq 0.05$). Weight increase and feed conversion efficiency were highest for the 44% crude protein diet and were significantly greater than the 36 and 32% diets (0.39 versus 0.33 and 0.27, respectively). These data indicate that optimal crude protein levels are likely to be in excess of 40% for hybrid sunfish in recirculating culture systems. The poor feed conversion efficiencies observed may be due to the experimental animals being sexually mature and directing considerable amount of their food intake towards gamete production and reproductive behavior. Proximate analysis of feeds and fish whole bodies is now under way.

Pond culture

Juvenile hybrid sunfish (mean weight = 12 g; 0.04 oz), were stocked (May 23, 1995) at a rate of 5,504 fish/ha (2,228 fish/acre), into 16 ponds averaging 0.04 ha (0.10 acre) (four treatments/four replicates per treatment). Ponds were supplied with one of four practical diet formulations containing crude protein levels of 32, 36, 40, or 44%. Feeding rate was initially 3% of the estimated biomass once a day except on days of sampling. All ponds exhibited nest building activities by June 6 and recruitment of F_2 hybrids in some ponds was apparent by July 18. Feeding rates were reduced to 2% (August 15 through September 26, 1995) when a large amount of feed was noticed left from the previous feedings. This reduction in feeding activity coincided with high temperatures of 30°C (86°F). Resulting data was of limited use due to natural recruitment of F_2 offspring.

Year 1 adult hybrid sunfishes (source: Fountain Bluff Fish Farms, Illinois; mean initial weight = 40 g; 1.41 oz) were stocked April 16, 1996, into 16 ponds averaging 0.04 ha (0.10 acre) (four treatments/four replicates per treatment). Stocking density was 13,875 fish/ha (5,615 fish/acre). All ponds were limed and fertilized 2 weeks prior to stocking to promote plankton blooms. Feeding to apparent satiation was carried out two times per day except during times of rain and strong winds. Aeration to ponds with dissolved oxygen levels of less than 2.0 mg/L was applied with a tractor driven paddlewheel. Harvest is to be carried out October 29, 1996, following a complete draw down.

Researchers at MSU have empirically determined the optimal energy level for growth and protein retention in 125 mm (4.9 in) hybrid sunfish utilizing a saturation kinetics model for curve fitting. Results demonstrate the semi-purified diet developed for these trials is well accepted by these fish; this results in a slightly lower but comparable growth to that obtained using a commercial control diet. There were no significant differences in growth or net protein utilization (NPU) between the experimental diets and the control diet; hence the semi-purified diet is suitable for the remaining phases of these trials.

The whole body indispensable amino acid (IAA) profile of 50 and 125 mm (2.0 and 4.9 in) hybrid sunfish, green sunfish, and bluegill has been determined. The data obtained has been used for predicting the IAA requirements for these species using the A:E ratio ([individual IAA content/total IAA content+Cys+Tyr] (1000)) of whole fish tissue. These predicted IAA requirements will be used in the preparation of diets for the remaining phases.

MSU researchers are currently beginning a trial evaluating growth, NPU, protein retention, and energy retention in 125 mm (4.9 in) hybrid sunfish fed graded levels of protein in isocaloric diets using the optimal energy level predicted in the previous trial. Diets have been formulated to meet IAA requirements for hybrid sunfish determined by researchers at Purdue University (Purdue) with the unknown requirements incorporated at levels predicted by the A/E ratio. This trial will be completed the first of the year; results will be used to predict the optimal P:E ratio for 125 mm (4.9 in) fish.

Research at Purdue was initially focused on quantifying key nutritional requirements of hybrid sunfish. Through three separate studies with the hybrid sunfish, growth was relatively low despite offering a broad variety of diets. Prior to conducting the next series of studies on critical nutritional requirements, an evaluation of pure bluegill was conducted. Growth of pure bluegill was double the growth observed with hybrid bluegill. The studies were conducted in the same experimental systems in the same conditions with the same broad variety of feeds. There was also differential use of commercial diets. Results of those studies clearly indicated that diets formulated for trout and salmon were better than diets formulated for catfish. Further, there were clear distinctions within the trout diets. That is, all trout diets are not the same nor is the response in the hybrid sunfish comparable to the pure bluegill. Both the optimum lipid:carbohydrate ratio and quantitative phosphorus requirements are underway. The optimum lipid study was expanded to include both hybrid sunfish and bluegill. Results will be known by December 1996.

Researchers at the University of Missouri have examined the potential to increase growth rates of hybrid sunfish during growout by using feeding schedules that bring out these fishes' compensatory growth response (increased growth following a period of fasting). Hybrid sunfish were held individually in experimental enclosures submerged in larger water recirculation tanks. Water temperature was maintained at 24°C (75.2°F) as was a 15-h light/9-h dark photoperiod regime. Mealworms (*Tenebrio molitor*) were used as the food in these initial experiments so that daily consumption by individual fish could be accurately determined. Over the 105-day experiment, mean growth rates of hybrid sunfish in the 2 and 14 day no feeding cycle groups were 2.1 and 1.5 times faster than the controls that were fed *ad libitum* every day.

These results represent the first demonstration that fish can be grown significantly larger than daily-fed controls over identical time periods by eliciting the compensatory growth response. Growth improvements from compensatory growth appeared to result from increases in both consumption rate and growth efficiency. While best results were observed for the shortest off/on feeding cycle, there was some suggestion from growth responses that longer off/on cycles (>14 days) may be of value.

The primary goal of the University of Wisconsin-Milwaukee (UW-Milwaukee) researchers was to utilize the early life stage feeding technology developed for yellow perch and apply this approach to centrarchids, specifically, black crappie. The researchers selected two early life stages as their starting points for the development of intensive aquaculture strategies. Young-of-the-year (YOY) Wisconsin pond-raised black crappie (*N* = 1,200) were obtained in fall 1994. Under laboratory conditions these fish accepted adult frozen brine shrimp as a transitional food within 3 days and were habituated to commercial starter feed within 14 days. Survival to present was greater than 65%. In addition, UW-Milwaukee researchers obtained several hundred YOY black crappie from a commercial producer in Iowa. Initially these fish were fed "green tank" water organisms, which included copepods, ostracods, and smaller cladocerans. These organisms are all much larger than those fed to yellow perch at first feeding. Later on, brine shrimp nauplii (BSN) (*Artemia franciscana*) and a beef liver mixture was added to the feeding schedule. This group of black crappies habituated to a formulated starter diet within 26 days. This group of fish (*N* = 73) was terminated on September 25, 1995; mean length and weight was 66.8 mm (2.63 in) and 3.92 g (0.14 oz), respectively.

Since the last report, UW-Milwaukee researchers have continued to expand their efforts to habituate YOY black crappie to formulated diets. Past efforts to spawn adults in the laboratory or to collect wild adults have not been successful. They have continued to maintain the group of YOY black crappies acquired in October 1994 for use as a captive brood stock. These fish were habituated to commercial formulated diet within 14 days of arrival and have been maintained on a rearing regime that is intended to promote gonadal development. It is anticipated that these fish will be fully mature and available for spawning in the spring of 1997.

As a back-up to their efforts to produce YOY from laboratory and wild spawns, UW-Milwaukee researchers obtained 2,741 pond-spawned YOY black crappies (mean length = 26 mm; 1.0 in; mean weight = 0.1-0.5 g; 0.004-0.018 oz) from the Gavin's Point National Fish Hatchery in Yankton, South Dakota. The fish were stocked into a circular flow-through rearing tank and the photoperiod was set at 13-h light. When offered BSN on the day of arrival approximately half the fish accepted the food. Trial feedings with formulated diets on the day of arrival were unsuccessful. These fish took longer to habituate to formulated diet than either the slightly larger YOY brought to the lab in October 1994, those habituated to a formulated diet within 14 days, or the larval crappies tested in July 1995 that habituated to formulated starter diet within 26 days. These results suggest that there is a strong preference for BSN, and that habituation is not readily achieved by merely offering the formulated diet along with the transitional live food. This group of YOY crappie was very reluctant to feed in the presence of observers. Although there was limited interest in formulated foods as early as 6 days after the beginning of the trial, the general population consumed mainly the BSN. Full habituation to formulated diet appeared to closely follow the forced restriction of the live food. Survival during the trial was excellent, 99% over a rearing period of 103 days. UW-Milwaukee researchers intend to continue rearing this group of fish to demonstrate the growth that can be achieved under intensive flow-through culture with formulated diets. Growth information has been obtained at 0 days (26 mm; 1.0 in), 12 days (34 mm; 1.3 in), 57 days (55 mm; 2.2 in), and 105 days (75 mm; 3.0 in) since the start of the trial.

One objective of the ISU researchers was to spawn sunfish out-of-season through temperature and photoperiod manipulation under laboratory settings (bluegill and hybrid sunfish). ISU researchers stocked adult fish at a ratio of two males to four females (170 g; 6.0 oz) per 640-L (169-gal) tanks in a recirculation system. After an acclimation period, temperature and photoperiod were maintained at 24°C (75.2°F) and 14-h light/10-h dark. They were able to spawn bluegills during a 6-month period (December 1994 - May 1995); 40 spawns averaging 20,000 larvae each were obtained from 24 females. Hybrid sunfish were successfully produced the following fall.

The second objective of the ISU study was to develop a procedure for tank-rearing larval bluegill and larval hybrid sunfish. In the first set of experiments, seven commercial diets were used for feeding larval bluegill from the onset of exogenous feeding to 28 days posthatch. Although all diets were consumed by the larvae, none were digested and survival was essentially zero. In the next set of experiments, bluegill larvae were able to digest commercial diets by feeding them BSN for an initial 7-day period and then switching to commercial feed over a 3-day period. Using this protocol, three feeds (Fry Feed Kyowa® B-250, Hatchery Encapsulon® Grade II, and Larval AP-100®) were compared over a 28-day interval. There were no significant ($P \leq 0.05$) differences in growth (length and weight) among the three diets at the end of 28 days, but survival was significantly higher for fish fed Fry Feed Kyowa® B-250. In another experiment, Fry Feed Kyowa® B-250 was fed to larval bluegill after feeding them BSN for 3, 7, or 14 days with an additional 3-day weaning period with mixed feeding. Larvae fed BSN for 14 days had significantly higher growth and survival than did larvae in the 3-day and 7-day treatment groups. In a final experiment, Fry Feed Kyowa® B-250 was fed to larval hybrid sunfish after feeding them brine shrimp for 0, 3, or 7 days with an additional 3-day weaning period of mixed feeding. The larvae fed brine shrimp for only 0 or 3 days initially grew slower than did the larvae in the 7-day treatment; however, by the end of the experiment (28 days posthatch), there were no significant differences among lengths or weights in the three treatments. At 28 days posthatch, larvae fed brine shrimp for 7 days had a significantly higher survival rate than larvae in either the 0- or 3-day treatments. Results indicate that the protocol for tank-rearing larval bluegill and larval hybrid sunfish should include using brine shrimp prior to using a commercial diet. It appeared that larval hybrid sunfish could digest the commercial diet at the onset of exogenous feeding. However, without BSN much lower survival rates resulted. Survival rates of about 25 and 37% can be expected for bluegill and hybrids, respectively, by following this protocol.

Work planned

UNL will produce videos in 1997 related to the upcoming sunfish culture guide. This guide will be competed during 1997.

Critical nutritional requirements for targeted species reduces feed costs and overall cost of production of fishes will continue to be defined by Purdue and MSU researchers. SIUC researchers will compile data from their recirculation and pond studies. These data will be important pieces of information for manufacturers of feed.

UW-Milwaukee researchers will attempt the laboratory spawning of their captive black crappie brood stock by manipulating temperature and photoperiod. If necessary they will use spawning induction substances in spring 1997. If successful, the YOY black crappie produced from this brood stock will be used in the new NCRAC sunfish project. Researchers at ISU will continue to do research into sunfish culture by growing hybrid sunfish up to food-size and to evaluate a sunfish hybrid produced by crossing a female redear sunfish (*L. microlophus*) with a male bluegill.

Impacts

- Coupled with the NCRAC-sponsored development of improved intensive larval sunfish culture techniques at ISU under the direction of Morris, commercial fish farmers have the tools to establish stocks of polyploid sunfishes.
- NCRAC funding permitted SIUC to leverage funding from the American Fishing Tackle Manufacturing Association to evaluate benefits of triploid sunfish in recreational fishing ponds. The supply of triploids to recreational fisheries could provide a new market for regional producers.
- Developing diets specifically for targeted species results in maximum performance at the lowest possible cost. Purdue research directed at minimizing costs of feeds will help to maximize profit to the producer.
- It now appears that the intensive culture technology developed for yellow perch can be applied to black crappie. Also, YOY (30-60 day-old) pond-produced black crappie can habituate to prepared diets within 26 days; YOY (100 day-old) pond-produced black crappie can habituate to prepared diets within 14 days. The potential for the intensive culture of black crappie looks very promising.
- It is now possible to produce bluegills and hybrid sunfish in the laboratory out-of-season by manipulation of temperature and photoperiod

without the use of hormones. This protocol allows for the production of these fish, regardless of season, for both laboratory studies and aquaculture stocking.

- The potential for the intensive culture of black crappie will provide an alternative to seasonal pond rearing and could expand the growth and production to an annual basis in conjunction with recirculating aquaculture system technology.

Publications, manuscripts, or papers presented

See the Appendix for a cumulative output for all NCRAC-funded sunfish activities.

Support

Years	NCRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1994-96	$174,999	$177,300	$12,012[a]			$189,312	$364,311
Total	**$174,999**	**$177,300**	**$12,012**			**$189,312**	**$364,311**

[a]Farmland Industries, Inc.

NCRAC has funded three sunfish projects. This progress report is for the third project which is chaired by Donald L. Garling. The project continues and builds upon the first two projects. The 2-year third project began September 1, 1994.

Salmonids

Project Component Termination Report for the Period June 1, 1990 to August 31, 1996

NCRAC funding level

$79,799 (June 1, 1990 to February 28, 1994)

Participants

Anne R. Kapuscinski, University of Minnesota, Minnesota

James E. Seeb, Southern Illinois University-Carbondale, Illinois

Robert J. Sheehan, Southern Illinois University-Carbondale, Illinois

Extension liaison

Ronald E. Kinnunen, Michigan State University, Michigan

Non-funded collaborator

Hugo Kettula, Seven Pines Trout Hatchery, Lewis, Wisconsin

Reason for termination

The objective for this work on Salmonids was completed.

Project objective

Evaluate all-female diploids and all-female triploids, and use brood stock developed in the region to produce all-female diploid and all-female triploid trout populations.

Principal accomplishments

Efforts culminated in a 265-day grow-out trial at Southern Illinois University-Carbondale (SIUC) in which the performance of all-female triploid, all-female diploid, and mixed-sex diploid rainbow trout were compared. The results of the growout trial vindicated the NCRAC interest in all-female and all-female triploid rainbow trout.

The growout trial was initiated with approximately 100 g (3.53 oz) fish. Progeny from three families of all-female triploid and progeny from three corresponding full-sib families of all-female diploid trout were used in the trial. The mixed-sex diploid trout were progeny of three families that were half-sibs of the corresponding all-female diploid and all-female triploid families. Trout used in the growout trial were from crosses made at the University of Minnesota (UM), where they were also reared to 10 to 20 g (0.35 to 0.71 oz) prior to shipping to SIUC for the growout trial.

A water re-use system and twelve raceways were used in the growout trial, four raceways per treatment. Each raceway was stocked with 25 trout, but stocking densities were reduced to 15 trout per raceway on day 180 of the trial. Mean initial weights were 93.5, 84.2, and 111.6 g (3.30, 2.97, and 3.94 oz) for the mixed-sex diploid, all-female diploid, and all-female triploid, respectively. Mean initial lengths and weights did not differ among the three groups.

Growth was linear during the growout trial. Absolute growth rate was highest for the all-female triploid, intermediate for the all-female diploid, and lowest for the mixed-sex diploid, 2.38, 1.78, and 1.58 g/day (0.08, 0.06, and 0.05 oz/day), respectively ($P \leq 0.025$). Mean final weights were 520.5 g (1.15 lb) for the mixed-sex diploids, 567.5 g (1.25 lb) for the all-female diploids, and 748.9 g (1.65 lb) for the all-female triploids.

No significant differences ($P \leq 0.05$) were found in survival, food conversion ratios, condition factor, liver somatic index, visceral fat weight, or dress-out percentage yield among treatments. By day 180 of the growth trial, most of the males in the mixed-sex diploid group were sexually mature, while the mixed-sex diploid females and the all-female diploids were still maturing. Based on subsamples of trout sacrificed at that time; mean gonadosomatic index for mixed-sex diploid males was 3.13, while values for the mixed-sex diploid females, all-female diploid and all-female triploid trout were 1.13, 1.86, and 0.38, respectively.

All-female diploid and all-female triploid trout show promise for practical trout farming. All-female trout production eliminates the problem of early maturation in males which leads to poor flesh quality and undesirable appearance, and results indicate that all-female diploid trout grow better than mixed-sex diploid trout. All-female triploid trout, however, grew the fastest. Farmers should consider all-female triploid trout production, especially those targeting markets utilizing larger trout.

The all-female triploid trout grew faster than the all-female diploids and mixed-sex diploids in growth trials, but aquaculturists also need to know how triploids perform in other respects to make decisions regarding their production and use. Many culturists produce food fish, but fingerling production for recreational fish stocking programs provides another market outlet for cultured fish. Harvest, crowding, handling, and hauling are problems inherent to fish-farming as well as to fish stocking programs.

Survival of triploids was evaluated during simulated transportation in one experiment with 33 g (1.16 oz) chinook salmon, another with 14 g (0.49 oz) coho salmon, and a third with 1.5 g (0.05 oz) rainbow trout. Triploids were produced via heat shocks. Both diploids and triploids were stocked into replicate containers in each experiment at densities recommended for transporting salmonids. Mortality was recorded every 30 min. Diploids had been exposed to heat shocks in the chinook salmon experiment, but not in the other two experiments.

Triploid chinook salmon died faster than diploids ($P \leq 0.005$). The maximum difference between mortality distributions (D_{max}) was 21.7%. Coho salmon triploids also died faster than diploids ($P \leq 0.005$). D_{max} occurred at 660 to 690 min, when 74% of the triploids were dead but only 47% of the diploids were dead. D_{max} in the rainbow trout experiment was only 6.7% ($P \leq 0.05$), indicating no difference. These results indicate that triploid rainbow trout can tolerate extreme environmental conditions about as well as diploids.

The reduced survival found for triploid chinook and coho salmon indicates that survival may be lower for triploids of these two species under some aquacultural conditions, and survival may also be reduced after stocking. Diminished survival, however, does not necessarily preclude the use of triploids in situations where natural stock protection is an important consideration in stocking programs or in site-selection for an aquaculture installation.

Early growth and survival was also examined for mixed-sex diploid, all-female diploid, and all-female triploid rainbow trout at UM and SIUC during earlier periods of this research. Mixed-sex diploids and all-female diploids early growth and survival was also examined by Seeb at the Fort Richardson State Fish Hatchery, Anchorage, Alaska, under practical fish culture conditions.

Survival through the eyed stage was relatively high (83 to 97%) for mixed-sex diploid, all-female diploid, and all-female triploid eggs in the UM study. Survival through the eyed stage was significantly lower for all-female triploids, in comparison to mixed-sex diploids, but only by approximately 13%. All-female triploids survived as well or better than mixed-sex diploids and all-female diploids after hatching, and growth through 14 weeks did not differ between mixed-sex diploid, all-female diploid, and all-female triploid trout ($P \leq 0.05$).

Cumulative mortality from fertilization through hatching, yolk-sac absorption, and up to 0.5 g (0.02 oz) was about 30% higher ($P \leq 0.05$) for all-female triploids, as compared to mixed-sex diploid and all-female diploid trout in the SIUC trial. Differences in mortality appeared to primarily occur prior to hatching and, secondarily, during yolk-sac absorption. These results, in conjunction with those obtained at UM, suggest that the triploidy induction procedure, the heat shock, was the primary factor responsible for the increased mortality. Mortality did not differ ($P \leq 0.05$) after hatching through growth to 0.5 g (0.02 oz) at SIUC.

A 240-day growth trial initiated with the mixed-sex diploid, all-female diploid, and all-female triploid trout once they reached 0.5 g (0.02 oz) was then conducted at SIUC. There were no mortalities in mixed-sex diploid, all-female diploid, and all-female triploid trout during the 240-day growth trial. This pattern of similar survival after yolk-sac absorption between diploid and triploid rainbow trout was confirmed in the UM study, in the simulated transportation experiment (above) and in the growout trial. The trout grew from 0.5 g (0.02 oz) to approximately 2.7 to 3.0 g (0.10 to 0.11 oz) during the trial. Growth did not differ between mixed-sex diploid, all-female diploid, and all-female triploid trout ($P \leq 0.05$). Feed conversion efficiencies (wet weight of fish/dry weight of feed) did not differ during the 240-day growth trial; they ranged from 93% for the mixed-sex diploids to 99% for the all-female diploids. Feed conversion efficiency was 96% for the all-female triploid trout.

Findings in the UM and SIUC studies show that survival in triploid trout is somewhat diminished prior to the onset of exogenous feeding. However, the economic loss associated with this additional mortality in all-female triploid trout prior to exogenous feeding is minor, since numbers of eggs generally are not limiting in rainbow trout culture, and relatively little investment in rearing costs occurs prior to feeding. The additional production costs associated with this early mortality is more than offset by the better growth during growout. All-female triploid trout do not undergo sexual maturation, so the retention of good flesh quality and appearance is reason enough for producing them.

The Fort Richardson State Fish Hatchery study confirmed no differences in survival between mixed-sex diploid and all-female diploid trout following yolk-sac absorption and through 349 days of age; survival for mixed-sex diploid and all-female diploid trout exceeded 90% during the trial. The growth trial was divided into three phases (83, 148, and 349 days) because numbers of trout per replicate were reduced twice as they grew. Mean weights for the mixed-sex diploid and all-female diploid trout, respectively, were 4.1 g (0.14 oz) and 3.7 g (0.13 oz) at 83 days; 32.4 g (1.14 oz) and 27.8 g (0.98 oz) at 148 days; and 81.0 g (2.86 oz) and 67.8 g (2.39 oz) at 349 days of age. Growth did not statistically differ between mixed-sex diploid and all-female diploid trout. Food conversion efficiency also did not differ between mixed-sex diploid and all-female diploid trout; it ranged from 65.3 to 73.7% during the first 2 phases.

The results of the Fort Richardson State Fish Hatchery study were consistent with findings in the UM and SIUC pre-maturation trials with rainbow trout. Prior to the onset of sexual maturation, mixed-sex diploid and all-female diploid trout differ little in survival and growth.

To determine why all-female diploid and triploid rainbow trout grow faster than mixed-sex trout during growout, two lines of investigation were pursued at SIUC. One investigation examined daily activity patterns and activity intensity in mixed-sex diploid and all-female diploid trout, and the other studied muscle cell growth dynamics in diploid and triploid trout.

Although adult all-female diploid trout showed activity levels higher than mixed-sex diploids at lower water temperatures, the reverse was true at the higher temperatures (above 12.5°C; 54.5°F) at which this species is typically cultured. This means that all-female diploid trout have more dietary energy available for growth at culture temperatures, because they waste less energy on non-essential swimming. Another, and perhaps the most important, reason why all-female diploids outgrow mixed-sex diploids is that rainbow trout males mature and slow their growth earlier than females, due to the investment of energy into gonadal tissues and development of secondary sexual characteristics.

Muscle fiber growth dynamics in triploids is of interest, because whole-body growth occurs via two processes: (1) increased size of muscle fibers or hypertrophy and (2) increased numbers of fibers or hyperplasia. Fish show what has been referred to as indeterminate growth; i.e., they are capable of hyperplastic and hypertrophic growth even after adulthood, whereas postnatal growth occurs only by hypertrophy in other vertebrates. In fish, however, hyperplastic growth eventually ceases, but the longer a species is capable of hyperplastic growth, the larger its ultimate size and the faster its growth.

Muscle fiber growth dynamics were examined in triploid rainbow trout using both biochemical (RNA, DNA, and protein measurements) and histological (muscle fiber diameter sizes) approaches. It is believed that this is the first time that muscle cell growth dynamics has been investigated in any triploid animal.

Triploid trout less than 30 cm (11.8 in) in total length showed muscle fiber size distributions which differed from diploids. Specifically, triploid hyperplastic muscle fibers were larger than those of diploids. However, the difference in fiber size distributions diminished as the trout grew, and it disappeared in larger trout where hyperplasia plays only a small role in growth. This increase in hyperplastic muscle fiber size results in a decrease in the cellular surface area to volume ratio which may be unfavorable to metabolic exchanges between the cell and its external milieu. Poorer survival in triploids during early life may be linked to the increase in hyperplastic muscle fiber size. Another potential disadvantage for triploids is that their muscle cells (which are multinucleate) appear to have fewer nuclei per muscle cell. This study also showed that larger diploid and triploid rainbow trout have similar growth capacities; i.e., they are capable of growing to the same maximal size and at the same rate, all else being equal. This suggests that the superior growth in all-female triploid trout is not due to any inherent differences in growth capacity. Rather, it is probably because triploid female rainbow trout do not direct dietary energy into gonadal growth and the development of secondary sexual characteristics.
SIUC researchers also found that RNA concentrations did not differ between diploids and triploids growing at the same rate and that protein concentrations did not differ in diploid and triploid muscle tissues. This indicates that the rate of protein synthesis does not differ between diploids and triploids, despite the latter having fewer nuclei per cell. This further suggests that genes of the third set of chromosomes in triploids are expressed to compensate for the reduced number of nuclei in triploid muscle cells. Meiotic gynogenesis, followed by sex reversal, is an important initial step in the production of brood stock for all-female rainbow trout production, because it ensures all XX progeny. However, meiotic gynogens exhibit poor viability and growth, because they are highly inbred; a level of inbreeding roughly equivalent to several generations of full-sib matings. Gynogenesis followed by sex-reversal does ensure the production of 100% all-female progeny, but it is inefficient to use gynogenesis for the continuing production of brood stock. A far better approach is to sex-reverse all-female progeny produced from an outcross between an XX sex-reversed male gynogen and a normal XX female, because the outcross eliminates inbreeding depression. The progeny can thus be much more successfully and efficiently raised to sexual maturation.

SIUC researchers shipped about 500, 5 cm (2.0 in) sex-reversed XX males to the Seven Pines Trout Hatchery. Since these were the progeny from an outcross between XX Isle of Mann males and XX Seven Pines females, their viability should be excellent. However, only about 20 pairs of trout were used to produce the 500 progeny, substantially lower than the number of brood stock required to ensure sufficient genetic diversity for aquacultural purposes. Genetic diversity needs to be increased in the XX male brood stock at some future time before it can truly be said that a regional brood stock for all-female production has been established.

Impacts

Studies of all-female diploid rainbow trout demonstrate that:

- all-female diploid trout grow and survive as well as mixed-sex diploid trout during early life;
- declines in flesh quality and appearance due to sexual maturation occur earlier in mixed-sex diploid than all-female diploid trout;
- all-female diploid trout grow faster than mixed-sex diploids through grow out, and survival is similar;
- all-female diploid trout show reduced non-essential activity at culture temperatures above 12.5°C (54.5°F), possibly accounting in part for their better growth as compared to mixed-sex diploid trout.

Studies of all-female triploid rainbow trout showed that:

- all-female triploid trout show somewhat reduced survival through yolk-sac absorption; production of all-female triploids via crosses of tetraploids with diploids may reduce or eliminate this problem;
- survival beyond yolk-sac absorption in all-female triploid trout is similar to mixed-sex diploid trout under normal conditions, and it was also similar under adverse conditions in simulated transportation tests;
- all-female triploid trout showed the anticipated reduced gonadal growth;
- all-female triploid trout were clearly superior to mixed-sex diploids and all-female diploids during grow out through market size;
- studies of muscle cell growth dynamics indicate that there is no inherent difference in the capacity for growth between diploid and triploid rainbow trout; the superior growth in all-female triploid trout appears to be primarily due to their failure to undergo sexual maturation;
- all-female triploid trout production may be the best choice for regional farmers, given their superior growth over mixed-sex diploid and all-female diploid trout;
- all-female triploid trout production appears to be an especially strong option for farmers interested in producing a larger trout, since they grew faster than mixed-sex diploid and all-female diploid trout in these studies, and all-female triploids should not show declines in flesh quality and appearance which accompany sexual maturation in mixed-sex diploid and all-female diploid trout.

Recommended follow-up activities

All-female diploid and triploid rainbow trout show considerable promise for commercial aquaculture, especially in regions where breeding programs have not selected for stocks which mature at a larger size. Female rainbow trout mature at a larger size than males, so all-female diploid production reduces problems such as the declines in flesh quality and appearance prior to market size. All-female diploid trout also grew faster than mixed-sex diploid trout to market size in these studies. Producers interested in producing larger trout should give strong consideration to all-female triploid production, since the problems associated with sexual maturation appear to be forestalled indefinitely, and all-female triploid trout grew the best through growout in these studies.

Cost-effective all-female triploid and all-female diploid production in the NCR will necessitate farmers to develop brood stocks for producing all-female diploid and all-female triploid fry. This will require production of sex-reversed gynogens for all-female production and tetraploid production for crosses with diploids to produce triploids. Field trials which compare all-female triploid, all-female diploid, and mixed-sex diploid trout would enable farmers to determine the best choice for production stocks in commercial aquaculture settings. The following activities are suggested for follow-up:

- further production of sex-reversed gynogen brood stocks,
- production and evaluation of tetraploid brood stocks, and
- production trials for mixed-sex diploid, all-female diploid, and all-female triploid trout in commercial aquaculture settings.

Publications, manuscripts, or papers presented

See the Appendix for a cumulative output for all NCRAC-funded salmonid activities.

Support

Years	NCRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1990-91	$39,299	$22,669		$3,000[a]		$25,669	$64,968
1991-92	$20,500	$13,265		$5,000[b]		$18,265	$38,765
1992-93	$20,000	$14,960				$14,960	$34,960
Total	**$79,799**	**$50,894**		**$8,000**		**$58,894**	**$138,693**

[a]Seven Pines Trout Hatchery for time, use of rearing facilities, feed, and fish

[b]Alaska Fish and Game for time, use of rearing facilities, feed, and fish.

NCRAC has funded three salmonid projects. This project component termination report covers work undertaken for the first and second projects. Both projects were chaired by Paul B. Brown.

Salmonids

Progress Report for the Period September 1, 1994 to August 31, 1996

NCRAC funding level

$200,000 (September 1, 1994 to August 31, 1996)

Participants

Terence B. Barry, University of Wisconsin-Madison, Wisconsin

Paul B. Brown, Purdue University, Indiana

Konrad Dabrowski, Ohio State University, Ohio

Donald L. Garling, Michigan State University, Michigan

Terrence B. Kayes, University of Nebraska-Lincoln, Nebraska

Jeffrey A. Malison, University of Wisconsin-Madison, Wisconsin

Ronald R. Rosati, Illinois State University, Illinois

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Extension liaison

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Non-funded collaborators

Hugo Kettula, Seven Pines Trout Hatchery, Lewis, Wisconsin

T.R. Muench, Purdue University, Indiana

I. Navarro, University of Barcelona, Spain

Nebraska Game & Parks Commission, Calamus State Fish Hatchery, Burwell, Nebraska

Forrest Sawlaw, Archer Daniels Midland, Peoria, Illinois

K. Warner, National Center for Agricultural Utilization ARS, USDA, Peoria, Illinois

M. Randall White, Purdue University, Indiana

Wisconsin Department of Natural Resources, Lake Mills State Fish Hatchery, Wisconsin

Y. Victor Wu, National Center for Agricultural Utilization, ARS, USDA, Peoria, Illinois

Michael Wyatt, Sandhills Aquafarm, Keystone, Nebraska

Project objectives

1. Develop practical rainbow trout diets using regionally available feed ingredients, including fish meal analogs.

a. Evaluate the effects of feed binders in diets formulated from locally available plant ingredients on trout performance and on the stability of trout feces to enhance the removal of solids from hatchery effluents.

b. Evaluate the effectiveness of phytase treatment of plant feed ingredients on phosphorus and protein availability to trout.

c. Develop and evaluate fish meal-free diets using regionally available feed ingredients.

2. Use the stress response as a selection tool for developing strains of trout having improved performance under conditions found in the North Central Region (NCR).
3. Use stress and performance responses in trout to evaluate culture system design and operation under practical conditions.

Anticipated benefits

The development of less-polluting, lower-cost diets from regionally available ingredients will benefit existing aquaculturists facing stricter regulatory pressures to reduce waste nutrients in effluents, as well as new aquaculturists facing increasingly complex permitting processes. Using regionally available plant protein and animal by-product protein sources as substitutes for fish meal in trout diets should reduce the cost of feed manufacture (by reducing both ingredient and transportation costs) and help produce diets that are less polluting. The development of regional trout strains selected for superior growth and stress resistance when reared under the distinctive aquaculture conditions found in the NCR (i.e., relatively small-sized farms, low water flows, and variable water temperatures) will improve the overall production efficiency in both private and public sector facilities. In addition, sources of quality trout eggs from within the region will reduce the region's reliance on imported eggs, and help alleviate concerns about disease transmission. An increased understanding of how rearing density, loading, and water turnover rates influence fish growth, feed conversion, and disease resistance will improve overall production efficiency and help reduce effluent wastes. The improved feeds, fish strains, and rearing methods identified in this study will benefit private fish farmers, public sector hatchery managers, feed manufacturers, aquaculture facility designers, and other user groups.

Progress and principal accomplishments

Objective 1

Investigators at Michigan State University (MSU) conducted research to determine if mineral and protein availability could be improved in plant-based rainbow trout diets by pre-treating dietary soybean meal and/or corn gluten meal with the enzyme phytase. Phytase hydrolyzes phytate, a molecule which binds minerals (such as Zn and P) and proteins in the intestine. Soy-corn gluten meal-based diets were or were not pre-treated with phytase, and were or were not supplemented with 50 ppm zinc. The activities of the digestive proteases carboxypeptidase A (CPA) and B (CPB) were measured in pyloric fecal extracts because the enzymes contain a zinc atom at the active site. Intestinal alkaline phosphatase (ALP) activity was measured because ALP is also a zinc-dependent digestive enzyme and is associated with phosphorus digestion in vertebrates. Plasma was also collected for insulin assay because insulin is stored on a zinc-based crystal in the pancreatic cell. Insulin is important for protein utilization in fish. The feeding phase of the experiment was completed in July 1996. Tissue extracts were tested for CPA and CPB and ALP activities. Whole body, gill filament, and bone samples are currently being prepared for mineral analysis. Plasma samples have been sent to a collaborator, Dr. I. Navarro at the University of Barcelona, for insulin assay. The insulin results should be available by January 1997. Standard assays for CPA and CPB used with other fish species were modified for rainbow trout—the first CPA and CPB assay methods for this species. Preliminary results indicate that diets had no effect on fish moisture content, condition factor (k), or length-weight relationship. The evaluation of growth data was complicated by mortalities during the study; analysis of growth data is not yet available. Mortalities were not diet-related.

Researchers at Ohio State University (OSU) compared the growth rates of rainbow trout fed five different diets in which fish meal protein was replaced by an animal by-product mixture (i.e., replacement of 0, 25, 50, 75, and 100%). No differences were found among the five treatment groups in fish growth, dressing percentage, fillet quality, or gamete quality. Contrary to expectations, however, mineral analysis of fecal samples indicated that diets containing animal by-products were not less-polluting in terms of phosphorus levels.

Purdue University (Purdue) researchers found in the first year of this project that a fish meal-free diet containing soybean meal, corn gluten meal, and corn grain as the predominate ingredients could promote weight gains in rainbow trout within 90% of fish fed a control diet. In the second year, improvements were made in this diet. Lysine was identified as the first-limiting essential amino acid, meat meal was successfully incorporated into the diet, and a combination of canola and fish oils were found to be better than either lipid source alone. A commercial astaxanthin product successfully masked the yellow pigmentation in the muscle of trout. Fish fed any of the fish meal-free diets were preferred by a trained taste panel over fillets from fish fed a commercial diet.

Objective 2

University of Wisconsin-Madison (UW-Madison) investigators identified a physiological measure of stress that was well correlated with growth in rainbow trout—serum cortisol levels 3-hours following an acute handling stressor. Individual fish that consistently showed low 3-hour post-stress cortisol levels (i.e., fish that recovered rapidly from stress, defined as "low" fish) had a mean specific growth rate (SGR) of 0.54, as compared to a mean SGR of 0.41 in unselected fish. Fish with consistently high cortisol levels at 3-hours post-stress and a low SGR ("high" fish) were also identified. Selected fish were to be spawned in the autumn of 1995 and the offspring of selected and non-selected fish compared in terms of growth rates, stress responsiveness, and other indicators of performance. However, not enough selected individuals were available to complete the experiment, primarily because of a problem with tag retention. In December 1995, therefore, 160, 2-year-old fish were obtained from Seven Pines Trout Hatchery to begin a new round of selection. All fish were bled three times over a 6 month period to identify both "low" and "high" individuals. This selection process was much more efficient than that used previously, since only one physiological endpoint (3-hour cortisol level) was measured (compared to the nine endpoints evaluated in the earlier selection process). By September 1996, five female and five male "low" fish, and nine female and five male "high" fish had been identified. Spawning started in early October 1996. Sperm from two selected "low" males chosen at random was used to fertilize eggs from each selected "low" female, and likewise for the "high" fish. Eggs and milt from brood stock chosen randomly from the original Seven Pines population were fertilized in an identical manner to serve as non-selected controls. Five groups of larval fish from each population ("low," "high," and control) will be reared for subsequent performance evaluations.

Objective 3

In 1996, a 10-week production-scale field trail was performed at the Calamus State Fish Hatchery by University of Nebraska-Lincoln (UNL) researchers with help from personnel of the Nebraska Game and Parks Commission comparing the growth, performance, mortality rates, health, and stress responses of rainbow trout in raceways versus oxygen-supplemented cylindrical tanks. Six of the latter were each equipped with a sealed packed column supplied with oxygen, and assigned fingerling trout at a Piper rearing density of 0.45 or 0.9 (three tanks per treatment). Six raceways equipped with conventional packed columns were also each assigned fish at a Piper density index of 0.45 or 0.9 (three raceways per treatment). Turnover rates were kept constant between all four treatment groups. Parameters measured during the course of the study were dissolved oxygen, carbon dioxide, ammonia-nitrogen, pH, total dissolved gas pressure, P, and temperature. At the conclusion of the study, a stress challenge test and Goede health assessment were performed. Blood samples were collected and prepared for analyses of serum cortisol, glucose, and chloride levels.

Work planned

MSU investigators anticipate completing all of their experiments by the end of February 1997. In 1997, UW-Madison investigators will be comparing the growth and performance of selected and control fish reared under identical conditions. The selected brood stock will also be kept and respawned in the fall of 1997 to evaluate the effects of the selection process on gamete quality as well as on subsequent offspring performance. UNL investigators will statistically evaluate the growth, production, Goede health-assessment, and water chemistry data collected during the 1996 field trial. The blood samples collected at the end of this field trial will be analyzed for serum cortisol, glucose, and chloride levels by UW-Madison investigators. The findings of all the Nebraska studies will then be compiled and submitted for publication in a peer-reviewed journal, and as part of a NCRAC project termination report.

Impacts

- Trout diets devoid of fish meal can be used to produce marketable size rainbow trout, with no impact on fish quality. Basal diets can be formulated that use regionally-available feed ingredients. Growout fish fed that diet exhibited feed conversion ratios of 1.0-1.1 (fed to satiation). Ingredient costs of the diet were 15% less than a standard commercial trout diet containing fish meal; price comparisons were based on 5-year average commodity prices. Thus, as fish meal prices rise, alternative diets have been identified that result in similar weight gain of trout.

- The availability of rainbow trout strains with improved growth rate, feed conversion, and disease resistance will greatly improve the production efficiency of private and public fish hatcheries throughout the NCR. The availability of quality trout eggs from within the region will help reduce the need that regional trout farmers currently have for importing eggs from the West Coast. The stress hyperresponsive, slow-growing fish identified in this study have characteristics typical of "wild" trout, and thus may have advantages for stocking recreational fisheries.
- The field trials conducted by UNL investigators, both in the present and past salmonid projects, have verified that rainbow trout can be readily produced under both laboratory and practical rearing conditions at much higher rearing densities than is traditionally recommended. The Nebraska studies have also demonstrated that by using pure oxygen supplementation, trout can be produced in cylindrical tanks at as high a rearing density as in raceways, but at a significantly lower water turnover rate than is normally used in the latter. These findings are particularly important to trout farmers in the NCR who are often constrained by limitations in water and rearing space.

Publications, manuscripts, or papers presented

See the Appendix for a cumulative output for all NCRAC-funded salmonid activities.

Support

Years	NCRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1994-95	$102,042	$103,987		$8,723[a]	$15,000[b]	$127,710	$229,752
1995-96	$97,958	$104,096		$9,867[a]		$113,963	$211,921
Total	**$200,000**	**$208,083**		**$18,590**	**$15,000**	**$241,673**	**$441,673**

[a]University of Wisconsin Sea Grant

[b]International Collaborative Program for OSU to work jointly with the National Fisheries University of Pusan, Korea

NCRAC has funded three salmonid projects. This progress report is for third project which is a 2-year study that began on September 1, 1994. Ronald R. Rosati originally chaired the project until his departure from Illinois State University; after which Terence B. Barry became chair. The third project continues and builds upon the first two projects.

North Central Region Aquaculture Conference

Project Termination Report for the Period June 1, 1990 to March 31, 1992

NCRAC funding level

$7,000 (June 1, 1990 to March 31, 1992)

Participant

Donald L. Garling, Michigan State University, Michigan

Reason for termination

The objective for this project was completed and proceedings of the Conference published.

Project objective

To provide a forum for exchange of information and technology transfer between aquaculturists in the private and public sectors in the North Central Region (NCR) as well as surrounding states and provinces of Canada.

Principal accomplishments

Funds provided for this project were used for the initial costs of planning and advertising the first NCR Aquaculture Conference that was held March 18-21, 1991, in Kalamazoo, Michigan. Approximately 240 people participated in the first NCR Aquaculture Conference which was co-hosted by North Central Regional Aquaculture Center (NCRAC); the Michigan Department of Natural Resources, Fish Division; Illinois Department of Conservation, Fish Division; Michigan Fish Growers' Association; and Michigan Cooperative Extension Service. Proceedings from that conference were published in late 1991. Copies were sent to all registrants and distributed in accordance with the National Coordinating Council on Aquaculture's Publication Policy. Additional copies of the proceedings were made available at cost from the Michigan Department of Natural Resources, Wolf Lake State Fish Hatchery, Mattawan, Michigan.

Impacts

Persons from both the public and private sectors were provided with the most recent information and technologies pertaining to aquaculture in the NCR.

Recommended follow-up activities

Funds provided by NCRAC were to cover up-front expenses for planning and advertising this first regional aquaculture conference. These costs were to be recouped and revenues generated for additional regional conferences that would be held every 2 years thereafter at sites rotating throughout the region.

Publications, manuscripts, or papers presented

See Appendix.

Support

Years	NCRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1990-92	$7,000						$7,000
Total	**$7,000**						**$7,000**

Crayfish

Project Termination Report for the Period, September 1, 1992 to August 31, 1995

NCRAC funding level

$49,677 (September 1, 1992 to June 30, 1995)

Participants

Paul B. Brown, Purdue University, Indiana
Harold E. Klaassen, Kansas State University, Kansas
Robert J. Sheehan, Southern Illinois University-Carbondale, Illinois

Extension liaison

Jeffrey L. Gunderson, University of Minnesota-Duluth, Minnesota

Non-funded collaborators

Carl Richards, University of Minnesota-Duluth, Minnesota
Robert Wilkinson, Southwest Missouri State University, Missouri

Reason for termination

The objectives for this project were completed.

Project objectives

1. Complete a study of the status of the crayfish industry in the North Central States, relative to its extent, culture operations in use, market characteristics, and problems which need to be addressed by research.
2. Complete a report on indigenous crayfish species appropriate for culture in the North Central Region (NCR), to include species life histories, ranges of distribution, economic assessment of appropriate culture production systems, a bibliography of pertinent literature, and a summary of critical information gaps.
3. Conduct preliminary trials evaluating the performance of several promising indigenous species in pond culture.

Principal accomplishments

Objective 1

Within the NCR, 73 crayfish aquaculturists were identified by state extension contacts; they were sent a survey form and asked to respond to a series of questions. Based on the responses, crayfish production in the region appears to be under 10,000 kg (22,046 lb) per year. It is felt that this may be an underestimate of total production as several of the larger producers did not respond despite numerous mailings of the survey. The majority of those who responded (71%) indicated they grew crayfish in polyculture with other finfish. The primary market for crayfish was bait, as a hard-shell product (78% of respondents). Respondents felt there was opportunity for expansion in both the bait (hard- and soft-shell) and human food market. They also indicated that the best return on investment was as tail meat or as a hard-shell bait product. The principal problem areas identified were markets for their products and growth rates of the various species native to the region.

Objective 2

A report on the life history and culture potential of four indigenous crayfish species (*Orconectes immunis*, *O. virilis*, *O. nais*, and *Procambarus acutus*) is nearing completion and will serve as an important source of information for new culturists interested in crayfish aquaculture in the NCR. Information is presented on the life history, biology, distribution, and an assessment of appropriate culture systems. A bibliography and summary of critical information gaps for each of the four species is also included.

Objective 3

Research was conducted at Purdue University (Purdue), Southern Illinois University-Carbondale (SIUC), and Kansas State University (KSU) to evaluate the growth, production, and survival of several indigenous crayfish species in various pond culture systems.

Research at Purdue was designed to compare growth of several of the region's native species of crayfish in side-by-side comparisons. In the first year of the study, *O. virilis*, *O. immunis*, *O. rusticus*, and *P. acutus* were evaluated. In the second year of the project, *O. virilis*, *O. propinquus*, and *O. longidigitus* were compared. *O. virilis* grew better than the other crayfish in both years and their yield was higher than the other crayfish in the first year. However, yield was similar in the second year among all crayfish species evaluated.

Research at SIUC was conducted to compare the growth and production of three species of crayfish (*P. acutus*, *O. virilis*, and *O. immunis*) under polyculture conditions, and compare growth and production of crayfish under two production strategies: (1) artificial destratification/aeration, use of prepared feeds, perpetually filled ponds, and seining (first year) and (2) winter cover-crop production, fall-winter draw down, and harvest via baited traps (second year).

Four ponds (0.06 ha; 0.15 acre) were aerated and four were not in year 1. Each pond was stocked with about 8,340 young-of-the-year (YOY) crayfish. Only *O. immunis* and *O. virilis* were stocked in the first study year. All three species were examined in the second year. Heavy rains in November precluded planting in the fall prior to year 2, so cover-crop ponds were planted with Clark Wheat at a rate of 120 kg seed/ha (107 lb/acre) in April of year 2. The wheat reached a height of 20 cm (7.9 in) prior to flooding. The following were the specific findings over the 2 years of study by SIUC.

- Bottom mean dissolved oxygen (DO) concentrations were significantly lower in non-aerated ponds and in cover-crop ponds than in aerated ponds.
- Bottom temperatures were about 1°C higher on average in aerated versus non-aerated ponds, and the difference was significant.
- Average daily weight gain was significantly higher in aerated versus non-aerated ponds and cover-crop ponds.
- *P. acutus* grew faster than the other two species and *O. virilis* grew significantly faster than *O. immunis*. YOY crayfish began reaching harvestable size (70 mm total length [TL]; 2.76 in) in appreciable numbers by July.
- Mean weights were significantly greater in aerated (15.9 g; 0.56 oz) versus non-aerated ponds (11.8 g; 0.42 oz).
- Harvest from the cover-crop ponds was extremely low (8 kg/ha on average; 7.1 lb/acre) versus the aerated ponds (221 kg/ha on average; 197.2 lb/acre).
- *O. nais* appears to be a subpopulation of *O. virilis*, rather than a true species, based on starch-gel electrophoresis.
- The percent edible tail meat was higher for *O. immunis* (21.9%) than for *P. acutus* (19.3%), *O. virilis* (19.6%) and a sample of *P. clarkii* (16.1%) that had been obtained.

At KSU growth, survival, and harvest of the crayfish *O. nais* were evaluated in three 0.20 ha (0.5 acre) farm ponds. The water quality of these ponds varied considerably but was typical of many Kansas farm ponds.

Each pond was to be stocked in mid-summer with a low density (3/m^2; 0.3/ft^2) of YOY crayfish to allow for maximum growth rate. Ponds were not fed or aerated. Growth, survival (both summer and winter), and harvest were evaluated through two growing cycles, 1993-94 and 1994-95. Prior to stocking, the ponds were to be poisoned to remove existing crayfish. Due to unusually wet weather during 1993, only one pond was poisoned and stocked at the low density. The other two ponds were intensively seined and trapped; crayfish that remained in the pond were used for the study. During 1994-95 all three ponds were poisoned and stocked as proposed.

Edible size crayfish

The size of crayfish considered edible or the minimum marketable size varied somewhat among the three research groups. Crayfish were judged to be edible size in the KSU study if they were larger than 38 mm (1.5 in) carapace length (CL) (approximately 76 mm TL; 3.0 in). This is somewhat larger than the 70 mm (2.8 in) TL (approximately 11-12 g or 38-41 crayfish/lb) judged as edible size in the SIUC research. Crayfish exceeding 47 mm (1.9 in) CL (approximately 94 mm TL; 3.7 in) were designated jumbo size in the Kansas State study. Crayfish weighing 15-18 g (approximately 25 to 30 crayfish/lb) were considered minimum marketable size in the Purdue study.

Crayfish did not generally reach edible size during their first growing season, but attained edible and jumbo size by the following June. At the end of the growing season (both years), average CL of YOY crayfish varied significantly among ponds and ranged from 23 to 41 mm (0.9 to 1.6 in).

During June of 1994 and 1995 all three ponds were intensively trapped with minnow traps over a 2-week period. The catch was high at first, but fell off rapidly. Generally, after ten trap nights at least 90% of the harvested crayfish had already been captured. Ponds with larger crayfish trapped out more quickly. The weather was cool during the beginning of June 1995 and trapping success was low, but success increased rapidly as water temperatures warmed. Size of yearling crayfish harvested in June varied from pond to pond and year to year and ranged from 8% edible size (no jumbo) to 100% edible size (87% jumbo).

Crayfish survival was variable. Summer survival (stocking time to fall) ranged from 12% to 78%. Winter survival (fall to spring harvest) ranged from 3% to 55%. Winter survival was consistently lower than summer survival.

Impacts

- The crayfish producer survey was the first attempt at defining the status of crayfish aquaculture in the NCR, the potential for expansion, and the current crayfish culture problems/impediments.
- A manuscript is being written that succinctly summarizes the biological characteristics and examines the aquaculture potential of four native crayfish species. The document will be a valuable tool for aquaculturists and extension personnel in the region.
- Growth of several species was compared and *O. virilis* appears to be the best of those studied when reared in pond monoculture.
- Several species grew to minimum marketable size for human consumption in one growing season and many attained jumbo size by the following June.
- Aeration improves growth and production in crayfish ponds, but providing a cover crop did not.
- All three species evaluated at SIUC have their advantages: *P. acutus* reaches harvestable size early in the production season, *O. virilis* exhibited good growth and survival, and *O. immunis* had the highest percentage of edible tail meat.
- YOY crayfish can be successfully stocked.
- Most of the marketable-size crayfish can be harvested from small ponds within 2 weeks.
- Survival is quite variable and dependent on weather and pond conditions. Winter is a critical period. Aeration would improve survival.

Recommended follow-up activities

The two primary problems identified by current aquaculturists raising crayfish in the region were market assessment and development and crayfish growth rates. Marketing studies for freshwater crustaceans are lacking and are needed for developing business plans. Numerous factors can effect growth and virtually all need to be explored with crayfish. Crayfish exhibit density-dependent growth and survival, that is, as density increases, growth and survival decrease. This happens with various species of fish as well, but is usually solved by in-depth studies. There is also a need for a biocide registered for use on crayfish which would allow for more active management of production ponds.

Publications, manuscripts, or papers presented

See Appendix.

Support

Years	NCRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1990-95					$61,960[a]	$61,960	$61,960
1992-95	$49,677	$58,049				$58,049	$107,726
Total	**$49,677**	**$58,049**			**$61,960**	**$120,009**	**$169,686**

State of Indiana, Business Modernization and Technology Center, through the Purdue University New Crops Center

This project was chaired by Paul B. Brown.

National Aquaculture Extension Workshop

Project Termination Report for the Period, October 1, 1991 to September 30, 1992

NCRAC funding level

$3,005 (October 1, 1991 to September 30, 1992)

Participants

Carole R. Engle, University of Arkansas at Pine Bluff, Arkansas
James T. Davis, Texas A&M University, Texas

Collaborators

Donald W. Webster, University of Maryland, Maryland
Joseph E. Morris, Iowa State University, Iowa
Fred Conte, University of California Davis, California
Paul Olin, University of Hawaii, Hawaii

Reasons for termination

The objectives for this project were completed.

Project objectives

1. Improve Extension's information and delivery system for aquaculture in concert with industry areas of high priority.
2. Enhance the development of new state Extension educational programs in aquaculture by highlighting innovative programs.
3. Examine the scope of Extension efforts in aquaculture at the state, regional, national, and international levels.
4. Evaluate the role and effectiveness of Extension activities within the USDA Regional Aquaculture Centers (RACs) program and pursue actions of enhancement.
5. Identify opportunities and needs to enhance the development of regional and interregional Extension programs in aquaculture.
6. Strengthen communication networks and exchanges of materials, programs, and expertise among aquaculture Extension staff.
7. Examine team building and multidisciplinary approaches to Extension programming in aquaculture.
8. Address national and regional issues impacting educational programs and state and federal legislation.
9. Examine major trends in commercial aquaculture and emerging opportunities.
10. Explore use of new communication technology and innovative reporting techniques.

Principal accomplishments

Specialized training was provided for over 90 extension scientists from at least 43 states during the National Extension Aquaculture Workshop held March 3-7, 1992, at the 4-H Center in Ferndale, Arkansas. Featured were speakers from many agencies and organizations that impact on aquaculture production, marketing, and federal policy in the United States.

This training was jointly sponsored by the five RACs and the states involved. In addition to speakers from within the group, representatives from several universities, the U.S. Departments of Agriculture, Commerce, and Interior, U.S. Army Corps of Engineers, and the Food and Drug Administration presented papers and led discussions. "Networking with other Professionals," "Use of Therapeutants," "Aquaculture Waste Management," and "The National Aquaculture Information Center" occupied participants on the first day of the workshop. There was also time for other discussion groups and visits to a very well stocked and maintained media center. Many participants got an opportunity to discuss points of view with experienced extension scientists throughout the workshop. In addition, they were exposed to many resource materials of immediate use as well as becoming acquainted with other extension specialists to contact for information when it is needed.

On the second day, the discussion switched to "Fish and Shellfish Inspections," "Marketing and Processing," "Production and Marketing Economics," and an excellent panel discussion on "You and the Law." Later a discussion of how to interface with extension efforts of the Fish and Wildlife Service alerted many of the participants to the possibilities of funds available and other joint efforts. Though this day was concerned with economics rather than biology, the learning opportunities were tremendous. “Time Management,” which is a concern for all professionals, led off the discussion on the workshop’s third day. This session was followed by a discussion of “Environmental Impacts, Endangered Species, and Non-tidal Wetlands.” Facility design covered ponds, raceways, and recirculating systems in a manner that all non-engineers could easily follow. Next on the agenda was a session on “Improved Communications” with discussions ranging from electronic information networks to teleconferencing. Guidance on the use and misuse of these systems was particularly enlightening.

The final day was spent in group discussions on how the RACs can better serve their clientele, followed by a tour of two premier aquaculture installations. One of these, Anderson Minnow Farms, is reputed to be the largest such installation in the world. The other, Keo Farms, allowed many to see first hand some of the complexities of commercial spawning and rearing of hybrid striped bass and triploid grass carp. Over 60 of the participants stayed for an additional day to receive in-depth training in production and marketing of either hybrid striped bass or bait minnows. These sessions featured speakers from the commercial sector as well as researchers and extension specialists.

Evaluations from the participants were tabulated, and the workshop was rated 4 on a scale of 1 to 5. Two objectives of the workshop were for participants to receive training in current subject matter and to understand new information transfer capabilities. These received very high evaluation scores, as did an improved understanding of national initiatives and guidance in enhancing coordination with other agencies. When asked, “What did you get from the program?”, 41 of 42 respondents indicated that they got the names of other people to contact for help in difficult situations, and 40 said they received new, usable resource material. Over one-half stated that they received answers to questions, ideas that they could try immediately, and most indicated a better understanding of RAC programs. As this was the first of these ever held, these indicate that the participants received excellent training as well as taking part in a varied and intensive program.

Impacts

The total effect of this workshop will not be realized for 5 to 10 years. At this stage it is known that there has been increased cooperation across regional lines in planning of projects and better communication between specialists. Knowing aquaculture specialists in other parts of the United States has enabled many specialists to secure assistance with specialized problems.

Recommended follow-up activities

Participants rated the need for future workshops at 4.6 out of a possible 5.0 after considering the time and travel costs involved, and most preferred 2 or 3 years between workshops. A few of the participants commented that the program was too intense and too long, but even these indicated that if it was shortened and the format modified they would recommend the workshop to others.

Publications, manuscripts, or papers presented

See Appendix.

Support

Years	NCRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1991-92	$3,005				$12,020[a]	$12,020	$15,025
Total	**$3,005**				**$12,020**	**$12,020**	**$15,025**

[a]Each of the four other Regional Aquaculture Centers contributed $3,005 for the workshop.

Baitfish

Project Termination Report for the Period September 1, 1992 to September 30, 1995

NCRAC funding level

$61,973 (September 1, 1992 to September 30, 1995)

Participants

Frederick A. Copes, University of Wisconsin-Stevens Point, Wisconsin
Daniel W. Coble, University of Wisconsin-Stevens Point, Wisconsin
Leroy J. Hushak, Ohio State University, Ohio
Extension liaisons
Daniel A. Selock, Southern Illinois University-Carbondale, Illinois
Joseph E. Morris, Iowa State University, Iowa
Non-Funded Collaborators
Charles Berry, Jr, South Dakota Coop. Fishery & Wildlife Unit, South Dakota
Carl Gollon, Gollon Brothers Fish Farm, Wisconsin
Dirk Peterson, Minnesota Department of Natural Resources, Minnesota
Charles Rabeni, Missouri Cooperative Fishery & Wildlife Unit, Missouri

Reasons for termination

The objectives for this project were completed.

Project objectives

1. Conduct a comprehensive survey of the status of the baitfish industry in selected North Central states to determine: (a) species used; (b) sizes of species marketed; (c) sources of species; (d) seasonal availability; (e) shortfalls in supplies; (f) relative value of various fish and nonfish species; and (g) common problems of the industry that may need to be addressed by research.
2. Estimate the costs of culturing bait species commonly used in the North Central Region (NCR) in selected types of production facilities, e.g., extensive and intensive pond culture, tanks, and raceways.
3. Estimate the economic contribution (output, employment, and income) generated by the bait industry to selected state economies.
4. Assemble a list of rules and regulations for each state affecting the baitfish culture industry.
5. As time permits, summarize biological life cycle information for several underused or unused species that have culture potential and which may match needs of the regional industry.

Principal accomplishments

Objective 1

The many species and sizes used were identified; the most important baitfish was the fathead minnow and non-fish bait was the night crawler. About two-thirds of baitfish were harvested from the wild; the rest were cultured. Non-fish bait was about 50:50 wild vs. cultured. Availability varied seasonally and shortages were identified. Values of various fish and non-fish baits were estimated; baitfish composed 64%, and non-fish bait, 36% of the estimated value of bait. Bait mortality was a problem for 50% or more of wholesalers and retailers. Better temperature control and handling and transport would probably reduce mortality.

Objective 2

The 107 respondents who reported growing baitfish on the 1990 survey of fish growers in the Economics and Marketing Project were resurveyed about their baitfish enterprises, the sales of baitfish, and the costs of producing those baitfish during 1993. After three mailings and numerous follow-up telephone calls, a total of 33 surveys were completed, of which only 10 were useable. The remaining respondents were no longer in the baitfish business or handled only wild-caught species. Even for the 10 useable responses, the data provided was not sufficient for detailed budget analysis. Four of 10 reported sales of less than $10,000 during 1993 while only one reported baitfish sales in excess of $40,000. Nearly all operations could reach break even, i.e., cover their reported costs, within the sales class they reported.

Objective 3
The value of the industry was estimated in six state economies. For all six combined, the total minimum estimated value in 1992 was about $165 million for baitfish and $92 million for non-fish bait.

Objective 4
A list of rules and regulations affecting the bait industry was assembled for the 12 states in the NCR.

Objective 5
Several species of important baitfish that are harvested from the wild were identified for investigation of potential for fish culture. At least one, the emerald shiner, will be studied with funding from another source.

Impacts

- Identification of the most important baitfish species, fathead minnows, and non-bait, night crawlers, was accomplished. Protection and research may be needed in the future.
- Identification of supply shortages indicates species for which increasing the supply would aid the industry. Baits commonly in short supply include: fathead minnows, lake shiners, golden shiners, night crawlers, leeches, and crayfish.
- Identification of disease and handling problems indicates areas for fruitful future research and extension efforts.
- Estimates of economic value, $165 million for baitfish and $92 million for non-fish bait for six states, emphasize the importance of the industry to those state economies.
- Inconsistent state regulations identified as problematic to the industry.
- Study has generated more than 50 inquiries on baitfish culture and markets.
- Aquaculture shortcourse offered, March 1993.
- Copes served as moderator of afternoon session of the Governor's Conference on Agriculture: Wisconsin Aquaculture 1994, University of Wisconsin-Stevens Point, February 1994.

Recommended follow-up activities

- Study problems identified by the survey respondents and increase extension educational information on proper bait handling procedures.
- Investigate culture methods for important non-propagated species and potentially valuable unused species.
- Study ways to alleviate supply shortages.

Publications, manuscripts, or papers presented

See Appendix.

Support

Years	NCRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1992-95	$61,973	$44,482	$2,000[a]	$16,132[b]		$62,614	$124,587
Total	**$61,973**	**$44,482**	**$2,000**	**$16,132**		**$62,614**	**$124,587**

[a]Various bait dealers

[b]U.S. Fish and Wildlife Service and National Biological Service (Wisconsin Cooperative Fishery Research Unit)

This project was chaired by Daniel W. Coble.

Wastes/Effluents

Project Termination Report for the Period September 1, 1992 to August 31, 1996

NCRAC funding level

$153,300 (September 1, 1992 to February 29, 1996)

Participants

Fred P. Binkowski, University of Wisconsin-Milwaukee, Wisconsin
James M. Ebeling, Ohio State University, Ohio
Konrad Dabrowski, Ohio State University, Ohio
Reginald D. Henry, Illinois State University, Illinois
Kyle D. Hoagland, University of Nebraska-Lincoln, Nebraska
Terrence B. Kayes, University of Nebraska-Lincoln, Nebraska
Joseph E. Morris, Iowa State University, Nebraska
Ronald R. Rosati, Illinois State University, Iowa

Extension liaison

LaDon Swann, Purdue University, Illinois/Indiana

Non-funded collaborators

John Hyink, Glacier Springs Trout Hatchery/Alpine Farms, Wisconsin
Iowa Department of Natural Resources (DNR), Fairport State Fish Hatchery, Iowa
Iowa DNR, Rathbun State Fish Hatchery, Iowa
Myron Kloubec, Kloubec's Fish Farm, Iowa
Bill Johnson, Rushing Waters Fisheries, Wisconsin
Dave Smith, Freshwater Farms of Ohio, Inc., Ohio
John Wolf, Glacier Springs Trout Hatchery/Alpine Farms, Wisconsin
Michael Wyatt, Sandhills Aquafarm, Nebraska

Reason for termination

The objectives for this project were completed.

Project objectives

1. Characterize aquaculture effluents from four types of aquaculture production systems: pond culture, flow through culture (raceway), cage culture, and recirculating systems.
2. Generate a data base from these four types of production systems to help promote a reasonable choice of effluent discharge regulations by government agencies.

Principal accomplishments

Objective 1

Pond production systems

Fairport State Fish Hatchery, Iowa

Water quality was monitored in four culture ponds stocked with channel catfish (*Ictalurus punctatus),* fingerlings at Fairport State Fish Hatchery near Muscatine, Iowa during 1993. Data were collected during the culture season and at harvest to analyze pond and effluent water quality. During the course of the growing season, water temperature, nitrates, and total suspended solids levels decreased while dissolved oxygen (DO), ammonia, un-ionized ammonia, and 5-day carbonaceous (organic) biological oxygen demand ($CBOD_5$) increased.

Analysis of data collected at harvest revealed that total phosphorus and total solid levels increased substantially in the pond effluents compared to those within the ponds. Towards the latter stages of fish harvest, $CBOD_5$ levels significantly increased within the ponds; effluent quality significantly deteriorated, having increased levels of total phosphorus, total nitrogen, $CBOD_5$, total solids, and total suspended solids. Fish biomass was a positive influence on $CBOD_5$.

Kloubec Fish Farm, Iowa

Samples were obtained from Kloubec's channel catfish and hybrid sunfish ponds in early and late October 1993. During the sampling period, two ponds had elevated levels of nitrites and three ponds had elevated levels of nitrates compared to earlier sampling periods. However, $CBOD_5$ levels decreased in all ponds during this sampling period. The two ponds with the highest levels of $CBOD_5$ at this time had been harvested the previous month. The act of seining probably resulted in direct increase in $CBOD_5$ levels compared to those ponds that had not been harvested.

Ponds at Kloubec's Fish Farm had higher $CBOD_5$ levels than did the flow-through situation at the Rathbun State Fish Hatchery, but were similar to pond levels at the Fairport State Fish Hatchery. However, the nitrogenous compounds levels were low. The two ponds with the highest feed levels had the highest $CBOD_5$ levels.

Raceway (flow-through) production systems

Rathbun State Fish Hatchery, Iowa

The effects of a flow-through aquaculture facility, Rathbun Fish Hatchery, Iowa, were assessed in 1993. Significant differences ($P \leq 0.10$) were determined in both water quality and invertebrate parameters at six sample sites. Sites closest to the culture facility had elevated levels of several nitrogenous and phosphorus compounds compared to sites at the water intake and Chariton River. Main production factors influencing water quality parameters at sites were those taking place within the main hatchery building (flow, fish biomass, feed quantity, and quality). Invertebrate groups, both zooplankton and other macroinvertebrates, did not differ between the upstream and down-river stations.

The overall conclusion concerning this data set is that the effects of aquaculture effluents from this hatchery are minimal at best on both chemical and biological factors. High flows resulting from flood conditions caused increased dilution of aquacultural effluents. The 1993 field season had the worst flooding in the state's history. Thus, data collected during this period may not be representative of a typical year where some hatchery effects may have been seen under more normal conditions.

Sandhills Aquafarm, Nebraska

The goal of the University of Nebraska-Lincoln (UNL) research was to monitor key water quality parameters above and below Sandhills Aquafarm, a modern trout production facility on Whitetail Creek in Western Nebraska. Whitetail Creek is a spring-fed, first order stream with relatively constant flow and good water quality. Sandhills Aquafarm consists of 12, 2.4- 33.5 m (8-100 ft) raceways with total flows of 23.5 m^3/min (6,200 gpm) and annual production rates of rainbow trout of 77,100 kg/year (170,000 lb/year). Four sites were established above the facility and four below to obtain reliable, representative physicochemical measurements and water samples for laboratory analyses.

It was clear that several water quality parameters continued to differ consistently above versus below the aquaculture facility, particularly DO, pH, ammonium-nitrogen, total nitrogen, orthophosphate, and phosphorus. Total suspended solids and turbidity showed no consistent trends. While temperature and biochemical oxygen demand (BOD) appeared to exhibit relatively little difference above and below the facility (although even these differences were consistent), downstream decreases in DO and pH, and increases in ammonium-nitrogen, total nitrogen, total phosphorus, and orthophosphate were evident. These data clearly indicate that water quality was altered downstream from the facility in both 1993 and 1994.

Rushing Waters Fisheries, Wisconsin

Rushing Waters Fisheries is one of the most productive commercial rainbow trout hatcheries in Wisconsin. It has earthen raceways and ponds with a total flow approximately half that of the Nebraska Sandhills operation. As such, it is representative of the more typically-sized private trout production facilities in the North Central Region (NCR). This facility is supplied by groundwater wells and springs of moderate conductivity (between 400-600 mols) and is located at the head of a small creek that is a tributary to Blue Springs Creek in Jefferson County, Wisconsin.

Alterations in water quality occurred in the effluents of the three chains of raceways as compared to the source waters entered at the head of each raceway chain, and the water quality of the combined effluent in the creek leaving the property. Increases in BOD, total suspended solids, total ammonia nitrogen, nitrite-nitrogen, soluble reactive phosphorus, and total phosphorus were evident. Under typical production conditions these changes were slight, but on at least one occasion raceway cleaning activities created more elevated conditions of total suspended solids and total phosphorus in the creek leaving the property.

The effluents from the earthen production raceways had slightly lowered levels of nitrate-nitrogen compared to the source water. It seems reasonable that the natural primary and secondary productivity in the earthen bottomed rearing units would utilize nitrate. Dissolved oxygen levels in the groundwater well sources tended to be slightly lower than in the effluents from the raceways. Use of aerating devices in the rearing units kept DO levels high, and the level in the newly pumped well water probably had not yet had enough contact with the atmosphere to reach full saturation before sampling. Source water samples were taken from an open reservoir rather than from groundwater wells, and water from this reservoir had slightly higher levels of solids, ammonia, and phosphorus than the well water sources. This difference was slight, however, in comparison to the general differences between the source waters and the effluents.

Alpine Farms, Wisconsin

Tank rearing of yellow perch and whitefish at Alpine Farms, Sheboygan Falls, Wisconsin was investigated to characterize the effluents produced by alternative regional aquaculture species. Yellow perch and whitefish tank effluents produced changes in water quality parameters similar in direction and magnitude to those of the other flow through rearing situations. Differences appeared to be controlled by production conditions (water exchange, loading, and ration level) rather than by the species reared.

Glacier Springs Hatchery, Wisconsin

The intended opportunity to examine the changes in effluent water quality during the renovation of a former hatchery that had been inactive for over a decade did not materialize during the project period, due to changes in the owner's plans. A representative set of before renovation water quality data was gathered, which would be suitable for comparison if future renovation occurs. Emphasis was shifted to the Rushing Waters Hatchery study when plans for renovation were delayed.

Cage culture production systems

Trout culture

Freshwater Farms of Ohio's trout cage culture facility is located near Urbana, Ohio in an abandoned quarry. The site consists of four separate quarry lakes, two of which discharge into a third, which together with the fourth, discharge into the Mad River. A total of 10 sampling sites were monitored, including spring inflow into two lakes, the cage culture site at two depths, the discharge from the production lake into a settling lake, the discharge into the Mad River from the settling pond, discharge from an unused lake into the Mad River, and the Mad River upstream and downstream from the discharges.

For all measured parameters, there were no significant differences. There was no negative impact of Freshwater Farms of Ohio's trout cage culture operation on the water quality of the quarry lakes or the receiving water of the discharge. In fact, in most cases significant improvement occurred due to the diluting effect of the quarry discharges.

Channel catfish culture

The Piketon Research and Extension Center (PREC-OSU) has a small demonstration cage culture operation in a 1.8 ha (4.4 acre) reservoir located at the facility. The cage culture operation reflects what a small farmer could easily build in a farm pond for the production of channel catfish and for trout grow out in the winter months. The system was lightly stocked over the spring months with trout and yellow perch fingerlings and then heavily stocked with channel catfish (850 kg; 1,874 lb) in mid-summer.

The impact of the small scale cage catfish cage culture operation at PREC-OSU is not easily characterized due to the input from the Center's wastewater treatment plant. Still most water quality parameters were typical of catfish production ponds.

Recirculating systems

The facilities studied at Illinois State University included two recirculating aquaculture systems (RAS) stocked with Nile tilapia (*Oreochromis niloticus*). The first system consisted of a 18,927 L (5,000 gal) culture tank, a settling tank particle filter, a vertical screen submerged media biofilter, and an oxygen column. The second system consisted of a 170,343 L (45,000 gal) culture system, a drum microscreen particle filter, a submerged media biofilter, and oxygen columns. The second system is similar in design, although smaller in scale, when compared to operating commercial systems found in the private sector. The second system was producing 226.8-453.6 kg (500-1,000 lb) of live tilapia each week during the time of these trials.

Data collected on two different RASs demonstrate that RAS effluents contain elevated levels of total solids, settleable matter, BOD, forms of nitrogen (excluding non-ionized ammonia), phosphorus, and reduced concentrations of DO, which agree with previous RAS studies.

Objective 2

The combined data sets from these investigations have been tabulated and attached, along with an extensive bibliography concerning aquaculture effluents, as appendices to the Project Termination Report Part II.

This data set and references provide a single source overview of effluent water quality from representative regional aquaculture production facilities.

Impacts

Data from the recirculating system study has already been used by a private sector aquaculturist in developing a new large recirculating system that meets USEPA compliance. It is anticipated that as this database is made available to the industry, many more actual applications will occur. Aquaculturists can use this data to take a proactive stance in helping environmental regulatory agencies compose practical aquaculture discharge regulations. Aquaculturists may also use the data collected as baseline values in research to determine the efficiency of newer effluent treatments and best management practices (BMPs) designed to reduce the impact of effluent discharges. Practices that alleviate effluent problems may result in more efficient operation of the hatchery facility and economic savings, and also decrease environmental impacts. This information will help protect the quality of water resources and may alleviate the fears of the general public as to the perceived polluting aspects of aquaculture systems.

Recommended follow-up activities

This work has measured the levels of solids produced by representative regional facilities as settleable solids, total suspended solids, and dried solids. The more dramatic changes in water quality from aquaculture operations are associated with clean out events for removal of settled aquaculture waste materials. Solid wastes are most easily removed from culture systems by conventional water treatment processes, while nutrients, once they become dissolved, require treatment technologies that are cost prohibitive. Strategies to improve commercial fish foods from the perspective of waste management need to be evaluated. Better understanding of the influence of commercial diet formulations on the integrity of fecal solids and the consequent impact on nutrient release, holds promise of reducing the release of phosphorus into aquaculture effluents. This is one of the aspects of greatest regulatory concern due to its potential role in the eutrophication of receiving waters. The addition of fiber to the diet can potentially influence fecal durability in water and permit easier removal. The practicality of this strategy needs to be investigated and demonstrated.

In recirculating aquaculture systems the size distribution of suspended solids particles shifts to smaller sized particles that are the most difficult to remove, and more information is needed on the impact of such particulate solid matter on fish health and the performance of recirculating systems. Recycle systems also produce expectedly more concentrated waste, but they also permit more opportunities for innovative reuse or disposal. Efficient solids removal and management provides an avenue for the utilization of waste by-products as potentially beneficial resources in the context of integrated resource management plans.

Wise resource management calls for finding beneficial use for these concentrated aquaculture by-products. These types of practices can promote aquaculture as a beneficial or at least a benign influence on water resources when good stewardship is practiced. In this regard, comparison of real-life aquaculture production situations to other common land and water use practices will be helpful in arguing for realistic and just regulatory and permit situations. Comparing aquaculture waste production within the context of other contemporary land use practices with regard to impact on regional water quality, should be useful for demonstrating that current and prospective regional aquaculture is relatively benign environmentally.

Using the results of this project, a report needs to be prepared that contrasts the potential impact of regional aquaculture development with other contemporary agricultural, municipal, industrial, and natural resource land uses. There is a need for this information to be organized into an easily understood format so that normal aquaculture operating conditions can be viewed against the existing general background of water quality fluctuation and environmental impact. This report should also review and evaluate current research on alternative beneficial uses of aquaculture by-products, emphasizing integrated resource management and aimed at developing sustainable aquacultural practices. Options to be examined should include constructed wetlands, irrigation uses, hydroponics, and even biogas production strategies. Often the situations and examples of such practices, as presented in the existing literature, deal with species, climates, and situations, which may or may not be applicable to the environmental and regulatory situations in our region.

There is a need to review and present this information in relation to its relevance and application to aquaculture in the NCR.

Publications and papers presented

See Appendix.

Support

Years	NCRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1992-93	$77,064	$54,427	$15,000[a]			$69,427	$146,491
1993-94	$76,236	$43,261	$20,000[a]		$10,000[b]	$73,261	$149,497
Total	**$153,300**	**$97,688**	**$35,000**		**$10,000**	**$142,688**	**$295,988**

[a]Glacial Springs Hatcheries
[b]Archer Daniels Midland

This project was chaired by Fred P. Binkowski.

National Aquaculture INAD/NADA Coordinator

Progress Report for the Period September 1, 1992 to August 31, 1996

NCRAC funding level

$17,000 (September 1, 1993 to May 14, 1997)

Participants

Ted R. Batterson, Michigan State University, Michigan
Henry S. Parker, USDA/CSREES/PAPPP, Washington, DC
Robert K. Ringer, Michigan State University, Michigan
Rosalie A. Schnick, Michigan State University, Wisconsin

Project objectives

1. Ensure effective communications among groups involved with Investigational New Animal Drug/ New Animal Drug Applications (INADs/NADAs), including Canada.
2. Serve as an information conduit between INAD/ NADA applicants and the U.S. Food and Drug Administration's Center for Veterinary Medicine (CVM).
3. Identify and encourage prospective INAD participants to become involved in specific investigational studies and NADA approval-related research.
4. Seek the support and participation of pharmaceutical sponsors for INAD studies and NADAs and coordinate with INAD/NADA sponsors to achieve CVM approval more quickly.
5. Guide prospective and current INAD holders on the format for INAD exemption requests and related submissions to CVM.
6. Identify existing data and remaining data requirements for NADA approvals.
7. Review, record, and provide information on the status of INADs and NADAs.
8. Encourage and seek opportunities for consolidating the INAD/NADA applications.
9. Coordinate educational efforts on aquaculture drugs as appropriate.
10. Identify potential funding sources for INAD/ NADA activities.

Anticipated benefits

Investigation and approval of safe therapeutic and production drugs for use by the aquaculture industry are some of the highest priorities currently facing the industry. At present, only a few approved compounds are available to the industry and further development of the aquaculture industry is severely constrained by a lack of approved drugs essential for treating more than 50 known aquaculture diseases. CVM has afforded the aquaculture industry throughout the U.S. with a "window of opportunity" to seek approval of legal drugs to be used in their production practices. The need for additional drugs is great, but securing data necessary to satisfy the requirements of CVM for drug approval is time consuming, costly, and procedures are rigorous. The INAD/NADA process is the one method that allows the industry to provide CVM with data on efficacy and also aids producers in their production practices.

Coordination and educational efforts directed toward potential INAD/NADA applicants will save time and effort for both the industry and CVM. The National Coordinator for Aquaculture INADs/NADAs serves as a conduit between an INAD/NADA applicant and CVM. The National Coordinator helps to alleviate time demands on CVM staff, thus allowing more time to process a greater number of applications, as well as increasing the breadth of research endeavors within the industry. The grouping of INAD applicants should help to alleviate redundancy, amalgamate efforts, and increase the amount of efficacy data, all of which should result in greater progress toward developing available, approved therapeutic, and production drugs.

Progress and principal accomplishments

In September 1992, Ringer, Professor Emeritus of Michigan State University, was hired on a part-time basis as National Coordinator for Aquaculture INAD Applications. He served in that capacity through August 31, 1994.

As National Coordinator for Aquaculture INADs Ringer participated with CVM in educational workshops on INAD procedures and requirements. These workshops were conducted throughout the U.S. This included workshops held in conjunction with the U.S. Trout Farmers Association, Boston Seafood Show, and Aquaculture Expo V in New Orleans. The workshop at the Boston Seafood Show was videotaped and is now available on cassette from the Northeastern Regional Aquaculture Center. In addition to the workshops, talks were presented on aquaculture drugs at the request of several organizations, including the World Aquaculture Society.

Ringer also helped in the preparation of a letter that CVM used in requesting disclosure information from those holding aquaculture INADs. By law, CVM cannot release any information about an INAD without such permission. A table containing information about these disclosures was made available to the general public. This included the names and addresses of the INAD holders as well as the drug and species of fish intended for use of the drug. It is intended that this table will be periodically updated after additional disclosure permissions have been obtained.

On May 15, 1995, Schnick, recently retired Registration Officer from the National Biological Service's Upper Mississippi Science Center (UMSC), was hired on a three-quarter time basis as National Coordinator for Aquaculture New Animal Drug Applications (National NADA Coordinator). On May 15, 1996, her position was increased to a full-time basis.

As National NADA Coordinator, she organized and coordinated a major INAD/NADA workshop in November 1995, under sponsorship of CVM, that led to increased communications between INAD coordinators, better coordination of the data generation for each drug, and consolidation of several INADs.

New INAD/NADA sponsors

Schnick helped gain a new INAD/NADA sponsor for amoxicillin (INAD #9659) and met with Vetrepharm Limited (United Kingdom) in May 1996 in Fordingham, UK, to discuss an action plan for the development of the INAD/NADA on their broad spectrum antibacterial product. Schnick also helped obtain and is working with INAD/NADA sponsors for hydrogen peroxide (microbicide, INAD #9671), luteinizing-hormone releasing hormone (spawning aid, INAD #9318), common carp pituitary (spawning aid, INAD #9728), and Aqui-S (anesthetic, INAD #9731).

Progress on therapeutic drugs

Schnick and representatives of the Upper Mississippi Science Center (UMSC), La Crosse, Wisconsin, held a special session at the Mid-continent Warmwater Fish Culture Workshop in February 1996 to consider label claims and identify potential pivotal study sites for chloramine-T under the federal-state drug approval partnership program (a project of the International Association of Fish and Wildlife Agencies = IAFWA Project).

Based on residue and environmental data, CVM determined on July 11, 1996, that there are no human food or environmental safety concerns over the use of copper sulfate as a therapeutant, thus making approval relatively easy. Two meetings were held in July and August 1996 with a potential NADA sponsor and CVM to discuss the data requirements for approval and develop an action plan needed to obtain approval of copper sulfate as a therapeutant.

On July 18, 1996, CVM accepted the data and conclusions of a target animal safety study on the toxicity of formalin to warm- and coolwater fish eggs that was submitted along with a proposed formalin label by UMSC in December 1995. CVM will soon issue a notice in the Federal Register inviting sponsors to amend their labels to include the extended claims for both the fungicide (based on UMSC studies) and parasiticide uses (based on studies at Auburn University, Auburn, Alabama). These extensions of the formalin NADA to additional species will remove the need for INADs on formalin for these claims.

Progress on anesthetics

Two meetings in June and August 1996 were held with representatives of Aqui-S™, an anesthetic approved for use on fish in New Zealand, to discuss the potential for development of Aqui-S™ in the United States. Aqui-S™ is approved in New Zealand with a zero withdrawal time and offers a potential alternative to benzocaine. UMSC decided to evaluate the comparative efficacy and regulatory requirements needed for approval on both benzocaine and Aqui-S™. Work on benzocaine through the IAFWA Project has been put on hold until the new anesthetic, Aqui-S™, can be evaluated. After an evaluation has been made on efficacy and regulatory requirements, UMSC will decide along with its state partners in the IAFWA Project and U.S. Fish and Wildlife Service whether to pursue Aqui-S™ or benzocaine for approval as an anesthetic/sedative.

Progress on hormones

A meeting was held at CVM headquarters on April 11, 1996, with Stoller, users of common carp pituitary (CCP), and researchers to determine a course of action for gaining approval of CCP. As a follow-up to that meeting, CVM coordinated a conference call on May 15, 1996, that covered: (1) the identification of researchers and the design of target animal safety studies; (2) the writing of the environmental assessment through the National Research Support Program Number 7 (NRSP-7); and (3) potential funding sources of the target animal safety studies.

The National NADA Coordinator contacted all the holders of disclosed INADs on human chorionic gonadotropin (hCG) at the urging of CVM to send all the data to the sponsor, Intervet, Inc., that was incorporated in a February 1996 Intervet submission to CVM. CVM ruled on February 12, 1996, that enrollment in an INAD will not be required to use hCG as a spawning aid. CVM will defer regulatory enforcement if used by or on order of a veterinarian. Any hCG product may be prescribed, but CVM strongly encourages the use of Intervet’s product, Chorulon®.

Schnick worked with CVM, Auburn University, Rangen, Inc., and tilapia producers to develop INAD #9647 on 17- methyltestosterone (MT) for tilapia (obtained January 25, 1996) and then worked to obtain authorization from CVM and permission from Auburn University to allow the use of MT on yellow perch under Auburn’s INAD (obtained February 22, 1996). NCRAC provided $25,000 to Southern Illinois University-Carbondale and the University of Wisconsin-Madison to conduct a target animal safety study on MT with walleye and has requested $5,000 for Auburn University to conduct a literature review of the environmental data on MT for NADA submission to CVM.

Progress on the IAFWA project

Several meetings were held at UMSC in May and June 1996 to review the whole IAFWA Project related to the following topics on each of the 10 study plans: (1) remaining data requirements; (2) tasks and jobs; (3) assignments for each job; (4) a time table for completing each assigned task; (5) budget projections by study plan and year; (6) budget shortfalls for the original IAFWA Project; and (7) assessment of the potential products at the end of the IAFWA Project.

UMSC has reprogrammed its effort and direction under the IAFWA Project due to changes in requirements and circumstances for benzocaine, chloramine-T, hydrogen peroxide, oxytetracycline, and sarafloxacin. Efforts were made to save the entire IAFWA Project during government downsizing and budget reductions. Based on the assessment of the remaining data requirements and the funds available, UMSC determined that the IAFWA Project was short a total of $1.4 million and 2 years of effort.

Work planned

The National NADA Coordinator developed an action plan that centers on coordinating all drugs of high priority for aquaculture toward NADAs through the INAD process. In particular, Schnick plans to: (1) develop a major initiative on amoxicillin to obtain approval for its use as a broad spectrum antibacterial in all fishes; (2) determine the potential of fumagillin to control or prevent whirling disease in salmonids and Hamburger Gill Disease in catfish and pursue an INAD/NADA if feasible; (3) help determine the potential for approval of two anesthetics, benzocaine and Aqui-S™; (4) assist the efforts of the NRSP-7 to complete the approval process for sarafloxacin to control enteric septicemia in channel catfish; (5) identify potential funding sources for INAD/NADA activities; and (6) continue to coordinate efforts to obtain approvals for all 19 high priority aquaculture drugs.

Several meetings and workshops are planned that will benefit aquaculture drug approvals. A meeting will be held in Kansas City, Missouri, on November 7-8, 1996, to discuss the protocols and select the pivotal study sites for chloramine-T. The National NADA Coordinator arranged the agenda and speakers for a special session entitled “Partnerships for Aquaculture Drug Approvals: Models for Success” to be held at World Aquaculture ‘97, Seattle, Washington, February 19-23, 1997. An International Harmonization Workshop for Aquaculture Drugs/Biologics is scheduled as part of World Aquaculture ‘97 to be held in Seattle, Washington on February 24, 1997, that will create an international forum, identify potential actions, and develop implementation strategies in cooperation with other countries to facilitate approvals of aquaculture drugs.

Impacts

Establishment of the National NADA Coordinator position in May 1995, has resulted in coordination, consolidation, and increased involvement in the INAD/NADA process on 17 of the 19 high priority aquaculture drugs and activities on two new drugs of interest to aquaculture. Six new INAD/NADA sponsors have initiated new INADs and progress has been made toward unified efforts on existing and new INADs/NADAs.

This enhanced coordination will help gain extensions and expansions of approved NADAs and gain approvals for new NADAs. In fact, data on formalin have recently been accepted by CVM and amended NADAs are expected soon from the three current NADA sponsors of formalin.

Publications, manuscripts, papers presented, or reports

See Appendix.

Support

Years	NCRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1992-93				$17,000[a]		$17,000	$17,000
1993-94	$2,000			$12,180[b]	$4,000[c]	$16,180	$18,180
1994-95	$5,000		$23,750[d]	$70,000[e]	$10,000[f]	$103,750	$108,750
1995-96	$10,000		$20,000[g]	$56,920[h]	$5,000[i]	$81,920	$91,920
Total	**$17,000**		**$43,750**	**$156,100**	**$19,000**	**$218,850**	**$235,850**

[a]USDA funding through a Cooperative Agreement with NCRAC
[b]USDA funding through a Cooperative Agreement with NCRAC ($8,500) and FDA's Office of Seafood Safety ($3,680)
[c]Northeastern Regional Aquaculture Center ($2,000) and Southern Regional Aquaculture Center ($2,000)
[d]American Pet Products Manufacturers Association ($7,500), American Veterinary Medical Association ($10,000), Catfish Farmers of America ($2,000), Fish Health Section of AFS ($1,000), Florida Tropical Fish Farm Association, Inc. ($500), Natchez Animal Supply ($1,000), National Aquaculture Council ($1,000), and Striped Bass and Hybrid Producers Association ($250)
[e]USDA funding through a Cooperative Agreement with NCRAC ($23,000), CVM ($22,000), and USDI/NBS International Association of Fish and Wildlife Agencies Project ($25,000)
[f]Northeastern Regional Aquaculture Center ($5,000) and the Center for Tropical and Subtropical Regional Aquaculture ($5,000)
[g]American Pet Products Manufacturers Association ($1,000), Catfish Farmers of America ($10,000), Fish Health Section of AFS ($1,000), Florida Tropical Fish Farms Association, Inc. ($1,500), Striped Bass & Hybrid Producers Association ($1,500), Simaron Fresh Water Fish, Inc. ($2,500), and Abbott Laboratories ($2,500)
[h]CVM ($18,400) and USDI/NBS International Association of Fish and Wildlife Agencies Project ($28,520)
[i]Center for Tropical and Subtropical Aquaculture ($5,000)

Ted R. Batterson serves as the facilitator for this multi-year project interacting with Henry S. Parker and a steering committee in overseeing the Coordinator's activities.

Appendix

Extension

NCRAC extension fact sheet series

Garling, D. L 1992. Making plans for commercial aquaculture in the North Central Region. NCRAC Fact Sheet Series #101, NCRAC Publications Office, Iowa State University, Ames.

Harding, L. M., C. P. Clouse, R. C. Summerfelt, and J. E. Morris. 1992. Pond culture of walleye fingerlings. NCRAC Fact Sheet Series #102, NCRAC Publications Office, Iowa State University, Ames.

Kohler, S. T. and D. A. Selock. 1992. Choosing an organizational structure for your aquaculture business. NCRAC Fact Sheet Series #103, NCRAC Publications Office, Iowa State University, Ames.

Swann, L. 1992. Transportation of fish in bags. NCRAC Fact Sheet Series #104, NCRAC Publications Office, Iowa State University, Ames.

Swann, L. 1992. Use and application of salt in aquaculture. NCRAC Fact Sheet Series #105, NCRAC Publications Office, Iowa State University, Ames.

Morris, J. E. 1993. Pond culture of channel catfish in the North Central Region. NCRAC Fact Sheet Series #106, NCRAC Publications Office, Iowa State University, Ames.

Morris, J. E. In review. Pond culture of hybrid striped bass fingerlings. NCRAC Fact Sheet Series #107, NCRAC Publications Office, Iowa State University, Ames.

Cain, K. and D. Garling. 1993. Trout culture in the North Central Region. NCRAC Fact Sheet Series #108, NCRAC Publications Office, Iowa State University, Ames.

Mittelmark, J. In review. Fish health management. NCRAC Fact Sheet Series #109, NCRAC Publications Office, Iowa State University, Ames.

Rosscup Riepe, J. In review. Managing feed costs: Limiting delivered price paid. NCRAC Fact Sheet Series #110, NCRAC Publications Office, Iowa State University, Ames.

Rosscup Riepe, J. In press. Costs for pond production of yellow perch in the North Central Region, 1994-95. NCRAC Fact Sheet Series #111, NCRAC Publications Office, Iowa State University, Ames.

Morris, J. E. and C. C. Kohler. In press. Pond culture of hybrid striped bass fingerlings in the North Central Region. NCRAC Fact Sheet Series, NCRAC Publications Office, Iowa State University, Ames.

NCRAC technical bulletin series

Thomas, S. K., R. M. Sullivan, R. L Vertrees, and D. W. Floyd. 1992. Aquaculture law in the North Central

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Swann, L. 1992. A basic overview of aquaculture: history, water quality, types of aquaculture, production methods. NCRAC Technical Bulletin Series #102, NCRAC Publications Office, Iowa State University, Ames.

Kinnunen, R. E. 1992. North Central Region 1990 salmonid egg and fingerling purchases, production, and sales. NCRAC Technical Bulletin Series #103, NCRAC Publications Office, Iowa State University, Ames.

Hushak, L. J., C. F. Cole, and D. P. Gleckler. 1993. Survey of wholesale and retail buyers in the six Southern states of the North Central Region. NCRAC Technical Bulletin Series #104, NCRAC Publications Office, Iowa State University, Ames.

Lichtkoppler, F. P. 1993. Factors to consider in establishing a successful aquaculture business in the North Central Region. NCRAC Technical Bulletin Series #106, NCRAC Publications Office, Iowa State University, Ames.

Swann, L. and J. Rosscup Riepe. 1994. Niche marketing your aquaculture products. NCRAC Technical Bulletin Series #107, NCRAC Publications Office, Iowa State University, Ames.

Tetzlaff, B. and R. Heidinger. In review. Basic principles of biofiltration and system design. NCRAC Technical Bulletin Series #109, NCRAC Publications Office, Iowa State University, Ames.

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Brown, P. and J. Gunderson, eds. In press. Culture potential of selected crayfishes in the North Central Region. NCRAC Technical Bulletin Series #112, NCRAC Publications Office, Iowa State University, Ames.

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Swann, L. 1992. Something fishy: Hybrid striped bass in cages. VHS format, 12 min. NCRAC Video Series #101, NCRAC Publications Office, Iowa State University, Ames.

Pierce, R., R. Henderson, and K. Neils. Aquacultural marketing: A practical guide for fish producers. 1995. VHS format, 19 min. NCRAC Video Series #102, NCRAC Publications Office, Iowa State University, Ames.

Swann, L., ed. 1993. Investing in freshwater aquaculture. VHS format, 120 min. NCRAC Video Series #103, NCRAC Publications Office, Iowa State University, Ames.

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NCRAC Culture Series

Summerfelt, R., ed. 1996. Walleye culture manual. NCRAC Culture Series #101, NCRAC Publications Office, Iowa State University, Ames.

Other videos

Kayes, T. B. and K. Mathiesen, eds. 1994. Investing in freshwater aquaculture: A reprise (part I). VHS format, 38 min. Cooperative Extension, Institute of Agriculture and Natural Resources, University of Nebraska-Lincoln.

Kayes, T. B. and K. Mathiesen, eds. 1994. Investing in freshwater aquaculture: A reprise (part II). VHS format, 41 min. Cooperative Extension, Institute of Agriculture and Natural Resources, University of Nebraska-Lincoln.

Situation and outlook report

Hushak, L. J. 1993. North Central Regional aquaculture industry situation and outlook report, volume 1 (revised October 1993). NCRAC Publications Office, Iowa State University, Ames.

Workshops and conferences

Salmonid Culture, East Lansing, Michigan, March 23-24, 1990. (Donald L. Garling)

Midwest Regional Cage Fish Culture Workshop, Jasper, Indiana, August 24-25, 1990. (LaDon Swann)

Aquaculture Leader Training for Great Lakes Sea Grant Extension Agents, Manitowoc, Wisconsin, October 23, 1990. (David J. Landkamer and LaDon Swann)

Regional Workshop of Commercial Fish Culture Using Water Reuse Systems, Normal, Illinois, November 2-3, 1990. (LaDon Swann)

North Central Aquaculture Conference, Kalamazoo, Michigan, March 18-21, 1991. (Donald L. Garling, Lead; David J. Landkamer, Joseph E. Morris, and Ronald Kinnunen, Steering Committee)

Crayfish Symposium, Carbondale, Illinois, March 23-24, 1991. (Daniel A. Selock and Christopher C. Kohler)

Fish Transportation Workshops, Marion, Illinois, April 6, 1991, and West Lafayette, Indiana, April 20, 1991. (LaDon Swann and Daniel A. Selock)

Regional Workshop on Commercial Fish Culture Using Water Recirculating Systems, Normal, Illinois, November 15-16, 1991. (LaDon Swann)

National Aquaculture Extension Workshop, Ferndale, Arkansas, March 3-7, 1992. (Joseph E. Morris, Steering Committee)

Regional Workshop on Commercial Fish Culture Using Water Recirculating Systems, Normal, Illinois, November 19-20, 1992. (LaDon Swann)

In-Service Training for CES and Sea Grant Personnel, Gretna, Nebraska, February 9, 1993. (Terrence B. Kayes and Joseph E. Morris)

Aquaculture Leader Training, Alexandria, Minnesota, March 6, 1993. (Jeffrey L. Gunderson and Joseph E. Morris)

Investing in Freshwater Aquaculture, Satellite Videoconference, Purdue University, April 10, 1993. (LaDon Swann)

National Extension Wildlife and Fisheries Workshop, Kansas City, Missouri, April 29-May 2, 1993. (Joseph E. Morris)

Commercial Aquaculture Recirculation Systems, Piketon, Ohio, July 10, 1993. (James E. Ebeling)

Yellow Perch and Hybrid Striped Bass Aquaculture Workshop, Piketon, Ohio, July 9, 1994. (James E. Ebeling and Christopher C. Kohler)

Workshop on Getting Started in Commercial Aquaculture Raising Crayfish and Yellow Perch, Jasper, Indiana, October 14-15, 1994. (LaDon Swann)

Aquaculture in the Age of the Information Highway. Special session, World Aquaculture Society, San Diego, California, February 7, 1995. (LaDon Swann)

North Central Aquaculture Conference, Minneapolis, Minnesota, February 17-18, 1995. (Jeffrey L. Gunderson, Lead; Fred P. Binkowski, Donald L. Garling, Terrence B. Kayes, Ronald E. Kinnunen, Joseph E. Morris, and LaDon Swann, Steering Committee)

Walleye Culture Workshop, Minneapolis, Minnesota, February 17-18, 1995. (Jeffrey L. Gunderson)

Aquaculture in the Age of the Information Highway. Multimedia session, 18-month meeting of the Sea Grant Great Lakes Network, Niagra Falls, Ontario, May 6, 1995. (LaDon Swann)

AquaNIC. Annual Meeting of the Aquaculture Association of Canada, Nanaimo, British Columbia, June 5, 1995. (LaDon Swann)

Yellow Perch Aquaculture Workshop, Spring Lake, Michigan, June 15-16, 1995. (Donald L. Garling)

Rainbow Trout Production: Indoors/Outdoors, Piketon, Ohio, July 8, 1995. (James E. Ebeling)

Hybrid Striped Bass Workshop, Champaign-Urbana, Illinois, November 4, 1995. (Christoper C. Kohler, LaDon Swann, and Joseph E. Morris)

Economics and Marketing

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Lipscomb, E. R. 1995. The biological and economic feasibility of small scale yellow perch (*Perca flavescens*) production. Master's thesis. Purdue University, West Lafayette.

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O'Rourke, P. D. 1996. The economics of recirculating aquaculture systems. In Proceedings of successes and failures in commercial recirculating aquaculture, Roanoke, Virginia, July 19-21, 1996.

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Rosscup Riepe, J. In review. Managing feed costs: limiting delivered price paid. NCRAC Fact Sheet Series #110, NCRAC Publications Office, Iowa State University, Ames.

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O'Rourke, P. D. and A. M. T. Edon. 1995. Economic analysis of advanced walleye fingerling production in an intensive recirculating system. Combined North Central and 9th Annual Minnesota Aquaculture Conference and Trade Show, Minneapolis, Minnesota, February 17-18, 1995.

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Rosscup Riepe, J. 1994. Getting started in commercial aquaculture: Economics. Workshop on getting started in commercial aquaculture raising crayfish and yellow perch, Jasper, Indiana, October 14-15, 1994.

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Yellow Perch

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Binkowski, F. 1995. Intensive yellow perch fry rearing. Yellow Perch Aquaculture Workshop, Spring Lake, Michigan, June 15-16, 1995.

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Hybrid Striped Bass

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Walleye

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Northeastern Regional Aquaculture Center Compendium Report *for the Period May 1, 1989 to August 31, 1996*

Table of Contents

Genetic Improvement of the American Oyster (*Crassostrea virginica*) for Commercial Culture in the Northeast (88-1)

Project period January 1, 1992 to December 31, 1992

NRAC funding
$510,000

Work group chair
Susan E. Ford, Rutgers University, New Jersey

Participating investigators/ cooperative agencies
Harold Haskin, Rutgers University, New Jersey
Robert Hawes, University of Maine, Maine
Herbert Hidu, University of Maine, Maine
Dennis Walsh, Aquacultural Research Corporation, Massachusetts
Carey Matthiessen, Cotuit Oyster Co., Massachusetts
Patrick Gaffney, University of Delaware, Delaware
William Fisher, University of Maryland, Maryland
John Ewart, University of Delaware, Delaware
Stewart Tweed, Rutgers University, New Jersey
Karl Rask, University of Massachusetts Amherst, Massachusetts

Overview
Since 1984, the protozoan parasite *Haplosporidium nelsoni*, the causative agent of the MSX disease of the American oyster, has been discovered in new areas of the Northeastern United States. Steady declines in the size of oyster landings have accompanied the spread of this pathogen.

The oyster industry relies heavily upon hatchery-produced seed supplies as the base for replenishing local stocks. Thus, there is a clear need for a seed supply that is reliable both in quality and quantity.

The goal of this project is to produce genetically improved broodstock which meet the criteria of being disease resistant, having good growth rates, and producing oyster meat of high quality. The precise genetic mechanisms involved in resistance to MSX disease are also under investigation.

Objectives
- To evaluate survival, growth, and meat quality of existing MSX-resistant strains.
- To produce new strains of the American oyster with improved growth and survival against MSX disease through hybridization and polyploidy techniques.
- To develop an information base pertaining to the genetic basis for MSX-disease resistance.
- To disseminate experimental results to the aquaculture community.

Extension component
- Assemble and distribute technical and other relevant information on the genetic improvement of *Crassostrea virginica*, and field performance of oyster stocks to individuals or groups from industry, extension and the research community through extension publications and workshops.
- Organize a workshop session on genetic improvement of *Crassostrea virginica* at the Milford Shellfish Biology Seminar to present final reports and summarize overall results of the laboratory research and field evaluation carried out during the project's 3-year term.
- Prepare and distribute fact sheets summarizing important information on oyster diseases, underlying principles of genetic improvement and disease resistance, and significant project accomplishments; and formulate practical recommendations for the production, deployment, and management of hatchery produced disease resistant stocks in the Northeastern Region.

Principal accomplishments
The major objective of this final year of the project was to complete the evaluation of diploid and triploid resistant and susceptible crosses for disease resistance and growth in Delaware Bay. During the first year of the test (1991), the groups were tested for resistance to MSX disease. The general conclusion was that pure-resistant stocks were most resistant, followed by the hybrids between resistant and susceptible stocks, and

then by susceptible oysters. Within each of these groups, the diploids performed better than the triploids.

During 1992, the surviving oysters were challenged by Dermo Disease in addition to MSX. There was relatively little difference in survival among diploid groups, although the homozygous-resistant and susceptible oysters had somewhat lower mortality (16% - 18%) than did the hybrids (22% - 24%). On average, mortality of triploid groups was two to three times greater than that of their diploid counterparts.

Growth varied according to genetic make-up. Pure resistant triploid oysters were 21% larger, by weight at the end of the study, than were pure-resistant diploids. The diploid hybrids, however, were 7 to 14% larger than the triploid hybrids and 22% larger than homozygous diploids.

A wrap-up session to present the results of the entire study to industry members and other researchers was conducted at the Milford Shellfish Symposium, Milford, CT in February 1992. Four presentations were made by principal investigators and the session was concluded by a panel discussion, including investigators and industry members, on applicability of the results to oyster culture in the Northeastern and Mid-Atlantic Regions of the United States.

Usefulness of findings

The success of triploid oysters in commercial culture elicited a great deal of interest in their potential for enhancing disease resistance. The results of the triploid study clearly show that creating triploids, which entails expense and risks beyond that incurred in the production of diploid oyster larvae, provides no advantage in defense against either MSX or Dermo Diseases. Calculations of total surviving biomass, which takes into account final size of survivors as well as survival rates, will provide more exact information on the relative value of the various genetic combinations.

Work planned

Results of the project, which showed good performance of the MSX-resistant lines, stimulated a great deal of interest on the part of oyster growers and led directly to an ongoing project "Commercial Field Trials of MSX-resistant Strains of the American Oyster (*Crassostrea virginica)*." A commercial hatchery in Maine has produced and sold seed from a fast-growing line developed by the University of Maine. Host-parasite interaction studies begun under this project led directly to the funding of another grant entitled "Cellular Responses of Oyster hemocytes to Protozoan Pathogens" by USDA's National Research Initiative Grant Program.

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Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1988-89	$170,000						$170,000
1989-90	$170,000						$170,000
1990-91	$170,000						$170,000
Total	**$510,000**						**$510,000**

Hybridization, Genetic Manipulation and Sex Control in Striped Bass (*Morone saxatilis*) (88-2)

Project period June 1, 1988 to March 15, 1993

NRAC funding

$200,000

Work group chair

Reginal M. Harrell, University of Maryland, Maryland

Participating investigators/ cooperative agencies

Howard J. Kerby, U.S. Fish & Wildlife Service, West Virginia

Harold L. Kincaid, U.S. Fish & Wildlife Service, Pennsylvania

Jamie Geiger, U.S. Fish & Wildlife Service, West Virginia

Merrill Leffler, Maryland Sea Grant College, Maryland

Walnut Point Farm, Maryland

Ritchloam Hatchery, Florida

Overview

The striped bass, *Morone saxatilis*, is an excellent food and sport fish along the Atlantic Coast. Declines in natural populations and recent state laws either banning or restricting commercial fishing have maintained high wholesale prices.

Striped bass have been successfully hybridized with both white bass and white perch. A great deal of commercial interest exists in the commercial culture of these hybrids due to their increased vigor over pure striped bass.

Limitations in culture technology are imposed by the limited availability of wild broodstock females. A further concern is that fertile hybrids may backcross and significantly affect the gene pool of the parental species.

This project investigates the role of polyploidy in enhancing marketability through improved growth rates and feed conversion. The desirability of triploids also rests in their functional sterility, which would allay the fears of natural resource managers.

Objectives

- To develop technologies for the commercial scale production of triploid striped bass larvae.
- To study the relative growth and survival of triploid larvae as compared to normal diploid larvae.
- To refine techniques for producing tetraploid larvae.
- To disseminate these technologies throughout the U.S. aquaculture industry.

Principal accomplishments

This project was designed to determine if genetic manipulation of striped bass hybrids improved performance expression from an aquaculture perspective. The project had three objectives: 1) develop technology that will enable production of commercial scale numbers of triploid larvae and conduct relative growth and survival studies comparing them to normal diploids; 2) refine techniques for producing sterile striped bass (hybrids); and 3) transfer technologies developed in the first two objectives to culturists to stimulate further development of a new major aquaculture industry in the United States.

We were successful in producing triploid hybrid striped bass using pressure induction at levels that would be commercially viable. Although polyploidy was achieved, inconsistencies in success was evident and believed to be due to variations in ambient incubation conditions of the gravid females prior to spawning. We successfully reared triploid progeny at two separate facilities, a freshwater site in West Virginia, and a saltwater site in Maryland. Both diploid and triploid hybrids reached commercial market size (> 500 g) in the second year of growout. However, triploid performance was less than that of its diploid counterparts through the second year of growth with triploids being significantly smaller than the diploids at both culture sites.

Efforts to induce sex reversal (produce XX males) in pure striped bass through the use of hormones (17 *a*-methyl-testosterone and estradiol) was unsuccessful. Two years after treatment, gonadal development was minimal with only 7% of the fish showing sexual differentiation and only one of the control group was a female. Treatment group females were from the estradiol tests and the males were from both hormone treatments. The remaining undifferentiated fish (93%) may have been males, but it was unknown whether these undifferentiated fish were expressing delayed sexual development or were sterilized by the use of the hormones.

Considerable effort was undertaken throughout the course of this project to transfer the technology as it became available. Three annual culture workshops were held in which participants experienced hands-on training in all aspects of striped bass culture, including polyploidy induction. Additionally, three extension videos were produced.

Usefulness of findings

Although triploid induction methods resulted in fish that grew slower than its diploid counterpart, commercial market size was achieved within 2 years growth. Likewise, all triploid fish tested were functionally sterile while both male and female diploids were mature during their second year of growth. Comparative performance of triploid was still better than pure striped bass reared under similar conditions, and the fish appear to be sterile. This sterility greatly increases the value of hybrids for use in open water culture situations where culture would otherwise be prohibited due to potential outcrossing. We do not recommend disregarding the potential of triploid induction as a viable alternative based on this study's growth and performance. The hybrid we used was the sunshine bass hybrid (white bass female x striped bass male) which, in affect, resulted in our working with a fish that had 2/3 of its genetic make-up coming from white bass while the diploids only had 1/2 of its genome from the white bass. This may account for the differences seen. Future work should examine the palmetto bass (female striped bass).

Work planned

Results of this research project have already stimulated the growth of the striped bass industry in the Northeastern Region, and Maryland in particular. Several of the participants in the workshops have started their own commercial aquaculture operations and are into the market at this time. Part of the findings of this project were integrated into the recent revision of the striped bass culture manual sponsored by the American Fisheries Society. The manual entitled "Culture and Propagation of Striped Bass and its Hybrids" is proclaimed to be the one resource striped bass culturists have, and is already in its second printing.

The success of the short courses associated with this project has prompted additional funding from NRAC and other sources to continue offering the course, with the most recent being offered in the Spring of 1993.

The most important benefits derived from this project concern the facts that although triploid sunshine bass did not perform as well as the diploid counterparts, they did reach market size within a normal growing period. Also, because it appears the fish are indeed sterile, one of the major hurdles preventing open-water culture of hybrid striped bass in the Northeastern Region has been removed (the concern of genetic adulteration of the native striped bass stocks).

Publications and presentations

Publications

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Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1988-89	$65,000					$104,677	$169,677
1989-90	$65,000						$65,000
1990-91	$65,000						$65,000
1991-92	$5,000						$5,000
Total	**$200,000**						**$304,677**

Analysis of the Economics and Marketing of Farmed Finfish in the Northeast (88-3)

Project period October 1, 1988 to March 30, 1992

NRAC funding

$230,476

Coordinator

James L. Anderson, University of Rhode Island, Rhode Island

Participating investigators/ cooperative agencies

Ivar Strand, University of Maryland, Maryland
Joe M. Regenstein, Cornell University, New York
Douglas Lipton, University of Maryland, Maryland
Eugene German, Cornell University, New York
Carole Bisogni, Cornell University, New York
John Gates, University of Rhode Island, Rhode Island
Jon Sutinen, University of Rhode Island, Rhode Island
Maryland Department of Agriculture, Maryland

Overview

The aquaculture sector contribution to the world's supply of salmon is expected to continue to increase. Although the U.S. is the world's largest producer and exporter of salmon, imports from farmed fish have increased steadily. Canada has recently launched a highly successful program to support an emerging pen-raised salmon industry in New Brunswick. The U.S. counterpart suffers from inadequate investment capital, unclear state policies, and general lack of government support.

If aquaculture is to succeed in the U.S. it will have to be justified on an economic basis. This study provides a much-needed analysis of the finfish aquaculture production, marketing, and distribution system in the Northeast to evaluate the justification for aquaculture investments in the region. Results of this research will be used by practicing aquaculturists, policy makers, and those who would invest in the emerging aquaculture industry.

Objectives

- To characterize status and trends within the finfish aquaculture industry in the Northeast.
- To determine production costs and conduct market analyses for striped bass, hybrid striped bass, and ocean pen-reared salmon.
- To publicize and disseminate project results.

Principal accomplishments

1. Researchers at the University of Rhode Island completed a comprehensive in-person survey for the pen-reared salmonid industry in the Northeast. The researchers found that the industry is presently confined to Maine, that rapid growth occurred since 1981, and that despite regulatory and marketing uncertainties, growers expect that production will nearly triple in the next 5 years. Results of the study are presented in a 204-page technical report entitled "Pen-Reared Salmonid Industry in the Northeastern United States."
2. Researchers at Cornell University completed and published a study entitled "An Economic Analysis of Freshwater Finfish Aquaculture in the Mid-Atlantic States." The study provided a descriptive analysis of the Mid-Atlantic finfish aquaculture industry, estimated profitability of current enterprises, and assessed the potential for expansion.
3. Researchers at the University of Maryland completed a survey of the hybrid striped bass industry in the Northeast and prepared a draft of a report summarizing their results. They found that the industry is presently concentrated in Maryland where 18 growers produced an estimated 250,000 pounds in 1990. Expectations are that the industry will expand, particularly as current regulatory restrictions are eased.

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Villalobos, M. E. 1992. Beliefs, practices, and needs for information about fresh finfish among people of Hispanic origin living in New York. M. S. Thesis. Cornell University, Ithaca. 228pp.

Vukina, T. and J. L. Anderson. 1992. Price forecasting with state-space models of nonstationary time series: The case of the Japanese salmon market. URI/AES Report 2893.

Vukina, T. and J. L. Anderson. (Unk.). Forecasting approach to optimal intertemporal hedging. American J. of Agricultural Economics. (Forthcoming).

Vukina, T. 1991. Hedging with forecasting: A state-space approach to time-series modeling. Ph.D. Thesis. Department of Resource Economics. University of Rhode Island, Kingston, RI. 190pp.

Weld, M., W. Knoblauch, and J. Regenstein. 1990. An economic analysis of the freshwater finfish aquaculture in the Mid-Atlantic states. A. E. Res. p. 90-8. Department of Agricultural Economics, Cornell Agricultural Experiment Station. Cornell University. Ithaca, NY. 41pp.

Weld, M., W. Kroblauch, and J. Regenstein. June 1990. Freshwater finfish aquaculture in the Mid-Atlantic States. AES Research Report 90-8. Department of Agricultural Economics. Cornell University, Ithaca, NY. 43pp.

Weld, M. 1990. An economic analysis of freshwater aquaculture in the Mid-Atlantic states. M. S. Thesis. Agricultural Economics. Cornell University. Ithaca, NY. 99pp.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1989-90	$58,406	$69,050					$127,456
1990-91	$90,690	$73,050					$163,740
1991-92	$86,380	$77,030					$163,410
Total	**$230,476**	**$219,130**					**$454,606**

Aquaculture Extension Workshop (88-4)

NRAC funding

$25,000

Objectives

- Identify constraints affecting the development of a strong aquaculture industry in the Northeast.
- Obtain a broad spectrum of input from all groups in the Northeast interested in the development of an aquaculture industry.
- Seek input for establishing regional priorities for NRAC programming.

Accomplishments

The workshop was held on October 17-18, 1988, at the Tara Hyannis Hotel, Hyannis, MA. There were 97 attendees with 37 from industry, three from county and town governments, six from state governments, eight from the federal government and 42 from academia. Dr. Nick C. Parker, Scientific Director, SE Cultural Fish Laboratory, US Fish and Wildlife Service, was the keynote speaker discussing “Future of the Aquaculture Industry in the Northeast: Issues, Opportunities, and Problems.”

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1988-89	$25,000						$25,000
Total	**$25,000**						**$25,000**

Governmental Regulation of Growth and Development: Improving the Legal Framework for Aquaculture in the Northeastern United States (90-1)

Project period January 1, 1992 to December 31, 1992

Total funding

$93,780

Work group chair

Alex W. Wypyszinski, Rutgers University, New Jersey

Participating investigators/ cooperative agencies

Norman K. Bender, University of Connecticut, Connecticut
Timothy Eichenberg, University of Maine School of Law, Maine
James M. Falk, University of Delaware, Delaware
Bruce E. Lindsay, University of New Hampshire, New Hampshire
Marilyn A. Altobello, University of Connecticut, Connecticut

Overview

Legal and regulatory obstacles are commonly cited as major impediments to aquaculture growth and development in the United States. Although the economic potential for aquaculture is promising, the permitting process is difficult, particularly when operations involve the use of public waters.

A complex legal framework confronts the potential aquaculturist. Policymakers who have the responsibility of striking a balance between protection of environmental and public interests should be made aware of the operational needs of the aquaculture industry.

This project is designed to assess existing regulations, analyze basic legal and policy issues and develop recommendations for changes in the legal framework. The ultimate goal is to improve public decision making within a climate that is supportive of aquaculture ventures.

Objectives

- To assist aquaculture policy makers in the Northeast by collecting and categorizing existing laws dealing with management of the public trust.
- To prepare guidebooks for each state in the region describing current laws, regulations, and permitting procedures.
- To design case studies illustrating the socio-economic impact of government regulations on the aquaculture industry.
- To develop recommendations for legal changes to enhance decision making and protect aquaculture ventures in the Northeast.

Principal accomplishments

Phase 1 of the project continues; most of the information has been placed in computer files. Given the rapid changes taking place in the area of aquaculture law, regulation, and policy, this phase should continue until the end of the project. In assembling this material, the suggestion has been made that a procedure for periodic updates be designed for the future.

Phase 2 Analysis of basic legal and policy issues and preparation of background papers has been completed by the University of Maine School of Law participants. Among specific questions which were addressed in phase 2 were: the relationship of aquaculture to the public trust doctrine (state ownership of coastal waters and trusteeship of public rights of fishing, commerce, and navigation): to riparian rights; to water quality and waste disposal; and to state responsibility for enforcement of wild stock finfish and shellfish regulations. Project participants defined the issues which were addressed in consultation with state officials, industry representatives, and government agency personnel.

Phase 3 Preparation of short case studies to document and assess the costs and impact of government regulation on the aquaculture industry in New Hampshire has been completed by University of New Hampshire participants. Their report has been circulated through the TIAC liaison, and will be included as part of the final report. Case study selection was based on criteria including the following: (a) species or type (marine or freshwater); (b) size of operation; (c) production methods employed; and (d) extent of vertical integration. Case studies were prepared through field research and interviews with aquaculturists, agency personnel, and local government officials, and through review of application and decision documents in the administrative record.

Phase 4 This phase required development of recommendations for changes in the legal framework to improve public decision making and to protect aquaculture ventures. These recommendations have been developed and are contained in the report filed by the University of Maine School of Law participants.

Phase 5 Development of recommendations for changes in the legal framework to improve public decision making and to protect aquaculture ventures, including model legislation and procedures.

Usefulness of findings

The status of aquaculture legislation/regulations throughout the region has been in a state of flux since the inception of this project.

On June 6, 1992, Alex W. Wypyszinski made a presentation entitled "Permitting for Aquaculture: What You Don't Learn From Textbooks" at the Aquaculture Center in the Mid-Atlantic Symposium in Annapolis, MD.

The New Jersey Aquaculture Plan recommended as a result of work conducted by project participants will begin following the Governor's signature of an executive order drafted by the Rutgers Fisheries and Aquaculture Technology Center's Aquaculture Plan Subcommittee. Drafting of the Plan should take place in 1993.

Work planned

Issues of aquaculture policy will be discussed at a regional conference planned for 1993 by project participants. This conference will be part of the outreach component of this project.

Publications and presentations

Ajuzie, E. I. S. 1994. Pollution and public regulation: their effect on social welfare, productivity growth, and policy in the Long Island Sound oyster industry. Dissertation.

Ajuzie, E. I. S. and M. A. Altobello. 1994. Input substitution possibilities in the Long Island Sound oyster industry: A dual approach to Allen and Morishima elasticities. Paper presented at the 1994 NAREA Annual Meeting, June 27-29, 1994, University of Delaware, Newark, DE.

Ajuzie, E. I. S. and M. A. Altobello. 1995. Effects of environmental pollution and public regulations on productivity growth in the Long Island Sound oyster industry: A dual translog cost function approach.

Eichenberg, T. and B. Vestal. 1992. Improving the legal framework for marine aquaculture: The role of water quality laws & the public trust doctrine. Technical Report by the Marine Law Institute for the Northeastern Regional Aquaculture Center/USDA. Publication #NRAC 92-007. North Dartmouth, MA. 53pp.

Wypyszinski, A. W., M. A. Altobello, B. E. Lindsay, J. Falk, T. Eichenberg, and K. Riaf. 1994. Governmental regulation of growth and development: improving the legal framework for aquaculture in the Northeastern United States. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 94-005. North Dartmouth, MA. 489pp.

Support

Years	NRAC USDA funding	Other support: University	Other support: Industry	Other support: Other Federal	Other support: Other	Other support: Total	Total Support
1989-90	$34,420	$53,109				$53,109	$87,529
1990-91	$39,450	$55,548				$55,548	$94,998
1991-92	$19,910	$51,755				$51,755	$71,665
Total	**$93,780**	**$160,412**				**$160,412**	**$254,192**

Northeast Regional Aquaculture Extension Program for a More Viable, Profitable Industry (90-2)

Project period January 1, 1992 to December 31, 1992

NRAC funding

$131,780

Work group chair

Joseph K. Buttner, State University of New York, Brockport, NY

Participating investigators/ cooperative agencies

Karl Rask, University of Massachusetts Amherst, Massachusetts

Overview

A primary goal of the Cooperative and Sea Grant Extension Programs is the timely and efficient transfer of information to prospective and practicing aquaculturists. The need for information is particularly acute in the Northeast where, despite an abundance of opportunities, definitive aquacultural data have not yet been standardized and made readily accessible. In addition, aquaculture information generated in different states and regions is often not transferred, resulting in lost opportunities and inefficient allocation of limited resources as states duplicate each others' efforts.

This program will compile existing Northeast aquaculture information and develop 10 publications which will address the highest priority concerns of the region's aquaculture community. The program also includes an industry and staff training component which will introduce extension personnel to aquaculture through workshop experiences.

Objectives

- To facilitate transfer of information to user groups in the Northeast through the development and distribution of aquaculture extension publications.
- To provide hands-on training opportunities to Extension/Sea Grant staff and to industry representatives. Extension/Sea Grant staff and to industry representatives.
- To establish an aquaculture information network among Cooperative Extension and Sea Grant Marine Advisory personnel.

Principal accomplishments

Expressed objectives of this project were to develop fact sheets for novice and established aquaculturists, to set up and hold workshops that address important needs of the aquaculture industry, and to facilitate networking between extension specialists and their clientele throughout the Northeast. The project in 1991/92 achieved all of its stated objectives: 10 extension publications have been produced, two conferences that addressed Fish Health Regulations were held, support was provided for two important regional meetings (Milford Finfish and Shellfish Seminar, NRAC Industry Summit), and networking between extension people in different states and their clientele was enhanced (e.g. list of aquaculture related publications available in each state and an updated list of aquaculture specialists by state that includes name, telephone number, address, specialty, FAX number, and electronic mail box).

Usefulness of findings

Information and exchange mechanisms established by this proposal serve as a foundation for closer cooperation and continued growth in the future. Networking within extension and with clientele has been enhanced. A cadre of relatively few extension people has been expanded to include all Northeastern states and the District of Columbia. Perhaps more importantly, industry people have become more familiar with the role and capabilities of extension. Increasingly, priorities and methodologies are defined through interactions between industry, research, and extension. The recently published NRAC fact sheets illustrate how the products of this project benefit aquaculture. Extension benefits because county agents can now respond directly to many initial inquiries about aquaculture; they have become part of the NRAC outreach effort. Industry benefits because inquiries by potential and novice culturists are handled by county agents thus affording extension specialists more time to address the needs of established producers.

Work planned

The project has terminated, but momentum generated by the project will continue in at least three major avenues.

1. Under the leadership and coordination of John Ewart (DE) a follow-up extension project, "Development of a Northeastern Regional Aquaculture Extension Network," has been assembled and approved. Many participants in the original extension project are involved in the second project. Significantly, collaboration has grown substantially and the new project includes many new participants.
2. Concerns identified by the Workshop on Fish Health Regulations have been incorporated into the next Extension Project and a follow-up conference is scheduled. Karl Rask (MA) will continue to coordinate extension efforts in the area of fish health regulations.
3. Each NRAC supported research project now includes an extension person to facilitate communication with the Extension Work Group and timely transfer of information to industry. The approach initiated during the concluding extension project has become NRAC policy and will continue with future projects.

Publications and presentations

Baptist, G., D. Meritt, and D. Webster. 1993. Growing microalgae to feed bivalve larvae. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 93-002 (Formerly NRAC Fact Sheet #160). North Dartmouth, MA. 8pp.

Bowser, P. R. and J. K. Buttner. 1991. General fish health management. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 91-002 (Formerly NRAC Bulletin #111). North Dartmouth, MA. 12pp.

Buttner, J. K. and G. Flimlin. 1991. Is aquatic farming for you? Northeastern Regional Aquaculture Center/USDA Publication #NRAC 91-001 (Formerly NRAC Fact Sheet #101). North Dartmouth, MA. 2pp.

Buttner, J., G. Flimlin, and D. Webster. 1992a. Aquaculture systems for the Northeast. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 92-002 (Formerly NRAC Fact Sheet #120). North Dartmouth, MA. 4pp.

Buttner, G. Flimlin, and D. Webster. 1992b. Aquaculture species for the Northeast. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 92-003 (Formerly NRAC Fact Sheet #130). North Dartmouth, MA. 4pp.

Buttner, J. K., R. W. Soderberg, and D. E. Terlizzi. 1993. An introduction to water chemistry in freshwater aquaculture. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 93-003 (Formerly NRAC Fact Sheet #170). North Dartmouth, MA. 4pp.

Flimlin, G. 1992. Initial questions the county agent can ask the prospective fish culturist. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 92-001 (Formerly NRAC Publication #102). North Dartmouth, MA. 4pp.

Flimlin, G. and B. F. Beal. 1993. Major predators of cultured shellfish. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 93-004 (Formerly NRAC Fact Sheet #180). North Dartmouth, MA. 6pp.

Regenstein, J. M. 1992. Processing and marketing aquacultured fish. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 92-004 (Formerly NRAC Fact Sheet #140). North Dartmouth, MA. 4pp.

Rice, M. A. 1992. The Northern Quahog: The biology of *Mercenaria mercenaria*. Jaworski. C. and M. Schwartz, eds. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 92-006 (Published cooperatively Rhode Island Sea Grant, and University of Rhode Island Cooperative Extension). North Dartmouth, MA. 60pp.

Strombom, D. B. and S. M. Tweed. 1992. Business planning for aquaculture - is it feasible? Northeastern Regional Aquaculture Center/USDA Publication #NRAC 92-005 (Formerly NRAC Fact Sheet #150). North Dartmouth, MA. 12pp.

Miscellaneous Presentations

"Aquaculture extension specialists in the Northeast," September 1992, is a list of extension professionals by state that are actively involved in NRAC activities.

"Aquaculture extension publications," June 1991, is a list of state university, federal, state, and industry publications that may be useful to prospective and/or practicing aquaculturists.

"Industry summit" provided approximately $10,000 support to facilitate participation by commercial producers.

Developed and held a Workshop on Fish Health and Regulations (August 5, 1992) that included members of most state aquaculture associations in the Northeast, the National Aquaculture Association, and the United States Trout Farmers Association. A position statement was developed and approved.

Conference (July 5, 1992) was held with federal officials and concerns about federal guidelines for fish health were discussed

Support

Years	NRAC USDA funding	Other support: University	Industry	Other Federal	Other	Total	Total Support
1989-90	$65,475						$65,475
1990-91	$66,305						$66,305
Total	**$131,780**						**$131,780**

Genetic Manipulation of Oysters Through Hybridization and Polyploidy (90-3)

Project period January 1, 1992 to December 31, 1992

NRAC funding

$134,759

Work group chair

Patrick M. Gaffney, University of Delaware, Delaware

Participating investigators/ cooperative agencies

Standish K. Allen, Rutgers University, New Jersey
Don Merritt, University of Maryland, Maryland
Donald Webster, University of Maryland, Maryland

Overview

Hatchery production of bivalve seed stock has become an essential component of commercial aquaculture operations. Controlled hatchery production also offers the potential for the development of genetically superior lines. Shellfish industries in locations rendered unproductive or marginally productive because of disease or environmental degradation can possibly be revitalized by introduction of genetically improved lines.

In addition to developing commercial bivalve strains of demonstrated superiority through conventional selective breeding techniques, interspecific hybridization may by used to produce new, superior genetic types. In this project polyploids will be induced to create further modified oyster strains. Polyploidy is a condition created where individuals carry more than the normal number of chromosomes. Lines of these genetically altered species, tailored to particular environmental conditions, could be of tremendous benefit to the shellfish industry.

Objectives

- To develop and refine methods for the production of interspecific diploid and polyploid hybrid oysters.
- To implement experimental methods in small-scale hatchery systems.
- To prepare educational materials and sponsor a workshop on oyster culture.

Principal accomplishments

This project contained two components, research and extension. It began in summer 1990 and continued through 1992, with the majority of experimental crosses conducted in summer 1990 and summer 1991.

The research component was aimed at developing two tools, hybridization and polyploidy, for genetically manipulating oysters to produce improved lines for culture.

The extension segment was designed to bring general information about oyster culture to current and prospective culturists by means of an oyster culture workshop and extension fact sheets.

Hybridization

Broodstock of five *Crassostrea* species - the Japanese oyster *C. gigas*, the Suminoe *C. rivularis,* the native oyster *C. virginica*, the Caribbean mangrove oyster *C. rhizophorae*, and the Kumamoto oyster *C. sikamea*, were maintained and conditioned for spawning in quarantine. We were not able to spawn the Kumamoto oysters successfully, and could only obtain limited numbers of *C. rhizophorae*, so we concentrated on crosses involving the other three species. We made large numbers of crosses, both as pair matings and mass spawns, to ensure that our results are broadly applicable.

In brief, our findings are straightforward. Most crosses yielded adequate to good fertilization and early larval development, but the larvae were inviable, dying at 10-12 days after fertilization.

C. virginica formed inviable hybrids with *C. gigas* and *C. rivularis*. Larvae from the cross with *C. virginica* and *C. rhizophorae* survived longer, but failed to set. However, this work was limited in scope and does not necessarily mean that this cross cannot produce viable hybrids.

The Pacific oyster *C. gigas* formed inviable hybrids with *C. virginica*, but crosses with *C. rivularis* yielded a good set of spat in 1991. However, subsequent analysis (summer 1992) by enzyme electrophoresis showed patterns resembling pure *C. rivularis* in these animals. We hope to use nuclear and mitochondrial DNA markers to determine whether they are simply contaminants (which seems unlikely, since they appear to be *C. rivularis*), or true hybrids with suppressed expression of *C. gigas* enzymes.

Hybridization with Pacific oyster species thus appears to have little potential role for the genetic manipulation of the eastern oyster. *C. rhizophorae* may prove useful in this regard, but further work is required.

Hybridization among the Pacific oyster species appears to be possible, and should be pursued further. If hybrids are sterile (by virtue of their hybrid status, or induced triploidy) as well as resistant to the diseases that plague the eastern oyster, they might eventually find a niche in Atlantic coast aquaculture.

Triploids

In some cases, induction of triploidy (possession of three sets of chromosomes rather than the usual two) enables otherwise inviable hybrids to be "rescued." We crossed *C. gigas* females and *C. virginica* males and induced triploidy in the hybrid offspring. The resulting larvae showed a pattern of early mortality indistinguishable from that of diploid hybrids. However, this experiment should be repeated using various stocks of the two species before we can confidently conclude that triploidy cannot rescue hybrids.

Dissemination of results

The results of our research have been presented at annual meetings of the National Shellfisheries Association and the World Aquaculture Society. Details of the hybridization experiments, as well as a critical review of attempts at hybridization among species of the genus *Crassostrea*, will soon be published as three articles in the journal Aquaculture.

Extension component

A fact sheet on the remote setting of oyster larvae is nearing completion. This will include information on the use of this technique throughout the Mid-Atlantic region, as well as results from another NRAC-sponsored study on larval development and mortality. A second fact sheet, on algal production for hatchery operators, will also be issued soon.

A video on hatchery techniques and remote setting of oysters is also being prepared, with completion in 1994 anticipated.

Two remote setting demonstrations were held in Maryland in 1992. Personnel from this project also participated in education seminars at the Fish Farm Expo held in New Orleans in January 1992.

Usefulness of findings

Although the attempts at hybridization have not proven successful, the work undertaken in this project has pointed towards practical difficulties in producing hybrid oysters. Attempts at hybridization should continue with the eventual goal of producing genetically improved oysters, which would benefit the Northeastern oyster industry by resulting in increased yearly production.

Work planned

The experiments conducted make it very likely that earlier published and unpublished reports of hybrids were based on artifacts such as contamination. These results are important in evaluating the potential impacts of introducing exotic *Crassostrea* species into Mid-Atlantic waters: any impact of *C. gigas* for example, on the native oyster *C. virginica* will more likely be ecological than genetic. The one successful hybrid, *C. gigas* female x *C. rivularis* male, has potential for aquaculture but may be of limited use in the Northeast because of concern over the use of exotic species.

Publications and presentations

Allen, S. K., Jr. and P. M. Gaffney. 1993. Genetic confirmation of hybridization between *Crassostrea gigas* (Thunberg) and *Crassostrea rivularis* (Gould). Aquaculture. Vol. 113(4):291-300.

Allen, S. K., Jr., P. M. Gaffney, J. Scarpa, and D. Bushek. 1993. Inviable hybrids of *Crassostrea virginica* (Gmelin) with *C. rivularis* (Gould) and *C. gigas* (Thunberg). Aquaculture. 113(4):269-289.

Allen, S. K., Jr. and P. M. Gaffney. 1991. Hybridization among three species of *Crassostrea*. J. Shellfish Res. 10:301. Presented at the 1991 Annual meeting of the National Shellfisheries Association in Portland, Maine.

Gaffney, P. M. and S. K. Allen, Jr. 1992. Genetic aspects of introduction and transfer of molluscs. J. Shellfish Res. 11: 535-538.

Gaffney, P. M. and S. K. Allen, Jr. 1993. Hybridization Among *Crassostrea* Species: A Review. Aquaculture. 116:1-13.

Gaffney, P. M., C. M. Bernat, and S. K. Allen, Jr. 1993. Gametic incompatibility in wild and cultured populations of the eastern oyster, *Crassostrea virginica* (Gmelin). Aquaculture. 115:273-284.

Scarpa, J. and S. K. Allen, Jr. 1992. Comparative kinetics of meiosis in hybrid crosses of Pacific oyster *Crassostrea gigas* and Suminoe oyster *C. rivularis* with the American oyster *C. virginica*. J. of Exp. Zool. 263: 316-322.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1990-91	$71,345						$71,345
1991-92	$63,414						$63,414
Total	**$134,759**						**$134,759**

Northeastern Regional Aquaculture Center Newsletter-1990 (see also 91-5, 92-8) (90-4)

Project period 1 year beginning February 1990

NRAC funding

$15,000

Work group chair

Joseph K. Buttner, State University of New York at Brockport, NY

Participating investigators/ cooperative agencies

Gregg Rivara, Cornell University, New York

Overview

A regional newsletter is being published to address the need for timely and accurate dissemination of information about aquaculture in the Northeast and to describe the activities and projects of NRAC. This quarterly publication deals with recent developments in aquaculture research and commercial operations in the region. It also publicizes the contribution of NRAC to regional aquaculture activities.

Objectives

- To publicize the contributions of NRAC to regional aquaculture development.
- To disseminate results of aquaculture research and extension activities supported by NRAC.
- To provide a forum for the regional and national exchange of aquaculture information.
- To encourage support for aquaculture development.

Principal accomplishments

The Newsletter originated in 1989 as a mechanism to inform the aquaculture community and others in the Northeast about NRAC activities. Since 1990, the Newsletter has been edited by SUNY Brockport and Cornell Cooperative Extension. Number of issues printed and mailed has gradually increased from slightly over 1,500 to nearly 3,200. The format and layout has been standardized with two colors on the front and back. Articles highlight industry activities and NRAC projects.

The Newsletter represents perhaps the most effective, efficient, and economical mechanism to facilitate the timely transfer of information, to ensure that all parties are kept informed of important developments, and to promote the efforts of NRAC. Feedback from the readership has increased dramatically. More articles are being submitted and revision of the mailing list is a dynamic event. Additions, deletions, and changes are common. Between 20-50 new names are added between issues.

Publications and presentations

Developed, printed, and distributed three issues of "Northeastern Aquaculture," newsletter for the Northeastern Regional Aquaculture Center: Volume 3(3) Fall 1991; Volume 3(4) Winter 1991; and Volume 4(1) Spring 1992.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1989-90	$15,000						$15,000
1990-91	$11,415						$11,415
1991-92	$18,000						$18,000
Total	**$44,415**						**$44,415**

Increasing Aquaculture Production in the Northeast through Nutrition (90-5)

Project period May 17, 1990 to November 17, 1993

NRAC funding

$293,270

Chair

Susan Goldhor, Center for Applied Regional Studies, Massachusetts

Participating investigators/ cooperative agencies

David Bengtson, University of Rhode Island/E.P.A., Rhode Island
Hsiang-tai Cheng, University of Maine-Orono, Maine
Pavinee Chinachottee, University of Massachusetts-Amherst, Massachusetts

Radu Giurca, Center for Applied Regional Studies, Massachusetts
Tom Handwerker, University of Maryland-Eastern Shore, Maryland
Reginal Harrell, University of Maryland-Horn Point, Maryland
Steven Hughes, Monell Chemical Senses Center/USDI, Pennsylvania
Linda Kling, University of Maine-Orono, Maine
Robert Levin, University of Massachusetts-Amherst, Massachusetts
George Nardi, New England Fisheries Development Foundation, Massachusetts
Paul D. Maugle, University of Rhode Island, Rhode Island
Steven Mulvaney, Cornell University, New York
Michael Rice, University of Rhode Island, Rhode Island
Kenneth Simpson, University of Rhode Island, Rhode Island

Objectives

1. To estimate the nutrient requirements for striped bass and hybrid striped bass.
2. To develop nutritionally complete, cost-effective feeds using alternative protein and energy sources.
3. To develop commercially feasible processes for producing aquaculture feeds while maintaining appropriate bioavailability of nutrients.
4. To transfer technology and management practices to industry.

Principal accomplishments

Objective 1

A. Larval requirements:

1. Inland silverside (*Menidia beryllina),* initially used as a surrogate species for larval striped bass, were shown to be not comparable to that species. Histology shows the larval silverside gastrointestinal tract is far more developed, while growth and survival data show that silversides are much better able to digest and absorb nutrients from artificial diets than are striped bass at the same stage.
2. Ascorbic acid supplementation does not appear necessary in larval striped bass diets, and ascorbic acid-2-sulfate may actually lower survival.
3. Practical “microbound” diets, in which ingredients are very finely pulverized and bound together into minute balls, gave survival rates and weight gains significantly better than those achieved with microencapsulated diets.
4. No artificial diet gave survival rates or weight gains comparable to those achieved with live food. However, during the course of this project, incremental improvements brought survival rates and weight gains up from initially negligible to approximately one-half of those achieved with live food. A series of attractants (sucrose, sucrose + glutamic acid, sucrose + glutamic acid + glycine, glutamic acid, taurine, and betaine) all showed no or very small effects on growth and survival, as did the commercial feed stimulant, Finn-Stim™, at all levels of inclusion. Because the striped bass larval intestinal tract is so poorly developed, protease inclusion was tested; this gave negative results which were regarded as inconclusive because protease activity was lost so rapidly from the feed. If protease could be protected, its inclusion could be valuable. A similar rationale governed the inclusion of hydrolysates, which are partially pre-digested, in the feed. Both herring and dogfish hydrolysates appeared to improve survival, but had a negative effect on growth. Again, more work needs to be done here. Perhaps most interesting was an experiment on inclusion of vitamins B6 and E (in lecithin), omega-3 HUFA (in algae), and carotenoid (in algae) in larval diets. Although the percent increase in survival was not statistically significant, it was intuitively impressive, with HUFA inclusion raising survival from 33% to 45%.

B. Adult fatty acid requirements:

1. Growth and survival of striped bass, white bass, and palmetto bass (striped bass female x white bass male) improved significantly with HUFA supplementation, especially EPA and DHA. These fish do not appear able to elongate shorter chain fatty acids into HUFAs. Although sunshine bass (white bass female x striped bass male) did not respond to HUFA supplementation, body HUFA profiles of unsupplemented fish decreased over the length of the study period, suggesting that the 6-month study period may have been insufficient to deplete endogenous HUFAs. (Good growth performance may also have been due to hybrid vigor.)

Objective 2

1. Vitamin A has been found to be highly toxic to sunshine bass; anecdotal evidence in the literature suggests that this may be true for other bass as well.

2. Processing wastes were liquefied by enzymatic digestion, followed by pasteurization, screening to remove bone, vacuum evaporation to 50% solids, and preservation either by freezing or acidification. All wastes were treated with antioxidant prior to heating. Both cod (*Gadus morhua*) and dogfish (*Squalus acanthias*) wastes were utilized. Dogfish livers were removed prior to processing to keep oil at manageable levels. Since cod is gutted at sea, digestion was carried out by adding papain. Dogfish were autolysed by endogenous enzymes. Hydrolysates were incorporated into moist diets for Atlantic salmon at the 40% level; control moist diets utilized raw herring. (Diets containing cod had herring oil added to make lipid levels comparable.)

Cod hydrolysate preserved by freezing was accepted by Atlantic salmon as well as the control diet. Despite the fact that all feeds incorporating acidified hydrolysates were neutralized up to pH 5.5, feeds containing acidified cod hydrolysate showed reduced palatability. Different acids reduced palatability differentially. Contrarily, dogfish hydrolysates performed best when acidified, matching the control diet in acceptability between pH 5.5 and 6.1. Neutral dogfish hydrolysate showed reduced palatability, presumably caused by the generation of ammonia from urea above pH 6.1.

Human taste panelists judged salmon fed control or dogfish hydrolysate-containing diets superior to salmon fed cod hydrolysate-containing diets. While statistically significant, this result was unexpected and the work should be repeated.

Atlantic salmon dry feed ingredients were made by co-drying dogfish hydrolysate with one of the following: (1) high quality, low temperature-dried fish meal; (2) high quality, low temperature-dried poultry meal; (3) soybean meal. The co-dried ingredients were 50% hydrolysate on a dry weight basis and were included in dry diets at a 60-70% level, replacing 3/4 of the diet's fish meal. Although results were statistically insignificant due to using only 14 fish/treatment and a 68-day feeding period, the performance of the co-dried dogfish hydrolysate ingredients was impressive, with both the hydrolysate-fish meal and the hydrolysate-poultry meal equaling (and possibly out-performing) the control diet. (We thank the WRAC nutrition group who alerted us to the possibilities of poultry meal in salmonid diets.)

Objective 3

Both enzymatic hydrolysis and co-drying are commercially viable processes carried out at multiple industrial sites in the U.S. and other countries. Because companies are somewhat secretive about enzymatic hydrolysis, and because the use of processing wastes which must be collected from multiple sites poses real problems, project researchers, aided by an engineer from a commercial company manufacturing evaporators for fish meal stick water, developed and costed out a process for producing a concentrated hydrolysate. This process starts at outlying cutting plants where wet wastes are stabilized for later collection and transported to a central facility for further processing.

Impacts

a. Findings on vitamin A toxicity in sunshine bass, and the fatty acid requirements of striped bass and its hybrids have been communicated to feed manufacturers.

b. Relevant Northeastern fish processors have been informed of the results of the study on hydrolysates. A computerized spread sheet is being developed which will allow feed manufacturers, processors, investors, or entrepreneurs to look at the economics of a hydrolysis plant. Feed manufacturers and aquaculture producers have been informed through multiple information outlets of the results of the hydrolysate trials.

c. An NRAC extension fact sheet on the nutritional requirements of adult and larval fish has been prepared and includes as many of this project's findings as are relevant.

Suggested future work

Given the steady, incremental improvement of survival and growth rates by striped bass larvae on the diets formulated in the course of this project, this work should be continued and its possible extension to endangered marine species explored.

Work on pasteurized hydrolysates to replace raw fish in moist feeds should be carried further and cost-benefit analyses performed. Alternate waste species should be considered. The possibility of wild-caught fish carrying pollutant loads should be noted.

The use of high quality poultry meal in salmonid diets is extremely promising and deserves further study.

Publications and presentations

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Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1990-91	$97,770	$199,752				$199,752	$297,522
1991-92	$106,916	$255,024				$255,024	$361,940
1992-93	$88,584	$184,440				$184,440	$273,024
Total	**$293,270**	**$639,216**				**$639,216**	**$932,486**

Commercial Field Trials of MSX-Resistant Strains of the American Oyster *(Crassostrea virginica)* (90-6)

Project period March 21, 1990 to August 15, 1995

NRAC funding

$194,941

Work group chair

Susan E. Ford, Rutgers University, New Jersey

Participating investigators/ cooperative agencies

Karl Rask, The Cape Cod Resource, Massachusetts
Mijin Lee, State University of New York, Stony Brook, New York
Monica Bricelj, State University of New York, Stony Brook, New York
Gordon Taylor, State University of New York, Stony Brook, New York
Christine Paillard, Universite De Bretagne Occidental, Brest, France
William Barnish, William Barnish Shellfish, New Jersey
Richard Drew, Yarmouth Oyster Farms, Massachusetts
Gef Flimlin, Rutgers University, New Jersey
Joel Fox, Old Wharf Shellfish, Massachusetts
Harold Haskin, Rutgers University, New Jersey
Richard Kraus, Aquaculture Research Corporation, Massachusetts
Mark Luckenbach, Virginia Institute of Marine Science, Virginia
George Mathis, Mathis and Mathis, Ltd., New Jersey
Don Merritt, University of Maryland, Maryland
William Mook, Mook Sea Farms, Maine
Michael Naughton, Yarmouth Oyster Farms, Massachusetts
Richard Nelson, Cotuit Oyster Company, Massachusetts
Chip Petre, Intertidal Marine Aquaculture, Virginia
Irving Puffer, Wellfleet Oyster and Clam Company, Massachusetts
David Relyea, F.M. Flower and Sons Oyster Company, New York
Gregg Rivara, Cornell University, New York
Sam Shriver, World's End Aquaculture, Maryland
Robert Wallace, Billingsgate Shellfish, Massachusetts

Objectives

- To compare survival, growth, market value, and MSX incidence in MSX-resistant strains and local controls, using commercial quantities and commercial methods.
- To evaluate performance of the MSX-resistant strains in different parts of the region, under different growing conditions.
- To make results available to industry, extension agents, and other investigators.

Progress and principle accomplishments

Commercial scale quantities of MSX-resistant and control oysters were tested for growth and survival by 11 growers in five states from Massachusetts to Virginia. Comparisons between the two stocks were made using 2-year classes, tested in different years and under a variety of growout conditions ranging from poor to good. MSX disease was rare in all locations during the study period, thus the primary "stressor" to be examined was not present. The relative and absolute performance of the two stocks varied much more among locations (and growout conditions) than according to genetic background. When the stocks received good care (i.e., were thinned regularly and appropriately), they performed similarly, but under conditions of crowding, the resistant strain had slower growth and lower survival than the unselected controls.

Juvenile Oyster Disease (JOD) and Dermo Disease became more important than MSX in the Northeastern U.S. during the study period. Investigations into the two diseases were incorporated into the Field Trials Project because they affected the test stocks and because both are serious threats to oyster culture in the Northeastern U.S. Pathological studies of JOD, in conjunction with other NRAC funded projects, helped to: 1) describe the disease syndrome, 2) establish diagnostic criteria for the disease, and 3) provide continuing evidence that bacteria, but no recognizable protozoan pathogens, are associated with the disease.

Diagnostic assays for the parasite that causes Dermo Disease (*Perkinsus marinus*) were incorporated into the routine measurements made on the field trials stocks. Along with similar assays on additional oysters collected throughout the Northeast, these documented the apparent spread of the parasite northward along the U.S. coast in the early 1990's, associated with a pronounced warming trend.

Neither MSX-resistant or control stocks performed well in commercial tests under heavy Dermo Disease pressure in Chesapeake Bay. More rigorous, small scale evaluations in lower Delaware Bay however, provided the first evidence that resistance to Dermo Disease has a genetic component and can be improved through selective breeding.

Dissemination of the results was on a casual basis. All sampling was done by extension agents working with the growers themselves. Information was circulated by agents and growers in direct one-to-one contacts or by talking among themselves at state aquaculture meetings and at regional meetings such as the annual Milford Aquaculture Seminar.

Impacts

Results of the study have enabled oyster growers in the Northeast to see first-hand and under their own growing conditions, how seed from a selected strain compares to that from wild parents. Growers are enthusiastic about the concept of field trials projects and would participate in future ones. Of at least equal importance, the study has demonstrated that a number of problems, including hatchery and growout operations, and Juvenile Oyster Disease, need to be solved before oyster strains selected for adult performance (e.g., MSX-disease resistance) become commercially-attractive. The appearance of diseases and growout problems other than MSX clearly demonstrate that a focus of future studies should be on improving overall health of cultured animal, not just dealing with a single pathogen or disease.

Recommended follow-up activities

1. Emphasize overall animal health in research and extension projects seeking to improve yield of cultured organisms.
2. Continue breeding to improve disease resistance.
3. Develop guidelines for field trials projects in which industry participation is essential, but may not provide results that can be scientifically evaluated.

Publications and presentations

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Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1991-92	$43,078	$24,407	$14,893		$4,500		$86,878
1992-93	$46,160	$24,407	$14,893		$4,500		$89,960
1993-94	$35,430	$24,407	$14,893		$4,500		$79,230
1994-95	$33,388	$24,407	$14,893		$4,500		$77,188
Total	**$158,056**	**$97,628**	**$59,572**		**$18,000**		**$333,256**

Support for Extension Education (90-7)

Project period October 10,1990 to October 1,1991

NRAC funding
$1,600

Coordinator
George E. Flimlin, Rutgers University, New Jersey

Participants
Aquaculture extension agents in the Northeast

Overview
Modest travel stipends were provided to pay for travel to the National Shellfish Association Annual Meeting by Northeast extension agents associated with NRAC's oyster genetics projects. The meeting included presentations by NRAC oyster genetics projects participants.

Objectives
- To provide additional training opportunities in oyster genetics and technology transfer for cooperative and Sea Grant extension personnel.
- To facilitate the transfer of information between research and extension personnel.

Principal accomplishments
Five Northeast extension agents attended the National Shellfish Association Annual Meeting held in Williamsburg, VA, April 1-5, 1990.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1990-91	$1,600						$1,600
Total	**$1,600**						**$1,600**

Systems Technology Workshop (90-8)

Project period February 13,1990 to February 13,1991

NRAC funding
$25,000

Coordinator
Henry S. Parker, NRAC

Overview
NRAC has identified Aquaculture Systems Technology as one of its funding priority areas. An NRAC objective is for the center to serve as a catalyst for the development of appropriate aquaculture technology that addresses the regional climate, political realities, orientation and characteristics of people, and legal constraints.

A workshop, designed to attract representatives from a broad spectrum of the aquaculture industry was organized to generate information and provide a direction for regional projects in systems technology.

Objectives

- To provide a forum for information exchange among key representatives of industry, research, and extension.
- To develop an information base concerning the present status of aquaculture systems technology in the Northeast.
- To identify and prioritize systems technology research interests and needs.
- To assess opportunities for inter-regional collaboration.

Accomplishments

1. An NRAC Systems Technology Workshop was held in Baltimore, Maryland, on February 9-10, 1990.

The purpose of the workshop was to identify principal priority areas for NRAC projects in aquaculture systems technology.

2. Two priority areas were identified for NRAC project development:

a) development of new technology for assessing finfish biomass in aquaculture enclosures; and

b) characterization and amelioration of aquaculture wastes through development and application of new technologies.

Problem statements were subsequently developed for each priority area, regional work sessions were held, Work Groups were established, and proposals have been prepared for consideration by NRAC.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1988-89	$25,000						$25,000
Total	**$25,000**						**$25,000**

Detection of Fish Pathogens for Fish Health Inspection by Non-lethal Methods (91-1)

Project period March 21, 1991 to March 20, 1993

NRAC funding

$310,000

Coordinator

Pei W. Chang, University of Rhode Island, Rhode Island

Participating investigators/ cooperative agencies

Eileen E. Sadasiv, University of Rhode Island, Rhode Island
Paul R. Bowser, Cornell University, New York
Joseph K. Buttner, SUNY-Brockport, New York
Frank M. Hetrick, University of Maryland, Maryland
S.K. Samal, University of Maryland, Maryland
Phillip E. McAllister, U.S. Fish and Wildlife Service, West Virginia
Bruce Nicholson, University of Maine, Maine
John T. Singer, University of Maine, Maine
Paul W. Reno, Oregon State University, Oregon

Objectives

- To develop a non-lethal tissue sampling method (biopsy technique) for obtaining kidneys or other body tissues and to compare the recovery of fish pathogens in fish tissues or body fluid (gonadal fluid, feces, mucus, and blood) collected by lethal versus non-lethal sampling methods.
- To develop and compare the rapidity, sensitivity, specificity, and cost of the IPNV detection in samples taken by lethal and non-lethal sampling methods.
- To develop and test the efficiency of a time-resolving fluoroimmunoassay for the detection of

R. salmonirarum in tissues and body fluids collected by non-lethal sampling methods of fish.

- To develop and test the efficiency of monoclonal antibody based indirect ELISA for the detection of *A. salmonicida.*

a) To develop a solid phase ELISA for the detection of IPNV-specific antibodies. To compare the sensitivity and specificity of the ELISA with a serum neutralization test.

b) To correlate the antibody production and virus production of adult Atlantic salmon to IPNV following infection at 6°C and 12°C.

c) To survey fish stocks in hatcheries and in the field for their IPNV antibody levels and correlate their immune status with virus isolation and current and past history of IPNV outbreaks among them and their progeny.

- To transfer the technology of non-lethal sampling and improved detection methods to workers doing fish inspections by means of workshops and scientific publications. To inform fish producers on the availability and benefits of fish health inspection procedures that include non-lethal sampling.

Principal accomplishments

Tissue biopsies can be obtained from adult salmonids using a non-lethal surgical procedure. Adequate quantities of tissue can be obtained and used for disease diagnosis and fish health inspection purposes.

Comparison of methods used for detection of infectious pancreatic necrosis (IPN) virus in tissues and fluids of virus-carrier salmonids were carried out. Specimens of kidney (by lethal sampling) and ovarian fluid pellet (by non-lethal sampling in spawning fish), provide the highest level of sensitivity for assessing the prevalence of IPNV. Specimens of surface mucus, feces, and fluids from male and female reproductive products collected by non-lethal sampling can be used to assess the prevalence of IPNV in populations of fish.

Stress was induced by elevated water temperature and treatment with immunosuppressant enhanced virus titer and virus prevalence in populations of fish.

Nested primers were developed for a polymerase chain reaction (PCR) assay. The PCR assay was sensitive for the detection of virus in clinical samples and, by combining primers, IPNV, infectious *hematopoietic necrosis* virus, and viral *hemorrhagic septicemia* virus could be detected and distinguished in a single assay. The PCR assay provides a more rapid and less expensive detection method for IPNV than the standard virus isolation assay using cell cultures.

A dot-blot nucleic acid hybridization test was efficient in detecting American strains of IPNV in infected cell culture using clones of Ds RNA. The probe was unable to detect viral RNA directly in infected fish but was 100% effective in detecting viral RNA in cells inoculated with infected fish tissues.

Renibacterium salmoninarium, the causative agent of bacterial kidney disease, is three times and seven times more frequently found in kidneys than in sex products and feces, respectively.

Serum antibodies to IPNV were assayed in Atlantic salmon by virus neutralization test. Equal numbers of two age groups (17 and 27 months) were kept at one of three temperatures (6, 10, and 16°C). At various times following IPNV infection, the fish were bled by non-lethal methods and their sera tested for anti-IPNV antibodies. The majority of the fish developed detectable immune responses at all three temperatures. The final titer after 6 months was similar in all groups.

Eight hatcheries containing brook trout, rainbow trout, and steelhead trout were studied for IPNV virus isolation (from kidneys, spleens, and feces) and serum antibodies. Serum antibodies were detected in seven hatcheries where the virus was isolated. In one hatchery (the steelhead trout) where virus was not isolated, no antibodies were detected. Screening for IPNV antibodies in blood serum from fish populations can be developed as a non-lethal sampling method for fish inspection.

The extension component of this project was to transfer technology of non-lethal sampling and improved detection methods to workers doing fish inspection. These objectives were achieved by two publications and one workshop.

Suggested future work

A hands-on workshop to demonstrate non-lethal surgical procedures should be held for interested individuals who have a need for such technique. The comparative intensity of the assay systems for IPNV using polymerase chain reaction and cell culture should be definitively established.

The inability of the dot-blot nucleic acid hybridization test to detect viral RNA directly in infected fish tissue was a disappointment. Studies to improve the sensitivity of the cDNA probes should be done by improving the RNA extraction procedure from the infected fish.

The techniques and methods of non-lethal sampling and diagnosis should be collected and identified. Approval to use these techniques in approved fish inspection protocols must be obtained from federal, state, and provincial regulatory agencies. Industry must be made aware of the procedures after they have been accepted as a standard method. Finally, procedures must be made available to industry in a cost-effective manner.

Publications and presentations

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McAllister, P. E., W. J. Owens, and W. B. Schill. 1994. Effects of elevated water temperature and immunosuppressant treatment on prevalence and titer of infectious pancreatic necrosis virus in naturally infected brook trout. J. Aquatic Animal Health 6:133-137.

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Wooster, G.A., H. M. Hsu, and P. R. Bowser. 1992. Surgical biopsies from rainbow trout for fish health inspection purposes. 1992 Annual Meeting of the Fish Health Section of the American Fisheries Society and Eastern Fish Disease Workshop. Auburn, AL. (Abstract).

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1991-92	$156,015	$153,630		$53,310			$362,955
1992-93	$153,985	$164,904		$55,340			$374,229
Total	**$310,000**	**$318,534**		**$108,650**			**$737,184**

Unexplained Oyster Mortalities of Hatchery-Reared Oysters, *Crassostrea virginica*, in the Northeast: A Preliminary Study (see also 92-5 & 92-9) (91-2)

Project period 1 year beginning May 1991

NRAC funding

$11,828

Work group chair

Susan E. Ford, Rutgers University, New Jersey

Participating investigators/ cooperative agencies

V. Monica Bricelj, State University of New York, Stony Brook, New York
Francisco J. Borrero, State University of New York, Stony Brook, New York

Objectives

- To develop and distribute an epidemiological questionnaire or survey among commercial oyster growers along the Northeast Coast that would allow documentation of mortality episodes occurring at each site during the 1991 growing season, as well as provide documentation on past mortalities.
- To design, coordinate and implement a sampling program at three key oyster growing locations; the Damariscotta River, Maine, Oyster Bay, New York, and Fishers Island, New York, that will provide an initial database for characterization of the problem.

Publications and presentations

Bricelj, V. M., S. E. Ford, F. J. Borrero, F. O. Perkins, G. Rivara, R. E. Hillman, R. A. Elston, and J. Chang. 1992. Unexplained Mortalities of Hatchery-Reared, Juvenile Oysters, *Crassostrea virginica*. J. Shellfish Res. 11:331-347.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1991-92	$11,828	$7,028	$5,000				$23,856
Total	**$11,828**						**$23,856**

Water Quality and Waste Management in Aquaculture Production (91-3)

Project period August 1991 to August 15, 1995

NRAC funding

$200,800

Work group chair

Thomas A. Hopkins, Center for Applied Aquatic Research, Maryland

Participating investigators/ cooperative agencies

Kenneth Bergstrom, Red-Wing Meadow Farm, Massachusetts
John Ewart, University of Delaware, Delaware
Conrado Gempesaw, University of Delaware, Delaware
Thomas Handwerker, University of Maryland Eastern Shore, Maryland

Reginal Harrell, University of Maryland, Horn Point, Maryland
Michael Moore, US Fish and Wildlife Service, Pennsylvania
Ernie Tresselt, Creek Fisheries, Inc., Maryland, Maryland
Fred Wheaton, University of Maryland College Park, Maryland

Objectives

- Develop a database describing water quality charac- teristics of representative aquaculture production facilities in the Northeastern U.S.
- Measure critical properties of culture systems dynamics and effluent waters in representative production systems.
- Develop criteria and methodology to test and evaluate production, waste, and system management technologies with a uniform format.
- Transfer information to industry and other production personnel.

Publications and presentations

Ewart, J. W., J. Hankins, and D. Bullock. 1995. State policies for aquaculture effluents and solid wastes in the Northeast Region. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 95-002 (Formerly NRAC Bulletin #300). North Dartmouth, MA. 24pp.

Harrell, R. M., W. Van Heukelem, and J. A. Urban. 1994. Wastewater effluent characterization in brackish water Maryland hybrid striped bass production ponds. Book of Abstracts World Aquaculture '94. New Orleans, LA. World Aquaculture Society. 1994:223.

Lussier, W., J. Bacon, and C. Gempesaw. 1994. A survey of waste management practices of aquaculture producers. Delaware Agricultural Experiment Station Bulletin, Department of Food and Resource Economics, University of Delaware.

Wheaton, F. W. and J. D. So. 1993. Environmental dynamics of commercial koi ponds. Presentation given before the Techniques for Modern Aquaculture Conference, Spokane, Washington. (This conference was jointly sponsored by World Aquaculture Society and the American Society of Engineers).

Wheaton, F. W. and J. D. So. 1994. Comparison of physical conditions in side-by-side fish ponds. Presented at the World Aquaculture Society/ Aquaculture Engineering Society meeting in New Orleans, LA. Book of Abstracts World Aquaculture '94. 1994:37.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1991-92	$150,500	$54,000	$28,000	$15,000			$247,500
1992-93	$50,300	$22,000	$5,000				$77,300
Total	**$200,800**	**$76,000**	**$33,000**	**$15,000**			**$324,800**

Support of the Fifth International Conference on Toxic Marine Phytoplankton (91-4)

Project period 1 year beginning March 1991

NRAC funding
$5000

Major participants
Theodore J. Smayda, University of Rhode Island

Objectives
- Provide partial funding support to publish the Conference proceedings; collate, prepare, and synthesize scientific material for discussion at Conference workshops; and prepare a bibliography for distribution at the Conference.
- The Conference objectives: a) provide a better scientific understanding of the environmental conditions triggering and regulating toxic bloom; b) facilitate site selection for sea farming activities and allow more effective management of these commercial ventures; and c) present findings from researchers and plan new interdisciplinary, multi-national collaborative research.

Principal accomplishments
The conference was held on October 28 to November 1, 1991, and was attended by researchers and industry representatives from around the world.

Publications and presentations
Smayda, T. J. and Y. Shimizu, eds. 1993. Toxic phytoplankton blooms in the sea. Proceedings of the 5th International Conference on Toxic Marine Phytoplankton. October 28 - November 1, 1991.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1991-92	$5,000						$5,000
Total	**$5,000**						**$5,000**

Northeastern Regional Aquaculture Center Newsletter-1991 (see also 90-4, 92-8) (91-5)

Project period January 1, 1992 to December 31, 1992

NRAC funding
$11,415

Work group chair
Joseph K. Buttner, State University of New York, Brockport, NY

Participating investigators/ cooperative agencies
Gregg Rivara, Cornell University, New York

Overview
A regional newsletter is being published to address the need for timely and accurate dissemination of information about aquaculture in the Northeast and to

describe the activities and projects of NRAC. This quarterly publication deals with recent developments in aquaculture research and commercial operations in the region. It also publicizes the contribution of NRAC to regional aquaculture activities.

Objectives

- To publicize the contributions of NRAC to regional aquaculture development.
- To disseminate results of aquaculture research and extension activities supported by NRAC.
- To provide a forum for the regional and national exchange of aquaculture information.
- To encourage support for aquaculture development.

Principal accomplishments

The Newsletter originated in 1989 as a mechanism to inform the aquaculture community and others in the Northeast about NRAC activities. Since 1990, the Newsletter has been edited by SUNY Brockport and Cornell Cooperative Extension. Number of issues printed and mailed has gradually increased from slightly over 1,500 to nearly 3,200. The format and layout has been standardized with two-colors on the front and back. Articles highlight industry activities and NRAC projects.

The Newsletter represents perhaps the most effective, efficient, and economical mechanism to facilitate the timely transfer of information, to ensure that all parties are kept informed of important developments, and to promote the efforts of NRAC. Feedback from the readership has increased dramatically. More articles are being submitted and revision of the mailing list is a dynamic event. Additions, deletions, and changes are common. Between 20-50 new names are added between issues.

Publications and presentations

Developed, printed, and distributed three issues of "Northeastern Aquaculture," newsletter for the Northeastern Regional Aquaculture Center: Volume 3(3) Fall 1991; Volume 3(4) Winter 1991; Volume 4(1) Spring 1992.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1989-90	$15,000						$15,000
1990-91	$11,415						$11,415
1991-92	$18,000						$18,000
Total	**$44,415**						**$44,415**

National Extension Workshop Support (91-6)

Project period 1 year beginning March 1991

NRAC funding

$13,000 + $5,000
Total $18,000

Work group chair

George E. Flimlin, Rutgers Cooperative Extension, New Jersey

Participants

One extension agent from Washington, D.C. and from each of 12 states in the Northeast U.S.

Objectives

- Improve Extension's information and delivery system for aquaculture in concert with identified national initiatives and critical issues.

- Enhance the development of new state extension educational programs in aquaculture by focusing on innovative programs that have enhanced commercial aquaculture development in the past.
- Examine the scope of Extension efforts in aquaculture at the state, regional, national, and international levels.
- Identify opportunities and needs to enhance the development of regional and interregional extension programs in aquaculture.
- Strengthen communication networks, sharing of educational materials, programs, and expertise among aquaculture extension specialists and agents.
- Inform extension aquaculture specialist to ongoing, cutting edge research efforts, linkages, research trends, research need identification, research databases, and strengthen research and extension linkages.
- Examine team building and multi-disciplinary approaches to extension programming in aquaculture.
- Address national, regional and state issues impacting on educational programs, and state and federal legislation.
- Examine major trends in commercial aquaculture and emerging opportunities.
- Define Extension's role in developing support programs (infrastructure) in aquaculture, targeting non-producer audiences (i.e., lending institutions, marketers, processors, consumers, and youth).
- Explore the use of new and innovative reporting techniques.

Principal accomplishments

Specialized training was provided for over 90 extension scientists from at least 43 states during the National Extension Aquaculture Workshop held earlier this month. The 4-day workshop was held at the 4-H Center in Ferndale, Arkansas. Featured were speakers from many agencies and organizations that impact on aquaculture production, marketing, and federal policy in the United States.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1993-94	$13,000						$13,000
Total	**$13,000**						**$13,000**

Alternative Marketing Options to Improve Profitability of the Northeast Aquaculture Industry (92-1)

Project period May 1, 1992 to December 30, 1994

NRAC funding

$200,000

Work group chair

Gregory Hanson, Pennsylvania State University, Pennsylvania

Participating investigators/ cooperative agencies

Robert Gempesaw, University of Delaware, Delaware
Catherine Halbrendt, University of Delaware, Delaware
Hsiang-Tai Cheng, University of Maine, Maine
James Wilson, University of Maine, Maine
Brad Powers, Maryland Department of Agriculture, Maryland
Alberto Manalo, University of New Hampshire, New Hampshire
Bruce Lindsay, University of New Hampshire, New Hampshire
Linda O'Dierno, New Jersey Department of Agriculture, New Jersey
Ken Gall, New York Sea Grant, New York
Robert Hermann, Pennsylvania State University, Pennsylvania

Milton Madison, Pennsylvania State University, Pennsylvania
James Anderson, Pennsylvania State University, Pennsylvania
Cathy Wessells, Pennsylvania State University, Pennsylvania

Objectives

- To identify consumer and seafood buyer preferences on product attributes, value added products, and seafood safety concerns affecting purchase decisions of Northeastern aquaculture products.
- To reduce institutional marketing constraints and to promote niche and value added marketing opportunities for Northeast aquacultural products.

Anticipated benefits

The project will increase the marketing effectiveness, efficiency and profitability of Northeast aquaculture producers.

Principal accomplishments

A. Consumer preferences and seafood safety.

Ten thousand surveys were mailed to random households dispersed across states according to the state's share of the total population for the region.

- The sampling procedure outlined above resulted in a return of 1,533 shellfish surveys and 1,529 finfish surveys; (ca. 30% response rate).
- Ninety-seven percent of respondents in each survey consume seafood in some form either at home or at restaurants.
- Thirty percent of the total survey sample consume fresh seafood as often as every week.
- Most consumers are likely to buy fresh seafood at supermarkets and seafood markets. The most frequently cited reason for purchasing these particular species products is that respondents like the taste and wish to add variety to their diets.
- Respondents also reported that they consider seafood a novelty and thought it was easy to prepare.
- Consumers in the New England states are, in general, more frequent consumers of seafood (in particular, of salmon, clams, and mussels).

B. Analysis of Demand for Finfish and Shellfish

Several important observations were found based on the survey and model results. First, among the three finfish products, hybrid striped bass was the least known followed by trout and salmon.

- It is critical that this information be known to producers because of the tremendous interest among aquaculture producers in the region to produce hybrid striped bass.

Based on the evoked set theory, if a product, e.g., hybrid striped bass is not part of the choice set or awareness set of the consumer, it does not have a chance of being purchased. Hence, producing this species for Northeast consumers who are not aware of the species will have immediate negative repercussions on the potential growth of the hybrid striped bass industry. In addition, there is a need to make consumers aware of the positive attributes of this species.

Second, among all the explanatory variables, experience, knowledge that seafood products are farm-raised, stories from the media were found to play a significant role in influencing perceptions, preferences, and choice of aquaculture products.

- One key question is how to provide the necessary "experience" to the potential consumer since it was found that if a consumer has purchased seafood in the past the chances of buying finfish or shellfish products are relatively high.
- It was also found that if consumers were aware that the seafood product was produced from an aquaculture farm, the perceptions and preferences of these consumers were positively affected.

C. Product attributes affecting wholesale clam buyers.

- Minimum shelf-life expectations vary among hard clam buyers; 21% require 6 or fewer days, 38% require 7-8 days, 25% require 9-12 days, and 15% require 14 days or more. Payment terms also vary with 29% paying on delivery, 6% within 2-7 days, 27% within 14-15 days, and 18% within 21-30 days.
- Of various attributes affecting hard clam purchase decisions "meat quality" (free of sand, mud, and shell bits) was ranked most important by 61% of buyers surveyed.

D. Consumer, retailer and food service business preferences.

- Consumers are not widely aware that many of the products that they purchase are aquaculture products. Public education and merchandising materials designed for use in retail stores and restaurants would help consumers identify and better understand aquaculture products.

- Aquaculture producers should be aware that taste and nutrition are two of the most important positive attributes of seafood products to consumers. Education and merchandising techniques that address these attributes could be effectively used to market aquaculture products.
- Consumers consider product “freshness” as the most important factor that determines what they buy. Educational and promotional materials that describe potential quality of “freshness” and advantages associated with controlled aquaculture production, could be used to more effectively market products.
- Aquaculture producers should consider how consumers prepare seafood most frequently, and should be aware that many consumers prefer products that are convenient and easy to prepare. Primary processed products such as fillets and steaks that can be readily prepared by baking, broiling, barbecuing, or stir frying, would be desirable in the New York and New Jersey marketplace.
- Aquaculture producers should consider the type of value-added or prepared seafood products that consumers purchase in this area. Soups, chowders, and both fresh and frozen, ready-to-cook entrees are the most frequently purchased products for every day use. Other alternative products such as fish cakes, smoked fish spreads, and pates that can be constructed using the by-products of the primary processing operation could be produced as specialty items.

E. Seafood counters in grocery stores.
From the aquaculture perspective, more seafood counters provide additional outlets for their product and expose potential new customers to an alternative fish food they may not have otherwise considered. A separate seafood counter increases visibility and improves handling practices. Results of the study include:

- A store with a rural location is only 0.75 times as likely to have a seafood counter and 0.75 times as likely to add one, as is a urban/suburban store.
- Chain stores (especially those with more than 40,000 sq. ft. of floor space) are more likely to have a seafood counter.
- A store with weekly sales of more than $99,000 is 1.80 times more likely to have a seafood counter, and 2.46 times more likely to add a seafood counter than a store with sales of less than $40,000.
- A store with high income clients is 3.54 times more likely to have a seafood counter and 1.86 times more likely to add a seafood counter than a store with primarily low income clients.

Impacts

a. The consumer preferences and seafood safety surveys conducted by Wessells, Manolo, and Gempesaw have generated numerous journal article submissions and reports. The survey responses to both the finfish and shellfish surveys now constitute the best source of marketing data available in the Northeast.

The NRAC/University of Rhode Island report “Consumer Preferences for Northeastern Aquaculture Products: Report on the Results from a Survey of Northeastern and Mid-Atlantic Consumers,” is likely to be a standard reference for marketing by aquaculturists both in the Northeast and throughout the U.S.

The seafood safety information in the surveys will be particularly useful in addressing producer and consumer concerns regarding the safety and healthfulness of shellfish and finfish products.

b. A more than 100-page report “Aquaculture Marketing Survey: Consumers, Retail Stores, and Food Service in New York and New Jersey,” by Gall and O’Dierno is likely to set a standard for consumer intercept and focus group market research. Of note is the chapter entitled: “Market Opportunities for Value Added Seafood Products,” which offers a practical guide to producers seeking to improve profit margins through additional service and/or processing. In addition, each chapter includes a “Summary of Market Opportunities” describing numerous market openings for aquaculturists in the Northeast Region.

c. The research conducted at the University of Maine provides comprehensive analysis of restaurant issues, including the potential for “white tablecloth” restaurants which are more likely to be a strong growth market for aquaculturists. This research also provides landmark analysis of barriers in the marketing chain that affect distributors of aquaculture products.

d. The University of Delaware analysis of retailer preferences for Atlantic salmon, tilapia, rainbow trout, and catfish points the way to market ideal aquaculture products with reference to product attributes including size, freshness, and price. The "ideal" fish analysis of this component of the project will better prepare Northeast producers for the competitive forces of the future.

Recommended follow-up activities

Focus on aquaculture product prices

Because price is such an important consideration in the seafood purchase decision, it would be helpful to determine whether middle income consumers are willing to pay a differential for aquaculture products.

Nation-wide marketing survey to better enable producers to understand marketing opportunities, and to update the 1988 marketing results

Conducting a national marketing survey would provide a much-needed comprehensive evaluation of the current demand for aquaculture products.

Hard clam market analysis

Further efforts in extending market research to Northeast hard clam aquaculturists is recommended, particularly focusing on methods to differentiate aquacultured products in the perspective of the consumer.

Value-added directory

The directory of value-added aquaculture products for Northeast producers was not adequately funded in this marketing project. Given the importance of development of niche markets for high profit, value-added products, additional research focusing on potential in the high income and densely populated Northeast is recommended.

White tablecloth restaurants

Higher costs of production and fresher product suggest that one of the best avenues for future growth of high value aquaculture is to focus on the relatively high price-per-meal restaurants. This area of marketing research provides a huge potential with high profit margins for producers. Problems that merit analysis are the provision of consistent and stable product supplies. Also, producers need better information on the prices that restaurants are willing to pay for a fresh, high quality, consistent product.

Publications and presentations

Gall, K. and L. O'Dierno. 1994. Aquaculture marketing survey: Consumers, retail stores, and food service in New York and New Jersey. New York Sea Grant and New Jersey Department of Agriculture.

Hanson, G. D., R. O. Herrmann, W. Delavan, and J. Qian. (Unk.) Aquaculture marketing in Pennsylvania and the Northeast. An Agricultural Economics and Rural Sociology Research Report (Forthcoming).

Hanson, G. D., Rauniyar, and R. O. Herrmann. 1994. Using consumer profiles to increase the U.S. market for seafood: Implications for aquaculture. Aquaculture. 127: 303-316.

Henderson, N. R. 1994. Product attributes affecting cultured hard clam purchase decisions. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 94-002 (also New Jersey Sea Grant-94-290 and Rutgers Cooperative Extension E-178). North Dartmouth, MA. 8pp.

Henderson, N. R. 1994. Aquaculture trade leads 1993. New Jersey Sea Grant-94-296 (also Rutgers Cooperative Extension Technical Report E-182).

Henderson, N. R. 1994. Wholesale buyer preferences for cultured hard clams. Abstracts of the 39th Annual Atlantic Fisheries Technological Conference, Hyannis, MA.

Henderson, N. R. 1992. AquaLine - Aquaculture Marketing Project Funded by NRAC. The Jersey Shoreline 14(3).

Herrmann, R. O., G. P. Rauniyar, G. D. Hanson, and G. Wang. 1994. Identifying frequent purchasers in the Northeastern U.S. Agricultural and Resource Economics Review. 23: 226-235.

Herrmann, R. O. and G. D. Hanson. (Unk.) A profile of aquaculture operations in Pennsylvania. An Agricultural Economics and Rural Sociology Research Report (Forthcoming).

Manalo, A. B., C. R. Wessells, and C. M. Gempesaw. Consumer attitudes toward quality and safety of Aquaculture products. Proceedings of the VIIth International Conference of International Institute of Fisheries Economics and Trade, July 1994, Taipei, Taiwan. (In Press).

Nauman, F. A. 1995. Modeling the Demand for Seafood Using the Evoked Set Framework. M.S. Thesis. Department of Food and Resource Economics, University of Delaware, DE.

Nauman, F. A., C. M. Gempesaw, J. R. Bacon, and A. Manalo. 1995. Consumers choice for fresh fish: Factors impacting purchase decisions. Mar. Resource Economics J. 10:117-142.

Wessells, C. R., S. F. Morse, A. Manalo, and C. M. Gempesaw II. 1995. Consumer preferences for northeastern aquaculture products: Report on the results of a survey of Northeastern and Mid-Atlantic consumers. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 94-004. North Dartmouth, MA, and Rhode Island Experiment Station Publication #3100, University of Rhode Island, RI. 54pp.

Papers presented

Bacon, J. R., C. M. Gempesaw II, A. Manalo, and C. R. Wessells. Quality and safety issues for farm-raised fish: Opinions of Northeast Regional consumers. Presented at the Mid-Atlantic Aquaculture Region Conference, Fredericksburg, VA, June 1994.

Henderson, N. R. 1994. Product attributes affecting cultured hard clam purchase decisions. Abstracts of World Aquaculture '94 Conference. World Aquaculture Society. New Orleans, LA.

Henderson, N. R. 1993. Marketing research and extension program to expand markets for New Jersey aquaculture products. Abstracts of 1993 South Atlantic Regional Aquaculture Conference. U.S. Chapter-World Aquaculture Society. Hilton Head, SC.

Manalo, A. B., C. R. Wessells, and C. M. Gempesaw II. Consumer attitudes toward quality and safety of aquaculture products. Presented at the VII[th] International Conference of the International Institute of Fisheries Economics and Trade, July 1994, Taipei, Taiwan.

Nauman, F. A., C. M. Gempesaw, J. R. Bacon, C. R. Wessells, A. Manalo, and W. W. Lussier. Consumer choice for fresh fish: Factors impacting purchase decisions. Paper presented at the World Aquaculture Society meetings, February 1995, San Diego, CA.

Wessells, C. R., C. M. Gempesaw II, and A. Manalo. Consumer preferences for farm-raised seafood: Results from a survey of Northeast consumers, presented at the Atlantic Fisheries Technological Conference, Hyannis, MA. September 1994.

Wessells, C. R., C. M. Gempesaw II, and A. Manalo. Consumer awareness and perceptions of farm-raised shellfish: Implications for marketing. Paper presented at the World Aquaculture Society meetings, February 1995, San Diego, CA.

Wessells, C. R., C. M. Gempesaw II, and A. Manalo. Effects of consumer preferences and attitudes regarding shellfish on product demand, to be presented at the Milford Aquaculture Seminar, February 1995, Milford, CT.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1992-93	$114,214	$119,237					$233,451
1993-94	$85,786	$119,237					$205,023
Total	**$200,000**	**$238,474**					**$438,474**

Domestication of Striped Bass for Aquaculture (92-2)

Project period May 8, 1992 to August 31, 1994

NRAC funding

$198,128

Work group chair

Bernard Petrosky, Delaware State College, Delaware

Participating investigators/ cooperative agencies

William Bason, Delmarva Aquatics, Delaware
Joseph Buttner, SUNY Brockport, New York
John Foltz, Aquafarm Products, Inc., Delaware

Reginal M. Harrell, University of Maryland, Maryland
Scott Lindell, Aquafuture, Inc., Massachusetts
Steven VanGorder, Fresh Culture Systems Inc., Pennsylvania
Donald Webster, University of Maryland, Maryland
L. Curry Woods, University of Maryland, Maryland
Yonathan Zohar, University of Maryland, Maryland

Objectives

- Develop techniques to effectively capture, hold, and spawn wild striped bass, and to raise their progeny and existing captive stocks to reproductive maturity under various commercial production scenarios.
- Develop reliable techniques (including the use of critical temperature and photoperiod cycles) for inducing spawning of striped bass and hybrids on a year-round basis.
- Transfer information and technology to the striped bass cultivation industry.

Anticipated benefits

The proposed project will benefit the striped bass industry by aiding in the development of captive broodstocks, which are a desirable facet of any aquaculture industry and necessary to any potential selective breeding program. Information on the performance of the various lines produced will be made available for evaluation. The second objective will provide valuable techniques to manipulate striped bass spawning. In addition, the results of this project will provide further information regarding handling, maintaining, and spawning these fish, and will be acquired in a commercial aquacultural milieu. The participants will also become a resource through their training and hands-on experience during this project.

Principal accomplishments

Nine different cohorts of striped bass were produced and evaluated through larval culture phases. Animals from five of these cohorts have been grown to sizes of about 1 kg and potentially form the basis for one or more lines of domesticated striped bass.

This project demonstrated that exposing striped bass to 12-month, phase-shifted environmental regimes results in a shift of gonadal cycles, which follow the imposed external regimes. The ovarian and hormonal patterns in the shifted groups appear to be normal and fully entrained to the new cycles after 12 to 18 months of exposure to the shifted conditions. Females in the shifted groups can be induced to spawn out of season using a GnRHa sustained-release delivery system.

A survey of members of the Striped Bass Growers Association was conducted to assess their opinions of which traits should be developed by future breeding and selection. Survey results were presented at an international meeting and will also be reported via other channels. The University of Maryland's "Striped Bass and Hybrid Short Course" was held during April 1993 (with several course participants remaining in striped bass production). Extension personnel will facilitate continued communication between producers and researchers on industry desires and fish performance.

Impacts

- Five families of striped bass are presently under cultivation and have shown differences in growth performance.
- Development of domestic broodstocks allows control over reproduction and selection for genetic traits.
- Techniques to manipulate striped bass spawning have been developed so that captive fish can be successfully spawned at various times throughout the year.
- Information on producer's desires for striped bass traits have been collected to guide future efforts in selective breeding.
- Several participants in the "Striped Bass and Hybrid Short Course" remain in striped bass production.

Recommended follow-up activities

There is considerable evidence from the data gathered here that pure striped bass may grow as well in a commercial aquaculture setting as hybrid striped bass. Given that experimental culture temperatures were below optimum, these striped bass may grow faster than hybrids. Factors for future exploration are: the effects of lower salinities, and higher densities on striped bass growth, and the susceptibility of striped bass to handling stress. One bright note for the future of striped bass in recirculating aquaculture is that they are significantly more tolerant of sub-lethal levels of ammonia than hybrids, however they may be more susceptible to oxygen depletion.

Selected families from the Crane Aquaculture Facility and families collected from wild strains should be examined side-by-side to explore the possible production advantages of different strains and Crane's domesticated stock when subjected to commercial densities and handling stress. A future breeding program would exercise the most advantage by selecting the best strain and families of striped bass and white bass (exploiting additive genetic variance) and then hybridizing these striped bass with the white bass (exploiting dominant genetic variance). Such a breeding program will require careful coordination between multiple commercial and academic collaborators in at least the NRAC and NCRAC regions, and possibly nationwide.

Although manipulation of gonadal development spawning time was successful, quality of eggs produced was not always satisfactory. Based on experience with other fish species, low egg quality may be caused by an inadequate broodstock diet. Thus, we highly recommend an emphasis on future research regarding striped bass broodstock nutritional requirements in order to enhance the development of striped bass broodstock diet.

Publications and presentations

Mylonas, C., Y. Gothilf, S. Hassin, J. Stubblefield, and Y. Zohar. 1993. The manipulation of spawning in striped bass: Endocrine and molecular approaches. In Proceedings of the Annual Symposium of the Maryland Biotechnology Institute. 54pp.

Papers Presented

Mylonas, C., L. C. Woods, P. Thomas, and Y. Zohar. 1993. Endocrine approaches for the manipulation of ovulation and sperm production of captive striped bass *(Morone saxatilis*). Proceedins of the '93 World Aquaculture Society Meeting.

Mylonas, C., L. C. Woods, P. Thomas, and Y. Zohar. 1993. Endocrine approaches for the manipulation of ovulation and sperm production of captive striped bass (*Morone saxatilis*). XII International Congress of Comparative Endocrinology, Toronto, Canada.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1992-93	$110,269	$17,539	$24,900			$42,439	$152,708
1993-94	$87,859	$8,340	$19,546			$27,886	$115,745
Total	**$198,128**	**$25,879**	**$44,446**			**$70,325**	**$268,453**

A Comprehensive Investigation of Larval Development and Mortality in the Eastern Oyster (*Crassostrea virginica*) (92-3)

Project period April 6, 1992 to April 5, 1994

NRAC funding

$198,698

Work group chair

Carter Newell, Great Eastern Mussel Farm Inc., Maine

Participating investigators/ cooperative agencies

Donald Meritt, University of Maryland Horn Point Environmental Lab, Maryland

Kennedy Paynter, University of Maryland Horn Point Environmental Lab, Maryland

Roxanna Smolowitz, University of Penn and Marine Biological Laboratory, Massachusetts

Dennis Walsh, Aquacultural Research Corp., Massachusetts

Scott Gallager, Woods Hole Oceanographic Institute, Massachusetts

Gary Wikfors, National Marine Fisheries Service, Connecticut

V. Monica Bricelj, State University of New York at Stony Brook, New York
Donald Webster, University of Maryland Horn Point Environmental Lab, Maryland
H. Karl Rask, University of Massachusetts Cooperative Extension, Massachusetts

Objectives

- To characterize the normal microscopic anatomy of *Crassostrea virginica* from fertilization through metamorphosis, to early juvenile.
- To document normal larval development and feeding activity through metamorphosis with non-invasive, time lapse video technology.
- To characterize physiological rate processes in normal and stressed organisms using both real-time and post-sampling techniques.
- To document changes in major catabolic energy substrates throughout development and under conditions of nutritional stress.

Anticipated benefits

Studies were designed to elucidate the key factors that predict survival and metamorphic success in hatchery-reared oyster larvae (*Crassostrea virginica*). Factors such as feeding behavior, nutrition, and culture site were examined as variables. A host of possible factors were evaluated as indicators of larval competence and setting success including; proximal biochemical composition, sterol and triglyceride content, gross morphological development, oxygen:nitrogen ratios, and enzyme activities.

Principal accomplishments

Larval levels of total protein, carbohydrate, or lipid were not correlated with metamorphic survival; however, triglyceride content of eyed larvae was strongly correlated with metamorphic success suggesting that nutritional stores are essential to the metamorphic process. Histopathological examination of the larvae revealed gross morphological differences between high and low mortality groups consistent with starvation or malnourishment in the groups with low survival. Starvation stress may have longer term effects than previously thought.

Triglyceride content (expressed as triglyceride/sterol (TG/ST) ranged from 3 to 30. These values were closely associated with broodstock origin and culture site, and in turn, to metamorphic success ($R^2 = 0.56$; $P \leq 0.0001$).

Impacts

- Following the experiments it was recognized that culture methods at HPEL were not providing adequate nutrition to the broodstock or the larvae to provide adequate TG/ST ratios to the larvae at metamorphosis. Other indicators such as larval traditional feeding, activity and growth rate falsely suggested that even the low TG/ST larvae were doing well until metamorphosis.
- Proper broodstock conditioning plays an important role in producing larvae with adequate initial levels of TG/ST. In any case, all broods had consumed all egg reserves of TG by day 4 and tried to accumulate sufficient TG from their diet over the next 12 days before metamorphosis.
- Triglycerides play an important nutritional role during the non-feeding metamorphic process, and they must be present at certain levels if the larvae are to survive metamorphosis. A triglyceride/sterol ratio below 8 was correlated with poor setting success; above 8 other nutritional or environmental factors may become important. Setting success rarely improved at TG/ST ratios above 8.
- Histological examinations revealed gross morphological differences that were associated with nutritional status, culture site, and metamorphic success. Organ development and lipid accumulation may be positive indicators of setting success.
- Stresses such as larval transport and starvation may have greater, longer lasting impacts than previously appreciated.
- Declining pH in the larval cultures (probably from bacterial respiration) may be an easily monitored indicator of larval culture performance.
- Post-set oysters are relatively resilient to starvation stress; however shell growth and tissue growth can become decoupled under nutritional stress such that shell growth continues at the expense of soft tissues.
- Oxygen:nitrogen ratios suggest that during metamorphosis (and to a lesser extent during starvation) there is an increase in ammonia excretion linked to protein catabolism and deamination.
- The activity of the electron transport system (ETS) appeared to track the overall patterns in post-metamorphic larval metabolic rate, and may provide a useful index of nutritional condition.

Recommended follow-up activities

It is clear that improving the nutrition of both broodstock and larval cultures is the key to improving larval metamorphic success. Further studies to improve broodstock and larval nutrition may have the greatest impacts on commercial hatchery production. A more detailed study is needed to confirm the influence of nutrition on larval development and setting "fitness." Additionally, more detailed studies on the process of setting itself might help define what triggers animals to set and how that process proceeds.

The apparent correlation of drops in pH with poor larval performance should be confirmed. This simple measure of bacterial activity may prove to be a valuable and easily employed indicator of failing cultures.

It remains to be proven whether the activity of enzymes of the electron transport system (ETS) or enzymes such as Glutamine Dehydrogenase (GDH) could provide a useful index of nutritional condition in post-metamorphic larvae.

Publications and presentations

Bohn, R. E., D. W. Webster, and D. W. Meritt. 1996. Producing oyster seed by remote setting. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 95-001 (Formerly NRAC Publication #220). North Dartmouth, MA. 11pp.

Garcia-Esquivel, Z., V. M. Bricelj, and M. A. Gonzalez Gomez. 1994. Physiological and biochemical changes associated with normal development and nutritional stress in post-larvae of *Crassostrea* spp. J. Shellfish Res. 13(1)288.

Paynter, K., C. Caudill, L. Van Heukelem, and D. Meritt. Metamorphic competence of larvae of the eastern oyster, *Crassostrea virginica*: Biochemical composition and metamorphic survival of larval broods cultured at different hatcheries. (In review).

Smolowitz, R., K. Paynter, and D. Meritt. Metamorphic competence of larvae of the eastern oyster, *Crassostrea virginica*: Successful and unsuccessful spawns. (In review).

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1992-93	$112,970	$55,675				$55,675	$168,645
1993-94	$85,728						$85,728
Total	**$198,698**	**$55,675**				**$55,675**	**$254,373**

Northeast Regional Aquaculture Industry Situation and Outlook Report (92-4)

Project Period April 6, 1992 to December 31, 1992

Total funding

$35,029

Work group chair

Cathy Wessells, University of Rhode Island, Rhode Island

Participating investigators/ cooperative agencies

James Anderson, University of Rhode Island, Rhode Island

Thomas Weaver, University of Rhode Island, Rhode Island

Nancy Balcom, University of Connecticut, Connecticut

Linda Marek-Howe, University of Vermont, Vermont
Brian Beal, University of Maine at Machias, Maine
Ralph Boragine, American Seafood Institute, Rhode Island

Overview

It is generally accepted that aquaculture production and its contribution to the economy is growing. However, in the Northeast, only a few states have evaluated their aquaculture sectors and, regionally, only the pen-reared salmonid industry has been comprehensively surveyed. In order to assist investors, bankers, and funding agencies in their distribution of capital and funding resources, it is important to identify areas of rapid growth and potential economic value of the various segments of the aquaculture sector. There is also a need to identify constraints to development and potential opportunities.

Objectives

- To compile preliminary estimates of private aquaculture production and value for the Northeastern Region.
- To identify future opportunities and current problems facing the industry.
- To assist identifying priority research directions based on industry need.

Progress and principal accomplishments

The project is being carried out by investigators at the University of Rhode Island, the University of Maine, the Sea Grant Marine Advisory Program at the University of Connecticut, the University of Vermont, and the American Seafood Institute.

A list of 1,174 potential aquaculture producers in the Northeast Region generated through various sources has been compiled. Various state and local government agencies were contacted in order to obtain lists of lease holders, fish culture permit holders, etc. This task was complicated by the heterogeneity of aquaculture regulatory agencies between states. However, most of the states that actually maintain lists of aquaculture producers were cooperative in supplying them. As a final source, various aquaculture industry directories were obtained and cross referenced with the existing list. Based upon work completed to this point, it is estimated that less than 50% of the list is comprised of active private aquaculture operations. Many of the individuals on the list are leaseholders with no current aquaculture production.

At this time, 137 in-depth interviews have been completed. Of this number, 75 have been completed in-person at the respective culture sites. The remaining 62 interviews have been conducted over the telephone. It is believed that most of the major producers have either already been contacted or will be personally contacted by the end of February. In addition, all potential producers on the list will be contacted through a brief mail survey and will be offered a free subscription to the American Seafood Institute Report in return for their cooperation. This mailing is presently being coordinated by the University of Rhode Island and the American Seafood Institute. The questionnaires will be mailed no later than February 15.

As the lead institution in the project, the University of Rhode Island has compiled the list of potential producers and coordinated the efforts of the project participants. In addition, University of Rhode Island researchers have made trips to Maine, Massachusetts, New York, New Jersey, Pennsylvania, and Maryland in order to conduct personal interviews with major private aquaculture producers. University of Rhode Island researchers have conducted 117 interviews as of this date.

Linda Marek-Howe, Extension Assistant Professor at the School of Natural Resources, University of Vermont, is the principal investigator for the project in the state of Vermont. As of this date, Ms. Marek-Howe has completed eight interviews out of a list of 10 potential private aquaculture producers in the state.

Nancy Balcom, with the Sea Grant Marine Advisory Program at the University of Connecticut, has assisted in interviewing producers within the state of Connecticut. Ms. Balcom has completed three interviews including the largest oyster producer in the region. Ms. Balcom is in the process of completing the remaining six shellfish interviews within the state.

Chris Van Orsdel, a Graduate Research Assistant under the advisement of Dr. James Wilson at the University of Maine, has participated along with a team of URI researchers in conducting interviews with major salmon producers in the state. Mr. Van Orsdel has also conducted an additional nine interviews with primary aquaculture producers.

The Aquaculture office of the Maryland Department of Agriculture has also been especially helpful in providing data from their own survey of aquaculture production in the state. These data, along with information gathered through personal interviews with key industry participants, will be useful in completing the Maryland portion of the Situation and Outlook Report.

Usefulness of findings

This project will yield a valuable report which can be used to identify the current status and trends in the industry, and in setting research priorities. This information will also be of value to industry participants, extension personnel, legislators, policy makers, researchers, and potential investors.

Publications and presentations

Bush, M. J. and J. L. Anderson. 1993. Northeast Region Aquaculture Industry Situation and Outlook Report (November 1993). Northeastern Regional Aquaculture Center/USDA Publication #NRAC 94-003. North Dartmouth, MA. 71pp.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1992-93	$35,029						$35,029
Total	**$35,029**						**$35,029**

Unexplained Mortalities of Hatchery-Reared Oysters, *Crassostrea virginica*, in the Northeast: Initiation of a Hematopoietic Study (see also 91-2 and 92-9) (92-5)

Project period March 23, 1992 to March 31, 1993

NRAC funding

$10,000

Work group chair

Susan Ford, Rutgers University, New Jersey

Participating investigators/ cooperative agencies

Francisco Borrero, SUNY-Stony Brook, New York
Frank O. Perkins, Virginia Inst. Mar. Science, Virginia
David Relyea, Frank M. Flower & Sons, Inc., New York
Craig Strong, Blue Points Oyster Co., New York

Objectives

- To determine whether juvenile oyster mortalities are associated with a particular broodstock or growout site.
- To determine whether mortalities can be stimulated by experimental temperature elevation.
- To document the association of tissue and shell abnormalities with mortalities.
- To determine whether mortalities are associated with a pathogen.

Principal accomplishments

Mortalities were not associated with a particular of broodstock obtained from the Connecticut shore of Long Island Sound. Native set from the Connecticut shore deployed in Oyster Bay developed a high incidence of abnormal conchiolin but had low overall mortalities. Cohorts spawned and deployed in Oyster Bay early in the season (by early April) survived well, whereas those deployed after late April suffered high mortalities.

There were some site-specific differences in mortality, although they were geographically widespread. High mortalities occurred at several sites, including top and bottom trays, in Oyster Bay, New York, and just offshore of Oyster Bay, in Long Island Sound. No mortalities occurred at the Great South Bay site. Although not part of the study, Juvenile Oyster Mortality was also reported from a site on the north shore of eastern Long Island and at a site in Point Judith Pond in Rhode Island.

Mortalities did not occur under elevated temperature in the hatchery. In fact, oysters maintained inside the hatchery at a constant 25°C in a mixture of saline well water and 25 um-bag filtered water suffered little or no losses.

Mantle lesions appeared first in affected oysters, followed about 2 weeks later by the deposition of abnormal conchiolin on the inner shell and the start of mortality. The prevalence of pre-mortality mantle lesions was, in general, highly correlated with the subsequent prevalence of conchiolin deposits and mortality in groups that suffered the highest death rates.

Some cohorts which had been deployed early developed high levels (50-60%) of abnormal conchiolin, but suffered relatively little mortality (25-30%), indicating that many individuals had been affected by the etiological agent but had survived.

Nearly 400 individual oysters were examined by tissue section histology without finding any evidence of a protozoan pathogen. As in last year's study, bacteria and ciliates of various kinds were seen in some oysters, but there was no consistency in their pattern and they may have been secondary invaders.

Publications and presentations

Ford, S. E., F. J. Borrero, and W. J. Blogoslawski. 1993. Studies of Juvenile Oyster Mortality on Long Island Sound, NY, in 1992. J. Shellfish Res. 12(1):108.

Ford, S. E., et al. Unexplained mortalities of cultured juvenile oysters, *Crassostrea virginica* in the Northeastern United States. Presented at the European Association of Fish Pathologists meeting, Brest, France. September 1993.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1992-93	$10,000			$10,000		$10,000	$20,000
Total	**$10,000**			**$10,000**		**$10,000**	**$20,000**

Fish Counting and Measurement *in Situ*: A Technology Assessment (92-6)

Project period January 1, 1993 to October 31, 1993

NRAC funding

$6,899

Work group chair

John E. Huguenin, Massachusetts Maritime Academy, Massachusetts

Objectives

• To document past and ongoing research, and evaluate and recommend the most promising approaches for accurate counting and measurement of fish *in situ*.

Anticipated benefits

A comprehensive evaluation of the widely scattered past and present efforts in accurate counting and measuring of fish is needed for proper research planning. This includes documentation of past and ongoing research, evaluation of the most promising approaches, and recommendations for future efforts. Without this perspective, research funds are likely to be wasted on redundant or unproductive approaches.

Principal accomplishments

The study has approached the current situation on *in situ* counting and measuring of fish from three perspectives

a. Data were collected on relevant past and ongoing research. This included literature searches and contacting research organizations and individuals throughout the world.b. Data on existing commercially available counting and measuring equipment were collected. This was also a world wide search.

c. The state-of-the-art with regards to alternate technical approaches was evaluated and short- and long-term recommendations for meeting the existing fish counting and measuring needs were developed.

The project developed as originally proposed and anticipated. The final report has been printed and is currently available for distribution.

Publications and presentations

Huguenin, J. E. 1993. Fish counting and measurement *in situ*: A technology assessment. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 93-008 (Formerly NRAC #221). North Dartmouth, MA. 59pp.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1992-93	$6,899						$6,899
Total	**$6,899**						**$6,899**

Creation of High Survival Resistant Lines of American Oyster Using MSX-Resistant Strains (92-7)

Project period January 1, 1993 to October 31, 1993

NRAC funding

$8,625

Work group chair

Standish K. Allen, Rutgers University, New Jersey

Objectives

• To create two new strains of resistant oysters, consisting of:

a. five founder populations derived from Delaware Bay MSX-resistant strains and

b. five founder populations from Long Island MSX-resistant strains.

These strains will collectively be called High Survival Resistant Lines (HSRL).

Anticipated benefits

Since 1958 Rutgers has been breeding American oysters for resistance to MSX-disease. Until about 10 years ago, this work has been relatively unnoticed. With the spread of MSX into the Northeast Region in the early 80s, Rutgers Resistant (RR) oysters have received increasing attention for their considerable potential to mitigate MSX-disease losses in commercial aquaculture stocks.

Rutgers maintains a commitment to continuing these strains and to strategies for further improvement in the American oyster. This work is accomplished principally at the Cape Shore Hatchery located toward the mouth of Delaware Bay. Here MSX-disease pressure is probably the most consistent of any location in the US. Consistent disease pressure is essential to continued development and maintenance of resistant stocks.

The second major East Coast disease, Dermo, has appeared recently in Northeast sites as well. In 1990 it appeared in Delaware Bay, and in 1991 -

Massachusetts. RR oysters are not resistant to Dermo. Additionally, because genetic variability in RR stocks has been constrained through intense selection pressure and population bottlenecks, they may be more susceptible. Almost certainly, there is enough loss of genetic variability in RR oyster to question the wisdom of selecting for Dermo resistance from any one of the RR strains per se.

However, among all the resistant strains, much of the original genetic variation still exists. Reconstituting that variation is possible by mating the strains among themselves creating a super-stock, a high survival line. Such a super-stock should be fully-resistant to MSX, as all the individual strains had been, with the additional advantage of increased genetic variation. This variation would be advantageous for two major reasons. First, it would eliminate the apparent inbreeding problems seen recently in various commercial hatcheries during propagation of certain strains. Second, it would reconstitute additive genetic variance which could be exploited for selection to Dermo resistance. In fact this strategy is our best hope for creating stocks of oysters resistant to both MSX and Dermo.

From June 10 to July 21, 1992, we produced two geographic races of HSRL: a Delaware Bay (DB HSRL) and a Northeast race (NE HSRL). Broodstock for DB HSRL comprised four strains of resistant oyster, now 3 years-old, and wild stock from Delaware Bay. All these populations have been exposed to Dermo pressure for at least one generation. Constituent populations for NE HSRL included two succeeding generations of Long Island RR strains (BLA and CLA), and Flowers, Inc. and Ocean Pond, Inc. varieties of the BLA line. We did not introduce Long Island wild stock genes into NE HSRL.

Founder populations were produced by controlled-matings among (but not within) each constituent population. Matings were made for both DB HSRL and NE HSRL using as many pairs as possible from each constituent population. Five founder sub-populations for each race (DB or NE) were produced, but one of the NE subpopulations was lost.

Through a series of mating among (but not within) strains, we produced a total of 2388 families comprising the five DB HSRL sub-populations and 3287 families comprising the five NE HSRL sub-populations. In 1993, we culled the number of oysters down to about 500 per group to better manage the populations as they grow. The inventory of strains as of the end of September 1993 is as follows: DB HSRL 10 — 1149; DB HSRL 20 — 476; DB HSRL 30 — 93; DB HSRL 40 — 1050; DB HSRL 50 — 969; NE HSRL 10 — 560; NE HSRL — 537; NE HSRL 30 — 625; NE HSRL 40 — 660. We have also set each of these groups up into two replicates to track disease mortality quantitatively over the next few seasons. Mortality over the first disease challenge season has been low. This low mortality probably reflects the stocks innate resistance to MSX; a reading on Dermo mortality will be more clear after the 1994 season, since Dermo infections are progressive.

Work planned

The stocks are scheduled for spawning for a second generation (DB HSRL 11 - 51 and NE HSRL 11 - 41) in the summer of 1995, following two full seasons of disease exposure.

Impacts

The effort of this project represents a new direction for the MSX-resistant stocks. It is expected that these new populations (already fully resistant to MSX-disease) will be available for industry use beginning 1994. Resistance to Dermo will be gradual, and as resistance is acquired, the stocks will be more and more useful to areas affected by either or both diseases.

Publications and presentations

Allen, Jr., S. K. 1993. Development of high survival resistant lines in oysters using MSX-resistant strains. J. Shellfish Research 12:105. (Abstract).

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1992-93	$8,625						$8,625
Total	**$8,625**						**$8,625**

Northeastern Regional Aquaculture Center Newsletter-1992 (see also 90-4, 91-5) (92-8)

Project period January 1, 1993 to October 31, 1993

NRAC funding

$18,000

Work group chair

Joseph K. Buttner, State University of New York, Brockport, NY

Participating investigators/ cooperative agencies

Gregg Rivara, Cornell University, New York

Objectives

- To publicize the contributions of NRAC to regional aquaculture development.
- To disseminate results of aquaculture research and extension activities supported by NRAC.
- To provide a forum for the regional and national exchange of aquaculture information.
- To encourage support for aquaculture development.

Anticipated benefits

The periodic newsletter, “Northeastern Aquaculture,” highlights NRAC activities and other developments important to the aquaculture industry in the Northeast. The newsletter is distributed to over 3,200 recipients. Most are located in the Northeast, but the mailing list includes people from most states and several foreign countries. Presently all people that request the newsletter are added to the mailing list regardless of location.

The newsletter provides a forum for useful information to be widely disseminated in a more or less timely and cost-effective manner. The primary user group, industry, is kept informed and abreast of NRAC activities. Results of NRAC supported studies and other important activities are summarized in the Newsletter. Source(s) for follow-up and detailed information are provided. Researchers and agency people also receive the Newsletter. They gain a better appreciation of what is happening outside their own laboratories and offices. A more-informed industry, research, and agency group results in better communication and cooperation.

Principal accomplishments/ publications and presentations

Three newsletters have been printed in 1993 and, a fourth is currently being developed for distribution before year-end.

Work planned

A new project is being developed for approval to produce three or four issues of “Northeastern Aquaculture” in 1994.

Impacts

Through the Newsletter, most people associated with aquaculture in the Northeast are kept informed of important developments. The transfer of information to interested individuals is greatly facilitated as recent “deliverables” are described in the Newsletter and methods of obtaining more comprehensive information provided.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1989-90	$15,000						$15,000
1990-91	$11,415						$11,415
1991-92	$18,000						$18,000
Total	**$44,415**						**$44,415**

Publication of Unexplained Oyster Mortality Findings (see also 91-2 and 92-5) (92-9)

Project period June 27, 1994

NRAC funding

$3,000

Work group chair

Monica Bricelj, Marine Sciences Research Center, New York

Participating investigators/ cooperative agencies

Susan Ford, Haskin Shellfish Research, New Jersey
Francisco Borrero, State University of New York, Stony Brook, New York
Frank Perkins, Virginia Institute of Marine Science, Virginia
Gregg Rivara, Cornell Cooperative Extension, New York
Robert Hillman, Batelle Ocean Sciences, Massachusetts
Ralf Elston, Batelle Marine Sciences, Massachusetts
Jeng Chang, State University of New York, Stony Brook, New York

Objectives

To cover costs associated with publication in the J. Shellfish Res. (JSR) of the manuscript "Unexplained mortalities of hatchery-reared, juvenile oysters, *Crassostrea virginica* (Gmelin)."

Principal accomplishments

The manuscript was published in the J. Shellfish Res.

Publications and presentations

Bricelj, V. M., S. E. Ford, F. J. Borrero, F. O. Perkins, G. Rivara, R. E. Hillman, R. A. Elston, and J. Chang. 1992. Unexplained mortalities of hatchery-reared, juvenile oysters, *Crassostrea virginica* (Gmelin). J. Shellfish Res. 11(2):331-347.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1993-94	$3,000						$3,000
Total	**$3,000**						**$3,000**

Milford Shellfish Seminar (93-1)

Project period February 22 to February 24, 1993

NRAC funding
$2,500

Work group chair
Dept. of Commerce, NOAA, NMFS, NEFSC, Milford Lab, Milford, Connecticut

Objectives
- Provide forum for technical exchange of recent research developments prior to publication.
- Distribute abstracts of all oral and posters presentations to attendees.
- Provide annual meeting where industry can discuss shellfish research and regulatory issues with federal, state, and academic shellfish biologists.
- Strengthen communication networks and exchanges of materials, programs, and expertise among aquaculturists.
- Examine team building and multidisciplinary approaches to regional programming in aquaculture.
- Examine major trends in commercial aquaculture and emerging opportunities.

Principal accomplishments
The 12th Annual Shellfish Biology Seminar was held in Milford, Connecticut from February 22 to February 26. The most recent information on aquaculture, recruitment, and regulatory activities affecting the aquaculture industry were made available to all attendees.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1993-94	$2,500						$2,500
Total	**$2,500**						**$2,500**

Assessment of the Impact of Stray (Neutral-to-Earth) Voltage on Finfish Aquaculture in the Northeast (93-2)

Project period August 12, 1993 to August 11, 1994

NRAC funding
$12,000

Work group chair
Gordon Wilder, Hy-On-A Hill Trout Farm, New Hampshire

Participating investigators/ cooperative agencies
Stephen Greaves, Greaves Dairy Equipment, Vermont
Fern Wilder, Hy-On-A Hill Trout Farm, New Hampshire
Shawn Wilder, Allied Electronics, New Hampshire

Objectives
1. To identify finfish aquaculture facilities in the Northeast that have experienced unexplained mortalities that may fit the pattern of stray voltage-related stress.
2. To determine the presence/absence of stray voltage at facilities identified in objective 1.
3. To document the patterns of behavioral changes in the fish that may indicate the presence of environmental stress.

4. To observe patterns in mortalities and review other rearing facility factors (water quality, stocking density, health inspection reports, etc.) to determine whether there is a correlation between stray voltage and finfish mortality.
5. To identify possible solutions to eliminate stray voltage problems at aquaculture facilities.

Principal accomplishments

Twenty-six letters with questionnaires were sent to state aquaculture associations and fish and wildlife agencies within the region. Recipients were asked to distribute the questionnaire among members of their associations' departments to growers that might have experienced unexplained losses of finfish that might be attributed to stray voltage. From 15 responses, two finfish rearing facilities with potential stray voltage problems were identified: one in New Hampshire and one in Massachusetts.

The grower in New Hampshire had been raising Brook and Rainbow Trout since 1953. For the past 8 years he had been pumping water from a dug well. A recirculating pump inside the tank supplemented the natural water flow. He had never experienced any significant losses until the fall of 1991 when the power company replaced a pole in front of his home. During the following 18 months, all trout introduced into his tank on three occasions from two different hatcheries died within 10-12 hours. A fourth introduction comprised of 24 shiners also died within hours within 10-12 hours. A fourth introduction, comprised of 24 shiners, also died within hours.

The Massachusetts grower had experienced devastating losses over a period of several years. During the period just prior to this investigation, the facility had experienced higher than normal losses of larger fish. A pathologist's examination proved negative for pathogenic agents. Larger fish may be more susceptible to voltage stress than smaller fish as their greater body size exposes them to larger electrical gradients. AC voltage checks between the electrical ground and the water in the tanks following the most recent fish loss revealed voltage differentials varying from 2.5 to 4 volts. On November 27, 1993, the New Hampshire grower utilized an AC voltmeter to detect the presence of stray voltage at the recirculating pump in the trout tank and at nearby electrical receptacles.

From January 6, 1994, through February 17, 1994, a Rustrack Ranger II Data Logger with accessories was used to measure the neutral-to-earth voltage at the Massachusetts location. Differentials were monitored at three locations: (1) between the outside fish tanks and the secondary neutral, (2) a separate reference rod and the secondary neutral, and (3) the fish tanks and the reference rod.

In early November 1993, the New Hampshire trout grower reported unexplained rainbow trout losses with rapid and extensive (100%) mortalities. Prior to the mortality, he had observed severe stress evidenced by erratic swimming, obvious discomfort, surfacing as if to get air, running the surface of the water, and poor feeding. All trout (between 1 and 2 years old) died within a 24-hour period.

Water testing at the New Hampshire facility revealed normal levels for trout propagation. Stocking density was heavy. Tiger stripes were present across the backs of some moribund specimens.

The Massachusetts trout grower noticed that, in addition to gill hyperplasia, the dying fish seemed to orient themselves facing the walls of the tanks and stayed on the bottom.

At the Massachusetts station, the overall husbandry and layout appeared to be adequate to support trout. Nevertheless, gills seemed to be very irritated, and the skin of all species showed a darkened, striped effect. Internally, there appeared to be no unusual characteristics. The fish had been checked annually since 1991 by a certified fish biologist, and appeared to be disease-free. Since the water supply, water chemistry, and fish pathology did not appear to explain the observed mortalities, the electrical sources supplying the fisheries were suspect.

Possible solutions for stray voltage include: voltage reduction, a four-wire system, new construction, isolation from primary and secondary neutrals, and identification and removal or repair of faulty wiring or motors.

Impacts

A correlation between stray voltage and fish mortalities has been determined. This information will be useful to all fish growers, making them aware of a problem that can easily be overlooked. Massive losses of fish can be prevented by simple electrical monitoring and modification of the electrical grounds in the vicinity of fish ponds, pools, or raceways, which has the potential to be a significant breakthrough for the entire fish rearing industry.

Recommended follow-up activities

Further scientific study about the effects of voltage on fish behavior and physiology are recommended. Tolerance levels of different species and sizes of fish to stray voltage and identification of measures that may be taken to prevent environmentally stressful situations at fish rearing facilities should be explored. Building fish tanks or raceways to reduce or eliminate the problem of stray voltage on a non-conductive surface, developing methods for monitoring stray voltage at hatcheries, and identifying stray voltage experts as sources of information are suggested follow-up activities.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1993-94	$12,000						$12,000
Total	**$12,000**						**$12,000**

Alternatives to Lessen the Economic Impact of Aquaculture Regulations (93-4)

Project period July 1, 1993 to November 30, 1995

NRAC funding

$86,260

Work group chair

Richard E. Bohn, University of Maryland, Maryland

Participating investigators/ cooperative agencies

James W. Dunn, Pennsylvania State University, Pennsylvania
Conrado M. Gempesaw II, University of Delaware, Delaware
Gregory D. Hanson, Pennsylvania State University, Pennsylvania
Susan Mangin, US Fish & Wildlife Service, District of Columbia
Eric B. May, Department of Natural Resources, Maryland
Joseph P. McCraren, US Trout Farmer's Association, West Virginia
Eileen C. Sadasiv, University of Rhode Island, Rhode Island
Alex W. Wypyszinski, Rutgers University, New York

Objectives

- To evaluate the costs and other economic burdens associated with regulations developed from existing policies.
- To compile through literature searches, questionnaires, and other means, information on the distribution of diseases and pathogens from both cultured and wild sources, and on existing fish health regulations in the Northeast Region.
- To assemble a committee charged with preparing and disseminating a "white paper" outlining the history and evolution of fish health policy within the Northeast Region, acknowledging regulations based on existing biological information, and identifying issues of fish health significance in need of resolution.
- To address an alternate approach to policy development and implementation that recognizes the missions of industry, agricultural agencies, and natural resource agencies.

- To prepare for regional and national meetings on fish health policy by assisting the working committees which are developing these meetings with information from the "white paper."
- To determine the types of costs of regulation and the parties that bear them as a result of fish health policies.
- To develop a mechanism for evaluating the economic impact of fish health regulations on the aquaculture industry.
- To use this mechanism to estimate the economic impact of selected fish health regulations on the aquaculture industry.

Anticipated benefits

For over a century, fish health regulations have been promulgated by resource agencies in the interest of protecting resident fish stocks. The regulations have developed into trade barriers to private aquaculture. Fish health certification became the legal and technical basis for eliminating or reducing fish health problems, but the technology and information which developed since do not appear to support this approach.

While most fish health studies have focused on hatchery problems, private industry has encouraged review of the assumed impact of pathogens on the resource. Regulators have agreed that this is a worth-while avenue of research, considering the original intent of the regulations. It is also generally agreed that better information is needed on pathogen distributions. Combined with a more standard assessment of the economic burdens placed upon private industry, this project provides a timely opportunity to offer information leading to far-ranging changes in the way both regulators and industry view fish health regulation.

Principal accomplishments

Over 3,000 citations have been obtained, reviewed, and compiled. The following databases have been searched: Fish and Wildlife Reference Service, Fish and Fisheries Worldwide, CRIS/USDA, CAB, and Biosis. Additional "gray literature" has also been gathered where encountered.

All information relative to diseases impacting the Northeastern Region of the United States and occurring elsewhere were retained. Any information concerning diseases and their impact on wild stocks, regardless of origin, were also retained. Few citations from the Northeast were located, so additional investigation was pursued nationally.

To seek information in addition to cited research, a questionnaire was developed in cooperation with the U.S. Fish and Wildlife Service and state regulatory personnel. Questions were developed to gain information about incidence and movement of fish pathogens which may have been experienced throughout the United States. One hundred seventy-eight questionnaires were mailed in September of 1993, primarily to state regulatory and wildlife personnel. Thirty-five states were surveyed in this manner, although some key states failed to respond. Answers to the original questions demonstrated that a follow-up questionnaire would be required for clarification as well as to gain more complete coverage by the survey, so a telephone questionnaire was completed and compiled in January 1995.

Information from previous NRAC projects compiling Northeast state regulations was utilized by the subcommittee and additional information was obtained from federal and state regulators. The data was summarized for the "white paper" on fish health regulations.

The subcommittee formed a working group for the "white paper" and met originally in July 1993. Assignments were given for assisting the economics subcommittee, state and federal regulations and economic involvement, database searches, development and collation of questionnaire, and drafting the "white paper." Most assignments have been accomplished to date.

The revised draft of the "white paper" is in progress. The subcommittee met in June 1994, to review the work on the draft. Drafting was delayed to include OIE (international) standards and regulations, released in preliminary form in July 1995, and further delayed by the death of the subcommittee chairman in September 1995.

Membership of the working group for the "white paper" was expanded to include representatives of the U.S. Fish and Wildlife Service and veterinary sciences. This provides a broader perspective in the discussion of alternative approaches to fish health policy development and implementation included in the "white paper." Comments and additional federal, state, and private resource and animal management representatives are being sought during the final drafts of the "white paper."

Information gathered for the project have been presented at the Wild Stocks/Hatchery Pathogen Symposium held at the US Trout Farmers Association (USTFA) meeting in October 1993, in Duluth, Minnesota; at the Aquaculture in the Mid-Atlantic 1994 in Fredricksburg, Virginia; at the Chesapeake Watershed Ad-Hoc Fish Health Committee in February 1995; and at the Maryland Fish Health Subcommittee Meeting in March 1995.

Members of the economic subcommittee met with industry representatives in 1993 and 1994 to determine cost factors associated with regulations. Factors determined by fish health policies were identified; these costs were clarified using the regulatory compilation from previous NRAC projects and the fish health subcommittee.

The mechanism for evaluating the economic impact of fish health regulations was established and applied to trout egg production. A case study was designed to evaluate the economic viability and regulatory impacts on a facility located in the Northeast Region. The hatchery (designed to have large economics of scale) was simulated using a dynamic, stochastic, capital budgeting simulation model. A paper describing this mechanism was presented at the 1995 "Northeastern Agriculture and Resource Economics Association Meeting," and is "in press."

An overall measurement of the costs of reduced trade due to existing fish health policies was applied to the trout egg industry model. Two scenarios were designed to capture the economic effects of current animal health regulations. The shutdown scenario required the hatchery to cease production for 2 years to ensure eradication of a serious disease. Another scenario required the destruction of trout eggs and treatment of the broodstock. The economic simulation results show that it is better to shut down to correct a serious disease problem rather than try to continue to operate and experience the destruction and loss of production due to a violation of a regulatory policy. A case study describing this impact was presented at "Aquaculture in the Mid-Atlantic" in June 1995.

Impacts

The information being generated has been requested by a number of policy-making bodies, reflecting the timely nature and need for this study. Both state and federal (U.S. Fish and Wildlife Service, USDA/APHIS) regulatory institutions are requesting the results, as well as state and regional groups (such as Chesapeake Watershed Ad-Hoc Fish Health Committee). Dissatisfaction with current policies, in particular the New England Fish Health Guidelines, and decreasing federal assistance for implementation and enforcement of fish health policies shows the willingness of regulators to weigh the costs and examine the alternatives.

Recommended follow-up activities

Future studies should establish training for veterinarian familiarity with fish health issues and practices, to provide better support for certification and drug-approval processes under their authority. Specific scientific research directions for fish health are contained in the "white paper."

Publications and presentations

In Print

Bacon, J. R., C. M. Gempesaw II, W. W. Lussier, and J. W. Dunn. 1995. The economics of a large-scale trout hatchery in the Northeast Region. (Abstract). Agricultural and Resource Economics Review 24:2 (In Press).

Papers Presented

Bohn, R. E. Current fish health regulations and progress. Aquaculture in the Mid-Atlantic Conference 1994, Fredricksburg, VA. June 1994.

Bohn, R. E. NRAC fish health watershed ad-hoc fish health West Virginia. January 1995.

Bohn, R. E. NRAC fish health survey results. Pennsylvania Fish Health Subcommittee meeting, Harrisburg, Pennsylvania. February 1995.

Bohn, R. E. NRAC fish health survey results. Maryland Fish Health Subcommittee meeting, Greenbelt, Maryland. March 1995.

Bacon, J. R., C. M. Gempesaw II, W. W. Lussier, and J. W. Dunn. The economics of a large-scale trout hatchery in the Northeast Region. Northeastern Agricultural and Resource Economics Association meeting, Burlington, Vermont. June 1995.

Dunn, J. W., C. M. Gempesaw II, and G. D. Hanson. The economic effect of restricting interstate movement of fish. Aquaculture in the Mid-Atlantic/Fish Expo 1995 meeting, Washington, D.C.. June 1995.

May, E. B. and R. E. Bohn. Fish health regulations and the spread of fish diseases. Wild Stocks/Hatchery Symposium, USTFA Annual meeting, Duluth MN. October 1993.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1993-94	$64,210	$20,510	$7,350	$500	$3,700	$32,060	$96,270
1994-95	$22,050	$19,900				$19,900	$41,950
Total	**$86,260**	**$40,410**	**$7,350**	**$500**	**$3,700**	**$51,960**	**$138,220**

Computer Network Communication System (93-5)

Project period July 15, 1995 to January 14, 1996

NRAC funding

$25,000

Work group chair

Thomas Handwerker, University of Maryland Eastern Shore, MD

Participating investigators/ cooperative agencies

H. Karl Rask, University of Massachusetts Cooperative Extension, Massachusetts

Objectives

- To survey the telecommunication resources within the NRAC community and formulate the basic BBS protocol and services.
- To install a PC-based, bulletin board system (BBS) with graphics and hypertext/hypermedia capabilities. This includes the necessary hardware, support software, communication linkages, addresses, and user documentation.
- To create appropriate packages of information that can be utilized by commercial growers, associations, and extension and research specialists with variable computer or commercial expertise. This includes: a) the development of methodology to support the acquiring of information; b) developing a working prototype for assimilating the data from several diverse cooperators; and c) selection of a hypertext architecture that enhances the information flow, retrieval, and navigation for critical decision making in commercial aquaculture.
- To enhance the telecommunication skills of growers and specialists to include access to additional databases, regional centers, and interest groups.
- To evaluate the protocol for submission of documents, reports, or announcements for utilization in Aquaculture Association Newsletters, the NRAC Newsletter, the National Association of State Aquaculture Coordinators (NASAC), and for general electronic distribution.

Anticipated benefits

A bulletin board system would provide the NRAC community with a vehicle to access information and facilitate real-time discussions among regional stakeholders on issues of regional importance.

Principal accomplishments

A needs assessment survey of industry, technical, and extension specialists in the region revealed that technical and extension specialists in the region had good Internet access; however, industry representatives had little experience or access capabilities.

Several commercial software systems were evaluated and MajorBBS (a Galacticomm product) was acquired and installed. The NRAC NETWORK was installed on a 486 PC platform with four 14,400 baud modems and 12 TELNET channels, permitting simultaneous access by up to 16 users.

An on-line help manual was made available.

Impacts

Unfortunately there were several delays in evaluating and acquiring the necessary software and establishing the BBS system. Subsequently the PI failed to deliver contracted training materials and never conducted on-site training and demonstration programs. A satisfactory base of informational and instructional materials was never established on-line; so users who accessed the BBS were essentially greeted by an empty shell. The BBS therefore failed to attract the necessary "critical mass" of users that would have stimulated further growth and acceptance.

At the same time, the rapid evolution of alternate technologies such as the World Wide Web and the Internet (AQUA-NIC etc.) has effectively rendered the BBS obsolete.

Recommended follow-up activities

NRAC is currently examining the possibility of rededicating the 486-PC for use as a FAX-on-demand server for informational bulletins. This could be accomplished by simply acquiring and installing new software. Many growers who do not have Internet access do have FAX capabilities and rely on them in their daily business. NRAC is also establishing a website for dissemination of relevant information.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1994-95	$25,000	$9,400				$9,400	$34,400
Total	**$25,000**	**$9,400**				**$9,400**	**$34,400**

The Role of Bacteria and Microalgae in Unexplained Juvenile Oyster Mortalities (93-6)

Project period May 1, 1993 to June 30, 1994

NRAC funding

$71,491

Chair

Susan Ford, Rutgers University, New Jersey

Participating investigators/ cooperative agencies

Gordon Taylor, SUNY, Stony Brook, New York
Monica Bricelj, SUNY, Stony Brook, New York
David Relyea, Frank M. Flower & Sons Co., New York
Joseph Grochowski, Nantucket Marine Laboratory, Massachusetts
Robert Garrison, Nantucket Aquafarm, Massachusetts

Objectives

- To monitor survival, growth, and pathology of juvenile eastern oysters grown under commercial conditions at the Frank M. Flower, Co. Nursery, in Oyster Bay, New York.
- To compare survival, growth, and pathology of juveniles grown on 1/4" and 1 mm screens.
- To compare survival, growth, and pathology of juveniles grown in various combinations of well salt- water, 25 um filtered bay water, and raw bay water.
- To attempt transmission of Juvenile Oyster Disease from affected nursery animals to unaffected oysters in the hatchery.
- To sample water, sediment, and culture container contents (including oysters) for presence of

Vibrio spp. at the FMF Co. site and at a control site in Oyster Bay.
- To perform challenge experiments with suspected pathogenic *Vibrio* spp. collected at the nursery site.
- To sample water in the vicinity of the FMF Co. Nursery for the presence and abundance of potentially noxious phytoplankton, and isolate and produce unialgal, non-axenic cultures.
- To perform challenge experiments with suspected noxious microalgae, if candidate species are associated with Juvenile Oyster Disease.
- To perform challenge experiments with potentially damaging chain- and colony-forming microalgae collected on Nantucket Island, Massachusetts.

Anticipated benefits

Mortalities of juvenile oysters are a major impediment to the development of oyster aquaculture in the Northeast. Identification of a cause should permit the design and implementation of measures to reduce or eliminate the losses.

Principal accomplishments

From the 1993 SUNY survey of the Frank M. Flower Co. Nursery floats, 1,749 individual bacterial isolates from oyster tissue, hatchery waters, algal cultures, bay waters, and sediments were archived. These isolates were grouped on the basis of source, time of collection, and growth characteristics. Those from oyster tissue (as well as shell liquor and inner shell scrapings) that preceded and coincided with the onset of mortality have been given the highest priority for identification. Presently, 200 of these have been subjected to a stringent series of identification procedures, including sensitivity to Vibriostat and oxytetracycline, BIOLOG GN micro plate ID system, API 20E test strips, and other supplementary confirming tests (e.g., haemolysis, growth at 0% NaCl, etc.). Most of the isolates associated with diseased oysters are closely aligned phenotypically to just a few species of *Vibrio: V. anguillarum; V. papahaemolticus; V. vulnificus; V. aliginolyicus*; and *V. metchnikovii*.

In two separate challenge experiments, seven *Vibrio* isolates were injected into the mantle cavity of separate lots of non-diseased oysters (N=~ 1200 oysters). All lots were replicated and held in separate containers at the SUNY Flax Pond Facility. Two *Vibrio* isolates produced significantly higher mortality than controls (*E. coli* inoculated or uninoculated). Mortalities produced by other isolates were similar to controls. At present, we do not know if the pathogenic isolates are unique strains of known bacterial species or unique species altogether. Further taxonomic characterization is required. We are currently analyzing bacteria re-isolated from oysters in high-mortality groups in order to fulfill Koch's postulates.

About 2,000 juvenile oysters required for the investigation of chain- and colony-forming phytoplankton in juvenile oyster mortality were produced at the Nantucket Marine Lab in the Spring, 1994. Three laboratory incubation systems, each receiving filtered sea-water, were constructed to reproduce summertime temperature regimes present on Nantucket Island during the 1989 juvenile oyster mortality episode.

Two hundred and fifty 10 mm seed were placed in each system and incubated at 20, 25, and 30°C for 3 weeks and fed unicellular cultures of *Isochrysis* and *Skeletonema* at rate of 100,000 to 200,000 cell/ml/day. Survival was 100% and growth was 60% in all systems, demonstrating that during the study period under the experimental conditions, survival was independent of temperature.

Attempts by the Nantucket Marine Lab to culture long-chained or large colonial populations of algae collected from the field have failed. In all cases, when pure cultures have been initiated from the field collections, unicells resulted. No colonial forms or chains could be maintained in the laboratory.

Suggested future work

Final identification of *Vibrio* isolates and histopathological examination of experimentally inoculated oysters will be conducted at SUNY and Rutgers, respectively. On Nantucket, juvenile oysters have been placed in the field where chain-forming or colonial algae occur. They will be monitored over the remainder of the summer.

Impacts

One finding of the summer of 1993 field study was a reduction in mortality of juvenile oysters held in 1/4" mesh trays at the Frank M. Flower Co. Consequently, that company has begun to use larger mesh trays in its nursery operation. Although juvenile oyster mortalities have not occurred at that site in 1994, oysters in the larger mesh are growing somewhat faster than those in the smaller mesh.

Publications and presentations

Ford, S. E. 1994. Unexplained mortalities of hatchery-reared oysters, *Crassostrea virginica,* in the Northeast: A hematopoietic study. Final Technical Report to NRAC. June 1994. 27pp.

Ford, S. E., et al. 1993. Unexplained mortalities of cultured juvenile oysters, *Crassostrea virginica,* in the Northeastern United States. Presented at the European Association of Fish Pathologists Meeting, Brest, France. September 1993.

Lee, M., et al. 1994. The roles of *Vibrios* and *Gymnodinium sanguineum* in unexplained juvenile oyster mortality at the F. M. Flower and Sons Oyster Hatchery in Oyster Bay, New York. 14th Milford Aquaculture Seminar. February 21-23, 1994.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1993-94	$71,491	$23,789	$12,700		$2,500	$38,998	$110,489
Total	**$71,491**	**$23,789**	**$12,700**		**$2,500**	**$38,998**	**$110,489**

Possible Cytotoxic Effects of the Dinoflagellate (*Gyrodinium aureolum*) on Juvenile Bivalve Molluscs (93-7)

Project period July 1, 1993 to June 30, 1994

NRAC funding

$2,489

Work group chair

Sandra E. Shumway, Southampton College, New York

Participating investigators/ cooperative agencies

Roxanna Smolowitz, University of PA/Laboratory of Marine Animal Health, Massachusetts

Objectives

- To determine the possible cytotoxic effects of exposure to *Gyrodinium aureolum* on juvenile, commercially important shellfish from the Gulf of Maine (surf clam, *Spisula solidissima*; blue mussel, *Mytilus edulis*; sea scallop, *Placopecten magellanicus*; softshell clam, *Mya arenaria*; eastern oyster, *Crassostrea virginica*; and European oyster, *Ostrea edulis*).
- To assess possible reversibility of any cellular damage.

Principal accomplishments

- Delineated the effects of a commonly occurring dinoflagellate, *Gyrodinium aureolum*, on juveniles of commercially important bivalve molluscs. The species were chosen because they are either cultured or fished commercially in Gulf of Maine waters. Only juveniles were used in the experiments as they were expected to be the most susceptible to any toxins present.

- Confirmed lethal effect of this dinoflagellate on juvenile bay scallops, *Argopecten irradians*, and noted cytotoxic effects on digestive gland tissues. While the most devastating effects were noted in bay scallops, mortalities were also noted in surf clams and quahogs, and possible cellular damage was noted in mussels, sea scallops, and European oysters.

Impacts

Aquaculturists should monitor for the presence of *Gyrodinium aureolum*. Given the large size of this dinoflagellate (approximately 25-30 mm), it could be easily be filtered out of the incoming water to hatcheries. No data are currently available on the effects of any toxins that might be present in the water during blooms of this dinoflagellate.

Publications and presentations

Lesser, M. J. and S. E. Shumway. 1993. Effects of toxic dinoflagellates on clearance rates and survival in juvenile bivalve molluscs. J. Shellfish Res. 12: 377-381.

Smolowitz, R. and S. E. Shumway. 1996. Possible cytotoxic effects of the dinoflagellate, *Gyrodinium aureolum* on juvenile bivalve molluscs. J. Shellfish Res. 14(1):248.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1993-94	$2,849	$7,786				$7,786	$10,635
Total	**$2,849**	**$7,786**				**$7,786**	**$10,635**

Analysis of Genetic Purity of Captive & Wild Striped Bass (93-8)

Project period July 1, 1993 to June 30, 1994

NRAC funding

$15,955

Work group chair

Reginal M. Harrell, University Maryland, Horn Point Environmental Laboratory, Maryland

Participating investigators/ cooperative agencies

L. Curry Woods, III, University of Maryland, Crane Aquaculture Facilities, Maryland

Objectives

- To complete the purity screening of CAF broodstock.
- To screen the 1993 broodstock fish associated with the NRAC Project 92-2 (Domestication of Striped Bass for Aquaculture).

Principal accomplishments

Genomic DNA was isolated from all family representatives or their parents of the Crane Domesticated Stocks, and a series of three single locus nDNA species diagnostic probes using Southern hybridization methodologies and four PCR-based assays. The molecular markers used in this study revealed, through agarose gel electrophoresis, different banding patterns for striped bass and white bass. Depending on the marker, one to five alleles were diagnostic for striped bass while white bass controls displayed one or two alleles. Based on the analyses of the molecular data, we found no evidence of introgressive hybridization present in the CAF founder stock or wild fish tested. Since we have not observed genetic linkage among any of the seven loci tested, results indicate that these white bass alleles are extremely rare, if present at all.

Suggested future work

Given the information that the Crane Domesticated Stocks and the fish collected from wild populations and used in the “Domestication of Striped Bass Project” are genetically pure, the next phase of these studies would be to initiate selective breeding studies and continue domestication efforts with the progeny.

No further purity evaluation with these progeny or parents is recommended at this time. However, any stocks taken from the wild should be evaluated for purity if the fish are to be used in a domestication and selective breeding effort.

Publications and presentations

Woods, L. C., III, B. Ely, G. Leclerc, and R. M. Harrell. DNA evidence for genetic purity of captive and

domesticated striped bass broodstocks. Aquaculture 137:41-44.
Woods, L. C., III and R. M. Harrell. 1994. Validation of the genetic purity of captive and domesticated striped bass broodstocks. Abstracts of the 5th International Symposium of Genetics in Aquaculture, June 19-25, Halifax, Nova Scotia, Canada. (Abstract).

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1993-94	$15,955	$10,675				$10,675	$26,630
Total	**$15,955**	**$10,675**				**$10,675**	**$26,630**

A Proposal for the Study of a Protozoan Disease Agent(s) Associated with Mortalities of Hatchery-reared Juvenile Oysters in the Northeastern United States (93-9)

Project period July 1, 1993 to June 30, 1994

NRAC funding

$10,000

Work group chair

Frederick G. Kern, USDA/NOAA/NMFS, Maryland

Participating investigators/ cooperative agencies

C. Austin Farley, USDA/NOAA/NMFS, Maryland
Earl J. Lewis, Jr., USDA/NOAA/NMFS, Maryland
Dorothy Howard, USDA/NOAA/NMFS, Maryland
Dr. Harry Danforth, USDA, Maryland
Dr. Eugene Small, University of Maryland, Maryland
Dr. Ana Baya, Maryland Department of Agriculture, Maryland
Phillip Rutledge, University of Maryland, Maryland
Don Meritt, University of Maryland Horn Point Lab, Maryland
David Relyea, F. M. Flower & Sons, Inc., New York
Joseph Zahtila, F. M. Flower & Sons, Inc., New York
Gregg Rivara, Cornell University, New York
Stan Czyzyk, Bluepoints Co., New York

Objectives

- To provide a continuous source of infectious disease agent for future identification.
- To study the role of salinity in the disease process to determine if salinity may be a barrier to the disease agent.
- To explore potential therapeutic and continue pathological studies of juvenile oyster mortalities concentrating on epizootiology, pathology, and parasitology.
- To continue the identification of the suspected protistan pathogen by use of electron microscopy to discern parasite-specific ultrastructural characteristics.
- To demonstrate etiologic relationships with known features of the disease - age, size, and conchiolin deposition.
- To continue transmission studies to prophylactic use of selected ciliate and bacterial medications to eliminate the disease.

Principal accomplishments

Six sequential weekly samples of F. M. Flower and Sons Co. cultured juvenile oysters were examined for Juvenile Oyster Disease (JOD) from June 15 - July 29, 1993. Progression of parasite prevalence and intensity, and gross and microscopic pathology were documented and correlated with subsequent mortalities. No significant presence of JOD was seen in naturally set oysters. Indications of JOD were not found in Flower's cultured clams, *Mya arenaria* or *Mercenaria mercenaria*. Data suggest that resistance to JOD may be developing in Flower's oysters. Some samples provided evidence that 1 mm seed may spread JOD to new growing areas once introduced.

Ultrastructural studies revealed rare intracellular bodies, resembling protists, in mantle epithelia of JOD-infected oysters. These presumed protists had mitochondria with tubular cristae, small dense bodies, and vesicles. One organism showed evidence of a pellicle and endogenous budding.

Attempts to isolate candidate ciliate organisms from infected oysters resulted in the isolation of over 25 species, many identified to genus by Dr. Eugene Small. An unidentified spirochaete has been repeatedly isolated from JOD-infected juveniles, but not from uninfected oysters.

Clear correlations between intracellular inclusions (which we consider to be a protist), mantle lesions, conchiolin, mortality, and shell checks in survivors have been noted. Mortality correlates with both size and age in populations affected by the disease. Evidence was found of healed conchiolinous lesions in larger, dead oysters (80-110 mm).

Due to the lack of available, uninfected juvenile oysters for transmission studies, only one transmission study was accomplished. Alternative methods using naturally-infected seed from the Flower's Hatchery were used for substitute experimental salinity studies.

Uninfected, cultured juveniles from the University of Maryland Horn Point Laboratory were exposed to survivors of the 1992 JOD epizootic for 6 weeks at salinities of 10-26 ppt. Disease onset was delayed and subsequent mortalities were reduced at salinities below 20 ppt. JOD-infected oysters were also subjected to 6-week salinity exposures of 10-30 ppt salinity. Mortalities likewise were reduced in salinities below 18 ppt.

Of five therapeutic agents tested, only erythromycin was effective in reducing mortality in infected animals. Minocycline, recommended by the manufacturer for treatment of gram-negative bacterial infections such as *Vibrio*, had no effect. Four species of *Vibrio* were isolated from eight of 18 JOD-infected animals. No evidence of an etiological relation was apparent.

Anticipated benefits

- Possible identification and clarification of the role the suspected causative agent plays in the mortalities of hatchery-reared juvenile oysters.
- Association of the above organism with the disease syndrome.
- The identification of management approaches based on acquired knowledge of the pathogen and its requirements.

Impacts

Reduction in JOD-associated mortalities of 1993 hatchery-reared seed has resulted from applying management techniques at the Flowers hatchery that were developed from field and laboratory studies [e.g., suggestions to spawn earlier to reach 30 mm or larger size before the onset of the disease; use survivors of the previous years' epizootic (ones with severe growth checks) as broodstocks].

Publications and presentations

Farley, C. A. and E. J. Lewis. 1994. Studies of Juvenile Oyster Disease, 1993. J. Shellfish Res. 13(1):314. (Abstract).

Lewis, E. J. and C. A. Farley. 1994. Effects of salinity and selected treatments on Juvenile Oyster Disease. J. Shellfish Res. 13(1):316. (Abstract).

Support

Years	NRAC USDA funding	Other support: University	Other support: Industry	Other support: Other Federal	Other support: Other	Other support: Total	Total Support
1993-94	$10,000			$19,000		$19,000	$29,000
Total	**$10,000**			**$19,000**		**$19,000**	**$29,000**

1994 Project Development (93-10)

Project period January 1, 1994 to December 31, 1994

NRAC funding
$38,740

Principal investigator
Dr. Victor J. Mancebo, Northeastern Regional Aquaculture Center, Massachusetts

Objectives
- Convene two meetings of the Technical/Industrial Advisory Council in 1994.
- Fund travel expenses for Technical and Industry Advisors to attend Work Group meetings.

Principal accomplishments
Two meetings of the Technical/Industrial Advisory Council were convened during the project period.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1994	$38,740						$38,740
Total	**$38,740**						**$38,740**

1994 Industry Summit (93-11)

Project period January 1, 1994 to March 31, 1994

NRAC funding
$15,360

Work group chair
Dr. Victor J. Mancebo, Northeastern Regional Aquaculture Center, Massachusetts

Objectives
- Work with state aquaculture associations in the Northeastern Region to select participants and develop an agenda for an Industry Summit to be convened in February 1994.
- Cover all expenses associated with holding a 2-day summit.

Principal accomplishments
Participants were selected to develop an agenda for an Industry Summit which was convened on February 29, 1994.

A set of priority topics were identified by the industry participants. These were listed by rank order of importance to the industry in the Northeast Region and problem statements were drafted. The problem statements became the basis for the “Annual Request for Proposals” issued by NRAC for the 1994 RFP and 1995 RFP.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1994	$15,360						$15,360
Total	**$15,360**						**$15,360**

Aquaculture and the Marine Environment: The Shaping of Public Policy (93-12)

Project period August 12, 1993 to March 12, 1994

NRAC funding

$10,000

Work group chair

Dr. Harlyn O. Halvorson, Policy Center for Marine Biosciences & Technology (PCMBT), Massachusetts

Participating investigators/ cooperative agencies

Dr. Hans Kornberg, Christ College, Cambridge, England
Dr. Alex Keynan, Hebrew University, Jerusalem, Israel
Mr. Eric Anderson, University of Massachusetts Dartmouth, Massachusetts

Objectives

- To conduct a workshop to address relevant issues in waste management and release of cultured organisms.
- To prepare proceedings of the workshop for different audiences.
- To carry-out an educational and implementation program that reaches the appropriate people as an extension component of the workshop.
- To recommend policy changes.

Principal accomplishments

The main objectives of the initial proposal have been met. PCMBT has been formed, a broadly-based steering committee selected, and with their help the mission of PCMBT was defined. A brochure describing the goals and activities of the Center has been prepared by Eric Anderson. The workshop on “Aquaculture and the Marine Environment: the Shaping of Public Policy” was held last August/September and a final report prepared and distributed.

The differences we have encountered since the original proposal have been to more sharply define the nature of the next workshop. Identifying the primary causes of failure of aquaculture in the U.S. has helped to focus more clearly areas in which PCMBT can play a more active role. For example, state and local regulations and permitting requirements are disincentives to aquaculture. Ways are being sought in which PCMBT can be a mediator in discussions between all the interested parties.

Mr. Eric Anderson prepared a report from the notes, documents, and tapes of the workshop. After review by staff of PCMBT, the early drafts were reviewed by the steering committee on September 21, 1993, and the final draft on November 9, 1993. Final approval was received at two steering committee meetings (November 17, 1993, in Washington, DC and November 19, 1993, at Kennedy School of Government, Harvard University). This report has been widely distributed, including members and staff of the House of Representatives Committee on Merchant Marine and Fisheries (who are considering legislation), the Office of Technology Assessment (OTA), and relevant federal and state agencies.

Numerous discussions have been made with individual members of the steering committee to focus planning for the next workshop.

Dr. Halvorson attended the World Aquaculture Meeting in New Orleans in January and the Coastal Science Session at the Cape Cod Community College "Aquaculture on Cape Cod: Economic Opportunities and Legal Constraints" on March 4 where there were opportunities to discuss the recommendations of the workshop. He attended a preplanning meeting for the International Marine Biotechnology Conference in Naples, Italy in February 1994, for which PCMBT is a sponsor. This meeting took place in Tromso, Norway in August 1994. PCMBT organized a second version of "Aquaculture and the Marine Environment: the Shaping of Public Policy" at this meeting.

Dr. Halvorson served on the Offshore Aquaculture Committee (OTA) and reviewed the OTA draft report, "Aquaculture Food and Renewable Resources from U.S. Waters - International Examples of Success and Failure and their lessons for the U.S." PCMBT received a grant from OTA for a study on Aquaculture to be conducted by Dr. Rollin Johnson.

Anticipated benefits

As noted in the final report, the conference "Aquaculture and the Marine Environment: The Shaping of Public Policy" was sponsored by a number of organizations knowledgeable about and interested in marine aquaculture. Participants represented government, academia, industry, research institutes, and public interest organizations.

This conference was designed to explore public perceptions about marine aquaculture, assemble relevant facts and realities surrounding these perceptions, and explore the economic impacts being achieved or lost as a result of these perceptions and regulations. Two major themes of the workshop were considered to be of public concern. The first was waste management (including water quality) and the second was interactions of aquatic stocks with native populations, which included risks, as well as the impacts of released animals. These topics were examined from various perspectives in order to create a comprehensive overview that will aid lawmakers in formulating fair regulations that are sensitive to environmental, economic, and social issues. It was made clear that current regulations are a severe hindrance to further aquaculture development, and that this issue must be addressed if the United States is going to reap the benefits of a strong, internationally competitive aquaculture industry. The topic is timely since Congress will be considering new legislation regarding aquaculture this year and in the next decade.

PCMBT has developed numerous contacts with people in the aquaculture industry and with organizations representing the industry. Their newsletters have covered our workshop and we are now getting requests for help and inquires from members of the aquaculture industry community. I expect this trend to continue.

One unexpected bonus, in part due to analysis of the causes of failures in aquaculture, was the planning for a short course to help corporate executives in the biotechnology industry enhance their knowledge and the skill base for decision making in biotechnology. This plan has grown out of cooperative planning with faculty and administration members at the University of Massachusetts Dartmouth, the Biotechnology Center for Excellence Corporation, the Southeastern Massachusetts Partnership, and PCMBT. This course illustrates the ability of PCMBT to leverage support from other organizations.

Suggested future work

Two main problems were identified from the workshop of the previous year:

1. Economic inability of aquaculturists to prepare a business plan and to analyze market possibilities. Aquaculture should be treated as part of biotechnology; as such it should emphasize job-creating possibilities or else downstream processing of products from it.
2. Regulations at both the state and local levels. Federal and state regulators are very poorly informed about aquaculture. A New England Aquaculture Authority is needed as this industry transcends state boundaries. Aquaculture needs to be placed on the agenda of the governors meetings. An education program is needed for regulators.

As a result of this activity, we recommend that another workshop be convened. The focus of this workshop should be how sustainable non-polluting aquaculture ventures can be achieved in the New England Region.

This workshop should:
• define the information needed,
• identify the available sources, and

• discuss how such information could be assembled in the most useful form.

An agenda for this workshop has been proposed. This agenda is based on interviews with various persons who are trying to promote aquaculture in this region who have been in contact with potential entrepreneurs. There is a general consensus that the technical questions, which have to be considered by potential applicants of aquaculture, are only part of the problem. Critical to the initiation of a project and its successful outcome are detailed considerations of:
• its economic feasibility and
• the requirements of the various regulatory agencies, which differ between and within states.

A list of potential participants in such a workshop who could help define the outline of an information book, has been developed as well as experts who could supply the necessary information and advice on the best approach to reach the desired audience.

The information book should be about 100-200 pages, and written in easily understandable language. It must be emphasized that this is not a textbook to teach people about aquaculture. It is intended to be a means of directing interested people to the information necessary to initiate a marine Aquaculture project. The sources of information may be publications, databases, reports, and academic consulting institutions. This book should inform the reader on the problems they may have to confront and where the information can be found to address them.

Publications and presentations

Halvorson, H. O. 1993. Aquaculture and the marine environment: The shaping of public policy. The Northeastern Regional Aquaculture Center/USDA Publication #NRAC 93-009. North Dartmouth, MA. 30pp.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1993-94	$10,000	$7,800			$19,200	$27,000	$37,000
Total	**$10,000**	**$7,800**			**$19,200**	**$27,000**	**$37,000**

Support for Production of INAD Videos (93-13)

Project period February 25, 1994 to February 24, 1995

NRAC funding

$2,000

Principal investigator

Ted R. Batterson, Michigan State University, Michigan

Participating investigators/ cooperative agencies

Robert K. Ringer, Michigan State University, Michigan

Objectives

• Ensure effective communications among groups involved with Investigational New Animal Drug (INAD) applications, including liaison relations with Canada.
• Serve as an information conduit between applicants and the US Food & Drug Administration/Center for Veterinary Medicine.

Principal accomplishments

As National Coordinator for Aquaculture INADs, Ringer participated with the Food and Drug

Administration (FDA) and the Center for Veterinary Medicine (CVM) in educational workshops on INAD procedures and requirements. These workshops were conducted throughout the U.S. This included workshops held in conjunction with the U.S. Trout Farmers Association, Boston Seafood Show, and Aquaculture Expo V in New Orleans.

Publications and presentations

The March 18, 1993, workshop at the Boston Seafood Show was videotaped. The videos are available on cassette from the Northeastern Regional Aquaculture Center.

Batterson, T. R. 1993. INAD workshop. Northeastern Regional Aquaculture Center/USDA Video #V-4. North Dartmouth, MA. 282:00.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1994-95	$2,000						$2,000
Total	**$2,000**						**$2,000**

Market Research for Nori Production (94-1)

Project period February 25, 1994 to February 24, 1995

NRAC funding

$9,500

Project period

January 1, 1994 to December 31, 1994

Principal investigator

Ira Levine, Coastal Plantations International, Inc., Maine

Participating investigators/ cooperative agencies

J. Eldredge, Commonwealth Marketing, Maine
J. Kupel, Commonwealth Marketing, Maine
P. Gartland, Commonwealth Marketing, Maine
L. Conneen, Commonwealth Marketing, Maine
S. Crawford, Coastal Plantations International, Inc., Maine
S. Erhart, Maine Coast Sea Vegetables, Maine
A. Bonner, Coastal Plantations International, Inc., Washington
R. Bisi, Matrix Marketing, Massachusetts
L. Bock, Yes Marketing, Washington
H. Burke, Commonwealth Marketing, British Columbia
J. Davis, California Marketing, California
D. Fiol, Matrix Marketing, Maryland
R. Flores, Ray Flores Associates, California
K. Jackson, Commonwealth Marketing, Ontario
L. Long, Lone Star Naturals, Texas
J. Maurer, Matrix Marketing, Pennsylvania
S. Saturn, Commonwealth Marketing, California
S. Shelton-Foley, Commonwealth Marketing, Illinois
P. Stein, Matrix Marketing, New Jersey

Objectives

- To determine the number, location, and identity of supermarket chains carrying (or interested in carrying) nori and sushi products.
- To determine identity, source, and retail price points of competitive products.
- To review current packaging and merchandising of competitive products.
- To identify appropriate food brokers for future sales representation of Coastal Plantation International's product line.
- To identify additional key players in the distribution channel (e.g., whole-salers/distributors).
- To confirm estimated $6 million market size within supermarkets.

• To develop a demographic profile of the supermarket consumer of nori and sushi products.

Anticipated benefits

- Obtained nori and sushi product information and identify the potential for market penetration of the supermarket distribution channels by American-produced nori.
- Research results will be incorporated into future product line configurations, packaging design, pricing strategy, in-store merchandising strategy, and the selection of specific geographical markets.
- Increase the potential for the success of American-produced nori in the U.S. marketplace
- Marketplace success will stimulate additional nori cultivation sites along the Atlantic Coast of the Northeast and Mid-Atlantic states.

Principal accomplishments

Research indicates a viable niche for an American-produced nori product in upscale supermarket chains, particularly in West Coast Markets. The supermarket audit indicates nori sales between $4 and $6 million with nori prices ranging from a low of $0.17/sheet to a high of $0.49/sheet. Average nori sheet price approaches $0.40.

The West Coast followed by the Midwestern markets appear to be the most active concerning nori sales. The typical nori consumer is either Asian American or a sophisticated Caucasian consumer, 25-59 years with a college education and a household income over $35,000.

Impacts

This project identified the specifics of one segment of the U.S. nori market - supermarket chains. As a result of the research, marketing strategies were modified and opportunities were identified. The ability of American-produced nori to penetrate this market segment was enhanced.

Recommended follow-up activities

Subsequent research on the development of an American nori industry is multiphasic. Additional funds are needed to: 1) improve nori cultivars through genetic enhancement; 2) continue the Japanese-American nori farmer exchange program; 3) improve cultivation, engineering and farming technologies; 4) conduct market research and development; and 5) provide consumer education.

Publications and presentations

A comprehensive listing of markets and supermarket chains appears in the NRAC Final Technical Report.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1994-95	$9,500		$8,000			$8,000	$17,500
Total	**$9,500**		**$8,000**			**$8,000**	**$17,500**

A Proposal to Study Potential Ciliate Pathogens of Hatchery-Reared Juvenile Oysters in Northeastern United States (94-3)

Project period February 21, 1994 to April 20, 1995

NRAC funding
$10,000

Principal investigator
Eugene B. Small, University of Maryland, Maryland

Objectives
- To collect and fix oyster tank protists at the collections sites.
- To stain the ciliates with appropriate diagnostic silver stains in the laboratory.
- To identify stained ciliates and designate (if applicable) potentially causal disease organisms.

Principal accomplishments
Protists were collected at F. M. Flower and Sons Co. in conjunction with Farley and Lewis collections of juvenile oysters. The experimental tank water in which juvenile oysters became infected at the Oxford Mary land Laboratory facilities were also sampled as was the filtered solids from the Oyster Bay Hatchery. Water in the floating oyster trays at the hatchery was also extensively sampled.

Filtered solids collected from oyster racks at Oyster Bay and from Oxford Laboratory experimental tanks, (particularly those retained by 5 micron filter material) contained a new species of the suctorian ciliate, *Endosphaera* spp. This new species was successfully isolated and we initiated culture efforts. This small (< 10 m diameter) ciliate has been found in those sites wherein one might expect to find a presumptive pathogen. The size of the suctorian organism is in the right order of magnitude to be the intracellular parasite (found in histological section by Farley and Lewis) of infectious JOD.

Conferences have been held between the PI, Farley, and Lewis to demonstrate the stained organisms. Research results were presented by the PI at the 1995 15th Milford, Connecticut Aquaculture Seminar (Small, 1995).

Impacts
Results establish a firm association of the suctorian ciliate with the presence of JOD syndrome. This gives an even stronger credence to the role of a suspected protist.

Recommended follow-up activities
1. Further studies to identify the disease agent.
2. Isolation of disease agent directly from the JOD-infected oysters.
3. Further filtration studies to determine the size of the infectious organism.
4. Cultivation of the suctorian in order to develop an innocula for uninfected oysters.
5. Transmission electron microscopy studies to better identify the etiological agent in the environment and in tissues of infected oysters.

Publications and presentations
Small, E. B. 1995. Ciliate protists associated with juvenile oyster disease. 15th Milford Aquaculture Seminar, Milford, CT, February 21-23, 1995. (Abstract). 20pp.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1994-95	$10,000						$10,000
Total	**$10,000**						**$10,000**

Continuing Studies of the Role of Bacteria (*Vibrio* spp.) and Microalgae (*Gymnodinium sanguineum*) in Unexplained Juvenile Oyster Mortalities (94-4)

Project period February 21, 1994 to April 20, 1995

NRAC funding
$11,145

Project period
July 1, 1994 to November 30, 1995

Principal investigator
Gordon Taylor, State University of New York, Stony Brook, New York

Participating investigators/ cooperative agencies
Monica Bricelj, State University of New York, Stony Brook, New York

Susan Ford, Rutgers University, New Jersey, New Jersey

David Relyea, Frank M. Flower and Sons Oyster Co., New York

Objectives
- To complete taxonomic identification of the pathogenic isolate, since only a tentative identification is likely from our current efforts.
- To evaluate optimal conditions for disease transmission.
- To consider a variety of countermeasures that might be applied to reduce the incidence of juvenile oyster mortality.

Principal accomplishments
We performed a series of challenge experiments with suspected pathogenic *Vibrio* isolates collected from symptomatic oysters at the Frank M. Flower and Sons Oyster Co. nursery site in Oyster Bay, New York. In additional to the current project, this series of challenge experiments was funded by two other grants. Although reported, in part, elsewhere, results from all experiments are discussed below in order to present a cohesive synopsis of our accomplishments.

Challenge experiments were performed on oysters from New Jersey and Long Island hatcheries where Juvenile Oyster Disease has never been reported. In three experiments, duplicate batches of oysters were injected with one of 10 strains of *Vibrio* that had been isolated from Frank M. Flower and Sons Oyster Co. Nursery. Two strains caused significant mortalities in the first and second experiments, although anomalous conchiolin deposits, typical of JOD, were not clearly and consistently evident. However, brown blister-like deposits were observed in all injected oysters. In three other experiments, oysters were infected by exposure to suspended bacteria instead of by injection. Frequent exchange of tank water was found to be important in conduction challenge experiments. Conchiolin deposits and ridges similar to early JOD symptoms were observed in some of the treatment oysters, and not in controls.

The numerically dominant strains of *Vibrio* were re-isolated from challenged oysters. The reisolates had the same physiological and metabolic characteristics as the *Vibrio* strains originally introduced to the experiment. The two strains which caused high mortalities within 7 to 14 days in the first and second challenge experiments and high mortalities in the forth and fifth challenge experiments most closely resembled *V. alginolyticus* and *V. anguillarum*.

Bacterial cultures were prepared for maintenance at -70°C. Frozen strains were periodically inoculated into two different media to confirm viability. Non-viable cultures were discarded. Viable cultures were refrozen in 20% glycerol at -70°C. Two algal isolates of *G. sanguineum* were maintained for use in future combined bacteria-phytoplankton exposures.

Impacts
Results of challenge experiments using *Vibrio* spp., isolated from Frank M. Flower and Sons Oyster Co., implicate these bacteria as an important factor in mortality of oysters which cannot be rejected as an etiological agent of juvenile oyster disease (JOD) at this time.

Recommended follow-up activities

Additional challenge experiments should be performed with other *Vibrio* and non-*Vibrio* species to confirm or reject whether a single species is responsible for the JOD syndrome and to fully satisfy Koch's postulate. In future challenge experiments, continuous exposure of bacteria and frequent changes of tank water are recommended. Also, these experiments should entail varying environmental factors such as continuous exposure to the food sources and addition of dinoflagellate *Gymnodinium sanguineum*, a possible stressor repeatedly observed in the nursery. Pathogenic strains must be identified unequivocally. Conditions favoring and discouraging JOD should also be identified to provide growers with additional means for controlling the disease.

Publications and presentations

Lee, M. 1995. The role of *Vibrios* in unexplained Juvenile Oyster Disease. MS thesis. Marine Environmental Sciences, State University of New York at Stony Brook, NY.

Lee, M., G. T. Taylor, V. M. Bricelj, S. E. Ford, and S. Zahn. 1996. Evaluation of *Vibrio* spp. and micro-plankton blooms as causative agents of Juvenile Oyster Disease in *Crassostrea virginica* (Gmelin). J. Shellfish Res. 15(2):319-329.

Papers presented

Lee, M., G. T. Taylor, V. M. Bricelj, and S. E. Ford 1995. Continuing studies of the role of bacteria (*Vibrio* spp.) in unexplained juvenile oyster mortalities. (Abstract). 15th Annual Milford Aquaculture Seminar. February 21-23, 1995.

Lee, M., G. T. Taylor, V. M. Bricelj, S. E. Ford, and S. Zahn. 1995. The potential role of bacteria (*Vibrio* spp.) and microalgae (*Gymnodinium sanguineum*) in Juvenile Oyster Disease (JOD) in hatcheries of Northeastern Untied States. Presented at the International Workshop of Shell Disease in Marine Invertebrates: Environment-Host-Pathogen Interactions Meeting. Brest, France. March 1995.

Lee, M., G. T. Taylor, S. Zahn, V. M. Bricelj, and S. E. Ford. 1994. The role of *Vibrios* in unexplained juvenile oyster mortality at the F. M. Flower and Sons Oyster Hatchery in Oyster Bay, New York. Long Island Sound Research Conference, State University of New York at Stony Brook, NY. September 30, 1994.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1994-95	$11,415						$11,415
Total	**$11,415**						**$11,415**

A Comprehensive Study of East Coast JuvenileOyster Disease in the Northeastern United States (94-5)

Project period July 18, 1995 to July 17, 1995

NRAC funding

$9,500

Principal investigator

Frederick G. Kern, NOAA, NMFS

Participating investigators/ cooperative agencies

Earl J. Lewis, NOAA, National Marine Fisheries Service, Maryland

C. Austin Farley, NOAA, National Marine Fisheries Service, Maryland

Dorothy W. Howard, NOAA, National Marine Fisheries Service, Maryland

Objectives

- To continue pathology and electron microscopy studies to identify the disease agent.
- To attempt to isolate the disease agent from infected oysters and aquaria by sieving and differential centrifugation.
- To test potential resistance of Flower's broodstocks with susceptible strains.
- To conduct field filtration experiments at a commercial JOD-affected facility to determine the size of the infectious particle responsible for the disease.
- To follow-up transmission studies of the disease using bacterial and protistan isolates of the 1993 study, as well as use salinity and therapeutic medications as a barrier to transmission.
- To make further attempts to isolate bacteria from experimentally infected oysters as warranted by data.

Principal accomplishments

One hundred forty nine samples of juvenile oysters were evaluated grossly for juvenile oyster disease during July to September 1994. Selected specimens were fixed for electron microscopy by several different methods in an effort to improve fixation. Rare intracellular bodies, resembling what we see in light microscopy, continue to be found. Histological sections of paraffin-embedded tissue were tested with monoclonal antibodies to *Bonamia ostreae* and *Mikrocytos mackini*. Tests were negative for *M. mackini* and inconclusive thus far for *B. ostreae*. Whole oyster pools from two samples of JOD-infected oysters tested negative for virus.

Because of an apparently limited number of JOD organisms present in any given oyster, the feasibility of sieving and differential centrifugation may not be applicable for isolation of the agent. However, since evidence suggests that a protist may be involved, special training in how to isolate individual protists was acquired. Attempts to isolate the JOD agent were also made in collaboration with Dr. Eugene Small. Samples were provided to Dr. Small from our work and the F. M. Flower and Sons Hatchery and experimental transmission aquaria.

Oysters from potentially resistant broodstock and susceptible broodstock were spawned and compared. Experimental data indicate resistance is being developed in Flower's broodstock.

Replicate groups of two spawning batches were exposed to filtered water at two JOD-infected locations. Oysters held in 25 µm filtered water became infected by JOD, but suffered lower cumulative mortalities and experienced a longer incubation period than those held in 50 µm or unfiltered water.

A transmission study at salinities of 14-30 ppt, to duplicate our 1993 work, showed infections were obvious only at salinities of 18 ppt and higher. An experimental laboratory study showed JOD was able to be transmitted by material filtered from infected water without the presence of JOD-infected oysters. A repeat study showed little transmission even though *Vibrio* counts were very high. Cultures of ciliates isolated from JOD-infected oysters last summer were not able to be maintained and utilized in transmission experiments. A spirochaete is being evaluated for possible use in transmission experiments.

Fifty experimentally infected and control oysters, plus 20 naturally infected oysters, were culture for vibrios and other bacteria. No correlation between JOD and a *Vibrio* spp. was found.

Oysters were exposed to JOD-infected water maintained at room temperature for 8 months without the presence of JOD-infected oysters. The water did not transmit the disease.

Impacts

Development of a JOD resistant strain of oyster has strong application for survival of cultured oysters and growth of the aquaculture industry. Survivability in juveniles thus far shows resistance to JOD two-seven times that of other juveniles currently used.

Results of filtration studies show the causative organism is a filterable agent. However, most hatcheries use 5 to 25 µm filtered water to spawn and rear early life stages of oysters. This level of filtration does not appear to be adequate to prevent transmission of JOD to young oysters. This conclusion is based on early test results which show the agent can pass a 5 µm bag filter.

Recommended follow-up activities

1. Continued follow-up of JOD resistance in Flower's resistant strains. Continue to compare effects of JOD

on F_1, F_2, and F_3 strains of these oysters against susceptible stocks in field trials at numerous locations around Long Island Sound.

2. Investigate the possibility of transmitting JOD from other bivalves (such as hard clams) which may act as carriers of JOD without themselves being affected.

3. Continue electron microscopy and *Bonamia* monoclonal antibody studies to identify the disease agent.

All three of these follow-up activities are being conducted currently by our team under a NOAA Oyster Disease Research grant.

Publications and presentations

Farley, C. A. and E. J. Lewis. 1995. Juvenile oyster diseases studies 1994: Epizootiology, geographic occurrence. (Abstract). 15th Annual Milford Aquaculture Seminar. February 21-23, 1995. J. Shellfish Res. 14(1):241. (Abstract).

Farley, C. A., E. J. Lewis, D. Relyea, and J. Zahtila. 1995. Studies of resistance in progeny of broodstock selected from juvenile oyster disease (JOD) survivors. (Abstract). 15th Annual Milford Aquaculture Seminar. February 21-23, 1995. J. Shellfish Res. 14(1)242. (Abstract).

Lewis, E. J., C. A. Farley, D. Relyea, J. Zahtila, and G. Rivara. 1995. Transmission and filtration studies of juvenile oyster disease (JOD). (Abstract). 15th Annual Milford Aquaculture Seminar. February 21-23, 1995. J. Shellfish Res. 14(1):243-244. (Abstract).

Lewis, E. J., C. A. Farley, E.B. Small, and A. Baya. A synopsis of Juvenile Oyster Disease (JOD) experimental studies in *Crassostrea virginica.* Submitted to Aquatic Living Resources to be published with other papers from the International Workshop on Shell Disease in Marine Invertebrates: Environment-Host-Pathogen Interactions. Brest France. March 29-31, 1995.

Small, E.B. 1995. Ciliated protists associated with Juvenile Oyster Disease. J. Shellfish Res. 14(1):247-248. (Abstract).

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1994-95	$9,500			$19,400		$19,400	$28,900
Total	**$9,500**			**$19,400**		**$19,400**	**$28,900**

Collection of Atlantic Halibut for the Regional Development of a New Aquaculture Species (94-6)

Progress report period July 18, 1994 to August 31, 1996

NRAC funding

$30,515

Project coordinator

Christopher Bartlett, University of Maine, Maine

Principal investigators/ cooperative agencies

Francis Ayres, Maine Salmon, Inc., Maine
Thomas Duym, Marine Technology Center, Maine
Michael Hastings, Maine Aquaculture Innovation Center, Maine

Objectives

- To establish a network of trained commercial fishermen for live halibut capture and transfer. Participation of several fishermen and completion of training workshops will effectively assess this portion of the study.
- To capture and transfer wild caught halibut to holding facilities at participating salmon aquaculture

operations. Success will be gauged relative to the number of fish facilitated, with the procurement of 75-120 individuals being possible.

- To design and construct a cage for flatfish cultivation which is compatible with existing salmon farm operations. Successful deployment and a review of performance during the growout trials will evaluate this objective.
- To maintain captive halibut for a 1-year period of performance, and collect biological information relevant for the domestication of this species. Documentation of these growth and health parameters over the course of the year will be used to assess this phase of the project.

Anticipated benefits

The project will gain valuable information on Atlantic halibut broodstock collection and containment, and anticipates the production of viable gametes for regional research on the culture of juvenile fish.

Principal accomplishments

The presence of two mature male halibut during the November inspection prompted project participants to investigate the transfer of fish to a land-based facility in preparation for potential spawners. Two halibut were trucked to an on-shore facility at Atlantic Aquafarms in Franklin, Maine, in December of 1995. Investigators chose two non-mature fish for this transfer in hopes of minimizing the risk of losing gravid fish during this trial run. The transfer was successful, however temperature fluctuations at the Franklin facility postponed further inspection and transfer attempts for several weeks. Two subsequent inspections in January and March found only eight mature males and no mature females. No additional halibut were transferred to the Franklin facility because of the lack of viable eggs.

The two halibut were administered a formalin bath upon arrival at the land based system to remove the presence of flukes, *Entobdella hippoglossi*, and any other non-apparent external parasites. The fish were contained in a 12 x 3.5 circular fiberglass tank with a recirculated supply of sea water. Water temperature was maintained at 3^{o}C after a brief incident where the temperature fell to 0^{o}C. During their 3-month containment, these halibut did not visibly accept feed, though their general appearance remained excellent. These fish were transferred back to Eastport during a delivery of Atlantic salmon smolt from the Atlantic Aquafarms Hatchery. The aeration equipment experienced difficulties during the drive, and the truck pulled over enroute for a 4-hour period while a replacement generator was obtained. The fish were returned to the containment pen at Maine Salmon, Inc., but were found dead 1 week later. Post-mortem examinations did not reveal the causative nature of their deaths because of partial decomposition.

Winter storms had a major impact on area fish farms, including the Maine Salmon Inc. operation where the holding pen for the halibut project is located. Twelve of the 17 existing salmon cages were damaged beyond repair at the Maine Salmon Farm between January and March. The halibut cage sustained minor damage to one hand rail and several net fastenings, which were quickly repaired after each incident. No halibut were lost immediately following these storm occurrences, but the fish fed poorly throughout the winter and early spring months.

Dive inspections during March, April, and May found eight additional dead halibut. Many remaining fish were suffering from a combination of external bacterial infections and fluke infestations. Consultation with Dr. Ken Waiwood, Department of Fisheries and Oceans Halibut specialist, resulted in treating the fluke infestations with a freshwater bath for 30 minutes. On June 8, 1996, fish were removed from the pen and placed into 30 cubic foot insulated, Dynoplast containers filled with well water. Many of the largest fish were difficult to manage with the project’s dip nets and containment boxes. Removal of the flukes was successful, but it was obvious to participants that larger tanks would be needed for future treatments of this nature. Because of the obvious stress to the fish during treatment it was decided to forego the scheduled weight measurements program. Samples weights were taken on six of the smaller fish and three floy anchor tags were replaced during the bath procedure. Five of the weighed fish had gained 5-12 pounds since the November 1995 measurements. These weights gains correlated to a 20%-34% gain in body weight. One of the halibut weighed was missing a tag, and thus no growth determination could be assessed.

Collection of wild halibut continued during the Spring of 1996, but with much less success. Commercial vessels did not catch any fish for the project until mid- May, approximately 1 month after their initial attempts. The price per pound for the fish was raised to $9.00 in hopes of encouraging further efforts. A total of five fish were transferred to the containment pen. One halibut died due to capture-induced trauma, bringing the total number of fish for the project to 27.

The halibut fed well through the late spring and summer months on a diet comprised primarily of fresh-caught mackerel and pollack. Supplemental vitamin and mineral capsules were provided by Dr. Linda Kling, University of Maine fish nutrition specialist, to ensure proper nutrition levels for gamete development. General husbandry practices and routine maintenance to the holding pen are continuing.

Work planned

On August 29, 1996, the Maine Aquaculture Innovation Center brought together a meeting of project participants and representatives of the University of Maine and the private sector to discuss the next phases of this project. It was decided that developing a small land based holding system would be essential for successful egg takes. The group anticipates using a sonar imaging unit from the University of Maine to assess the gonadal development of each halibut prior to transfer to the on-shore tanks. Nutritional studies and spawning trials would proceed through the winter months. The halibut would then return to the Maine Salmon Farm site for cost-efficient containment throughout the summer and fall months.

Impacts

The potential impact of this project on the research anddevelopment of Atlantic halibut culture in the Northeastern United States cannot be over stressed. This is currently the only captive population of these fish in our region.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1994-95	$30,000				$14,130	$14,130	$44,645
1995-96					$17,134	$17,134	$17,134
Total	**$30,000**				**$31,264**	**$31,264**	**$61,779**

Second Northeast Region Aquaculture Industry Situation and Outlook Report (94-7)

Project period November 15, 1994 to July 17, 1996

NRAC funding

$17,000

Project coordinator

James L. Anderson, University of Rhode Island, Rhode Island

Objectives

- To update the 1993 Bush and Anderson Northeast Region Aquaculture Industry Situation and Outlook Report by compiling estimates of private aquaculture production and value for the Northeastern Region for the period 1994-1995.
- To identify future opportunities and current problems facing the industry.
- To assist in identifying priority research directions based on industry need.

Anticipated benefits

The project will yield a valuable update of the situation of aquaculture in the Northeast United States which can be used to identify the current status and trends in the industry, and in setting research priorities. This information will also be of value to extension personnel, researchers, resource managers, and potential investors.

Principal accomplishments

- Prepared the, "Second Northeast Region Aquaculture Industry Situation and Outlook Report," which serves as an update to the 1993 Bush and Anderson Report, "Northeast Region Aquaculture Industry Situation and Outlook Report."
- Printed 600 copies and have distributed the majority. Requests are received weekly for this publication.
- Identified current constraints to aquaculture industry growth.
- Identified future aquaculture industry opportunities.
- Targeted priority research areas.

Recommended follow-up activities

"The Northeast Region Aquaculture Industry Situation and Outlook Report" should be updated on a regular basis.

Publications and presentations

Spatz, M. J., J. L. Anderson, and S. Jancart. 1996. Northeast Region Aquaculture Industry Situation and Outlook Report (1994-1995), Vol. 2. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 96-001 (Formerly NRAC #223). North Dartmouth, MA, [Rhode Island Agricultural Experiment Station Publication #33352, Kingston, RI]. 70pp.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1994-95	$17,000						$17,000
Total	**$17,000**						**$17,000**

Use of Artificial Spat Collectors to Assess and Enhance Recruitment of Scallops (*Argopecten irradians*) (94-8)

Project period November 1, 1994 to October 31, 1995

NRAC funding

$10,000

Principal investigator

Michael A. Rice, University of Rhode Island, Rhode Island

Participating investigators/ cooperative agencies

Jefferson T. Turner, University of Massachusetts Dartmouth, Massachusetts

Wayne Turner, The Waterworks Group, Inc., Massachusetts

Karin A. Tammi, University of Rhode Island, Rhode Island

Jean Lincoln, University of Massachusetts Dartmouth, Massachusetts

Scott Soares, The Waterworks Group, Inc., Massachusetts

Objectives

1. To determine the timing of scallop spatfall onto spat collectors in the Westport River Estuary.
2. To compare current regime and phytoplankton/seston availability in areas known for traditionally high scallop populations.
3. To begin making correlations between numbers of scallops on spat collectors with annual scallop harvests.

Anticipated benefits

There have been a number of impacts by the project on the community of Westport. Information as to optimum timing and location of spat collectors in the Westport River will be used by the Shellfish Commission of the town of Westport for continuing the project as part of their ongoing shellfish management activities (longer term studies related to objective 3). Coincidental to planned impacts, the project has become a focus for environmental education in the Westport Watershed area. Students from two high schools and a middle school have participated in the project as part of their science education. One of the project co-investigators (The Waterworks Group, Inc.) was recently awarded the 1995 First Prize in Environmental Education Excellence by the Secretary of Education and Secretary of Environment of the Commonwealth of Massachusetts.

Principal accomplishments

Research conducted in 1994 focused on improving the methods from a 1993 pilot study and to address objective 1. During the summer 1994, spatlines containing 20 spat collectors were sequentially deployed at eight study sites. A single spatline was deployed weekly to each area for a period of 10 weeks to determine peak settlement and recruitment times and evaluate fouling and mud crab predation. A total of 80 spatlines and 1,600 spat collectors were placed within the Westport Estuary (each study site containing a total of 10 lines and 200 spat collectors). The September to October 1994 harvest yielded 1,088 scallops. The most productive study sites were Corey's Island (492 scallops), Canoe Rock (182 scallops), Jug Rock (106 scallops), and Southard Shore (104 scallops). The best recruitment for any of the study sites was observed at Corey's Island which yielded a total of 492 scallops. In general, the greatest recruitment to all eight study sites occurred on spatlines deployed between June 21 and July 21. Both Speaking Rock and Hick's Cove had the poorest scallop recruitment, but displayed the greatest bio-volume of mud crabs, *Panopeus* spp. (16,520 ml and 14,375 ml, respectively).

Adult scallops were placed in surface and bottom rafts to determine growth and mortality. These scallops were sampled on a bi-weekly basis from March to October 1994 to monitor gonadal development. Histological analysis indicated that gonadal development commenced in late March. In late June and early July, scallops were at the peak of gonadal development. By August 25, gonads were empty indicating that the scallops were spawned out. Gametogenic activity of scallops ceased in October.

Efforts have been taken to assess the average water currents and food availability for scallops in the study sites. Results indicate that food flux (the product of food concentration measured in mg/L organic seston and average current speed or µg/L Chlorophyll-a and average current speed) both of which were highest at the Corey's Island site which has the traditionally highest scallop harvest.

A key feature of the Scallop Restoration Project is the involvement of community volunteers and students from local high schools and middle schools. The educational aspects of the project have been recognized by the Commonwealth of Massachusetts as a model program in environmental education by receiving a formal commendation from the Secretaries of Education and the Environment.

Impacts

There are four basic areas in which this project has contributed to the development of coastal aquaculture in the Southeastern New England Region. First, the demonstration of the technical feasibility of using artificial spat collectors for bay scallops will be of use to aquaculturists and fishery managers in the area. Second, knowledge of scallop larval timing will assist local shellfish managers in optimizing their stock enhancement programs. Third, the scallop program has served as a basis for an ongoing hands-on science education and aquaculture training program for elementary and secondary level students. These students are potentially the future aquaculturists of the region. Finally, the grass roots community involvement in Westport, Massachusetts has allowed many to participate in a program using the tools of aquaculture. This is important in Massachusetts where the doctrine of "home rule" or local management of coastal resources, is in place so local attitudes toward aquaculture will strongly influence the development of coastal projects whether public or private.

Recommended follow-up activities

The Waterworks group and the people of Westport are continuing with their efforts to use aquaculture techniques to restore shellfish to the Westport River, and to use shellfish as a focus for maintaining water quality. The following research topics have been identified by the Waterworks group for future studies:

1. spat collection of other commercially important shellfish species (oysters) in the Westport Estuary;
2. study of effects of nutrient overloading and algal fouling on bay scallop recruitment to eelgrass and spat collectors;
3. predation effects of mud crabs on newly settled bay scallops in spat collectors;
4. a performance comparison of commercial spat collectors with onion bag collectors;
5. economic study of the Westport River Shellfisheries to include secondary economic effects;
6. geographic Information System (GIS) mapping of the Westport River Watershed; and
7. expansion of student and teacher involvement in the "Living Laboratory" yearly marine science/ aquaculture curriculum.

Publications and presentations

Tammi, K. A., M. A. Rice, S. Soares, and W. Turner. 1994. Settlement and recruitment of bay scallops, *Argopecten irradians*, to artificial spat collectors in the Westport River Estuary, Westport, Massachusetts. J. Shellfish Res. 13:288. (Abstract).

Tammi, K. A., M. A. Rice, S. J. Soares, W. H. Turner, and M. Bumsted. 1995. The determination of optimal settlement and recruitment of bay scallops, *Argopecten irradians*, to artificial spat collectors in the Westport River Estuary, Massachusetts. J. Shellfish Res. 14(1):248-249. (Abstract).

Tammi, K. A., S. Soares, W. Turner, and M. A. Rice. 1994. Settlement and recruitment of bay scallops, *Argopecten irradians*, on artificial spat collectors in the Westport River Estuary, Westport, Massachusetts. J. Shellfish Res. 13:320. (Abstract).

Tammi, K. A., S. J. Soares, W. H. Turner, and M. A. Rice. 1996. Settlement and recruitment of bay scallops, *Argopecten irradians* (Lamarck 1819), to artificial spat collectors in the Westport River Estuary, Westport, Massachusetts. p. xx-xx. In G. E. Flimlin, ed. Proceedings of the 1994 National Shellfisheries Association Symposium on Shellfisheries Enhancement. Environmental Protection Agency, Washington, D.C. (Accepted).

Tammi, K. A., M. A. Rice, S. J. Soares, and W. H. Turner. 1995. Spawning and spat collection of the bay scallop, *Argopecten irradians*, in the Westport River Estuary. J. Shellfish Res. 14(1):279-280. (Abstract).

Tammi, K. A., W. H. Turner, M. Bumsted, and M. A. Rice. 1996. Veliger voodoo and other witchcraft in the Westport River: Bay scallop, *Argopecten irradians*, veliger abundance and the variability of spatfall and recruitment to artificial spat collectors in the Westport Estuary, Massachusetts. J. Shellfish Res. (Accepted abstract).

Tammi, K. A. 1996. Spawning and recruitment of northern bay scallops, *Argopecten irradians irradians*, in the Westport River Estuary. Masters Thesis. University of Rhode Island, Kingston, RI.

Tammi, K. S. and M. A. Rice. (In Prep.). Gonadal development, larval abundance, and recruitment of northern bay scallops, *Argopecten irradians irradians*, onto artificial spat collectors in the Westport River Estuary. (For submission, J. Shellfish Res.).

Tammi, K. A., J. Lincoln, and M. A. Rice. (In Prep.). Phytoplankton and bivalve larval populations in the Westport River Estuary, Massachusetts during summer of 1994. (For submission, Estuaries).

Tammi, K. A., M. A. Rice, and J. F. Heltshe. (In Prep.). Settlement and recruitment of northern bay scallops onto artificial spat collectors: A comparison of the performance on onion bags with monofilament and commercially produced spat collectors. (For submission, J. Applied Aquaculture).

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1994-95	$10,000			$6,750	$2,000	$8,750	$18,750
Total	**$10,000**			**$6,750**	**$2,000**	**$8,750**	**$18,750**

1995 Project Development (94-9)

Progress report period January 1, 1995 to August 31, 1996

NRAC funding

$43,024

Principal investigator

Dr. Victor J. Mancebo, (Jan. 1, 1995 - Aug. 31, 1995)
Dr. Robert Rheault, (Jan. 1, 1996 - Mar. 31, 1996)
Dr. Kim E. Harrison, (Apr. 1, 1996 - Aug. 31, 1996)

Northeastern Regional Aquaculture Center, Massachusetts

Objectives

- Convene two meetings of the Technical/Industry Advisory Council (TIAC) in 1995.
- Fund travel expenses for technical and industry advisors to attend project meetings.
- Convene one meeting of the TIAC proposal Review Panel in 1995.

Principal accomplishments

- Two meetings of the Technical/Industry Advisory Council were convened during the project period.
- The TIAC Review Panel met to review pre-proposals submitted for the 1995 request for proposal (RFP).

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1995-96	$43,024						$43,024
Total	**$43,024**						**$43,024**

Utilization of Off-Grade Salmon, Salmon Trim, and Mechanically Deboned Salmon in Fermented Sausages (95-9)

Project period November 28, 1995 to November 28, 1996

NRAC funding

$13,300

Principal investigator

Alfred A. Bushway, University of Maine, Maine

Participating investigators/ cooperative agencies

Terry M. Work, University of Maine, Maine
Kamil Belbez, University of Maine, Maine
Bob Bayer, University of Maine, Maine
Brian Beloin, University of Maine, Maine
Bill McLean, Stolt Sea Farm, Inc., Massachusetts

Objectives

- Development of low fat fermented fish sausage (pepperoni, summer sausage) using off-grade salmon, salmon trim, and mechanically deboned salmon.
- Evaluate the nutritional, chemical, physical, microbiological, and sensory characteristics of each fermented fish sausage.

Anticipated benefits

Production of fish sausage would reduce the processing waste stream (fish frames) while producing value-added products for the aquaculture industry. A high value, low fat, nutritious fish sausage resulted from this research. Development of fermented salmon sausages along with a market for these products will lead to the creation of jobs in the aquaculture and fish processing industries, particularly in economically depressed Washington County in Maine. It will also provide an alternative sausage product for ethnic consumers who are limited by dietary or religious constraints.

Principal accomplishments

Salmon pepperoni was prepared from cull salmon in one experiment using starter culture or encapsulated lactic acid. Acidification to a pH endpoint of 4.5 required 24 hours by fermentation and only 9 hours with encapsulated lactic acid. Chemical analysis revealed no significant difference for moisture, fat, protein, or ash content. There was a significant ($P \leq 0.05$) difference for several of the minerals. The Ca level was nine times higher in the encapsulated product because the salt used was calcium lactate. There was no significant difference in cholesterol level among the two treatments. The only significant ($P \leq 0.05$) differences in fatty acid content were for 16:1 and 22:5. The fermented pepperoni had more of the monounsaturated fatty acid while the encapsulated product had more of the polyunsaturated fatty acid. Lactic acid content was 4.43 and 4.54% for the encapsulated lactic acid and fermented pepperoni, respectively. No significant differences were noted for color or for mechanical texture analysis for compressibility and shear. Fermented pepperoni had significantly higher ($P \leq 0.05$) for flavor, texture, and overall acceptability. A break-even analysis demonstrated that salmon pepperoni would be more expensive than beef- or pork-based pepperoni, however the estimated price was in the low range for a gourmet product. For a production level of 20,050 pounds produced, a break-even cost per pound would be $4.98.

Based on the first experiment, additional research was undertaken to examine the use of salmon trim and mechanically deboned salmon in the preparation of sausage products (pepperoni & summer sausage). The use of these processing by-products would assist in reducing the formulation costs for these sausage products. As a result of the earlier research, encapsulated citric acid was used in the preparation of these products in an attempt to improve the sensory properties of the sausages. For comparisons, fermented sausage products were also prepared using similar conditions.

The moisture, fat, and caloric values of pepperoni products did not differ significantly between treatments. However, protein and ash content differed significantly ($P \leq 0.05$) between treatments. The acidified pepperoni had the highest moisture (27.8%) and fat (17.2%) content. Fermented pepperoni was higher in protein (42.1%) and ash (13.7%). There were no significant differences in color between treatments. The fermented pepperoni was significantly ($P \leq 0.05$) firmer then the acidified product. Panelists rated the fermented pepperoni significantly ($P \leq 0.05$) higher for flavor and overall acceptability.

The moisture, protein, fat, ash, and caloric content of the summer sausage products differed significantly ($P \leq 0.05$) between treatments. Fermented summer sausage had the highest moisture (29.1%), protein (41.5%) and ash (8.1%) content. Acidified summer sausage was higher in fat (22.1%). The fermented product was darker, less red and yellow than the acidified summer sausage. Instron texture analysis demonstrated that the fermented sausage was significantly ($P \leq 0.05$) firmer. The fermented summer sausage received significantly ($P \leq 0.05$) higher scores for flavor and overall acceptability.

Microbiological analyses of all salmon sausage products showed no detectable coliforms, *E. coli*, *S. aureus*, or *L. monocytogenes*.

Impacts

- Provides salmon processors with an outlet for by-products from a filleting operation.
- Demonstrates that fermented sausage products can be manufactured from salmon by-products.
- Demonstrates that starter culture fermentation leads to the best products when evaluated by consumer panels.
- Provides an opportunity for one or more of Maine's salmon processors to establish a line of value-added salmon sausage products.

Recommended follow-up activities

The major obstacle to the successful production of fermented salmon sausage products will be to reduce the raw product cost to a level which will make these products competitive with similar red meat and poultry products. If these products are marketed as gourmet foods, they may be able to command a higher market price, but this would limit the market. The use of underutilized species (herring, mackerel, etc.) in the preparation of fermented fish sausage products should be investigated. Direct acidification offers some advantages in terms of production throughout, but requires further research to improve the organoleptic characteristics of the finished products. A combination of starter culture with encapsulated acid may take advantage of the positive attributes of each.

Reasons for termination

The two objectives for this project have been completed and because no significant differences were observed in the lipid analyses of the fermented versus the directly acidified pepperoni, funds for these analyses were not spent. The outreach component will be initiated over the next 6 months, and we are presently working on the brochure that was part of the contract agreement.

Publications and presentations

Manuscripts

Beloin, B. W. 1996. Comparative analysis of encapsulated lactic acid versus starter culture fermentation in the acidification of salmon pepperoni. M.S. Thesis. University of Maine, Orono, ME. 1-84.

Belbez, K. B. 1997. Development of value added seafood products using salmon. M.S. Thesis. University of Maine, Orono, ME. 1-92.

Beloin, B.W., T. M. Work, M. E. Camire, and A. A. Bushway. Comparative analysis of encapsulated lactic acid versus starter culture fermentation in the acidification of salmon pepperoni. (In Prep., J. of Aquatic Food Product Technology).

Papers Presented

Belbez, K. B. 1997. Development of low fat fish summer sausage using off-grade salmon. Institute of Food Technologists Annual Meeting, Orlando, FL.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1995	$13,300		$6,700				$20,000
1996							
Total	**$13,300**		**$6,700**				**$20,000**

National Coordinator for Aquaculture New Animal Drug Applications (NADAs) (95-10)

Project period December 22, 1995 to December 21, 1996, Addendum: to May 30, 1997

NRAC funding

$15,000

Principal investigator

Ted R. Batterson, Michigan State University, Michigan

Participating investigators/ cooperative agencies

Henry S. Parker, USDA/CSREES/PAPPP, Washington, DC

Robert K. Ringer, Michigan State University, Michigan

Rosalie A. Schnick, Michigan State University, Wisconsin

Objectives

- Ensure effective communications among groups involved with Investigational New Animal Drugs/ New Animal Drug Applications (INADs/NADAs), including Canada.
- Serve as an information conduit between INAD/ NADA applicants and the U.S. Food and Drug Administration's Center for Veterinary Medicine (CVM).

- Identify and encourage prospective INAD participants to become involved in specific investigational studies and NADA approval-related research.
- Seek the support and participation of pharmaceutical sponsors for INAD studies and NADAs and coordinate with INAD/NADA sponsors to achieve CVM approval more quickly.
- Guide prospective and current INAD holders on the format for INAD exemption requests and related submissions to CVM.
- Identify existing data and remaining data requirements for NADA approvals.
- Review, record, and provide information on the status of INADs and NADAs.
- Encourage and seek opportunities for consolidating the INAD/NADA applications.
- Coordinate educational efforts on aquaculture drugs as appropriate.
- Identify potential funding sources for INAD/NADA activities.

Anticipated benefits

Investigation and approval of safe therapeutic and production drugs for use by the aquaculture industry are one of the highest priorities currently facing the industry. At present, only a few approved compounds are available to the industry and further development of the aquaculture industry is severely constrained by a lack of approved drugs essential for treating more than 50 known aquaculture diseases. CVM has afforded the aquaculture industry throughout the U.S. with a “window of opportunity” to seek approval of legal drugs to be used in their production practices. The need for additional drugs is great, but securing data necessary to satisfy the requirements of CVM for drug approval is time consuming, costly, and procedures are rigorous. The INAD/NADA process is the one method that allows the industry to provide CVM with data on efficacy and also aids producers in their production practices.

Coordination and educational efforts directed toward potential INAD/NADA applicants will save time and effort for both the industry and CVM. The National Coordinator for Aquaculture INADs/ NADAs serves as a conduit between an INAD/NADA applicant and CVM. The National Coordinator helps to alleviate time demands on CVM staff, thus allowing more time to process a greater number of applications as well as increasing the breadth of research endeavors within the industry. The grouping of INAD applicants should help to alleviate redundancy, amalgamate efforts, and increase the amount of efficacy data, all of which should result in greater progress toward developing available, approved therapeutic and production drugs.

Principal accomplishments

In September 1992, Ringer, Professor Emeritus of Michigan State University, was hired on a part-time basis as National Coordinator for Aquaculture INAD Applications. He served in that capacity through August 31, 1994.

As National Coordinator for Aquaculture INADs, Ringer participated with CVM in educational workshops on INAD procedures and requirements. These workshops were conducted throughout the U.S. This included workshops held in conjunction with the U.S. Trout Farmers Association, Boston Seafood Show, and Aquaculture Expo V in New Orleans. The workshop at the Boston Seafood Show was videotaped and is now available on cassette from the Northeastern Regional Aquaculture Center. In addition to the workshops, talks were presented on aquaculture drugs at the request of several organizations, including the World Aquaculture Society.

Ringer also helped in the preparation of a letter that CVM used in requesting disclosure information from those holding aquaculture INADs. By law, CVM cannot release any information about an INAD without such permission. A table containing information about these disclosures was made available to the general public. This included the names and addresses of the INAD holders as well as the drug and species of fish intended for use of the drug. It is intended that this table will be periodically updated after additional disclosure permissions have been obtained.

On May 15, 1995, Rosalie A. Schnick, recently retired Registration Officer from the National Biological Service’s Upper Mississippi Science Center (UMSC), was hired on a 3/4 time basis as National Coordinator for Aquaculture New Animal Drug Applications (National NADA Coordinator). On May 15, 1996, her position was increased to a full-time basis.

As National NADA Coordinator, she organized and coordinated a major INAD/NADA workshop in November 1995 under sponsorship of CVM that led to increased communications between INAD coordinators, better coordination of the data generation for each drug, and consolidation of several INADs.

New INAD/NADA Sponsors

Schnick helped gain a new INAD/NADA sponsor for amoxicillin (INAD #9659) and met with Vetrepharm Limited (United Kingdom) in May 1996 in Fordingham, UK, to discuss an action plan for the development of the INAD/NADA on their broad spectrum antibacterial product. Schnick also helped obtain and is working with INAD/NADA sponsors for hydrogen peroxide (microbicide, INAD #9671), luteinizing-hormone releasing hormone (spawning aid, INAD #9318), common carp pituitary (spawning aid, INAD #9728), and Aqui-S™ (anesthetic, INAD #9731).

Therapeutic Drugs

Schnick and representatives of the Upper Mississippi Science Center (UMSC), La Crosse, Wisconsin, held a special session at the Midcontinent Warmwater Fish Culture Workshop in February 1996 to consider label claims and identify potential pivotal study sites for chloramine-T under the federal-state drug approval partnership program (a project of the International Association of Fish and Wildlife Agencies = IAFWA Project).

Based on residue and environmental data, CVM determined on July 11, 1996, that there are no human food or environmental safety concerns over the use of copper sulfate as a therapeutant, thus making approval relatively easy. Two meetings were held in July and August 1996 with a potential NADA sponsor and CVM to discuss the data requirements for approval and develop an action plan needed to obtain approval of copper sulfate as a therapeutant.

On July 18, 1996, CVM accepted the data and conclusions of a target animal safety study on the toxicity of formalin to warm and coolwater fish eggs that was submitted along with a proposed formalin label by UMSC in December 1995. CVM will soon issue a notice in the Federal Register inviting sponsors to amend their labels to include the extended claims for both the fungicide (based on UMSC studies) and parasiticide uses (based on studies at Auburn University, Auburn, Alabama). These extensions of the formalin NADA to additional species will remove the need for INADs on formalin for these claims.

Anesthetics

Two meetings in June and August 1996 were held with representatives of Aqui-S™, an anesthetic approved for use on fish in New Zealand, to discuss the potential for development of Aqui-S™ in the United States. Aqui-S™ is approved in New Zealand with a zero withdrawal time and offers a potential alternative to benzocaine. UMSC decided to evaluate the comparative efficacy and regulatory requirements needed for approval on both benzocaine and Aqui-S™. Work on benzocaine through the IAFWA Project has been put on hold until the new anesthetic, Aqui-S™, can be evaluated. After an evaluation has been made on efficacy and regulatory requirements, UMSC will decide along with its state partners in the IAFWA Project and U.S. Fish and Wildlife Service whether to pursue Aqui-S™ or benzocaine for approval as an anesthetic/sedative.

Hormones

A meeting was held at CVM headquarters on April 11, 1996, with Stoller, users of common carp pituitary (CCP), and researchers to determine a course of action for gaining approval of CCP. As a follow-up to that meeting, CVM coordinated a conference call on May 15, 1996, that covered: (1) the identification of researchers and the design of target animal safety studies; (2) the writing of the environmental assessment through the National Research Support Program #7 (NRSP-7); and (3) potential funding sources of the target animal safety studies.

The National NADA Coordinator contacted all the holders of disclosed INADs on human chorionic gonadotropin (hCG) at the urging of CVM to send all the data to the sponsor, Intervet, Inc., that was incorporated in a February 1996 Intervet submission to CVM. CVM ruled on February 12, 1996, that enrollment in an INAD will not require the use hCG as a spawning aid. CVM will defer regulatory enforcement if used by or on order of a veterinarian. Any hCG product may be prescribed, but CVM strongly encourages the use of Intervet’s product, Chorulon™.

Schnick worked with CVM, Auburn University, Rangen, Inc. and tilapia producers to develop INAD #9647 on 17-a-methyltestosterone (MT) for tilapia (obtained January 25, 1996) and then worked to obtain authorization from CVM and permission from Auburn

University to allow the use of MT on yellow perch under Auburn's INAD (obtained February 22, 1996). The North Central Regional Aquaculture Center (NCRAC) provided $25,000 to Southern Illinois University-Carbondale and the UW-Madison to conduct a target animal safety study on MT with walleye and has requested $5,000 for Auburn University to conduct a literature review of the environmental data on MT for NADA submission to CVM.

IAFWA Project

Several meetings were held at UMSC in May and June 1996 to review the whole IAFWA Project related to the following topics on each of the 10 study plans: (1) remaining data requirements; (2) tasks and jobs; (3) assignments for each job; (4) a time table for completing each assigned task; (5) budget projections by study plan and year; (6) budget shortfalls for the original IAFWA Project; and (7) assessment of the potential products at the end of the IAFWA Project. UMSC has reprogrammed its effort and direction under the IAFWA Project due to changes in requirements and circumstances for benzocaine, chloramine-T, hydrogen peroxide, oxytetracycline, and sarafloxacin. Efforts were made to save the entire IAFWA Project during government downsizing and budget reductions. Based on the assessment of the remaining data requirements and the funds available, UMSC determined that the IAFWA Project was short a total of $1.4 million and 2 years of effort.

Work planned

The National NADA Coordinator developed an action plan that centers on coordinating all drugs of high priority for aquaculture toward NADAs through the INAD process. In particular, Schnick plans to: (1) develop a major initiative on amoxicillin to obtain approval for its use as a broad spectrum antibacterial in all fishes; (2) determine the potential of fumagillin to control or prevent Whirling Disease in salmonids and Hamburger Gill Disease in catfish and pursue an INAD/NADA if feasible; (3) help determine the potential for approval of two anesthetics, benzocaine and Aqui-S™; (4) assist the efforts of the NRSP-7 to complete the approval process for sarafloxacin to control enteric septicemia in channel catfish; (5) identify potential funding sources for INAD/NADA activities; and (6) continue to coordinate efforts to obtain approvals for all 19 high priority aquaculture drugs.

Several meetings and workshops are planned that will benefit aquaculture drug approvals. A meeting will be held in Kansas City, Missouri on November 7-8, 1996, to discuss the protocols and select the pivotal study sites for chloramine-T. The NADA Coordinator arranged the agenda and speakers for a special session entitled "Partnerships for Aquaculture Drug Approvals: Models for Success" to be held at World Aquaculture '97, Seattle, Washington, February 19-23, 1997. An International Harmonization Workshop for Aquaculture Drugs/Biologics is also scheduled as part of World Aquaculture '97, to be held in Seattle, Washington on February 24, 1997, to create an international forum, identify potential actions, and develop implementation strategies in cooperation with other countries to facilitate approvals of aquaculture drugs.

Impacts

Establishment of the National NADA Coordinator position in May 1995, has resulted in coordination, consolidation, and increased involvement in the INAD/NADA process on 17 of the 19 high priority aquaculture drugs and activities on two new drugs of interest to aquaculture. Six new INAD/NADA sponsors have initiated new INADs and progress has been made toward unified efforts on existing and new INADs/NADAs.

This enhanced coordination will help gain extensions and expansions of approved NADAs and gain approvals for new NADAs. In fact, data on formalin have recently been accepted by CVM and amended NADAs are expected soon from the three current NADA sponsors of formalin.

Publications and presentations

Publications in print

Schnick, R. A. 1996. Chemicals and drugs. In R. C. Summerfelt, ed. Walleye culture manual. NCRAC Culture Series #101, North Central Regional Aquaculture Center Publications Office, Iowa State University, Ames, IA. p. 347-354.

Schnick, R. A. 1996. Cooperative fish therapeutic funding initiative: States in partnership with federal agencies to ensure the future of public fish culture. Transactions of the 61st North American Wildlife and Natural Resources Conference 61:6-10.

Schnick, R. A., W. H. Gingerich, and K. H. Koltes. 1996. Federal-state aquaculture drug registration partnership: A success in the making. Fisheries 21(5):4.

Manuscripts

Gingerich, W. H. and Schnick, R. A. (In review). Federal-state aquaculture drug approval partnership program. Abstract for special session entitled “Partnerships for aquaculture drug approvals: Models for success” to be held at World Aquaculture ‘97, Seattle, Washington. February 19-23, 1997.

Schnick, R. A. (In Press). Approval of drugs and chemicals for use by the aquaculture industry. Veterinary and Human Toxicology.

Schnick, R. A. International regulatory aspects of chemical and drug residues. Proceedings of the International Conference on Fish Inspection and Quality.

Schnick, R. A. (In review). Overview of partnerships for aquaculture drug approvals. Abstract for special session entitled ”Partnerships for aquaculture drug approvals: Models for success” to be held at World Aquaculture ‘97, Seattle, WA. February 19-23, 1997.

Schnick, R. A. and R. D. Armstrong. (In Press). Aquaculture drug approval progress in the United States. Northern Aquaculture.

Papers Presented

Ringer, R. K. 1993. Workshop on INADs, NADAs, and the IR-4 Project. California Aquaculture Association, Oakland, CA October 11, 1993.

Ringer, R. K. 1993. INAD workshop: Proper drug and chemical use in aquaculture. 9th Annual Florida Aquaculture Association Conference, Fort Pierce, FL. November 6, 1993.

Ringer, R. K. 1994. National INAD Coordinator’s role in aquaculture. Aquaculture Expo VII/Annual World Aquaculture Society Meeting, New Orleans, LA. January 13, 1994.

Ringer, R. K. 1994. State of current USDA regulations on drug, therapeutic, and chemical use. North Carolina Aquaculture Development Conference, New Bern, NC. February 5, 1994.

Ringer, R. K. 1994. Investigational New Animal Drugs Workshop. Tropical and Subtropical Regional Aquaculture Center Industry Advisory Council Meeting, Honolulu, HI. March 14, 1994.

Schnick, R. A. 1995. Idaho Aquaculture Association Annual Meeting, Twin Falls, ID. May 19-22, 1995.

Schnick, R. A. 1995. Chemistry in Aquaculture Symposium. Convener and presenter, Cullowhee, NC. May 31-June 2, 1995.

Schnick, R. A. 1995. Joint Subcommittee on Aquaculture’s Working Group on Quality Assurance in Aquaculture Production. Washington, DC. June 23, 1995.

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Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1994-95	$5,000		$23,750[a]	$70,000[b]	$10,000[c]	$103,750	$108,750
Total	**$5,000**		**$23,750**	**$70,000**	**$10,000**	**$103,750**	**$108,750**

a = American Pet Products Manufacturers Association ($7,500), American Veterinary Medical Association ($10,000), Cat fish Farmers of America ($2,000), Fish Health Section of AFS ($1,000), Florida Tropical Fish Farm Association, Inc. ($500), Natchez Anima Supply ($1,000), National Aquaculture Council ($1,000), and Striped Bass and Hybrid Producers Association ($250)

b = USDA funding through a Cooperative Agreement with NCRAC ($23,000), CVM ($22,000), and USDI/NBS International Association of Fish and Wildlife Agencies Project ($25,000)

c = Center for Tropical and Subtropical Regional Aquaculture ($5,000) and North Central Regional Aquaculture Center ($5,000)

1996 Industry Summit (95-13)

Project period January 1, 1996 to March 31, 1996

NRAC funding

$15,360

Work group chair

Dr. Robert Rheault, Northeastern Regional Aquaculture Center, MA

Objectives

- Work with State Aquaculture Associations in the Northeastern Region to select participants and develop an agenda for an Industry Summit to be convened in February 1996.
- Cover all expenses associated with holding a 2-day summit.

Principal accomplishments

Participants were selected to develop an agenda for an Industry Summit which was convened in February 1996.

A set of priority topics were identified by the industry participants. These were listed by rank order of importance to the industry in the Northeast Region and problem statements were drafted. The problem statements became the basis for the annual request for proposals issued by NRAC for the 1996 and 1997 request for proposal (RFP).

Development of a Model Quality Assurance Program for the Aquaculture Industry in the Northeast (94-2)

Progress report period April 1, 1994 to August 31, 1996

NRAC funding

$60,000

Project coordinator

National Aquaculture Association

Participating investigators/ cooperative agencies

Gary Fornshell, University of Idaho Aquaculture Extension, Idaho
Walter Canzonier, New Jersey Aquaculture Association, New Jersey

Objectives

- To develop two introductory quality assurance programs designed to provide growers with a series of best management practices for continued production of safe, quality product; one program for finfish, the other for shellfish producers. These programs are viewed as providing industry with models or guidelines upon which future programs may be developed.

a) The BMP's for each manual will be tailored to reflect both the differences and similarities of finfish and shellfish culture. Examples of common BMP's might include site selection, water quality, waste management, proper drug/therapeutant use, feeds and feeding, etc.

b) Each program (i.e., manual) will contain a statement, for participating producers to sign and return to the National Aquaculture Association for record purposes. By signing and returning the card, the producer will have voluntarily committed to the principles of the program.

- To advise producers in the Northeast and elsewhere of the program's availability through use of the extension network, trade journals, media, association newsletters, and the Regional Aquaculture Center system.
- To forward requests for participation to the NAA which will serve as distributor of the programs and maintain a record of participation. Note: consideration should be given a nominal fee for grower participation in support of NAA-related costs for mailing, queries, record maintenance, and future revision. Other industries have noted too, that paying a fee is indicative of a producer's willingness to commit to a program, as opposed to simply receiving a gratis copy in the mail.
- To develop a brochure for producer use to promote public awareness of the quality assurance programs. Ensure that a half dozen sets of printer-ready copy are available for reprinting by producers who wish to do so.

Anticipated benefits

In 1992, the need for an aquaculture industry quality assurance program was identified by the Working Group on Quality Assurance (established in 1990 by the Joint Subcommittee on Aquaculture). Since then, both the catfish and trout industry have implemented their programs with adequate participation by producers.

Industry and consumers will benefit from implementing a QA program with consistently available, safe, wholesome, and fresh seafood products. It is industry's responsibility to ensure quality.

Participation will:

- heighten consumer confidence from an understanding of production practices, this will improve product image and marketability;
- assist producers in keeping abreast of information involving issues and developments that may impact their business;
- maximize economic returns for producers by applying best management practices, as defined in the QA program;
- tend to improve employee confidence and pride in knowing consistent management practices are followed;
- improve industry's position when complying with regulatory requirements; and
- poise the aquaculture industry to provide for the demand of a safe, healthy seafood supply and

thereby reduce the nation's trade deficit from imported seafood products.

Principal accomplishments

A no-cost project extension was approved October 31, 1995. Work to date has been directed toward the first objective that entailed development of two, introductory quality assurance programs: one program for finfish; another for shellfish. Both are in the final proof stage and blue lines will be forthcoming. The fourth objective (brochure development) has been accomplished and is currently film-ready. Fifteen thousand of the brochures will be printed for distribution to producers.

Time Line for Completion
First Draft - 3 months (completion: January 1996 Fish; February 1996 Shellfish)

Initial Review - 1-month (completion: February 1996 Fish; March 1996 Shellfish)

Initial Revision - 1-month (completion: March 1996 Fish; April 1996 Shellfish)

Second Draft (incorporate photographs) - 2 months (completion: July 1996 Fish; September 1996 Shellfish)

Review - 1-month (completion: August 1996 Fish; September 1996 Shellfish)

Completed manuals to be submitted to NRAC for approval.

Manuals will be printed and distributed, following approval of the final draft.

Work planned

Notification of availability and distribution is planned following publication.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1994-95	$60,000						$60,000
Total	**$60,000**						**$60,000**

Hard Clam Winter Mortality (95-1)

Progress report period June 1, 1995 to August 1, 1996

NRAC funding

$80,000

Project coordinator

John N. Kraeuter, Rutgers University, New Jersey

Participating investigators/ cooperative agencies

John Aldred, Town of East Hampton, Massachusetts
Paul Bagnall, Edgartown Shellfish Constable, Massachusetts
Richard Crema, R. F. Crema and Family, New Jersey
Gef Flimlin, Rutgers Cooperative Extension, New Jersey
Susan Ford, Rutgers University, New Jersey
Dale Leavitt, Woods Hole Oceanographic Institute, Massachusetts
Roxanna Smolowitz, LAAMP, University of Pennsylvania, Massachusetts
George Mathis, Mathis and Mathis Enterprises, New Jersey
Gregg Rivara, Cornell Cooperative Extension, New York

Objectives

- To evaluate methods for assessing condition of specific size classes of seed.
- To determine seed condition and experimentally manipulate for specific seed sizes.
- To retest seed condition and plant using overwintering field techniques.
- To test growth, survival, and condition in spring and subsequent periods.
- To evaluate incremental cost of size specific increases in condition of hard clam seed.

Anticipated benefits

1. Determination of optimum numbers of specific size clams required for testing condition and energy storage.
2. Detailed means of size specific testing of energy storage and "condition" for clam seed.
3. An examination of a means of manipulating and measuring condition and energy storage levels of clam seed.
4. Information on size specific effects of supplemental feeding on increased condition of clams to be planted or overwintered.
5. Initial cost estimates for supplemental feeding methodologies.
6. Analysis of whether energy storage level, disease, or their interaction are responsible for the observed mortalities in planted clam seed.
7. A recommendation of what level of condition is adequate for either late fall planting or overwintering seed.
8. An economic basis for deciding factors to be examined and their relative importance when considering supplemental feeding of clam seed to increase overwintering or planting survival.
9. A size specific decision matrix that will allow economic factors to be integrated with biological considerations to make management decisions on planting and overwintering of clam seed.

Principal accomplishments

1. Evaluate methods for assessing condition of specific size classes of seed.

Basic analyses have been completed. In general, 25 clams provides highly accurate condition information. As few as 10 clams will provide a statistically valid sample for most chemical measurements. Histology: cellular development vs. size is still being examined for potential.

2. Determine seed condition and experimentally manipulate for specific seed sizes.

The New Jersey experimental manipulation of seed has been completed. Four replicate lots of 8000 seed of four seed sizes were subjected to three treatments (lower food levels, ambient food levels, and supplemental food). Preliminary analysis did not indicate size related increases in glycogen due to supplemental feeding. Both the ambient and supplemental feeding replicates were larger than the low feeding levels of the same initial size. Examination of ambient water indicates that filtering did not reduce chlorophyll or particulate nitrogen as expected. In spite of the difficulty experienced in filtering the water due to filters clogging, most of the particles were below the limits of the filters. A significant set of sea squirts, *Moogula*, grew more rapidly in the supplemental food raceways. Cleaning and overgrowth may have increased mortalities in these raceways.

3. Re-test seed condition and plant, using overwintering field techniques.

Due to the exceptionally warm weather late into the fall, all plantings were delayed. This caused us to maintain the clams in the facilities longer than anticipated. All New Jersey groups were planted as indicated in the protocol. In New York, replicate groups of four seed sizes were placed in sand and mud trays for overwintering at two times, all trays were planted. Massachusetts was unable to raise enough 12 mm seed to provide for all replicates, so these were not placed in the field. Due to the unusual weather, Massachusetts plantings were delayed until after the New York and New Jersey plantings. The delay in cooling caused a rapid drop in temperature once cooling began and we missed the first target temperature. The first planting thus became the second in terms of temperature. In order to plant sufficient number of seed, we doubled the last planting. This planting involved clams that had been held at the planting site and a second group that had been held in another site that normally has better growing conditions.

4. Test growth, survival, and condition in spring.

All clams from New York and Massachusetts have been retrieved and counted. New York seed had generally higher glycogen when they were placed in the field than any other group. In general, survival appeared to be good at all sites, but larger seed performed better than smaller seed. In New York there were significant differences due to seed size, substrate, and the interaction of time with both of these variables. In general, larger seed survived better than smaller seed. Due to relatively poor survival of 12 mm seed placed on sand substrates late in the year, those planted on mud survived better. We are analyzing these clams to see if these differences can be attributed to differences in stored reserves or histological/pathological conditions. We believe that similar patterns hold for Massachusetts; but due to poor reliability of initial counts, we cannot determine with certainty whether these results are valid. In general it appears that those groups of clams that were transferred from the "good" growing areas did not survive as well as those from the overwintering site. We are currently discussing doing these tests again this coming winter. The New Jersey data are currently being analyzed, but size appears to be as significant as in other areas with larger seed performing better than smaller seed. The groups receiving ambient flow appear to have survived better than either of the other two groups. Final statistical analysis is underway to determine interactions between these various factors.

5. Evaluate incremental cost of size specific increases in condition of hard clam seed.

Data on hours required and production of algae has been collected. Analysis requires information from the above sampling.

Work planned

We have requested and received an extension until December 31, 1996, to allow more thorough analysis of the various aspects of this study. Given the importance of size at all sites, we have chosen to examine the condition of the largest and smallest groups of clams from the New Jersey experiment across all blocks and feeding types. This work should be completed by the end of August. Histology will be conducted on a reduced set of these samples. New York analysis is focusing on the size x sediment type interaction that affected the larger seed (12mm) in the second time period. These samples are currently being analyzed with completion expected by mid-September.

Impacts

The primary benefits of this work will not be realized until the spring sampling and subsequent analyses are completed. The primary benefit to date has been the development of a broad interest in assisting the hard clam aquaculture industry and closer ties between industry and municipal interests.

Publications and presentations

Two talks concerning the studies have been presented: one at the Milford Aquaculture Seminar and another at a New York Aquaculture Workshop.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1995-96	$80,000	$30,407	$9,700	$50,800*	$13,580	$ 99,787	$197,787
Total	**$80,000**	**$30,407**	**$9,700**	**$50,800***	**$13,580**	**$99,787**	**$197,787**

* NSF-SBIR Awarded to Mathis and Crema (Industry Members)

Monitoring Salmon Cages for Predators (95-2)

Progress report period July 1, 1995 to August 31, 1996

NRAC funding
$64,937

Project coordinator
Dehua Huang, Airmar Technology Corp., New Hampshire

Participating investigators/ cooperative agencies
Dehua Huang, Airmar Technology Corp., New Hampshire
Roger H. Tancrell, Airmar Technology Corp., New Hampshire
Robert K. Jeffers, Airmar Technology Corp., New Hampshire
Steven G. Christensen, Airmar Technology Corp., New Hampshire
Michael J. Simoneau, Airmar Technology Corp., New Hampshire
Christopher Bartlett, Marine Trade Center, Sea Grant, MAIC, Maine

Objectives
- To develop a means to detect the presence of seals and sea lions around salmon cages.
- To use these detection methods to trigger alarms to deter predators from encroaching close to the cages.

Anticipated benefits
The project will benefit the coastal fish farming aquaculture industry directly. Predation by seals and sea lions at coastal salmon farms is a continuing economy problem. A single attack from a seal or sea lion to a medium to large farm can result in $10K to $20K in lost revenue. The use of acoustical deterrent devices (ADD) is one of the predator control strategies employed by the salmon aquaculture industry. To deter predation from seals, the present technique is to operate high source level acoustic transmitter on a continuous basis. If a sonic field is always present, there is some risk of habituation.

The predator monitoring system around a salmon pen would greatly enhance the efficiency of acoustic deterrent devices, allowing for their use only when the presence of a seal is detected. Other potential applications for this technology might be determining fish size and monitoring fish health over long periods of time. Onset of disease can be detected by identifying abnormal behavior of caged fish.

Principal accomplishments
By the end of July 1996, the Airmar research team successfully collected audio recordings of sounds created by salmon and by seals during on-site visits to their natural habitats. Airmar originally outlined two approaches in its initial proposal (described in A and B in the following paragraphs). During the project research, the Principal Investigator (PI) extended the project by applying the currently available equipment for more objectives to encompass a broad range of detection methods (illustrated from C to F). The active CW-Doppler system is moved outside the salmon cage to detect any moving predators directly, and the hydrophone outside the cage listens to vocalization of the predator. Inside the cage, the Doppler system is used to monitor not only the average (mean) velocity of swimming fish, but also the speed difference from the slowest to fastest swimming fish. Further inside the cage, a hydrophone is used to monitor sounds produced by sudden jumping of fish when disturbed.

A. The CW-Doppler system inside the fish pen was originally proposed to monitor the fish mean speed when they are disturbed by predators. The Airmar research team successfully recorded the Doppler frequency data at a salmon farm. Preliminary laboratory data shows that Airmar's current CW-Doppler system is able to sense the salmon fish mean speed.

B. The original proposal also utilizes passive hydrophones outside the salmon cages to sense any acoustic signature of the existence of the predators (seals). However, our original focus was on the seal heart and flipper beat acoustic signals. So far, we have not been able to distinguish any acoustic signals for such marine mammal behavior due to high ambient noise of the ocean. The task of searching for distinct beat signals will continue during the extensive data analysis period.

C. With a CW Doppler system inside the fish pen, not only did we analyze the fish mean speed from the Doppler frequency, but we also discovered divergence of fish behavior. By monitoring the spread in Doppler frequency (the -3 dB and -6 dB spectral widths), we successfully analyzed the divergence of fish speed. This spectral width of the Doppler frequency is a promising parameter to monitor fish sizing and fish health over long periods.

D. Another new approach for detecting the presence of a predator was to monitor sounds emitted by the chaotic activity of fish when they become disturbed by mounting a passive hydrophone inside the fish cage. To generate a disturbance and to simulate chaotic fish swimming motion when predators exist, we threw fish food into the pen. During feeding, the fish changed their swimming pattern, increasing their speed dramatically. The acoustic sound generated by crowded chaotic fish was successfully recorded and analyzed. The Airmar research team was able to collect acoustic signals for salmon under "natural" conditions on a fully-operational Maine at-sea farm.

E Another new approach is to utilize the active CW-Doppler system outside the salmon cage where we can detect moving predators directly. The decision was made to monitor seals at nearby islands, instead of waiting for a seal attack at the pens. The probability of a seal attack on the pens was low because the fish farms are already equipped with Airmar's "dB Plus" seal deterrent system. We successfully recorded the Doppler signal from moving seals at sea.

F. While our boat was anchored near the island, we took the opportunity to record sounds emitted by the seals, by lowering a passive hydrophone a few feet below the water surface. The recordings show evidence of strong seal vocalization. This data is also currently being analyzed. During the extension period for this project, we plan to collect other acoustic signatures with seals in other locations, and preferably nearer the salmon cages.

The above preliminary experimental data show the positive acoustic cues that strongly support the decision made by the PI.

Work planned

A 6-month extension of the project has been granted to the Airmar research team. For the period from August 1 to December 31, 1996, an important task is the analysis of the extensive data already gathered to date. Detailed data analysis, signal processing, and application of automated pattern recognition techniques are planned. Each of the multiple modes of detection, listed from A. to F., has its advantages and disadvantages. Our objective is to reach conclusions, based on analysis of the current data, as to the most practical method for detecting predators. We believe the current recording contain sufficient information for us to draw distinctions as to the effectiveness of each approach.

After carefully reviewing test performance of Airmar's Doppler system in an "at-sea" environment, the Principal Investigator suggested that a higher power system, operated at a lower frequency, should be investigated. Currently, Airmar is applying for matching funds for a research project "Development of a High Power Doppler Fish Farm Underwater Predator Alert System." The Principal Investigator hopes to have one more field trial to apply this new equipment in a Maine fish farm as soon as practical. The test results will be included in the Northeastern Regional Aquaculture Center final report if the tasks can be performed in the time frame of the extension period.

Airmar has augmented its staff on this project by hiring a Manager of Research and Development. Dr. Roger H. Tancrell joined Airmar Technology at the beginning of June. His 26-year distinguished research experience from Raytheon Company in acoustics, transducer, and other close-related fields, provides a strong technical support for the project.

Impacts

- Predation by seals and sea lions at coastal salmon farms is a continuing problem (a single attack on a farm can result in $10,000 to $20,000 in lost revenue).

- Monitoring salmon cages for predators will provide a trigger system that will allow an Acoustic Deterrent Device (ADD) to operate efficiently, greatly reduce the potential for sea lion habituation, and directly benefit salmon farming aquaculture.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1995-96	$69,937		$84,476				$149,413
Total	**$69,937**		**$84,476**				**$149,413**

Development of a Northeastern Regional Aquaculture Extension Network (93-3) and; Addendum: Developmental Assistance for State Aquaculture Associations in the Northeastern Region (95-11)

Progress report period July 15, 1993 to August 31, 1996, Addendum to February 28, 1998

NRAC funding

$208,740

Project coordinator

John W. Ewart, University of Delaware, Delaware

Participating investigators/ cooperative agencies

Christopher Bartlett, Maine Aquaculture Innovation Center, Maine
Gef Flimlin, New Jersey Sea Grant Marine Advisory Service, New Jersey
Mike Opitz, University of Maine Cooperative Extension, Maine
Bill Burt, Massachusetts Cooperative Extension, Massachusetts
Mike Timmons, Cornell Cooperative Extension Service, New York
Don Webster, University of Maryland Sea Grant/Coop. Extension, Maryland
Nancy Balcom, Connecticut Sea Grant Marine Advisory Service, Connecticut
Roland Barnaby, NH Sea Grant Marine Advisory Service, New Hampshire
Craig T. Bast, West Virginia University Cooperative Extension, West Virginia
William Daniels, Delaware State University Cooperative Extension, Delaware
Leo Dunn, Pennsylvania Department of Agriculture, Pennsylvania
Reginal Harrell, University of Maryland Sea Grant/ Cooperative Extension, Maryland
Mike Hastings, Maine Aquaculture Innovation Center, Maine
Dale Leavitt, Massachusetts Sea Grant Program, WHOI, Massachusetts
Joseph B. McGonigle, Maine Aquaculture Association, Maine
Linda Marek Howe, University of Vermont Extension System, Vermont
Joe M. Regenstein, Cornell Cooperative Extension Service, New York
Mike Rice, University of Rhode Island, Rhode Island
Gregg Rivara, Cornell Cooperative Extension Service, New York
Lance L. Stewart, University of Connecticut Cooperative Extension, Connecticut
Dan Terlizzi, University of Maryland Sea Grant/ Cooperative Extension, Maryland
Aggie Spicer, West Virginia Cooperative Extension, West Virginia

Objectives

1. To develop regional educational programs on current topics identified by industry and assist state associations with educational outreach on local priority issues.
2. To improve the technical expertise and function of the regional extension network.

Anticipated benefits

A shared priority and specific goal of the USDA Regional Aquaculture Centers is the active involvement of Cooperative Extension and the Sea Grant Advisory Service to transfer useful information to aquaculturists and serve as a link or liaison between industry, researchers, state regulatory agencies, and the public. The Northeastern Regional Aquaculture Center, through its support of the current regional extension project (REP) (1993/96) "Development of a Northeastern Regional Aquaculture Extension Network" and its predecessor "Northeast Regional Aquaculture Extension Program for a More Viable, Profitable Industry" (1990/92), has significantly increased interaction and resource-sharing among Sea Grant Marine Advisory and Cooperative Extension Service agents and specialists from around the 12 Northeastern states and the District of Columbia. The Center's support of biennial Industry Summits (1992, '94, and '96) and the two regional extension projects (1990/92 and 1993/96) has increased regional industry/extension communication and has promoted a viable means for soliciting input and responding to industry priorities and concerns.

Principal accomplishments

This report summarizes the activities and accomplishments of the 1993/96 NRAC regional extension project "Development of a Northeastern Regional Aquaculture Extension Network." The 2-year project was originally scheduled to end in July 1995. However, with NRAC approval of a 1 year, no-cost extension until July 14, 1996, the REP continued to carry out its education and extension training activities. Between the second and third year of the project, an addendum proposal was developed to assist individual state aquaculture associations with local educational outreach needs and issues. The addendum was subsequently funded by USDA for a 1-year period (April 8, 1996-April 7, 1997). Because this additional work was developed as part of the 1993/96 REP, the termination date of the original project grant was also extended until April 7, 1997, for purposes of consistency.

Objective 1

A total of eight areas of activity (1. a-h) are summarized:

1. a) Industry Summits (1994 and 1996),
   b) Fish Health Regulations Workshop,
   c) Fish Health/Stress Management Workshop,
   d) Recirculating Systems Workshop,
   e) Aquaculture Effluent and Waste Management Workshop,
   f) Other Conferences, Workshops, and Regional Industry Meetings,
   g) Mobile Educational Programs, and
   h) Biosecurity Video for Aquaculture.

1. a) 1994 and 1996 Industry Summits

REP participants assisted the 1994 and 1996 Industry Chairs of the Technical/Industry Advisory Council (TIAC) to organize the agenda and site arrangements for biennial NRAC industry summits. Both summits were held at the Sheraton Airport Hotel in Providence, Rhode Island, on February 5-6, 1994, and January 13-14, 1996. The summit agendas addressed various topics including review and revision of research and project priorities, communication and interaction among state associations, the desired role and current status of the regional extension project, discussion of other state association concerns, and regional regulatory issues. Research priorities established by industry at both summits were used by the TIAC to develop the 1994 and 1996 Request for Proposals.

The 1996 Industry Summit was held concurrently with a meeting of the Regional Extension Work Group. The industry and extension groups met individually and jointly during the 2-day conference to discuss current and future industry/extension activities. The Extension Work Group included the suggestions and participation of regional industry in a follow-up proposal (1996/98 REP) to the current project. The proposal, approved by the NRAC TIAC and Executive Committee, was submitted to USDA in April 1996.

1. b) Fish Health Regulations Workshop

A Fish Health Regulations Workshop was included as part of the 1994 Industry Summit on February 5-6, 1994, in Providence, Rhode Island. The status and activities of state programs and the New England Salmonid Health Commission were reviewed. A draft resolution on industry fish health policy recommendations that was completed by a national ad-hoc committee at the Fish Farming Expo/WAS meeting in New Orleans, Louisiana, January 12-17, 1994, was introduced and discussed for endorsement and use by the Northeastern state associations to effect revisions to current policies.

1. c) Fish Health/Stress Management Workshop

A series of three annual New England Fish Health Workshops, organized by REP participants and industry representatives and partially sponsored by NRAC REP funds, were held each spring (April) from 1994-96 at the Marine Technology Center in Eastport, Maine. The workshops were planned in close cooperation with the Maine Aquaculture Association and the Maine Aquaculture Innovation Center. Workshop attendees included producers, researchers, students, regulators, and others from New England states and New Brunswick and Prince Edward Island, Canada. The success of the annual meeting has led to additional sponsorship from regional industry (Biomed, Inc., Moore Clark, Inc., Atlantic Salmon, Inc., and ShurGain, Inc.), and U.S. and Canadian government agencies (the National Biological Survey and the New Brunswick Department of Fisheries and Aquaculture).

1. d) Recirculating Systems Workshop

1. e) Aquaculture Effluent and Waste Management Workshop

REP participants organized, and NRAC REP funds provided support for, speakers and a joint proceedings for two individual day long special session programs "Intensive Recirculating Systems" and "Aquaculture Effluent and Waste Management." The special sessions were part of the 1995 joint meeting Aquaculture in the Mid-Atlantic/Aquaculture Expo VIII. Both programs were well attended (80 plus participants) and were offered on successive days at the conference which ran from June 24-28, 1995, in Washington, DC.

1. f) Other Conferences, Workshops and Regional Industry Meetings

A portion of REP resources allocated for meetings and workshops were set aside for ad hoc use during the term of the project. The purpose of this approach was to maintain a capability to respond and provide support to industry with regard to short-term problems, issues, or emergencies, and to help co-sponsor or otherwise support development of new or important continuing educational venues. During the course of the project, several continuing (annual) and other unanticipated meetings and workshops were supported with REP participation and/or sponsorship. These included:

Milford Aquaculture Seminar

Partial support was provided for the 14th (1994), 15th (1995), and 16th (1996) Milford Aquaculture Seminars held in Milford, Connecticut. REP funds were used for the publication of program abstracts and other expenses related to the educational program. Cooperative funding support from the National Marine Fisheries Service (NMFS) was also provided for the 1994 meeting. The NMFS has since become an annual sponsor.

State Industry Association/Extension Workshop

The REP organized a Northeast Aquaculture Industry Association/Extension Workshop in Boston, Massachusetts on March 15, 1995. The workshop brought together industry and extension representatives to identify options and determine the best approach to advance state association development, the number one priority of the '94 Industry Summit.

Workshop participants agreed to form an Industry/Extension Work Group to develop a proposal to request additional support from NRAC for state specific educational efforts. The proposal was submitted as an addendum to the current 1993/96 REP "Development of a Northeastern Regional Aquaculture Extension Network." All 12 aquaculture associations in the Northeastern Region participated in the development of the REP addendum proposal.

The addendum proposal revised objective 1 of the REP to include educational programming at the local level.

Each association collaborated with an institutional representative (IR) from their state to develop a plan of work and budget. As the state IR, REP participants, or the state Aquaculture Coordinator (Pennsylvania and New Hampshire) assumed the dual responsibility of managing grant accounts and funds and participating in proposed activities. The individual plans of work and budgets of participating state associations were compiled by the REP Coordinator into the addendum proposal. The proposal was approved by the NRAC Technical/Industry Advisory Council and Board of Directors and was submitted to USDA in August 1995. The addendum proposal was subsequently approved by USDA for a period of 1 year from April 8, 1996, to April 7, 1997.

Many of the state association activities for this new portion the project were planned to take place during the fall and winter months of 1995/96 when production demands are lower and time was more readily available to a larger percentage of association members. The REP addendum grant award was made by USDA in the Spring (April) of 1996 and several states experienced delays in getting accounts established at their respective supporting institutions. As a result, there has been limited activity and the associations have rescheduled planned activities to the fall and winter months of 1996/97. A complete review of accomplishments during this period will be included in the next progress report.

The New Jersey Aquaculture Association held its annual conference on December 2, 1995, in Egg Harbor City. There were 85 people in attendance for the program which contained presentations from the state Secretary of Agriculture and presentations on business aspects of aquaculture, insurance for aquaculture, aquaculture systems, New Jersey fish growers roundup, and the current status and future outlook for aquaculture in New Jersey. Project funds provided support for speakers from South Carolina, Maine, and Indiana's Oyster Industry Programs.

The REP provided support for a representative of the oyster industry in the Northeastern Region to participate in an industry oriented educational program entitled "Shellfish Aquaculture: Problems and Opportunities" held at Aquaculture in the Mid-Atlantic/Fish Farming Expo, Washington, DC on June 26, 1995. The session provided an update on the current status of the industry and highlighted issues affecting oyster aquaculture on the East, Gulf, and West Coasts through individual presentations and a panel discussion.

Pennsylvania Agriculture Show
At the request of the Pennsylvania Aquaculture Association (PAA), the REP provided partial support for an informational display at the Pennsylvania Agriculture Show held January 7-12, 1995, in Harrisburg, Pennsylvania. The Pennsylvania Agriculture Show is the largest annual public event in the state showcasing all forms of animal and plant agriculture. The week- long show is attended each year by an estimated 300,000 visitors.

Aquaculture Seminar for Regulators
A pilot program to educate regulators and others in the state of Massachusetts was developed (with partial REP support) by REP representatives from Barnstable County Cooperative Extension and the Massachusetts Sea Grant Program [Marine Biological Laboratory (MBL) and the Woods Hole Oceanographic Institution]. The resulting week-long residence course was held from May 15-19, 1995, at the MBL in Woods Hole, Massachusetts. The course targeted local regulators and other public and private sector agencies/groups within the Barnstable County area. The course was attended by 25 individuals representing 11 towns, two state agencies, one financial institution, and three private entrepreneur groups. Policy and technical aspects of aquaculture were covered: species, systems, industry profile, markets, financing, environmental, and other regulatory issues. These subjects were examined and discussed in formal (classroom) and informal settings during the week to offer a comprehensive view of the economic potential and constraints on industry expansion. Based on the success of the course, efforts are being made to expand the program to target a larger regional audience of regulatory and financial representatives.

Northeast U.S. Animal Health Association Meeting
Speaker support was provided for an REP participant representing NRAC and regional industry to give a presentation at the Northeast U.S. Animal Health Association (NE-USAHA) meeting held on April 23, 1996 in Frederick, Maryland. The purpose of the presentation was to educate NE-USAHA members about the aquaculture industry in the Northeastern Region and to review key health and policy issues affecting industry growth.

1. g) Mobile Educational Programs
Prior to development of the REP addendum, which allocated resources for speaker support and other educational outreach at the local level, all state aquaculture associations in the region were offered REP assistance for educational seminars and workshops in their states. The purpose was to facilitate education on subjects or issues of importance that would be otherwise unavailable for lack of local expertise, funding, or other reasons.

The following is a summary of REP support for speakers and other partial educational program costs to state aquaculture associations in the region:

New Hampshire Aquaculture Association
Speaker support (industry representatives) at New Hampshire Farm and Forest Day, February 1995.

Pennsylvania Aquaculture Association
Speaker support for a review of national fish health policies at the Pennsylvania Aquaculture Advisory Council Meeting, February 1995.

Maine Aquaculture Association
Support for REP participants to organize and conduct an introductory aquaculture session for commercial fishermen at the Maine Fisherman's Forum, March 1995.

Maryland Aquaculture Association
Speaker support (REP participant) at the MAA annual meeting, April 1995.

Ocean State Aquaculture Association (Rhode Island)
Speaker support (REP participants and researchers) for 1994 and 1995 meetings.

Support for two demonstration projects: remote setting (1994) and Chinese hat spat collectors (1995).

West Virginia Aquaculture Association
Speaker support (REP participants) for a Hybrid Striped Bass and Crawfish Culture Workshop, May 1995.

1. h) Biosecurity Video for Aquaculture
An instructional video entitled "Biosecurity in Aquaculture: Practical Steps for Healthy Fish" was developed and produced by Maine Cooperative Extension, University of Maine Sea Grant Marine Advisory program and the University of Maine Department of Public Affairs with industry participation and partial REP support. The video demonstrates common sense, cost-effective steps that can be taken in the hatchery and at the growout site to keep contamination by bacteria, parasites, and viruses as low as possible. The video was released for regional and national distribution in December 1994.

Objective 2
Project activities carried out under objective 2 are summarized below:

2. a) Regional Extension Survey (Situation and Outlook Report)
   b/c) Introductory and Technical Extension In-service Programs
   d) Aquaculture Industry Training Program

2. a) Regional Extension Survey
During the Fall of 1994, a questionnaire was distributed to compile information on the current status and needs of state extension programs in the region. Survey results (66% return rate) showed that extension personnel are often employed by, or affiliated with, the academic institution(s) in their state, through either the Sea Grant, Cooperative Extension, and other programs, or any combination thereof. Extension personnel in the Northeastern Region generally fall into three categories: full-time aquaculture agents/specialists, full-time extension with multiple program areas (i.e., marine resources, water quality, commercial fisheries, and aquaculture), and faculty (research and/or teaching) with partial extension appointments. Approximately 80% of the extension personnel responding worked part-time on aquaculture with 68% supported at a level of 0.25 Full Time Equivalents (FTE) or less. General aquaculture, fish, and water quality were most often cited as areas of expertise.

The survey also compiled information on in-service training needs, educational program topics, and computer use among extension programs and industry. Survey results were distributed to extension personnel at annual Regional Extension Workshops and to state industry associations at the Industry Summit. A review of REP goals, activities, and extension survey results was also presented at the annual meeting of the Northeast Extension Directors, February 6, 1995, in Philadelphia, Pennsylvania.

2. b/c) Introductory and Technical Extension Inservice Programs
REP participants organized and conducted two introductory training workshops on aquaculture at the annual meeting of the National Association of County Agricultural Agents (NACAA) held in Baltimore, Maryland from August 22- 26, 1993. Topics covered included NRAC program activities, national/regional information resources, and water quality management.

Annual Northeastern Regional Extension Training Workshops
Three annual Northeastern regional extension workshops were held for agents/specialists to facilitate networking, information exchange, and provide educational training on key subjects and issues identified by the extension survey. All extension training workshops (1994-1996) were held in

conjunction with other regional aquaculture or fishery conferences.

The first regional extension workshop was held in conjunction with the Fish Expo in Boston, Massachusetts, October 11-12, 1994. A second annual regional extension workshop was held at Aquaculture in the Mid-Atlantic/Aquaculture Expo VIII, June, 24-28, 1995, in Washington, DC. The third annual regional extension workshop was held at the Open Ocean Aquaculture Conference held in Portland, Maine from May 8-12, 1996. REP participants are also working on coordination and local arrangements to host the 2nd National Aquaculture Extension Workshop that will be held in Annapolis, Maryland from April 9-12, 1997.

2. d) Aquaculture Industry Training Programs

The goal of this portion of the project was to provide regional extension contacts with opportunities to develop practical experience and technical skills through hands on "internships" or training at commercial or other technical/research facilities. A package of information outlining the goals and objectives of the internship program was produced and mailed out to all aquaculture extension contacts in the Northeastern states. The package contained information on policies and procedures in effect for the program. Participation in the internship program was limited due to time availability of agents and specialists. Two extension training activities that did receive partial REP support included adaptation of mussel culture management software developed by the Atlantic Veterinary College of the University of Prince Edward Island, Canada, for hard clam culture in the Northeastern Region, and a review of the construction and operation of an innovative motorized floating nursery upwelling system for hard clams. Results of both training opportunities have been presented to industry via the New Jersey Aquaculture Association and will receive broader regional distribution during the next 6 months at other regional venues. To accommodate the time limitations of the majority of regional extension personnel, a "short course" approach was investigated as an alternative.

Short Course

The REP provided support for 10 extension personnel (and 10 individuals from the private sector) from the region to receive technical training at a week-long intensive short course entitled, "Closed Recirculating Systems." The course was held at the Agricultural Engineering Research Facility, Cornell University, Ithaca, New York, from July 10-14, 1995. Matching support for meals and lodging for REP participants was provided by individual state extension programs. A second annual Cornell short course on closed recirculating systems was held from August 20-24, 1996. Five additional extension representatives from the Northeastern Region received training along with 15 individuals from the private sector.

Work planned

Support of regional educational venues and programs and extension in-service training/network development covered under objectives 1 and 2 will continue during the no cost extension period (until April 7, 1997). Selected activities (NRAC information booth, technical presentations to regulators) described in a follow-up (1996/98) regional extension proposal currently pending before the USDA are also being initiated with remaining current REP funds.

Impacts

- REP activities have promoted increased interaction and resource sharing among Sea Grant Marine Advisory and Cooperative Extension Service agents and specialists from around the 12 Northeastern states and the District of Columbia.
- NRAC support and REP participation in the biennial Industry Summits (1992, 1994, and 1996) has increased regional industry/extension communication and has promoted a viable means for soliciting input and responding to industry priorities and concerns.
- REP support for the annual Milford Aquaculture Seminar has helped to maintain an important venue used for the last 16 years by the shellfish industry for networking and information exchange with research and extension.
- REP organization and support of the New England Fish Health Workshop has helped to establish an important new regional educational venue for the finfish industry.
- REP coordination of a Regional State Association/ Extension Workshop and subsequent REP addendum was in direct response to a top Industry Summit priority. The cooperation of REP personnel as collaborators and supporting institutional representatives strengthens industry-extension relations, has promoted team building and problem solving at the local level, and has advanced the

development of the regional extension network.

- REP sponsored educational programming - workshops on fish health, waste management, intensive production systems, short courses, a training course for regulators and bankers, speakers at state association meetings, and presentations on a variety of other fish and shellfish culture topics - has reached a large and diverse audience throughout the Northeastern Region. This programming was developed in direct response to issues identified as being of high priority to regional industry.
- REP speaker support and inclusion of visiting speaker support in the individual plans of work of all participating state associations has improved educational programming and outreach at the state/ local level.
- Annual Regional Extension Workshops and the Closed Recirculating Systems short course at Cornell have improved intra- and inter-state communication, extension network development and extension technical expertise on important regional subjects/ issues.
- Initial partial REP support to initiate the Cornell short course also provided the opportunity for 25 prospective culturists from the private sector to receive formal training.

Publications and presentations

Abstracts of technical papers presented at the 14th Annual Meeting, Milford Aquaculture Seminar, February 22- 24, 1994, Milford, Connecticut. J. Shellfish Res. 13(1):307-324.

Abstracts of technical papers presented at the 15th Annual Meeting, Milford Aquaculture Seminar, February 21- 23, 1995, Milford, CT. J. Shellfish Res. 14(1):233-252.

Abstracts of technical papers presented at the 16th Annual Meeting, Milford Aquaculture Seminar, February 26- 28, 1996, Milford, CT. J. Shellfish Res. 15(2):447-464.

Abstracts of technical papers presented at the 2nd Annual New England Farmed Fish Health Workshop, April 22, 1994, Marine Technology Center, Eastport, Maine. University of Maine Cooperative Extension, Orono, ME. 13pp.

Abstracts of technical papers presented at the 3rd Annual New England Farmed Fish Health Workshop, April 7, 1995, Marine Technology Center, Eastport, Maine. University of Maine Cooperative Extension, Orono, ME. 20pp.

Abstracts of technical papers presented at the 4th Annual New England Farmed Fish Health Workshop, April 11-12, 1996, Marine Technology Center, Eastport, Maine. University of Maine Cooperative Extension, Orono, ME. 15pp.

Aquaculture engineering and waste management. 1995. M. B. Timmons, ed. Proceedings from the Aquaculture in the Mid-Atlantic/Aquaculture Expo VIII Conference, June, 24-28, 1995, Washington, DC. Northeast Regional Agricultural Engineering Service. NRAES-90. 384pp.

Opitz, H. M. 1994. Biosecurity in aquaculture: Practical steps for healthy fish. Northeastern Regional Aquaculture Center/USDA Video #NRAC V-5 [University of Maine Cooperative Extension, Sea Grant and Dept. of Public Affairs MSG-VT-94-1]. North Dartmouth, MA. 15:35 min.

Support(93-3)

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1993	$77,000						$77,000
1994-95	$73,000						$73,000
Total	**$150,000**						**$150,000**

Support(95-11)

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1994-95	$77,000						$77,000
1995-96	$73,000						$73,000
1996-97	$58,740						$58,740
Total	**$208,740**						**$208,740**

Evaluating the Economic Impacts of Piscivorous Predator Damage and Control Methods at Finfish Aquaculture Facilities in the Northeastern States (95-3)

Progress report period April 24, 1995 to August 31, 1996

NRAC funding

$145,732

Project coordinator

Richard Bruggers, USDA/APHIS/ADC, Denver Wildlife Research Center, Colorado

Participating investigators/ cooperative agencies

Mark Tobin, Denver Wildlife Research Center, Colorado
James F. Glahn, Denver Wildlife Research Center, Colorado
Tommy King, Denver Wildlife Research Center, Colorado
Erica Egan, Denver Wildlife Research Center, Colorado
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James Forbes, USDA/APHIS/ADC, New York

Objectives

- Determine the distribution and relative magnitude of piscivorous bird presence and use of Northeastern finfish aquaculture facilities in Pennsylvania, New York, and New Jersey.
- Determine the extent of economic losses caused by piscivorous bird predation at Northeastern finfish aquaculture facilities.
- Evaluate the cost-effectiveness of two-strand electric fence barriers to prevent heron predation at finfish aquaculture facilities in the Northeastern states.

Anticipated benefits

The results of our research should provide aquaculture managers with more recent and accurate information on the impact of piscivorous birds to the industry and a possible technique to reduce these losses.

Principal accomplishments

During June 1995, we made initial site visits to 61 randomly selected aquaculture facilities in Pennsylvania, New York, and New Jersey to question managers about the nature of their facility and their bird predation problems, and to verify the present or absence of piscivorous birds, by species, using these facilities (objective 1). From mid-July through September 30, 1995, we conducted an intensive census of bird predator populations and made foraging rate observations at a subsample of 30 facilities initially visited (objective 2). From mid-April through June 1996, we continued our bird censuses and foraging rate observations under objective 2 at seven selected facilities to complete our understanding of bird predation problems during the spring and early summer. Based on their frequency of occurrence and the population densities observed, Common Grackles, Mallards, and particularly Great Blue Herons would appear to be the primary predators of concern to the industry. Preliminary analysis of foraging rate observations provided information about the potential impact of these primary predators. At trout-rearing facilities, Great Blue Herons consumed 2.2 trout (averaging 20 cm in length) per hour of observation.

Mallards and Common Grackles consumed trout at rates of 4.2 and 3.9 per hour of observation, but the average fish length was only 11 and 7.1 cm, respectively.

To more thoroughly understand the overall losses caused by primary predators, we initiated the second phase of objective 2 in June 1996 which involved directly measuring fish survival in paired raceways at five trout-rearing facilities with and without netting exclusion that we installed. Facility managers are cooperating with us on this study by stocking these raceways in a similar manner and monitoring removal of fish over time. After monitoring bird use of these raceways for 3 to 4 months, we plan to inventory fish in these raceways in October 1996.

In June and July 1996, we contacted 15 producers and made site visits in Pennsylvania and New Jersey to identify possible study sites for evaluating electric fence barriers for deterring heron predation (objective 3). Despite this effort, only two logistically feasible sites with more than one heron consistently present were identified. At these two sites, herons were initially censused in and around the raceways for 4 consecutive days before and again 4 consecutive days after a two-strand electric fence was erected around the raceways. The initial response of herons to the electric fencing was dramatic and heron activity in the raceways was reduced to almost nothing. Censuses are continuing twice monthly over the fall and winter to examine long-term effectiveness of these systems for deterring herons.

Work planned

Work under objective 1 is complete. Most of our field observations under objective 2 will be completed by November 1996. However, if sufficient funding remains after October, we plan to continue to census piscivorous bird populations at selected facilities periodically from November through March to ascertain the extent of predation problems that occur during this period. Most of our future work until the project completion date will involve further data analysis and reporting. A major part of our reporting effort will involve completing a leaflet on the impact and control of piscivorous bird predation and sponsoring workshops for aquaculture producers in March or April of 1997.

Donald F. Mott, who was co-principal investigator of this project, retired on March 30, 1996. On July 15, 1996, Dr. Mark Tobin took over Mr. Mott's position and is currently study director of the study concerning objective 3 of this project. Because we were unable to identify a sufficient number of suitable study sites in 1996 and the time constraints of the present subcontract, we will be unable to obtain definitive information about the cost-effectiveness of electric fencing barriers (objective 3), but will be able to provide information on their possible utility. With other work completed, it will be more logistically feasible to locate test sites and accomplish a definitive evaluation of electric fencing during the summer of 1997. To accomplish this task, we would need to extend the project termination date from June 24, 1997, to December 24, 1997, and receive a post-award budget increase in 1997 of $10,000.

Impacts

- A more thorough understanding of the distribution and relative magnitude of piscivorous bird predation at 61 finfish aquaculture facilities in the Northeastern United States.
- Definitive observational and experimental data on the extent of fish losses due to predation by primary predators that will include both direct and indirect losses.
- The potential utility of a two-strand electric fence barrier for preventing heron predation.

Publications and presentations

Glahn, J. 1997. Bird predation and its control at aquaculture facilities in the Northeastern United States. Northeastern Regional Aquaculture Center/ USDA Publication #NRAC 97-001 [APHIS 11-55-009]. North Dartmouth, MA. 17pp.

Glahn, J. F. and D. T. King. 1996. Assessing damage by fish-eating birds at Northeast aquaculture facilities- An integrated approach. Pennsylvania Fish Depredation Workshop. Kleinfeltersville, PA. July 10-11, 1996.

Glahn, J. F., T. Tomsa, E. Egan, and K. Preusser. 1997. A survey of piscivorous birds impacting finfish aquaculture facilities in the Northeastern United States. J. World Aquaculture Soc. (In Prep.).

Glahn, J. F., T. Tomsa, E. Egan, and K. Preusser. 1997. An assessment of economic losses caused by piscivorous bird predation at Northeastern finfish aquaculture facilities. (In Prep.).

Support

Years	NRAC USDA funding	Other support: University	Industry	Other Federal	Other	Total	Total Support
1995-96	$87,041			$32,250		$32,250	$119,291
1996-97	$58,691			$32,250		$32,250	$90,941
Total	**$145,732**			**$64,500**		**$64,500**	**$210,232**

Enhanced Digestibility of Fish Feeds to Reduce Waste Nitrogen, Phosphorus, and Solids (95-4)

Progress report period May 1, 1995 to August 30, 1996

NRAC funding
$141,840

Project coordinator
H. George Ketola, Cornell University, New York

Participating investigators/ cooperative agencies
Joseph H. Soares, Jr., University of Maryland, Maryland
Richard E. Austic, Cornell University, New York
Scott Lindell, Aqua Future, Inc., Massachusetts
Richard Colantuno, Limestone Springs Trout Co., Pennsylvania

Objectives
- To determine true digestibility of amino acids (the major sources of nitrogen) by salmon and striped bass fed fish meal and several plant proteins to formulate efficient diets to reduce nitrogen waste.
- To determine the minimum amount of phytase needed to digest undigestible phytin phosphorous in a practical-type diet fed to trout and striped bass to formulate efficient diets to reduce waste phosphorous.
- To determine the effect of nutrient dense diets and phytase on growth, feed efficiency, body composition, and retention and discharge of nitrogen and phosphorous by trout and striped bass.
- To determine the effects of different methods of pelleting practical diets on growth, feed efficiency, carcass composition, and digestibility of amino acids, nitrogen, and phosphorous by rainbow trout and striped bass.
- To determine the effect of a new diet (using knowledge gained from previous studies) on growth, feed efficiency, organoleptic characteristics, carcass composition, retention, and discharge of nitrogen (N) and phosphorous (P) by rainbow trout and striped bass reared under commercial conditions.

Anticipated benefits
Results of Salmon Study 1 show:

1. For corn gluten meal and soybean meal (with optimum toasting) digestibilities of crude protein and essential amino acids are as good or better than that for herring meal.
2. Growth of salmon fed corn gluten meal or soybean meal with supplemental amino acids to replace all fish meal (32.7%) was only slightly reduced (by 3-6%).
3. Soybean (with adequate toasting) and corn gluten meals appear to be well-suited for increased use in trout and salmon feeds with increased efficiency of retention of dietary nitrogen and reduced waste discharges.

Striped Bass, Study 1:

1. Average amino acid digestibility was very similar for herring meal, soybean meal (48%) and corn gluten meal at 89.2, 92.6, and 90.9%, respectively.
2. Dry matter digestibilities follow a similar pattern to amino acid digestibility as was 68.3, 72.2, 70.8, and 60.3% respectively for herring meal, soybean meal, Expo 96. TX, page 46.

corn gluten meal, and peanut meal.

Results of Study 2 are preliminary and it is premature to suggest benefits.

Principal accomplishments

Initiating of research was later than initially planned because of delays (from May to August 1995) in final completion of start contracts.

Study 1 (amino acid digestibility) was conducted with Atlantic salmon and striped bass.

A. *Atlantic salmon:* The study with salmon was completed and the results were described in a master's thesis by Julie Keene (1996), published, and presented as an oral report at the Cornell Nutrition Conference for Feed Manufacturers, Rochester, NY (October 24-26). Results showed that substitution of soybean meal, corn gluten meal, or peanut meal for 32.7% herring meal in an Atlantic salmon diet (without amino acid supplementation) depressed 14-week growth significantly. Without supplements, growth was depressed least with soybean meal (26%) and more by corn gluten meal (87%) and peanut meal (73%). When these diets were supplemented with amino acids to meet standards of the National Research Council's minimum requirements for the trout or the amino acid content of the trout egg, growth of salmon was significantly and markedly increased, and even more so for the egg standard. The amino acid supplements to meet the egg standard growth was 94 to 97% of that for salmon-fed herring meal. Apparent digestibilities of crude protein in herring, soybean, corn gluten, and peanut meals were 72, 81, 90, and 65%, respectively, with that for soybean and corn gluten being significantly greater than that for herring or peanut meals (data not shown). Apparent and true digestibilities of essential amino acids in soybean and corn gluten meals were as great or greater than those for herring. Apparent and true digestibilities of essential amino acids in peanut meal were as generally lower than those for herring meal.

B. *Striped bass:* This study was completed and a 14- week digestion trial was conducted using 0.5% added chromic oxide to the diet. The digestibility of dry matter was 68.3, 72.2, 70.8, and 60.3 for herring meal, soybean meal (48% protein), corn gluten meal, and peanut meal, respectively. No significant differences in dry matter apparent digestibility was detected. Amino acid apparent and true digestibility coefficients for the four protein supplements listed above showed striped bass had significantly reduced corn gluten meal and peanut meal.

Study 2 (phytase) was conducted with Atlantic salmon and striped bass.

A. *Atlantic salmon:* Diets were made commercially according to plan. Feeding of Atlantic salmon and striped bass commenced January 18 and January 22, 1996, respectively. At 6 weeks in the experiment with salmon, the study appeared to be compromised by the presence of rancid ingredients used to make the experimental feeds at the manufacturing plant. Slow growth observed in Atlantic salmon lead to making special chemical tests for rancidity (thiobarbituric acid and peroxide values) of diets. Tests revealed high oxidative rancidity (TBA and PV = approximately 100 mg or meq/kg) of dietary lipids apparently responsible for reduced growth.

Although growth of salmon was suppressed at 6 weeks, it was significantly increased (17%) by supplementation with phosphate, while supplementation with phytase enzyme tended to increase growth equivalent to 78% of that for phosphate, but the response was not significant ($P \leq 0.05$). Salmon were continued on the experiment until 14 weeks.

At 14 weeks, growth of salmon fed the experimental diets was not significantly increased by supplements of phytase enzymes from 500 to 4,000 units/kg feed. Growth was significantly increased by supplemental P (0.2%). In contrast feed efficiency (gain x 100/feed) was significantly increased by both P supplement and the highest level of enzyme (4,000 units/kg). Bone ash measurements were more sensitive measurements of P deficiency and responses to supplements of enzymes or P as previously demonstrated by Ketola (1994). Bone ash measurements did not appear to be influenced by rancid ingredients.

The influence of phytase and P supplements on P discharges and retention by salmon was measured. The concentrations of P in carcasses tended to increase with supplements of enzyme, but they were not significant.

The concentration was significantly increased by supplemental P. The retention of P in carcasses tended to increase with supplements of enzyme but was not significant except at the highest levels of phytase (2,000 and 4,000 units/kg) and with supplemental P. Discharges of phosphorus in effluents tended to decrease with supplements of enzyme and were significantly reduced at the highest levels of phytase (2,000 and 4,000 units/kg) but not with supplemental P.

Rainbow trout (Phytase): A confirmation experiment was conducted for 12 weeks with triplicate lots of 75 fingerling rainbow trout fed three rancid diets (the basal alone, and with supplements of phytase or P) in order to further examine the effects of these diets. Supplemental phytase (1,000 units/kg) or phosphorus significantly increased growth, feed efficiency, and bone ash. Supplemental phytase (1,000 units/kg) or phosphorus significantly increased retention of phosphorus in carcasses and significantly reduced discharges of phosphorus. The reductions in P discharges ranged from 13 to 19% depending on whether the discharges were expressed as grams of P discharges per kilogram of weight gain or feed fed.

Measurements with trout of digestibility of phosphorus in the rancid diets and remanufactured fresh (non-rancid) diets are in progress.

B. Striped bass: This study (including repeat study) is now complete. Four diets with four levels of phytase supplementation (0, 500, 1,000, 2,000 phytase units/kg feed) and a positive control phosphorus diet were assigned in duplicate to tanks and fed to juvenile striped bass for 16 weeks. An apparent P digestibility trial was conducted at the end of the growth period using 0.5% chromic oxide as a marker in the diets and after feeding the marked diet for 2 more weeks. Phytase (PU) was sprayed post-extrusion on the basal diet which contained over 70% plant sources and 0.58% total P, and 0.30% estimated available P. The positive control diet supplemented with 0.2% anhydrous NaH_2PO_4 with no added phytase, was analyzed to contain 0.73% total P. Significant improvements ($P \le 0.05$) were observed in mean weight gain in the 1,000 PU (53 g), 2,000 PU (49 g), and positive control (47 g) groups when compared to the 0.0 PU (34 g) supplemented group. There were no differences observed between the two highest phytase treatments and the positive control. Significant differences in mean feed conversions were also found between the 0.0 PU treatment (1.57) and the 1,000 PU, 2,000 PU groups (1.32 and 1.36 respectively, $P \le 0.05$). Bone ash concentrations (mg/kg DM) at the end of the experimental period were significantly greater in the 2,000 PU (34), positive control (33) ($P \le 0.05$), and 1,000 PU (32) ($P \le 0.06$) treatments, when compared to the 0.0 PU (25) treatment (pooled SEM=19.7). In addition, apparent P digestibility coefficients (%) were significantly greater in the 1,000 PU (67) and 2,000 PU (78) treatments, when compared to the unsupplemented group (42), (pooled SEM=6.2). Incidence of scoliosis was significantly greater in the unsupplemented (0.0 PU) treatment when compared to all other treatments.

Results of this experiment confirm that a phytase supplement in a high phytate diet will increase P utilization by the striped bass, and furthermore indicate that a level of approximately 1,000 PU is adequate to maintain proper growth and health comparable to an inorganic P supplemented diet.

Study 3 (High Nutrient Density Diets):

Diets were formulated by Drs. Ketola and Soares and will be manufactured in October commercially by Zeigler Brothers. Feeding of striped bass has begun.

Study 4 (practical formulations and pelleting technology):

In mid-November, the pelleting study will begin.

Work planned

Currently, we plan to complete the phytase experiment, making measurements of P digestibility in trout fed rancid and fresh feeds. This is necessary in order to better evaluate the effect of the enzyme. We need to consider an extension and perhaps request some further support.

Study 3 will be conducted in year 2 at Cornell University and the University of Maryland with supportive chemistry being done at Cornell University. Study 5 will be done as proposed.

Impacts

Results of study 1 show that corn gluten meal and soybean meal (with optimum toasting) have deficiencies of essential amino acids that can be very effectively corrected by supplementation or balancing the amino acids. Furthermore, the digestibilities of crude protein and essential amino acids in these plant meals is as good or better that for herring meal, thereby indicating that they are well-suited for increased use in trout and salmon feeds with increased efficiency of retention of dietary nitrogen and reduced waste discharges. Results also suggest that peanut meal was less effective in diets at high levels of supplementation. These data may provide a guide for greater use of plant proteins in place of fish meal in diets of trout and striped bass, thereby reducing excess dietary phosphorus and discharges.

Study 2 shows that phytase supplements increase digestion of otherwise underutilized phosphorus in salmon, trout, and striped bass, resulting in reductions in waste P discharges.

Publications and presentations

Dougall, D. S., L. C. Woods, III, L. W. Douglass, and J. H. Soares. 1996. Dietary phosphorus requirement of juvenile striped bass *Morone saxatilis*. J. World Aquacult. Soc. 27(1):82.

Hughes, K. H. and J. H. Soares. 1996. Efficacy of phytase on phosphorus utilization by the striped bass *Morone saxatilis*. (In Prep.).

Keene, J. C., R. E. Austic, and H. G. Ketola. 1995. Use of plant meals as primary protein sources in diets for juvenile Atlantic salmon (*Salmo salar*). 1995 Cornell Nutrition Conference for Feed Manufacturers (October 24-26). Rochester Marriott Thruway Hotel, Rochester, NY. p. 179-185.

Soares, J. H. and K. P. Hughes. 1995. Efficacy of phytase on phosphorus utilization. Maryland Nutrition Conference for Feed Manufacturers.

Presentations

Hughes, K. P. and J. H. Soares. 1996. Efficacy of dietary phytase on phosphorus utilization by the striped bass *Morone saxatilis*. World Aquaculture Society, Division U.S. National Aquaculture Society, Expo 96. TX. 46pp.

Keene, J. C., R. E. Austic, and H. G. Ketola. 1995. Use of plant meals as primary sources in diets for juvenile Atlantic salmon (*Salmo salar*). 1995 Cornell Nutrition Conference for Feed Manufacturers (October 24-26). Rochester Marriott Thruway Hotel, Rochester, NY.

Small, B. C. and J. H. Soares. 1996. Apparent digested ideal protein requirement of the striped bass (*Morone saxatilis*). To be presented at World Aquaculture '97, Seattle, Washington.

Soares, J. H. and K. P. Hughes. Improved dietary phosphorus utilization by striped bass fed phytase. 1996. 2nd World Fisheries Congress 1:105.

Papatryphon, E. and J. H. Soares. 1996. The use of phytase to increase phosphorus utilization in practical diet for the striped bass *Morone saxatilis*. To be presented at World Aquaculture '97, Seattle Washington.

Thesis

Keene, Julie Catherine. 1996. Protein nutrition of Atlantic salmon: The use of plant meals to replace fish meals as primary sources of dietary protein.

Support

Years	NRAC USDA funding	Other support: University	Other support: Industry	Other support: Other Federal	Other support: Other	Other support: Total	Total Support
1995-96	$69,931	$29,151				$29,151	$99,082
1996-97	$71,909	$27,756	$13,110			$40,866	$112,775
Total	**$141,840**	**$70,017**	**$13,110**			**$70,017**	**$211,857**

Dietary Modulation of Food Conversion Efficiency in Farmed Hybrid Striped Bass (95-5)

Progress report period August 1, 1995 to August 31, 1996

NRAC funding
$65,000

Project coordinator
Allen R. Place, Center of Marine Biotechnology, Baltimore, Maryland

Participating investigators/ cooperative agencies
Joshua Goldman, Aquafuture, Inc., Massachusetts
Mark Subramanyam, Zeigler Bros., Inc., Pennsylvania

Objectives
• To determine whether the addition of supplemental glutamine and/or highly unsaturated fatty acids (HUFAs) to the diet of hybrid striped bass increases the food conversion efficiency of the hybrid striped bass relative to conventional diets.

Anticipated benefits
If modulation of the diet is shown to enhance food conversion efficiencies it may lead to the development of new feeds for aquaculture that reduce the time and cost of bringing fish up to market size. In addition, increasing food conversion efficiencies may allow for fish to be kept at higher densities without decreasing water quality. Any benefits derived from research using hybrid striped bass may also be applicable to other species.

Principal accomplishments
In vitro experiments on the effects of the presence of glutamine on glucose and proline uptake by isolated hybrid striped bass intestine have been completed. Everted sleeves of fish intestines were incubated for 6 minutes in teleost ringers with a radio-labeled substrate, both with and without 25 mM glutamine in the incubation solution. Substrates tested included glucose at 5 mM and 25 mM and proline at 25 mM. In all three cases, the presence or absence of glutamine in the incubation solution did not affect the uptake rate for the substrate. This strongly suggests that if glutamine does up-modulate nutrient assimilation it does so by a mechanism other than active transport, such as pericellular uptake. We have also shown that the addition of DHA and EPA to the diet enhances proline uptake by as much as 15%.

We have recently communicated with Zeigler Bros. for the formulation of glutamine and DHA supplemented diets to be used in the *in vivo* studies. We have analyzed the soluble glutamine content of fish solubles (three batches), herring meal (six batches), and 60% fish meal (six batches). In all cases, the levels of endogenous glutamine are at least three orders of magnitude lower than our proposed lowest supplement (0.17% and 0.73%). We have also analyzed the fatty acid content of the menhaden oil (Zapata winterized) to be used and found the DHA/EPA ratio is 0.734. We have determined that we can change these ratios to 3.3 and 5.9 with addition of the DHAsco oil we currently have available. These diets are currently being formulated for the start of the feeding trials in mid October. Feed trials will be performed at both AquaFuture and COMB.

Work planned
Our immediate concern is to start the feeding trials by mid-October and have them finished by mid-December.

Impacts
As a result of this study, we anticipate to develop an improved formula to be used in the production of high performance feed for cultured hybrid striped bass.

Publications and presentations
Lund E. D. and A. R. Place. 1995. Fishes of the genus *Morone* exhibit enhanced intestinal uptake of proline when fed a diet high in highly unsaturated fatty acids. American Zoological Society. American Zoologist. Vol. 35:77A. (Abstract).
Lund E. D., A. R. Place, and C. V. Sullivan. 1995. Lipid contents of female striped bass plasma and oocytes exhibit seasonal changes associated with oocyte maturation. In 5th International Symposium

on Reproductive Physiology of Fish. Austin, TX, (Abstract). 371pp.

Ozkizilcik S., F. L. Chu, and A. R. Place. 1995. Complex microencapsulated diets as partial replacement of live food in larval fish culture. Larvi '95. Belgium, October 1995, (Abstract).

Ozkizilcik S., F. L. Chu, and A. R. Place. 1996. Ontogenetic change of lipolytic enzymes in striped bass (*Morone saxatilis*). Comp. Biochem. Physiol. 113B:631-637.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1995-96	$65,500	$65,254	$44,450			$109,704	$175,204
Total	**$65,500**	**$65,254**	**$44,450**				**$175,204**

Predator Exclusion Device (PED) to Control Predation by Conchs (*Busycon*) on Oyster Aquaculture Grounds (95-6)

Progress report period February 1, 1996 to August 31, 1996

NRAC total funding level

$25,100

Project coordinator

Dick Nelson, Cotuit Oyster Company, Massachusetts

Participating investigators/ cooperative agencies

Dave Ryan, Cotuit Oyster Co., Massachusetts
Al Surprenant, Cape Cod Oyster Co., Massachusetts

Objectives

- To exclude, or prevent all sizes of the channeled whelk, *Busycon canaliculatus*, and the knobbed whelk, *Busycon carica*, from entering the growing area.
- To allow oyster aquaculturists in the Northeast to grow oysters from 30 mm to 75 mm (market size) on the bottom with minimal predation by conch species.
- To eliminate to use of pens, trays, or bags in the oyster aquaculture industry for oysters greater than 30 mm.
- To develop an efficient trapping method for conch.
- To develop a cost benefit analysis for the device, comparing it to both cage systems and planting directly on the bottom with predator collection strategy.
- To develop blueprints and installation instructions for the device.
- To provide conch data for the device.

Anticipated benefits

- To develop a technique for bottom-planting oysters that yields low predation levels.
- To develop a technique for producing oysters at a lower cost than traditional aquaculture methods which include the use of trays, bags, and other protective structures.

Progress and principal accomplishments

PED systems were purchased, assembled, and deployed at the Cotuit and West Bay sites during April and May, 1996, according to the protocol in the grant application. Test oysters were planted in each of the four PEDs from May 20 to July 15. Controls were then set up when the first oysters were planted in each PED. The Pleasant Bay PED system and experiment was set up in late July and early August. Two PEDs were assembled and deployed at this location. The alternate Pleasant Bay site was used after approval by the regional NRAC office because of disease or algae problems at the Long Island site.

The PED systems at each location appear to be working very effectively at keeping conch from entering the planted area. Almost all control oysters at each PED and at locations removed from the PEDs have been consumed by conch.

Predation by conch inside the PEDs is minimal, with one exception at the West Bay location. At this location, due to an extreme tide or entanglement with recreational fishing gear enabled at least 13 conch to enter the protected area within the PED and eat to date, 68 oysters. The total number of conch found inside the perimeter of all other PEDs is 11. Total conch entrapped is as follows:

Cotuit 1	119	West Bay 1	172	Nauset 1	82
Cotuit 2	78	West Bay 2	134	Nauset 2	132

Interviews with several oyster growers have been conducted to gain information for developing the cost-benefit analysis of the PED system versus other systems.

While the PED system appears to be very valuable in protecting oysters from conch, it is unclear if the PED system has a value as a commercial conch fishing system. Greater than 75% of conch we have trapped in PED have been juveniles and/or knobbed whelk, a less valuable commercial product.

Work planned

- Harvest all oysters from each PED experiment to compute final results of oyster mortality in PED and in controls, and compile conch morphology.
- Finish interviews with other growers.
- Draw up plans for PED construction and installation.
- Prepare final report.

Impacts

A technique has been developed that effectively prevents conch from gaining access to bottom-planted oysters. Bottom planted oysters are less expensive to produce than oysters raised in cages or other structures.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1996-97	$25,100		$34,000				$59,100
Total	**$25,100**		**$34,000**				**$59,100**

Identification and Training for Aquatic Animal Health Services in Northeastern United States (95-7)

Progress report period January 1, 1996 to August 31, 1996

NRAC funding

$17,350

Project coordinator

John L. Pitts, Bellwether Consulting, Washington

Participating investigators/ cooperative agencies

Donald Abt, Aquavet Program, Massachusetts
Michael Opitz, Cooperative Extension, Maine
Bradley Powers, MD Dept. of Agriculture, Maryland
Joe Gloyd, American. Vet. Med. Assoc., Washington, DC
Otis Miller, USDA-APHIS, Maryland
Aggie Spicer, Cooperative Extension, West Virginia
Richard Bohn, USTFA, West Virginia

Objectives

- To expand laboratory, diagnostic, and certification services for private sector.
- To identify public and private laboratories in NRAC region providing services.

- To quantify need for producer services.
- To identify federally-accredited veterinarians to determine interest and education needs related to aquatic animal health.
- To provide laboratories with information on regional aquaculture production.
- To coordinate project to include federal and state regulations on aquaculture.
- To work with NBS Leetown Fisheries Academy regarding certification needs.
- To identify regional educational sites for aquatic animal health training.
- To develop action plan for workshops for regional training.

Anticipated benefits

- To provide a services directory for the private sector to access aquatic animal diagnostics and certification.
- To develop a compendium of state and federal regulations.
- To provide information and specific contacts for data and support.
- To identify qualified health care providers in the region and to develop health care training as needed.

Principal accomplishments

Final Draft Directory was completed following two reviews, and includes:

- diagnostic Laboratories,
- aquatic Animal Health Care Providers,
- information Resources on Farmed Aquatic Animals,
- state Agency Aquaculture Regulations,
- state Veterinary Practice Acts,
- federal Veterinarians in NRAC Region,
- seafood Inspection (HACCP) Contacts,
- new Animal Drug Approval Contacts,
- seven NRAC region training sites identified and characterized, with faculty, curriculum, and physical plant described, and
- aquatic animal health providers identified with training needs, 280 surveys returned from NRAC region, and 250 surveys from adjacent regions.

Work planned

- Final NRAC Directory prepared and submitted by November 15, 1996.
- Education and training segment completed and submitted by November 15, 1996.
- Supplemental documentation of regulations, federal and state documents to be prepared as a file to be housed at the NRAC office.

Impacts

Two hundred copies of the Directory will be circulated to the private sector, state and federal agencies, and interested parties through NRAC. The Directory will also be posted on NRAC's Home Page on the Internet. Impacts cannot be assessed at this time.

Publications and presentations

Pitts, J. L. Northeastern Aquatic Animal Health Directory: A Directory of Aquatic Animal Health Laboratory Services, Information Resource Contacts and Related State Regulations for the Private Sector Aquatic Farm Industry in the Northeastern Region of the United States of America. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 96-002 (Formerly NRAC #114). North Dartmouth, MA. 53pp.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1995	$17,350		$6,000		$6,500	$12,500	$29,850
1996							
Total	**$17,350**		**$6,000**		**$6,500**	**$12,500**	**$29,850**

Utilization of Off-Grade Salmon, Salmon Trim, and Mechanically Deboned Salmon in Fermented Sausages (95-9)

Progress report period November 28, 1995 to August 31, 1996

NRAC total funding level

$13,300

Project coordinator

Alfred A. Bushway, University of Maine

Objectives

- To develop low fat fermented fish sausage (pepperoni, summer sausage) using off-grade salmon, salmon trim, and mechanically deboned salmon.
- To evaluate the nutritional, chemical, physical, microbiological, and sensory characteristics of each type of fermented fish sausage.

Anticipated benefits

Production of fish sausages would reduce the processing waste stream (fish frames) while producing value- added products for the aquaculture industry. A high value, low fat, nutritious fish sausage would result from completion of this research. Development of fermented salmon sausages along with a market for these products will lead to the creation of jobs in the aquaculture and fish processing industries, particularly in economically depressed Washington County in Maine. It would also provide an alternative sausage product for consumers whose ethnic background limits their consumption of sausages for dietary and religious reasons.

Progress and principal accomplishments

As reported in the previous progress report, a fermented pepperoni has been successfully manufactured from cull salmon, and the chemical, physical, nutritional, microbiological, and sensory analyses completed. During the past 3 months, fermented pepperoni and summer sausage has been manufactured from salmon trim and mechanically deboned salmon (80:20 ratio). Pepperoni and summer sausage were manufactured by bacterial fermentation and by direct acidification with citric acid (3.0%). Citric acid was selected to overcome the poor sensory characteristics obtained with the encapsulated lactic acid in the earlier experiments. A pre-gelled carrageenan was used as the fat source in order to reduce the fat content of the final product. Each product was manufactured in triplicate and all chemical, physical, and microbiological analyses were performed on each replicate. Sensory analyses is currently being performed using one of the three replicates. Pepperoni and summer sausage acidified with encapsulated citric acid reached pH endpoint of 4.4 and 4.8, respectively after 12 hours. Bacterial fermentation produced a steadily decreasing pH and the endpoints were reached after 21 hours. Statistical analyses is currently being performed on the data, and will be completed in the next month. The moisture and fat content was higher in the encapsulated sausage products. Cholesterol and fatty acid analyses are being performed by Marge Gallagher, East Carolina University.

Work planned

All analyses and statistical analysis of the data will be completed in the next 2 months. Results will be prepared in a form suitable for presentation to potential user groups. Dissemination of the information will be performed by Bob Bayer.

Publications and presentations

We plan to report the results of this research at the June 1997 Institute of Food Technologists Meeting in Orlando, FL. The graduate student will complete the requirements for his M.S. degree.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1995	$13,300		$6,700				$20,000
1996							
Total	**$13,300**		**$6,700**				**$20,000**

1996 Project Development (95-12)

Progress report period January 1, 1996 to August 31, 1996

NRAC funding

$43,024

Principal investigator

Dr. Robert Rheault, (Jan. 1, 1996 - Mar. 31, 1996)
Dr. Kim E. Harrison, (Apr. 1, 1996 - Aug. 31, 1996), Northeastern Regional Aquaculture Center, Massachusetts

Objectives

- Convene two meetings of the Technical/Industry Advisory Council in 1996.
- Fund travel expenses for Technical and Industry Advisors to attend project meetings.
- Convene one meeting of the TIAC proposal Review Panel in 1996.

Principal accomplishments

- Two meetings of the Technical/Industry Advisory Council were convened during the Project Period.
- The TIAC Review Panel met to review pre-proposals submitted for the 1996 RFP.

Northeast Aquaculture Education And Awareness Video Program (96-1)

Progress report period June 10, 1996 to September 30, 1996

NRAC funding

$33,685

Project coordinator

Peter Mottur, President/Director, Mott Media, Inc., RI

Participating investigators/ cooperative agencies

Debra Lipsett, Mott Media, Inc., Rhode Island
William Sargent, Woods Hole-Bermuda Science Writing Program, Massachusetts

Aquaculture Sites:

Josh Goldman, Aqua Future, Inc., Massachusetts
Bob Wallace, Billingsgate Shellfish, Massachusetts
Ron Widmyer, Federal Hill Trout Farm, West Virginia
Merrill F. Lank, Connors Aquaculture, Maine
Richard Schuck, Maryland Aquatic Nursery, Maryland
Hill Bloom, Tallmadge Brothers, Inc., Connecticut

Objectives

- To produce a 30-minute video program about the aquaculture industry in the Northeast Region of the United States, which provides education and

awareness to the public concerning this growing business.
- To distribute this program throughout the Northeast to state and local aquaculture associations, educational institutions, local and regional television stations, and the general public.

Anticipated benefits

In order for the aquaculture industry to grow, state aquaculture associations and independent aquaculture businesses need increased support from their local communities. This video will promote aquaculture in the Northeast by featuring several private regional success stories and describing their positive economic impact. The program will emphasize the benefits of aquaculture expansion in local communities, including both jobs and development, and promote the feasibility of creating new ventures.

Principal accomplishments

A. Completed pre-production: June 10 - July 10, 1996, production coordination, scheduling, consultation with script writer, outline draft, and interview questions.

B. Completed field production (site nos. 1-3):

1. *Maryland Aquatic Nurseries,* Jarrettsville, MD 7/17 - (2) interviews, site tour, and extensive b-roll of operations, UW.
2. *Aqua Future, Inc.* Turners Falls, MA . 8/22 - 2 interviews, site tour, and b-roll of some operations. 9/10 - b-roll of operations (harvest, packing, UW).
3. *Tallmadge Brothers,* Norwalk, CT 9/11 - Bridgeport, CT, (2) interviews, site tour, and b-roll. 9/12 - Port Norris, NJ, (1) interview, b-roll (Peterson Packing, Bivalve). 9/20 - Norwalk, CT, b-roll of boats and docks, aborted boat ride.

Work planned

Schedule (proposed) field production (site nos. 4-7):

4. *Federal Hill Trout Farm*, West Virginia (10/25-10/26).
5. *Wellfleet Oyster & Clam, Co*., Wellfleet, MA (10/31-11/1).
6. *NRAC*, North Dartmouth, MA (11/7) interview Dr. Kim Harrison.
7. *Salmon Farm* (site TBD), Eastport, ME (12/2-12/4 proposed dates).

Off-line editing schedule

- 10/18/96 - 01/16/97
- Create select reels from raw footage, develop script drafts, music, graphics, narration, feedback from NRAC video review committee, design VHS sleeve, continue editing, and complete rough cuts for review.

On-line editing schedule

- 01/24/97 - 02/20/97
- Printing of sleeve design, record final voice over (narration) and music, complete final edit with all elements, dub VHS and Beta SP tapes, print press release (through NRAC), and distribute tapes and "press kit" to designated sources.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1995-96	$33,685						$33,685
Total	**$33,685**						**$33,685**

Proposal for the Study of QPX-Like Disease in *Mercenaria mercenaria* on the Coast of Massachusetts (96-10)

Progress report period February 15, 1996 to August 31, 1996

NRAC funding

$10,000

Project coordinator

Roxanna Smolowitz, Laboratory for Aquatic Animal Medicine and Pathology, University of Pennsylvania, Pennsylvania

Participating investigators/ cooperative agencies

Dale Leavitt, DFL Resources, Massachusetts
Frank Perkins, North Carolina State University, North Carolina
Robert Bullis, LAAMP, University of Pennsylvania, Massachusetts

Objectives

- Evaluate the occurrence and spread of the disease in the new seed just planted in the Provincetown and Duxbury flats twice during the year to follow the pathogenesis of the disease using condition index and gross and histopathologic examination of the tissues.
- Histopathologically examine seed from four hatcheries for QPX. These hatcheries have previously supplied seed to the Provincetown and Duxbury clam flats.
- Using condition index and gross and histopathologic methods, examine clam samples from two other culturists, on or just off the Cape, who have had or may be having problems.
- Utilizing light and electron microscopy classify the QPX-like organism by examining both cultured organisms and those taken from clam tissues.

Anticipated benefits

1. A beginning in the understanding of the biology of QPX in the clam flats by examining the pathogenesis of the disease in the clam flats (i.e., time, course, and severity of infection correlated with animal age and season/time post planting).
2. A determination of whether QPX is routinely present in seed purchased from different sources and may therefore be routinely imported to the plots in diseased seed clams.
3. An examination of clams from other areas around Cape Cod that have shown a high incidence of morbidity and mortality, poor growth to determine the occurrence, or extent of QPX as a possible cause.
4. Establishment of cultures of QPX which can then be used to further study its biology and its infectivity for clams.
5. Establishment of the correct classification of the organism for comparison to other similar organisms and to further understanding of the QPX's biology.

Principal accomplishments

1. Evaluate the occurrence and spread of the disease in the new seed just planted in the Provincetown and Duxbury flats.

Two groups of hard clams planted in Dee Osinski's lease in early (from Biosphere) and late [Aquaculture Research Corporation, (ARC)] summer of 1995 are being examined over the course of the grant. Originally we were going to collect animals only twice or so during the year, but have expanded the examination to four times per year for each group of animals in order to better follow seasonal effects on the occurrence and severity of the disease. Samples of animals were collected in January and June, and will be collected again in late October/early November 1996. Animals from both groups were negative for QPX in January. Animals from 1993 and 1994 plantings of hard clams (ARC) in William Bennett's lease in Duxbury were collected in July 1996 and both samples were positive for QPX spores in the mantles. Another sample from these groups will be collected again in November 1996. Evidence to date points to late development of disease in planted animals.

2. Histopathologically examine seed from four hatcheries for QPX.

Samples of clams have been collected from four different hatcheries. All four hatcheries have supplied seed to the Provincetown lease holders and two of the four (ARC and Bay Farms) have supplied seed to the Duxbury lease holder. Condition index will be done on one set of samples from each hatchery.

A. ARC provided 150, 5-6 mm shell height hard clams in June 1996 and were histologically negative for QPX. Fifty field plantable (17-20 mm shell height) hard clams were collected in September 1996 and remain to be examined histologically.
B. Biosphere supplied approximately 50 hard clams of shell height of approximately 1.5 cm on June 13, 1996. They also supplied another 150 hard clams of shell height of 9 mm which were negative for QPX.
C. Bay Farms supplied approximately 200 animals on August 28, 1996. They have not yet been histologically examined.
D. Cape Cod Oyster Co. (originating from Mooks Sea Farm) supplied 200, 3 to 5 mm shell height hard clams on June 21, 1996. They will provide an additional 100 field plantable animals in the next few week. Both samples will be examined histologically at a later time.

3. Using condition index and gross and histopathologic methods, examine clam samples from two other culturists, on or just off the Cape, who have had or may be having problems.

A. On April 11, 1996, a sample of hard clams, that had originate from Bay Farms in 1994, was collected from the lease of Mr. Mark Zivan in Pleasant Bay, Orleans, Cape Cod. He had experienced a sudden, severe, marked mortality of the hard clams on his lease in April. Gross and microscopic examination of the clam tissues did not show QPX in any animal. Nonspecific changes possibly consistent with fresh water upwelling (or some other environmental perturbation) were noted.
B. On August 8, 1996, two samples of clams were collected by Chris Clark from two plots forming part of the Barnstable Shellfish Project. In early July, the group had planted seed clams from three different hatcheries (ARC, Cape Cod Oyster, and Bay Farm) in several designated plots. By the first of August, significant mortality (approximately 20%) was noted in the plots planted with Bay Farm animals. Animals from two of the plots containing Bay Farm animals were examined. Additionally, a sample of Bay Farm hard clams that had been preserved upon arrival in July were examined. QPX was not found in any of the clams examined (approximately 100 animals). However, marked Idiopathic Gill Disease (cause unknown) was common in the affected clams.
C. Clams from a suspicious location (to be chosen by Mike Hickey, Division of Marine Fisheries, MA) will be examined both histologically and with condition index measurements in the following months.
D. Clams from Mitchell River, Chatham, Cape Cod (a location were QPX was identified in moribund clams in 1992) will be examined both histologically and with condition index measurements in the following months.

4. Utilizing light and electron microscopy classify the QPX-like organism by examining both cultured organisms and those taken from clam tissues.

The LAAMP microbiologist, Dr. Robert Bullis, is leading the culture work. We are now able to easily isolate the QPX organism from mantle tissue of the clams. But while QPX organisms can exist in saltwater mediums (Sterile Artificial Seawater or Vischniac's Media), they do not show sustained growth after about 2 days. So, we began using L15 media supplemented with lipids (Kleinschuster and Swink, 1993, The Nautilius 107:76). We have had good results in increasing sustained growth with this media. However, we still consistently face bacterial contamination in the cultures. Recently, Dr. Norman Wainwright, a microbiologist and a senior scientist at the MBL, became interested in the culturing attempt and donated several antibiotics, as well as technical expertise, to the project. We anticipate being able to repeatable culture QPX in pure uncontaminated cultures in a few months.

Light microscopic descriptions of the organism in the tissues have been made and will be published as part of a journal article (to be submitted to J.I.P.). Light microscopic descriptions of the organism in culture are being made by Drs. Bullis and Smolowitz. The appearance of QPX in culture mirrors its appearance in the tissue with the production of thalli surrounded by ectoplasmic net that mature into sporangia, containing approximately 40 spores. The sporangia then lysis releasing the spores as new thalli which then repeat the cycle. Dr. Frank Perkins has examined QPX in tissues from infected clams. He has identified features that place the organism in the Phylum Labyrinthomorpha but not in either the Thraustochytrid or Labyrinthulids. Once we supply him with cultured QPX, he will continue his ultrastructural identification and description of the parasite.

Work planned

We intend to finish collecting those samples (as described above) in 2 months. Histologically, examination and condition index determination will continue on the samples until completed. Once sufficient amount of cultured QPX is obtained, Dr. Perkins will examine the organism ultrastructurally.

Impacts

The primary benefits of this work are:

- the determination of whether seed is as a routine carrier of this disease,
- a preliminary examination and description of the pathogenesis of the disease in clams from infected leases, and
- investigation of other possible QPX infections in hard clams in the Cape Cod area.

The ability to culture the QPX organism will lead to classification of the organism, as well as infectivity studies in clams, and to the development of fast, accurate, and sensitive diagnostic tests.

Publications and presentations

Results of this work will be presented at the National Shellfisheries Association meeting in 1997 and will be made available for distribution on NRAC's home page.

Support

Years	NRAC USDA funding	Other support					Total Support
		University	Industry	Other Federal	Other	Total	
1995							
1996-97	$10,000		$150		$150	$300	$10,300
Total	**$10,000**		**$150**		**$150**	**$300**	**$10,300**

Northeastern Regional Aquaculture Center Publications

1. Publications (in Print)

(Listed in Order of New Publication Codes - rev. 8/97)

Old Code	New Code	
NRAC #100	NRAC 90-001	Pen-Reared Salmonid Industry in the Northeastern United States (available upon request from Dr. James Anderson, 401-874-4568) (203p.)
NRAC #101	NRAC 91-001	Is Aquatic Farming For You? (2p.)
NRAC #111	NRAC 91-002	General Fish Health Management (12p.)
NRAC #102	NRAC 92-001	Initial Questions the County Agent Can Ask the Prospective Fish Culturist (4p.)
NRAC #120	NRAC 92-002	Aquaculture Systems for the Northeast (4p.)
NRAC #130	NRAC 92-003	Aquaculture Species for the Northeast (4p.)

NRAC #140	NRAC 92-004	Processing and Marketing Aquacultured Fish (4p.)
NRAC #150	NRAC 92-005	Business Planning For Aquaculture - Is It Feasible? (12p.)
none	NRAC 92-006	The Northern Quahog: The Biology of *Mercenaria mercenaria* (53p.)
none	NRAC 92-007	Improving the Legal Framework for Marine Aquaculture: The Role of Water Quality Laws & the Public Trust Doctrine (60p.)

NRAC #113	NRAC 93-001	Fish Health Inspections: What are they? (2p.)
NRAC #160	NRAC 93-002	Growing Microalgae to Feed Bivalve Larvae (8p.)
NRAC #170	NRAC 93-003	An Introduction to Water Chemistry in Freshwater Aquaculture (4p.)
NRAC #180	NRAC 93-004	Major Predators of Cultured Shellfish (6p.)
NRAC #200	NRAC 93-005	History & Impact of MSX & Dermo Diseases on Oyster Stocks in the Northeast Region (8p.)
NRAC #210	NRAC 93-006	Genetic Improvement of the Eastern Oyster for Growth & Disease Resistance in the Northeast (8p.)
NRAC #112	NRAC 93-007	A Manual for Nonlethal Surgical Procedures to obtain Tissue Samples for use in Fish Health Inspection (28p.)
NRAC #221	NRAC 93-008	Fish Counting in Situ-A Technology Assessment (59p.)
	NRAC 93-009	Aquaculture and the Marine Environment-The Shaping of Public Policy (30p.)

NRAC #222	NRAC 94-001	Evaluation of Artificial Diets for Cultured Fish (4p.)
none	NRAC 94-002	Product Attributes Affecting Cultured Hard Clam Purchase Decisions (8p.)
none	NRAC 94-003	Northeast Region Aquaculture Industry-Situation and Outlook Report-November 1993 (71p.)
none	NRAC 94-004	Consumer Preferences for Northeastern Aquaculture Products: Report of the Results from a Survey of Northeastern and Mid-Atlantic Consumers (54p.)
none	NRAC 94-005	Governmental Regulations of Growth and Development: Improving the Legal Framework For Aquaculture in the Northeastern United States (489p.)

NRAC #220	NRAC 95-001	Producing Oyster Seed by Remote Setting (11p.)
NRAC #300	NRAC 95-002	State Policies for Aquaculture Effluents and Solid Wastes in the Northeast Region (24p.)

NRAC #223	NRAC 96-001	Northeast Region Aquaculture Industry-Situation and Outlook Report 1994-1995 June 1996 (70p.)
NRAC #114	NRAC 96-002	Northeastern Aquatic Animal Health Directory: A Directory of Aquatic Animal Health Laboratory Services, Information Resource Contacts, and Related State Regulations for the Private Sector Aquatic Farm Industry in the Northeastern Region of the United States of America (53p.)

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none	NRAC 97-001	Bird Predation and Its Control at Aquaculture Facilities in the Northeastern United States (17p.)

2. Videos

Code	Year	Title	Running Time (Min.)
NRAC V-1	(1990)	Cage Culture: Raising Fish in Ponds	24:38
NRAC V-2	(1991)	Farming Fish in Open Ponds	24:06

NRAC V-3	(1992)	Producing Striped Bass in Hatcheries	25:00
NRAC V-4	(1993)	INAD Workshop - Boston, MA, March 18, 1993	282:00
NRAC V-5	(1994)	Biosecurity in Aquaculture: Practical Steps for Healthy Fish	15:38
NRAC V-6	(1997)	Northeast Aquaculture	30:00

3. NRAC Citations (Publications & Videos) -rev. 8/97

Alphabetical Listing of Citations by Author

(Fact Sheets, Bulletins, Reports, Manuals & Directories, and Videos)

Allen, S. K., P. M. Gaffney, and J. W. Ewart. 1993. Genetic improvement of the eastern oyster for growth and disease resistance in the Northeast. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 93-006 (Formerly NRAC #210). North Dartmouth, MA. 8pp.

Baptist, G., D. W. Meritt, and D. W. Webster. 1993. Growing microalgae to feed bivalve larvae. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 93-002 (Formerly NRAC Fact Sheet #160). North Dartmouth, MA. 8pp.

Batterson, T. R. 1993. INAD workshop. Northeastern Regional Aquaculture Center/USDA Video #V-4. North Dartmouth, MA. 282:00 min.

Bengtson, D. A. and M. A. Rice. 1994. Evaluation of artificial diets for culture fish. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 94-001 (Formerly NRAC Fact Sheet #222). North Dartmouth, MA. 4pp.

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Bohn, R. E., D. W. Webster, and D. W. Meritt. 1996. Producing oyster seed by remote setting. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 95-001 (Formerly NRAC Publication #220). North Dartmouth, MA. 11pp.

Bowser, P. R. 1993. Fish health inspections: What are they? Northeastern Regional Aquaculture Center/USDA Publication #NRAC 93-001 (Formerly NRAC Bulletin #113). North Dartmouth, MA. 2pp.

Bowser, P. R. and J. K. Buttner. 1991. General fish health management. Northeastern Regional Aquaculture Center/ USDA Publication #NRAC 91-002 (Formerly NRAC Bulletin #111). North Dartmouth, MA. 12pp.

Bush, M. J. and J. L. Anderson. 1993. Northeast Region Aquaculture Industry Situation and Outlook Report (November 1993). Northeastern Regional Aquaculture Center/USDA Publication #NRAC 94-003. North Dartmouth, MA. 71Pp.

Buttner, J. K. and G. Flimlin. 1991. Is aquatic farming for you? Northeastern Regional Aquaculture Center/USDA Publication #NRAC 91-001 (Formerly NRAC Factsheet #101). North Dartmouth, MA. 2Pp.

Buttner, J. K., G. Flimlin, and D. W. Webster. 1992a. Aquaculture systems for the Northeast. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 92-002 (Formerly NRAC Fact Sheet #120). North Dartmouth, MA. 4pp.

Buttner, J. K., G. Flimlin, and D. W. Webster. 1992b. Aquaculture species for the Northeast. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 92-003 (Formerly NRAC Fact Sheet #130). North Dartmouth, MA. 4pp.

Buttner, J. K., R. W. Soderberg, and D. E. Terlizzi. 1993. An introduction to water chemistry in freshwater aquaculture. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 93-003 (Formerly NRAC Fact Sheet #170). North Dartmouth, MA. 4pp.

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Ewart, J. W. and S. E. Ford. 1991. History and impact of MSX and Dermo Disease on oyster stocks in the Northeast Region. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 93-005 (Formerly NRAC #200). North Dartmouth, MA. 8pp.

Ewart, J. W., J. Hankins, and D. Bullock. 1995. State policies for aquaculture effluents and solid wastes in the Northeast Region. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 95-002 (Formerly NRAC Bulletin #300). North Dartmouth, MA. 24pp.

Flimlin, G. 1992. Initial questions the county agent can ask the prospective fish culturist. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 92-001 (Formerly NRAC Publication #102). North Dartmouth, MA. 4pp.

Flimlin, G. and B. F. Beal. 1993. Major predators of cultured shellfish. Northeastern Regional Aquaculture Center/ USDA Publication #NRAC 93-004 (Formerly NRAC Fact Sheet #180). North Dartmouth, MA. 6pp.

Glahn, J. 1997. Bird predation and its control at aquaculture facilities in the Northeastern United States. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 97-001 [APHIS 11-55-009]. North Dartmouth, MA. 17pp.

Halvorson, H. O. 1993. Aquaculture and the marine environment: The shaping of public policy. The Northeastern Regional Aquaculture Center/USDA Publication #NRAC 93-009. North Dartmouth, MA. 30pp.

Henderson, N. R. 1994. Product attributes affecting cultured hard clam purchase decisions. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 94-002 (also New Jersey Sea Grant-94-290 and Rutgers Cooperative Extension E-178. North Dartmouth, MA. 8pp.

Huguenin, J. E. 1993. Fish counting and measurement *in situ*: A technology assessment. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 93-008 (Formerly NRAC #221). North Dartmouth, MA. 59pp.

Leffler, M., M. Nelson, and R. M. Harrell. 1990. Cage culture: raising fish in ponds. Northeastern Regional Aquaculture Center/USDA Video #NRAC V-1 [Produced in Cooperation with University of Maryland System Cooperative Extension Service Sea Grant Extension Program, and Maryland Sea Grant College]. North Dartmouth, MA. 24:38 min.

Leffler, M., M. Nelson, and R. M. Harrell. 1991. Farming fish in open ponds. Northeastern Regional Aquaculture Center/USDA Video #NRAC V-2 [Produced in Cooperation with University of Maryland System Cooperative Extension Service Sea Grant Extension Program, and Maryland Sea Grant College]. North Dartmouth, MA. 24:06 min.

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Rice, M. A. 1992. The Northern Quahog: The biology of *Mercenaria mercenaria*. Jaworski. C. and M. Schwartz, eds. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 92-006 (Published cooperatively with Rhode Island Sea Grant, and University of Rhode Island Cooperative Extension). North Dartmouth, MA. 60pp.

Spatz, M. J., J. L. Anderson, and S. Jancart. 1996. Northeast Region Aquaculture Industry Situation and Outlook Report (1994-1995), Vol. 2. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 96-001 (Formerly NRAC #223). North Dartmouth, MA, [Rhode Island Agricultural Experiment Station Publication #33352, Kingston, RI]. 70pp.

Strombom, D. B. and S. M. Tweed. 1992. Business planning for aquaculture - Is it feasible? Northeastern Regional Aquaculture Center/USDA Publication #NRAC 92-005 (Formerly NRAC Fact Sheet #150). North Dartmouth, MA. 12pp.

Wessells, C. R., S. F. Morse, A. Manalo, and C. M. Gempesaw II. 1995. Consumer preferences for Northeastern aquaculture products: Report on the results of a survey of Northeastern and Mid-Atlantic consumers. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 94-004. North Dartmouth, MA, and Rhode Island Experiment Station Publication #3100, University of Rhode Island, RI. 54pp.

Wooster, G. A., H. M. Hsu, and P. R. Bowser. 1993. A manual for non-lethal surgical procedures to obtain tissue samples for use in fish health inspections. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 93-007 (Formerly NRAC Bulletin #112). North Dartmouth, MA. 28pp.

Wypyszinski, A. W., M. A. Altobello, B. E. Lindsay, J. Falk, T. Eichenberg, and K. Riaf. 1994. Governmental regulation of growth and development: Improving the legal framework for aquaculture in the Northeastern United States. Northeastern Regional Aquaculture Center/USDA Publication #NRAC 94-005. North Dartmouth, MA. 489pp.

Southern Regional Aquaculture Center Compendium Report *for the Period May 1, 1989 to August 31, 1996*

Table of Contents

Analysis Of Regional And National Markets For Aquacultural Products Produced For Food In The Southern Region

Termination Report for the Period April 1, 1988 to June 30, 1990

Funding level
$346,038

Participants
C. Engle, U. Hatch, and H. Kinnucan, Auburn University
R. Pomeroy, Clemson University
L. Dellenbarger, Louisiana State University
J. Dillard, Mississippi State University
O. Capps, Texas A&M University

Administrative advisor
T. J. Helms, Assistant Director
Mississippi Agricultural and Forestry Experiment Station, Mississippi State, Mississippi

Project objectives
1. To obtain and analyze comprehensive market information from consumers, retail grocery stores, and restaurants.
2. To assess the effectiveness of advertising and promotion of farm-raised catfish.
3. To develop an overall assessment of potential for producing and marketing catfish and crawfish in the Southern Region.

Principal accomplishments
Objective 1

The survey of consumers revealed that catfish is not a product consumed primarily by low income, poorly educated persons living primarily in the deep South. Catfish is consumed in significant quantities by persons of all income and education levels, nearly all race and ethnic backgrounds, and in all major regions of the U.S., although a majority of consumption is still in the traditional consuming area. Changes in attitudes and perceptions of farm-raised catfish were evident from the survey. Differences in consumer ratings of catfish across regions are present, but not as large as anticipated. Although industry advertising and promotional programs are relatively new, they obviously have had an impact and should be continued at the highest level feasible. Many consumers outside the traditional catfish consuming region perceive that catfish are not readily available. Catfish received relatively low ratings on appearance and packaging, odor, and having few bones. These misconceptions should be addressed in future advertising and educational programs. Attributes of catfish that received relatively high ratings were nutritional value, flavor, and ease of preparation. Catfish were not perceived as being over-priced relative to other fish and meat. Marketers should take advantage of these favorable attributes.

Attempts to identify a profile for catfish consumers met with only limited success, mainly because catfish are being consumed in varying quantities by consumers in nearly all demographic categories studied. The majority of catfish consumers live in the four central regions of the U.S. Among occupational categories, households having a head classed as professional contained the largest number of catfish consumers. The household income category having the largest number of catfish consumers was $20,000 to $30,000. A majority of catfish consumers were white Protestants living in households containing 2-3 persons. A higher percentage of catfish consumers were in the 20-39 year-old age groups. A majority of catfish consumers had education levels of high school graduate, or above.

The national survey of grocery stores consisted of a random sample drawn from a population of 143,673 stores nationwide (did not include convenience stores). The survey revealed 45% of stores nationwide offered some form of catfish — ranging from a low of 27% in New England to a high of 59% in the West South Central division of the U.S. Twenty-one percent of store managers interviewed who did not sell catfish stated they were likely to add it in the next year. Given the number of stores nationwide, this suggests much potential for market expansion.

Generally, store characteristics associated with an increased likelihood of selling catfish included:

(1) members of a chain; (2) having a specialized fish market section; and (3) sales of more than $100,000 per month. Eighteen percent of the store managers reported that the national advertising campaign for catfish influenced their decision to add catfish to their product line. Regional impact of the national advertising campaign on catfish product adoption was greatest in the South Atlantic and Mountain regions. Stores in the Pacific and South Atlantic Regions reported the largest rate of catfish product adoption for the 2-year period prior to the time of the survey (a period overlapping The Catfish Institute's generic advertising campaign). Selected variables from the grocery store survey were included in a logit model that produced probabilities of stores adding catfish. Ranking of regional markets was quantified by a market potential index that incorporated the estimated logit probabilities, regional population and the percentage of stores not selling catfish. The top three prospects in terms of new market development, in decreasing order of potential, were found to be the South Atlantic, East North Central, and Pacific Regions.

Data from the national restaurant survey were used to evaluate market potential for the expanded use of aquaculture products with specific emphasis on developing market information regarding the restaurant use of catfish. Nationwide, 29% of restaurants reported that catfish was included on their menu. Of restaurants not serving catfish, 39% stated that adding catfish would not be difficult, while 19% stated they would consider adding catfish to their menu within the next year (1989). Restaurant managers in the two South Central Regions, the South Atlantic Region and the Pacific Region, expressed the greatest interest in adding catfish to their menus. Outside these regions, unfamiliarity with catfish seemed to be the most important constraint to adding the product in restaurants. The random sample of 1800 restaurants was drawn from a national population of 321,667 full-service restaurants. Assuming the random sample was representative, there were over 40,000 restaurants considering adding catfish to their menu. Regions outside the South which promise the greatest return to catfish market promotion and development expenditures include: New England, Middle Atlantic, East North Central, and Pacific. Restaurants which characterize their cuisine type as seafood, combination, and steak hold the greatest promise for market expansion. Other restaurant characteristics such as location, seating capacity, or type of ownership were not statistically significant to be used as a basis for recommendation. Research at Texas A&M utilized scanner data made available by a retail food firm (43 supermarkets) in Houston to: (1) evaluate marketable product forms of catfish and crawfish and (2) to estimate retail demand relationships for catfish and crawfish. Data were analyzed in econometric models emphasizing price and advertising elasticities of both fresh and convenience catfish and crawfish products. Price elasticities (percent change in purchases due to unit change in prices) for convenience catfish ranged from -5.5 to -12.8, and from -1.3 to -6.5 for fresh catfish products. The price elasticity of fresh crawfish was -3.3. Cross-price and advertising elasticities were also estimated. The authors warn against generalizing results from this one local market to regional or national levels. This study constitutes a pilot test of use of scanner data to investigate demand for catfish and crawfish products for a local market. The methodology needs to be replicated in other geographic areas.

Objective 2

Analysis of survey data strongly suggests that advertising and promotion have significantly contributed to the growth in sales of catfish, for both at-home (grocery store sales) and away-from-home (restaurant) consumption. Nationwide, approximately 37% of consumers who had eaten catfish had seen or heard some form of advertising of catfish. Several econometric models were designed to isolate the effect of generic advertising. The first model, which contained three equations, showed only a weak statistical significance of advertising, probably the result of the newness of the generic advertising program. A second eight equation model was estimated in an attempt to describe a hierarchy of effects of advertising. Not surprisingly, the results show the nascent advertising program exerting its influence through heightened consumers' awareness and improved perceptions of catfish. A third model, which included generic advertising expenditures as a variable, was used to project wholesale demand for catfish to 1995. This model projected sales in 1995 would be 60 million pounds lower without the assumed annual 1 million dollars of generic advertising. The demand analysis of scanner data from a local market (Houston, Texas) revealed a significant relationship between advertising and purchases of fresh catfish.

Objective 3
The Work Group generally agrees there is potential for steady growth in the market for both catfish and crawfish. From a purely physical standpoint, the potential for expanding production in the Southern Region is great. From an economic standpoint, production will ultimately be limited by both institutional (e.g., environmental, water use regulations), and market (demand) constraints.

There were no findings from this research to suggest the market for farm-raised catfish is nearing saturation, even in traditional consuming areas. If the current trend toward more fish and seafood consumption continues, there is sufficient evidence to suggest catfish can gain a larger market share of the total food budget, provided industry continues to advertise and promote its product. More research is needed to aid catfish marketers in identifying specific market niches where advertising and promotion will be the most cost-effective.

Impacts

There has been tremendous interest in the results of this research. The supply (2000 copies) of the first bulletin printed was exhausted within 6 months. One catfish processor alone requested 100 copies for use by salesmen and brokers. A long mailing list has been developed from requests for bulletins now in the publication process. The survey results, and other research contained in these bulletins, should be of much interest to marketers of farm-raised catfish and crawfish, as well as to their advertising agents.

The national surveys of households, grocery stores, and restaurants provide, for the first time, a national database on catfish and crawfish consumption by major regions of the U.S. Summaries and analyses of the survey data should be of much interest to catfish processors, marketers, industry organizations, and public institutions that have interest in the continued growth and development of this industry. Results indicate much potential for further expansion of the market, particularly in the South Atlantic, East North Central, and Pacific Regions. While the research conducted under this project identified broad areas of market potential, more detailed surveys of market segments will be needed to help identify specific catfish market niches.

The impact of industry advertising and promotion was studied and found to be significant. If the industry is to continue expanding its markets, effective advertising and promotion will be required.

A pilot study utilizing scanner data from supermarkets located in Houston, Texas, produced an own-price demand elasticity for catfish products that is highly elastic. An elastic demand suggests that lowering the retail price would result in greater total revenue for the industry. Thus, efforts to further improve efficiency, particularly in marketing, should continue so that retail prices are no higher than necessary to maintain growth of the industry. This research was limited to one market, so results may not lead to drawing broad nationwide or regional inferences. The methodology used in this research should be replicated in other geographic regions.

Publications

Journal articles

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Kinnucan, H. and R. Nelson. 1990. Market Segmentation Research for Food Products: The Case of Catfish. Southern Journal of Agricultural Economics 45:20-24.

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Dellenbarger, L. E., J. Dillard, and A. R. Schupp. 1989. Socio-economic Factors Associated with Catfish Consumption in the United States. Southern Journal of Agricultural Economics, July (Abstract).

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Zidack, W. 1989. A Dynamic Monthly Econometric Model of the U.S. Processed Catfish Industry.

Presented paper to the American Agricultural Economics Association Annual Meeting, Baton Rouge, LA, July.

Theses and dissertations

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Israel, Danilo Cano. 1990. Total, At-Home, and Away-From-Home Catfish Consumption in the United States: A Dichotomous and Ordered Logit-Probit Analysis. M.S. Thesis. Clemson University.

Preparation Of Southern Regional Aquaculture Publications

Termination Report for the Period March 24, 1988 to June 30, 1990

Funding level

$150,000

Participants

J. Jensen, Auburn University
L. Gray, University of Arkansas
C. Cichra, T. Wellborn, University of Florida
G. Lewis, R. Gilbert, University of Georgia
M. Masser, Kentucky State University
L. de la Bretonne, G. Jensen, Louisiana State University
J. McGilberry, Mississippi State University
J. Hinshaw, R. Hodson, North Carolina State University
A. McGinty, University of Puerto Rico
T. Schwedler, Clemson University
J. T. Lock, B. Higginbotham, G. Chamberlain, R. Miget, J. T. Davis, Texas A&M University
J. Rakocy, University of the Virgin Islands

Administrative advisor

Milo J. Shult, Associate Director
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Project objectives

1. Prepare a series of reference manuals on priority issues in Southern aquaculture for use and distribution by Cooperative Extension Services and other information-purveying agencies throughout the Southern Region.
2. Prepare video productions to demonstrate succinct points in the production of aquacultural products grown in the Southern Region.
3. Catalog all of the computer software available on aquaculture production in the Southern Region and evaluate the possibilities of developing a common format.
4. Perform all management services necessary to develop and produce the extension materials developed in objectives 1-3.

Principal accomplishments

The preparation of regional aquaculture publications is a direct result of Regional Aquaculture Center legislation. In the Southern Region less than 1/2 the states had fact sheets covering the major species in their state. By pooling regional expertise, over 50 fact sheets were made available covering most aspects of culture of the major species in the region.

The use of videos seems destined to become the major educational medium for the coming decade. With this in mind, videos on production of individual species and information common to all species were prepared. Again this was a regional effort which brought together the best expertise available to produce and direct these educational products. The 11 videos produced as part of this project constitute over half of the aquaculture production videos available at the time the project was terminated. The videos are being widely distributed and are being put to good use in homes as well as the high school and college classroom. Producers as well as the general public can begin to understand the many technical aspects of being involved with aquaculture.

Impacts

Providing the best information from which to make decisions is one facet of Extension responsibility. The materials made available through this project will assist producers and potential producers to make informed decisions. The economic value of this is difficult to measure but is estimated to exceed $1,000,000 annually in cost savings in just the state of Texas. The other 14 regional states and territories should realize similar savings. Another facet served by educational publications is to make the general public aware of the immensity and complexity of the aquaculture industry. No method has been devised to measure the value of an informed public making rational, intelligent decisions affecting land use planning, water allocations, and food safety, but most professionals consider it even more important to the future of the industry than information to the target audience.

Publications

A complete list of all SRAC publications, videos, and computer software is presented in the Appendix at the end of the project summaries for the Southern Regional Aquaculture Center.

Performance Of Aeration Systems For Channel Catfish, Crawfish, And Rainbow Trout Production

Termination Report for the Period March 1, 1988 to September 30, 1990

Funding level

$124,990

Participants

C. E. Boyd, Auburn University
F. E. Baker, J. D. Bankston, T. B. Lawson, R. P. Romaire, Louisiana State University
C. S. Tucker, Mississippi State University
J. M. Hinshaw, North Carolina State University
J. T. Davis, Texas A&M University

Administrative advisor

David H. Teem, Associate Director
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Project objectives

1. Evaluate the effects of paddlewheel aerators on oxygenation, water circulation, crawfish growth and maturity, and production in ponds. Demonstration of results in commercial crayfish ponds will be provided as an extension effort.
2a. Design, fabricate, and evaluate an aerator with the option of introducing pure oxygen for emergency aeration.
2b. Design, fabricate, and evaluate a device for circulating water in channel catfish ponds.
3. Evaluate the effects if oxygen enrichment by packed columns and pure oxygen in production experiments with rainbow trout; pure oxygen systems will also be compared with mechanical surface aerators.

Principal accomplishments

Objective 1

Paddlewheel aerators were evaluated in commercial crawfish ponds and in experimental crawfish ponds on the Louisiana State University Ben Hur Research Farm in Baton Rouge, Louisiana. Dye studies confirmed several theories: (1) water tended to flow through areas of least resistance (i.e., areas of less dense vegetation) and tended to flow more rapidly through deeper water than through shallow water (hugging deep water areas around levees) and (2) internal baffle levees were beneficial for better distribution of water and dissolved oxygen throughout ponds when circulating water with paddlewheels; however, tops of baffle levees must extend above water surface to prevent the water from overtopping the levees and short-circuiting.

Capacity of paddlewheels to move water varies with size, vegetative biomass, and positioning in the pond. Baffling is required around paddlewheel aerators to limit “backflow.” Flow capacity of the 10-hp units ranged from 2,284 to 5,145 gpm. The kw demand on these units was 6.5 and operating cost was $0.46/hr. It required 3-4 days to recirculate water throughout these large pond systems. In 5-acre ponds at LSU, two, 3-hp paddle wheel aerators produced a flow of about 2,500 gpm, recirculating the entire pond in 26 hours.

Early morning dissolved oxygen in ponds at LSU with paddlewheels was significantly higher than unaerated ponds. The spatial distribution of dissolved oxygen and temperature was more uniform in aerated ponds than in unaerated ponds. The paddlewheel aeration provided a definite advantage where dissolved oxygen was concerned. Flushing of non-aerated ponds was not as effective in maintaining satisfactory water quality in comparison to paddlewheel aerators.

Crawfish producers in Louisiana have installed recirculation systems with paddlewheel aerators. Two of these systems have been studied to assess the water volume flow and the flow patterns within the ponds. Adjustments in the way the paddlewheel devices are mounted within the internal levees have shown significant results related to water flow volume. By placing structures that restrict the return opening to the width of the paddles, the volume of water circulated more than doubled compared to tests conducted the first year. As an example, in a 22-acre, heavily-vegetated pond, a 5-hp paddlewheel aerator moved 6,150 gpm.

A pond was constructed as part of the Vocational Agriculture Program at Crowley High School. LSU provided the design and furnished a 3-hp aerator. The site is being used for teaching the principles and operation of this new crawfish pond system. Approximately 25 ponds have been constructed or modified to utilize the technology developed in this project. Production increases, product quality increases, and operating cost decreases have been reported. Some ponds report doubling gross dollar return per acre while others report only livability and size gains. Utility operating costs are reduced in all cases, some by as much as 75%.

In work conducted at Texas A&M University, over 1,100 people visited the demonstration project on a commercial crawfish installation to view the design and effectiveness of three treatments: (1) aerators versus flow-through water, (2) recirculated water and no added recirculation, and (3) aeration alone. Paddlewheel aerators, which also established recirculation patterns, were superior to other methods in this demonstration.

Horsepower requirements for aerators were less than that required for pumps to realize the same dissolved oxygen concentration. Catch rates between these two systems were not sufficiently different to recommend one over the other.

Paddlewheel aeration or recirculation using pumps were both superior to non-aerated or recirculated ponds. Fresh, flow-through water was equal to or better than all other methods, but the expense of water at $8.00 per acre-foot made this method uneconomical.

Based on the data developed from the four ponds in this study, 1.25 hp per acre of paddlewheel aeration was the most efficient and cost-effective method of aerating crawfish ponds both during the summer season and the winter season.

Objective 2a

A hooded paddlewheel aerator was designed and constructed to utilize pure oxygen. Initial tests of this design indicated a number of potential problems. After an attempt was made to correct these design flaws, a new series of trials were run. This second series of experiments again brought out problems that appear to be inherent in the application. We were still unable to fabricate a hood for the paddlewheel that did not collapse during operation. Also, when flow rates of oxygen were increased to levels calculated to be sufficient to provide the desired amount of oxygenation of water, large gas bubbles were lost in the effluent. Even when the effluent water was discharged at a depth of 3 feet, there still was a loss of large gas bubbles. It appears that pure oxygen supplementation of a paddlewheel aerator is not feasible.

Objective 2b

A water circulator was designed, fabricated, and tested. This 3-hp (2.2 kW) device consists of a large casing, fan-blade impellers, flow stabilizer surfaces, bearings, drive system, motor, and support frame. It was tested at several combinations of shaft speeds, fan-blade sizes, and fan-blade widths. The best results were obtained with a 6-inch wide fan blade of 24-inches in diameter. Results for this fan-blade width are summarized as follows:

Speed (rpm)	No. fan blades	Electric power consumed per hour (kw)	Discharge (gpm)
90	1	0.47	7,200
	2	0.58	7,400
	3	0.61	7,500
	4	0.67	8,000
120	1	1.40	9,500
	2	1.68	10,600
	3	1.79	11,000
	4	1.73	10,400
144	1	2.77	12,900
	2	3.40	12,400

One, 2.2-kw (3-hp) water circulator was placed in each of three ponds (1.6 ha; 4 acres) at the Delta Research and Extension Center, Stoneville, Mississippi, and three ponds served as non-circulated control ponds. A researcher from Auburn University made water movement measurements by the gypsum block or clod card technique. In this technique, the increase in the rate of dissolution of gypsum blocks in mechanically-circulated ponds over the rate of dissolution of gypsum blocks in non-circulated control ponds is taken as a measure of increased water movement. Blocks were placed at 48 positions in each of the ponds. Near the discharge of water circulators, gypsum blocks dissolved 3.5 times faster than in non-circulated ponds. At the furthest point from the water circulator, the dissolution rate was 1.2 times faster than in non-circulated ponds. Therefore, water circulators were effective in increasing water circulation. Energy input was not great — 0.75 hp per acre.

The effect of water circulation on channel catfish production was evaluated over 2 years in six, 1.6-ha, 1-m deep ponds at the Delta Research and Extension Center, Stoneville, Mississippi. Ponds were stocked with 15,750 channel catfish/ha in spring of 1991 and 1992, and fish were harvested in the fall and winter each year. Fish were fed to satiation once daily and supplemental aeration with 7.5-kw electric paddlewheel aerators was provided when dissolved oxygen concentration declined to between 2 and 3 mg/L. One, 2.2-kw water blender was installed in each of three randomly selected ponds. Blenders were fabricated with three, 5-bladed propellers operated at 90 rpm. Beginning in May, blenders were operated daily between 0900 and 1600. Three ponds without blenders served as controls. Relative to ponds without circulators, mid-day water temperatures and dissolved oxygen concentrations varied little with depth, demonstrating that circulators effectively mixed pond waters. Circulation did not reduce the incidence of critically low nighttime dissolved oxygen concentrations ($P \leq 0.05$), but did dramatically reduce (by a factor of 0.58; $P \leq 0.05$) the total hours of supplemental aeration needed to support fish production. Phytoplankton biomass, total and un-ionized ammonia-nitrogen, and nitrite-nitrogen concentrations did not differ between ponds ($P \leq 0.05$). Overall net fish production averaged 7,640 kg/ha in circulated ponds and 7,940 kg/ha in uncirculated ponds; the means did not differ ($P \leq 0.05$). Although the reduction in aerator use in circulated ponds was impressive, the power cost savings for aeration were largely offset by the cost of daily circulator operation.

Objective 3

Columns, which were built for preliminary trials, were made of rolled aluminum with perforated screen in-flow areas, and were filled with 1-inch Jaeger Tri-Pack media. Each column contained approximately 18 cubic feet of packing media. Typical flow rates through each column ranged from 250 to 500 gpm. In field trials, the oxygen content of the water was increased to approximately 145% of saturation with a calculated oxygen transfer efficiency averaging 62%. When tested on three production farms where the water was re-used serially, these devices quickly lost efficiency and capacity due to bacterial fouling of the packing media. A typical column could be operated no more than 14, and usually less than 7 days before the media would need to be removed and cleaned to remove bacteria and debris.

After consulting with industry cooperators, a decision was made to switch to pressurized packed columns for the addition of oxygen. At this time, one system has been constructed with a capacity to produce up to 350 gpm of water containing approximately 400 mg/L dissolved oxygen. It has recently been put into operation on a commercial farm in North Carolina. The theoretical efficiency of this type system is 100% for oxygen put into solution, however, the actual efficiency for this use will be somewhat less, due to bubble formation at the sites of discharge of the enriched water into the culture system. While testing the system, problems were encountered with lethargic feeding of fish due to elevated carbon dioxide levels in the lower tanks of the farm. The carbon dioxide levels reached a maximum of 19 mg/L and reduced the pH to 6.2. The problem was corrected by using perforated metal screens between tanks for more efficient gravity aeration, and by increasing water flow rate for greater dilution. Preliminary data indicate an increased production (per unit water flow) of approximately 170%, with an oxygen cost approaching $0.10 per pound of fish produced. Liquid oxygen costs during this portion of the test were $35 per 1,000 pounds, delivered.

The biological effects of supplemental oxygen were evaluated in a field study conducted on a commercial trout farm equipped with a pressurized packed column oxygen system. From September 14 - October 31, 1989, paired raceways at Jenning’s Trout Farm in North Carolina were stocked with commercial densities (75 kg/cubic meter) of rainbow trout with one of each pair of tanks receiving oxygen enriched water. Total weights of each group of fish were provided by the farm managers. Prior to the experiment, and at 2-week intervals during the test, tissue and blood samples were taken from the groups of fish for analyses of selected physiological parameters, including hematocrits, total hemoglobin, plasma cortisol, osmolality, glucose, and lactate levels. Samples of each group of fish were measured for length and weight prior to the oxygen enrichment and at the bi-weekly samples. Tissue samples were stored for future analyses of tissue energy levels.

The physiological parameters monitored in rainbow trout in tanks receiving supplemental oxygen (dissolved oxygen approximately 11.9 mg/L) did not differ from fish in tanks not receiving supplemental oxygen (dissolved oxygen approximately 9.4 mg/L). During the study, water temperatures declined from 12 to 8°C, and oxygen levels, even in non-supplemented tanks, were well above “stressful” levels for trout in culture systems. Fish exposed to supplemental oxygen exhibited a 18.5% increase in weight compared to the 24.5% increase in fish not receiving supplemental oxygen.

Impacts

Results of research conducted at Louisiana State University showed that paddlewheel aerators are effective in improving water quality in crawfish ponds by significantly reducing frequency and severity of critically low DO (less than 3 mg/L or 25% oxygen saturation), and by eliminating zones that either are low or devoid of oxygen and not suitable for optimal crawfish production. Paddlewheel aerators more effectively mix and circulate aerated water in crawfish ponds compared to the conventional water management practice of flushing ponds with fresh water.

Extension personnel in Louisiana have worked closely with farmers, and paddlewheel aerators have been installed in many crawfish ponds. The economic benefits have been estimated at $200 per acre. It is felt that water recirculating systems, with paddlewheel aerators, will become a standard management tool which will save water, reduce pumping costs, and improve profits.

Work done at Texas A&M University with water recirculation by paddlewheel aerators in crawfish ponds agrees with the LSU results. An estimated 75% of all farmers in the state are using either paddlewheels or pumps to recirculate water in their crawfish ponds. This is a marked change from the 25-30% who were using these methods in 1985. Because previous applied research studies have reported that harvests increase by 50 to 100% in recirculated ponds, individual farmers have benefited directly from this effort.

Research done at Auburn University demonstrated that a low-head high-discharge water circulator can be fabricated for a reasonable price (probably about $2,000 per unit), that the water circulator will significantly improve water circulation in catfish ponds, and that the power consumption of the aerator is less than that of paddlewheel aerators. Research with the water circulator at the Delta Research and Extension Center suggests that the water circulator will reduce the amount of paddlewheel aeration necessary in catfish ponds. At least two commercial aerator manufacturers now offer water circulators based on the design developed in this project.

Oxygen supplementation for rainbow trout in commercial trout raceways and in an experimental facility have not produced significant improvements in trout growth rates or food conversion. However, oxygen enrichment does allow increased carrying capacity on commercial trout farms, but with a slightly higher cost of production. Further research will be needed to ascertain if oxygen supplementation can improve profits in trout farming in North Carolina.

Publications

Journal articles

Boyd, C. E. and B. J. Watten. 1989. Aeration Systems in Aquaculture. CRC Critical Reviews in Aquatic Sciences 1:425-475.

Hinshaw, J. 1991. Validation of solid phase enzyme immunoassay technique for the measure of plasma cortisol in rainbow trout. Journal of Aquatic Animal Health.

Howerton, R. D. and C. E. Boyd. 1992. Measurement of water circulation in ponds with gypsum blocks. Aquacultural Engineering 11:141-155.

Howerton, R. D., C. E. Boyd, and B. J. Watten. 1993. Design and performance of a horizontal, axial-flow water circulator. Journal of Applied Aquaculture 3:163-183.

Tucker, C. S. and J. A. Steeby. 1995. Daytime mechanical water circulation of channel catfish ponds. Aquacultural Engineering 14:15-27.

Extension publications

Baker, F. E., D. J. Bankston, and T. Lawson. Undated. Recirculating crawfish ponds with paddlewheel aerators. Louisiana State University Extension Service, Louisiana State University Agricultural Center.

Abstracts and papers presented

Bankston, D. J., F. E. Baker, T. Lawson, and J. Roux. 1989. Demonstration of paddlewheel aerators in crawfish ponds. 1989 International Summer Meeting jointly sponsored by the American Society of Agricultural Engineers and the Canadian Society of Agricultural Engineering.

Tucker, C. S. 1994. Mechanical water circulation of aquaculture ponds. Proceedings of the 124th Annual Meeting of the American Fisheries Society.

Tucker, C. S. 1994. Using mechanical water circulation in catfish ponds. Annual Meeting of the Catfish Farmers of Arkansas, Hot Springs, Arkansas, February 1994.

Develop A Statistical Data Collection System For Farm-Raised Catfish And Other Aquaculture Products In The Southern Region

Termination Report for the Period June 1, 1989 to November 30, 1990

Level of funding

$13,771

Participants

J. E. Waldrop, Mississippi State University
L. de la Bretonne, K. J. Roberts, G. Jensen, Louisiana State University
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D. L. Gray,University of Arkansas
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G. W. Lewis, University of Georgia
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T. E. Schwedler, Clemson University
T. Wellborn, University of Florida
T. Hill, University of Tennessee

Administrative advisor

Verner G. Hurt, Director
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Mississippi State, Mississippi

Project objectives

1. Establish data needs by aquaculture enterprises by industry segment including: a) hatchery, b) fingerlings, c) growout, d) processing/marketing, and e) feed manufacture.
2. Identify sources of the data needs established in objective 1, above.
3. Identify appropriate collectors for each category including collection methods, data analysis, and form and timeliness of publication.

4. Establish a budget, including sources of funds, to accomplish the tasks identified in objectives 1-3, above.

Principal accomplishments

The Steering Committee for Data Collection Systems met at Delta Processors, Indianola, Mississippi, on August 4, 1989. Those present were Sam Hinote, J. E. Waldrop, Fred Tyner, Larry de la Bretonne, V. G. Hurt, and C. G. Shepherd.

A general discussion was held regarding the need to establish protocol, method of data collection, follow-up meetings, and identification of individuals recommended to assist with development of procedures to address objectives outlined in the Problem Statement developed earlier by this Steering Committee and subsequently approved by the SRAC Board of Directors.

Sam Hinote reviewed similar data they currently receive for catfish which includes a monthly processing report from NASS/USDA, Washington, D.C. Information is supplied by different processors and lacks consistency. Processors voluntarily submit information on a monthly basis, therefore, much information is lacking since not all processors participate. There was concern expressed about the degree of accuracy of information reported and whether there was any standardization of the data submitted.

There was a general feeling among the group that a national government agency should be designated to collect data, primarily because some sources would not be willing to report sales, distribution, and similar data, etc., to private or possibly regional agencies. The need for consistency of data, and for good definitions of the information requested by any survey, was repeatedly stressed.

Mr. Hinote also indicated that the Office of the Mississippi Commissioner of Agriculture has started collecting some processing data, but this too is presently somewhat limited.

It is very important that the charge be made to the agencies designated to collect aquaculture data to adequately identify the specifics of the data needed by different segments of industry.

This Steering Committee needs to identify and summarize the types of information now available, the frequency of reporting, and the types of information needed. This summary should be distributed to committee members and others who will attend the next meeting.

The reports previously prepared by the Mississippi Cooperative Extension Service need to be reinstated. This information was submitted three times a year and included estimates of the number and size of: (1) ponds in production; (2) ponds under construction; (3) ponds being renovated at each of the reporting intervals; and (4) an estimate of the number of acres involved in fingerlings and food fish. The group suggested it would be best to get this information from county agents and send it to one person in each state, perhaps the Extension Fisheries Specialist. Thus, information from the states could come from the grassroots level, be compiled by each state CES, and then possibly be coordinated by a central reporting agency. It was mentioned that Marty Brunson (MCES) is presently trying to reinstate this reporting system for Mississippi.

Sources now available:

1. Catfish Report—Mississippi only. Distributed by the Mississippi Agricultural Statistics Service (Dick Knight), Jackson, Mississippi.
2. Mississippi Weekly Processors' Report—distributed by Commissioner Jim Buck Ross' office.
3. Monthly Farm-Raised Processed Catfish Report—distributed by the Economics Research Service/ National Agriculture Statistics Service, Rockville, Maryland.
4. The report already mentioned which was previously distributed by Dr. Wellborn three times a year.
5. Aquaculture Outlook—Situation and Outlook Report—distributed in October and updated in March. These were prepared by the USDA/ Economic Research Service. It is uncertain as to whether this is a continuing effort.

Mr. Hinote identified the types of information needed by the industry at this time. These are:

1. Acreage and production statistics—what is happening in each state at the current time. These reports are needed in July and December of each year. They could be submitted to contact individuals in each state (Cooperative Extension Service) and

then possibly coordinated, compiled, and distributed on a regional level by an organization similar to Dick Knight's (NASS) and his counterparts in Mississippi. Information included in this category needs to be broken out by fingerlings, food fish, renovations, construction, and hatcheries. Recreational and fee fishing acreages also need to be identified.

2. Catfish feed report—feed manufacturing information could be correlated with the fish production and processing reports needed to serve the industry. It was felt that this may need to be developed by the state Departments of Agriculture for effective, reliable, and official reporting. Information is needed on the amount and types of feed being manufactured and distributed in the different states.
3. A state-by-state breakdown on the amount of fish processed and sold is needed. At the present time, there is no true supply and demand picture. This information is urgently needed by the industry. The National Marine Fisheries Report was discussed. Catfish as a commodity is not identified in this report.
4. It was suggested that the poultry database model could possibly be modified and used as guidelines to develop a format for aquaculture. There is certainly a need to get a 300 million pound commodity such as catfish properly identified in any aquaculture statistical report.

The group felt it would be appropriate for Drs. Waldrop and de la Bretonne to check with sources distributing the information previously discussed and get a current update on exactly what presently is available. They should contact the state Statistical Reporting Services, state Departments of Agriculture and Commerce, National Agriculture Statistical Service, and USDA/ERS. They should also contact key Extension representatives in major states producing finfish (catfish, etc.) and crustaceans (crawfish, etc.). A meeting was scheduled for October 10, 1989, in Jackson, Mississippi, to discuss how the current data are collected and to identify additional types of information needed and recommend procedures to collect these data.

It was agreed that this committee should develop a "model" data collection system for farm-raised catfish. Following model development, modifications, adaptations, and other improvements would be solicited from all interested parties. This "catfish" model should serve as a "suggested" approach for other aquacultural species.

Attending the October 10, 1989, meeting in Jackson, Mississippi were:

Verner Hurt - Director of MAFES and Administrative Advisor of this task force
C. G. Shepherd - Director of Southern Regional Aquaculture Center
Larry de la Bretonne - Aquaculture Specialist with the Louisiana Cooperative Extension Service
John Waldrop - Agricultural Economics Professor at MSU and Chairman of the task force
Harold Ishee - Mississippi Agriculture Statistics Service
Robbin O. Roark - National Aquacultural Statistics Service—Livestock Branch and in charge of aquaculture program for NASS, Washington, D.C.
Don Bay - Director of the Estimates Division of NASS, Washington, D.C.
Fred Tyner - Assistant Director of MAFES
Dick Knight - State Statistician for NASS in Mississippi

Following a review of the history of the task force and a discussion of currently available information, the group agreed to proceed as a work group that would use the catfish industry as a model to determine data needs, identify agencies or individuals best qualified to collect it, estimate funding requirements, and develop a plan to secure support for an expanded effort.

The representatives of the work group should include research, Extension, National Agricultural Statistical Service, Mississippi Agricultural Statistical Service, Cooperative State Research Service, Southern Regional Aquaculture Center, feed mills, producers (fingerling and foodfish), Catfish Bargaining Association, Processors, and Economic Research Service.

It was agreed that should the effort need to be expanded to other centers, then the appropriate contact would be Meryl Broussard, Cooperative State Research Service.

Representatives of the Mississippi Agriculture Statistics Service and the National Agriculture Statistics Service were most cooperative and supportive of this effort and indicated their willingness to work with the task force to develop specific plans for both data collection and funding effort. The committee generally agreed that there was a need for development of a plan that would be national in scope that would provide consistent data across all political and other special interest subdivisions.

The Steering Committee reviewed the data currently collected and developed recommendations in three categories:

(1) “Catfish Production and Processing Data,”
(2) “Catfish Feed Data,” and
(3) “Catfish Price-Quantity Data.”

The type of data needed, the source of this data, and the appropriate agencies to collect the data have been identified, along with the form and timeliness of publication. At this time the agency (NASS) is assessing the changes necessary and the new resources required to collect, analyze, and publish the needed data in a timely manner. This activity is expected to result in budget requirements for the data system.

After establishing a preliminary budget, the catfish model data collection system will be presented to the industry work group for refinement. At this point it should be available to other segments of aquaculture for their modifications, where needed, to meet any industry-specific needs.

Immunization Of Channel Catfish

Termination Report for the Period May 2, 1989 to April 30, 1991

Funding level

Year 1	$50,000
Year 2	$49,789
Total	$99,789

Participants

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Administrative advisor

Lowell T. Frobish, Director
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Objectives

1a. Identify and purify the immunodominant antigen of *Edwardsiella ictaluri*.
1b. Evaluate the protection of channel catfish provided by the immunodominant antigen of *E. ictaluri*.
2a. Map and clone the channel Catfish Herpes Virus (CCV) thymidine kinase gene to enable use in future studies as a selectable site for insertion of bacterial genes.
2b. Clone the S-layer protein gene of *Aeromonas hydrophila* as a candidate for insertion studies into the attenuated virus.
3a. Determine if specific immune resistance to *E. ictaluri* can be enhanced through dietary manipulation in non-immunized, bath and orally immunized, and oral-only immunized fish from each experimental diet.
3b. Compare the above groups for survival after challenge with virulent *E. ictaluri*.
3c. Evaluate fatty acid profiles and vitamin E content of the diets and tissues of fish from each group.
3d. If dietary enhancement is seen, determine least amount of time enhancing diets would have to be fed in order to balance optimal protection and cost-effectiveness.

Principal accomplishments

Objective 1a

Cell extract and crude membrane protein from *Edwardsiella ictaluri* were used to immunize channel catfish. The antibody titer of immunized fish to cell extract and crude membrane gradually increased the first week and then more rapidly thereafter until reaching a maximum titer at 4 weeks post intraperitoneal injection. An anamnestic response immediately followed a booster vaccination in which the antibody titer increased in a short period of time. The antibody titers gradually declined after this point, but were still detectable 11 weeks post-immunization. A whole cell formalin-killed preparation injected at 2.0 x $10e^4$ cells/fish produced lower antibody titers than 2.0 x $10e^6$ or 2.0 x $10e^8$ cells/fish, but there was no difference in antibody titers from 2.0 x $10e^6$ and 2.0 x $10e^8$ cells/injected fish. A crude membrane preparation produced similar results; antibody production resulting from 0.20 and 1.50 mg protein/fish were not different, but were higher than that stimulated by 0.02 mg protein/fish. In general, the higher the antigen concentration, the higher the antibody production, but an antigen saturation point was attained. The antibody response at 25 and 30°C were similar in rate of increase and did not differ. However, antibody titer of fish at 20°C was lower than those produced at 25 and 30°C. Channel catfish exposed to *E. ictaluri* by immersion for 2 and 5 minutes had similar antibody response, but fish exposed for 30 minutes and 8 hours had higher antibody response.

Objective 1b

Cells transferred from fish immunized with *E. ictaluri* were used to determine the cell-mediated immune response of channel catfish, *Ictalurus punctatus*, and compare this response to the humoral immune response. Channel catfish (average size of 28.7 g) were immunized intraperitoneally with cell extract of *E. ictaluri*, and 21 days later head kidney cells from immunized and non-immunized fish were removed and transferred to other immunized and non-immunized fish. Total white blood cell count from control fish was 2.5 x $10e^6$ cell/mL and 3.1 x $10e^6$ cells/mL in immunized fish at 21 days post-immunization. The mortality was 20% in non-immunized, non-cell transferred fish, 32% in immunized, non-cell transferred fish, and 8% in both immunized, cell transferred and non-immunized, cell transferred. Head kidney cells transferred from immunized and non-immunized fish to other immunized and non-immunized fish resulted in only 8% mortality, which indicated that cell transfer played a more important role in protection than immunization alone.

Channel catfish were vaccinated intraperitoneally with cell extract, crude membrane, and a 36 kDa purified outer membrane protein from *E. ictaluri*. Fish were boosted 14 days later, and then 28 days after initial vaccination they were challenged with *E. ictaluri*. When fish were vaccinated with the 36 kDa outer membrane protein and boosted, mortality was reduced from 54.5% in the control group to 24.0% in vaccinated fish. Fish vaccinated with cell extract, crude membrane (both boosted and non-boosted), and fish not boosted with the 36 kDa protein demonstrated no degree of protection compared to control. Survivors from a natural infection of *E. ictaluri* demonstrated a strong relationship between degree of protection. Intraperitoneal injection of 2.0 x $10e^7$ cells killed 100% of fish with antibody titers of 0 to 128; 77.8% of fish with titers from 256 to 512 (medium); and 57.7% of the fish with titers of >1024 (high). The second trial using 5.1 x $10e^5$ cells/fish gave 72.2% mortality in fish with no detectable antibody titers, 51.3% in low antibody titer fish, 25.0% in medium antibody titer fish, and 6.5% in high antibody titer fish. These results demonstrate that channel catfish had protective antibody after they were exposed to the pathogen, but if fish are challenged with large numbers of pathogens, this protective immunity can be overwhelmed.

Application of cell extract of *E. ictaluri* impregnated feed showed that vaccinated fish receiving the antigen-impregnated feed every 10 days, maintained their antibody titer.

Objective 2a

The gene encoding the previously identified unique channel Catfish Herpes Virus (CCV) thymidine kinase (Tk) was preliminarily located on the CCV genome. CCV genomic DNA libraries were constructed into plasmid pUC 19 and cosmid pHC 79. Analysis of CaCl2, DEAE and cationic liposome mediated transfection techniques using beta-galactoside expressing plasmid pON 105 on the channel catfish ovary cell line (CCO), a Tk deficient mutant of CCO (CCOBr), and the brown bullhead cell line (BB) revealed cationic liposome transfection of CCO cells to be the most effective combination. More importantly, cationic liposome mediated transfection of whole CCV-DNA onto CCO or CCOBr cells was the only method that effectively produced infectious viral progeny. This is the first account showing purified CCV-DNA to be infectious. Subsequently, cationic liposome mediated co-transfection of cloned wild type CCV-DNA with the Tk deficient mutant of CCV (CCVAr) in marker rescue assays mapped the mutation within the 18 Kb direct repeat ends of the genome.

In addition, the polymerase chain reaction (PCR) was used (Jack Numberg, Cetus Corporation) to amplify regions flanked by sequences with homology to degenerate primers corresponding to conserved amino acid sequences among herpes virus Tk's. Three PCR generated fragments were isolated, cloned into pUC 19, and sequenced. The corresponding amino acid sequence of the presumptive coding strand of one sequence (405) showed limited homology to the mammalian cytoplasmic and pox virus Tk.

A weak specific hybridization signal was located on the Eco RI L-fragment, which is located on the direct repeat ends of the CCV genome, when DNA-DNA hybridization analysis was performed using the purified 32P nick-translation labeled 405 fragment. The combined marker rescue and PCR data mapped the Tk gene within the direct repeat region of the CCV genome. With subsequent subcloning of restriction digested cosmid clones, the Tk gene was mapped to within a 3.1 Kb fragment of the 18 Kb direct repeat ends of the genome. This location is unique among herpesviruses, indicating significant divergence from previously identified herpes virus gene arrangements.

Objective 2b

The S-layer protein gene of *A. hydrophila* was cloned in the phagemid expression vector Lambda ZAP II (Stratagene, La Jolla, California). Genomic DNA from *A. hydrophila* was partially digested with Eco RI to yield fragments ranging in size from 2 to 10 kilobase pairs (Kb) and shotgun-cloned into the phagemid vector Lambda ZAP II. Under isopropylthio-B-D-galactosidase (IPTG) induction of the Lac Z promoter, approximately 25,000 plaque forming units (PFU) were screened on nitrocellulose membranes using an enzyme-linked immunosorbant assay (ELISA). Thirty positive clones were identified and purified. The p-Bluescript SK-plasmid was excised from the phagemid by co-infection with VCSM13 helper phage (Stratagene) and transformed into fresh host cells to produce double-stranded plasmid DNA. The transformed cells were then used for analysis of the inserts by agarose gel electrophoresis and determination of protein expression by Western blot analysis.

Two of the original 30 clones, which contained approximately a 9.4 Kb insert, expressed two proteins with molecular weights of 85 and 81 Kb. Both proteins were expressed with or without IPTG induction, indicating that the S-layer protein gene is contained within the Eco RI fragment and is under the control of its own promoter rather than the Lac Z promoter of the vector. Restriction enzyme digests of the 9.4 Kb Eco RI fragment were subsequently subcloned into p-Bluescript.

Objective 3a

Four laboratory prepared feeds were compared. These feeds were identical except for the lipid source which was beef tallow, soybean oil, menhaden oil, or an equal combination of all three lipid sources. Within each diet group, macrophage function and antibody production were compared in non-immunized, bath-immunized, orally-immunized, and bath followed by an oral boost.

There were significant differences among the groups in macrophage function. In general, macrophages from fish fed menhaden oil and the combination feed had an enhanced ability to kill engulfed, live *E. ictaluri*. Bath immunization further enhanced this killing, however, oral immunization, with our oral preparation, did not. Although the menhaden group had the highest macrophage killing activity, it had the lowest growth.

Objective 3b

An attempt was made to challenge fish from each group with a live, virulent strain of bacteria. Unfortunately, it was unsuccessful in that there were very few mortalities even in the non-immunized group.

Objective 3c

Liver and muscle were removed from representative fish from each group. Fatty acid profiles of the tissues reflected the dietary fatty acid profiles. Fish fed menhaden oil had the highest percentages of the long-chained polyunsaturated n-3 fatty acids. Tissue samples to be used for vitamin E analysis were unfortunately lost during a power outage.

Objective 3d

Because of the above results, three diets (commercial, beef tallow, and combination) were tested instead of the two diets proposed. Swim-up fry were received from LSU and divided into 24 groups, which included non-immunized and bath-immunized (which were later orally boosted).

All groups were fed commercial feed for varying times before switching to the lab diets in order to determine if time on the lab diets had any effect on protection. Groups were maintained on lab feeds 4, 2, or 1 week prior to the oral boost and on these feeds until challenged 3 weeks later.

Using a sub-sample of fish from each group, fish were bath-challenged with a strain of *E. ictaluri* which killed fish within 3 days of injection. Using 1012 bacteria and increasing the time of exposure to the bacteria to 2 hours, there were still very few deaths. A second challenge after a rapid temperature change was attempted. Again this was not successful in killing fish. It is believed water quality parameters, fish strain, and/or stress must play an important role in the bacteria's ability to enter the fish and overcome the defense mechanism. Some of these are currently being investigated.

To obtain some useful information from this experiment, it was decided to examine some of the actual macrophage killing mechanisms and the effects of both diet and vaccination on them. Phagocytes produce various oxygen radicals as a bactericidal mechanism against engulfed organisms. The production of intracellular superoxide anion as well as the extracellular secretion of superoxide anion and hydrogen peroxide can be measured.

It was found that vaccination enhanced the ability of macrophages to produce intracellular superoxide anion after phagocytosis of live *E. ictaluri*. The production was increased 8.5x in the fish fed combination feed, 6.5x in fish fed the commercial feed and only 2.3x in fish fed the beef tallow feed. The extracellular secretion of superoxide anion and hydrogen peroxide was increased in vaccinated fish from the combo and commercial groups but not in the fish fed beef tallow.

The conclusion is that nutritional manipulation can be used to potentiate the immune response. Certain lipids appear to be very useful in the immune response and macrophage function. A combination of menhaden oil, soybean oil, and beef tallow is probably the best regarding both disease resistance and growth. If lipid is used as a dietary enhancement prior to vaccination, these feeds would have to be fed for 3-4 weeks (at 24°C or above) prior to vaccination.

Impacts

Results of the research on objective 1 provide some basic knowledge to the immunity of channel catfish to *E. ictaluri*. It is important to know that catfish respond immunogenically to bacterial cell protein extract, and crude membrane material and that they do have an anamnestic response. Also >0.2 mg of protein was required to produce a significant immune response. Water temperature and length of exposure time to antigen are critical points to be determined. The role of cell-mediated immunity to the immune response was hinted but not proven. Protection of immunized catfish from infection of *E. ictaluri* was also an important key to antigen preparation (vaccine) and application. Fish exposed for 2 and 5 minutes had similar antibody response, but fish exposed for 30 minutes and 8 hours had higher antibody response.

In objective 2, the first steps of engineering CCV as a vaccine vector were accomplished. The thymidine kinase was cloned and mapped to a 3.1 Kb DNA fragment in the CCV genome. Also the immuno-dominant S-layer protein of *A. hydrophila* was cloned for insertion into the CCV-TK gene. This non-reverting mutant of CCV that expresses the bacterial antigen can provide protection against the bacterium and CCV. The research also establishes a foundation for developing a CCV based vaccine vector system that could be used with other catfish pathogens.

Work on objective 3 indicates that a combination of dietary immunopotentiators and vaccination programs could significantly reduce losses due to *E. ictaluri*.

Publications

Abstracts and papers presented

Awad, M. and R. L. Thune. 1991. Cloning and expression of the S-layer protein gene of *Aeromonas hydrophila*. Proceedings of Annual Meeting of Fish Health Section of American Fisheries Society, Portland, OR. p. 29.

Lingenfelser, J. T., V. S. Blazer, and R. E. Klinger. 1991. Metabolic activation of channel catfish macrophages. 16th Annual Eastern Fish Health Workshop. Martinsburg, WV. June, 1991.

Plumb, J. A. and S. Vinitnantharat. 1991. Kinetics of the immune response in channel catfish to *Edwardsiella ictaluri*. 16th Annual Eastern Fish Health Workshop. Martinsburg, WV. June, 1991.

Theses or dissertations

Hanson, L. A. 1990. Biochemical characterization and gene mapping of the channel catfish herpes virus (CCV)-encoded Thymidine Kinase, A selectable site for holologous recombination. Ph.D. Dissertation. Louisiana State University.

Vinitnantharat, S. 1991. Humoral and cell-mediated immune response of channel catfish, *Ictalurus punctatus*, to *Edwardsiella ictaluri*. Ph.D. Dissertation. Auburn University.

Enhancement Of The Immune Response To *Edwardseilla Ictaluri* In Channel Catfish

Termination Report for the Period May 2, 1989 to September 30, 1991

Funding level

Year 1	$46,559
Year 2	$51,804
Total	$98,363

Participants

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Project objectives

1. Determine if diets containing high concentrations of vitamin E will enhance the immune response of stressed and unstressed channel catfish to *Edwardsiella ictaluri*.
2. Determine if diets containing high concentrations of selenium will enhance the immune response of stressed and unstressed channel catfish to *Edwardsiella ictaluri*.
3. Determine if diets containing levamisole will enhance the immune response of stressed and unstressed channel catfish to *Edwardsiella ictaluri*.
4. Determine if diets containing combinations of vitamin E, selenium, and levamisole will enhance the immune response of stressed and unstressed channel catfish to *Edwardsiella ictaluri*.
5. Determine if dietary levamisole can prevent or ameliorate the immunosuppressive effects of dietary cortisol.
6. Determine if dietary selenium and levamisole can ameliorate stress-induced immunosuppression.
7. Develop an Extension Service publication concerning the relationship between nutrition and health in channel catfish.

Principal accomplishments

Objective 1

Channel catfish fingerlings were acclimated to laboratory conditions and fed diets containing 0, 60, or 2,500 IU/kg vitamin E for 3.5 months. Half of the fish in each treatment were immersion-vaccinated after 0.5 months using formalin-killed *E. ictaluri*. These fish also received an oral booster 2 months later.

After 3.5 months, the vaccinated fish had a significantly higher phagocytic index than the non-vaccinated fish (2-way ANOVA). Phagocytic index in the immunized fish also increased significantly in a diet-dependent manner in the vaccinated groups (1-way ANOVA). Bactericidal activity was significantly affected by diet in both vaccinated and non-vaccinated groups (1-way ANOVA); however, no pattern was evident. In general, bactericidal activity was higher in the non-vaccinated groups (2-way ANOVA). The groups (both vaccinated and non-vaccinated) fed the high vitamin E diet were significantly more resistant to red blood cell peroxidation than the groups fed the intermediate and low vitamin E diets. All groups responded similarly to challenge by injection of live bacteria.

Objective 2

Purified diets containing adequate vitamin E (60 IU/kg) were supplemented with 0, 0.25, and 10 mg/kg Se and fed to immunized and non-immunized fingerling catfish in aquaria to evaluate the effects of dietary selenium on immunocompetence and disease resistance to *E. ictaluri*. At the end of the 15-week feeding trial, selenium status of fish fed the various diets was confirmed by analysis of selenium-dependent glutathione peroxidase (SeGSH-Px) activity in liver. Fish fed the basal diet were selenium deficient as evidenced by significantly ($P \leq 0.05$) reduced SeGSH-Px activity as compared to fish fed diets supplemented with 0.25 and 10 mg/kg Se. However, supplemental selenium in the diet did not improve immunocompetence of catfish based on assessment of antibody titers, phage neutralization, peritoneal macrophage activity and resistance to a challenge by live *E. ictaluri*. In fact, selenium deficiency actually improved the resistance of catfish to bacterial challenge. Similar responses have been observed in some selenium-deficient mammalian species.

Subsequent experiments to evaluate the combined effects of dietary selenium and vitamin E on immunocompetence of channel catfish are proposed since these nutrients have complementary biochemical functions which may interact synergistically.

Objective 3

Initial studies revealed that bath immunization of catfish fingerlings and adults with formalin-killed-bacterins of *E. ictaluri* did not yield consistent immune responses with respect to protection and various serologic parameters. Hence, a pilot study was conducted comparing various formalin- and heat-killed preparations for efficacy in bath-immunization of catfish. The bacterin which proved superior was prepared from a two-broth culture, washed twice in saline, autoclaved, and the turbidity adjusted to Macfarland standard 4 and diluted 10x in a bath wherein fish were held for 20 minutes. Four groups of fish were studied, i.e., a control group maintained on a conventional diet containing no Se, a group maintained on 1% Carrisyn (an immunopotentiator), and a group which received levamisole after having been immunized. Each of the four groups were subdivided into subgroups, one of which had been immunized and the other which had not been immunized. All the fish which were immunized had significant agglutinin titers 2 weeks after immunization; the agglutinin titers of those fish which had received Carrisyn were 2- to 8-fold higher than control fish; levamisole treatment and selenium deprivation enhanced serologic response approximately 2-fold. Challenge studies using twice the LD50 of live organisms revealed that selenium deprivation and the incorporation of Carrisyn-enhanced protectiveness of the immunization protocol.

Objective 4

Channel catfish were fed five diets containing combinations of selenium and vitamin E (0 IU/kg E and 0 mg/kg selenium; 60 IU/kg E and 0 mg/kg selenium; 0 IU/kg E and 0.25 mg/kg selenium; 240 IU/kg E and 1.0 mg/kg selenium). Fish were fed the experimental diets for at least 120 days. Half of the fish receiving each diet were vaccinated by immersion in 5 x $10e^{9}$ formalin-killed cells per mL on day 90, given oral boosters (15.7 x $10e^{10}$ formalin-killed cells/kg) on days 104-106, and sampled beginning on day 120. Production of intracellular and extracellular superoxide anion by macrophages, glutathione peroxidase activity of liver, red blood cell resistance to peroxidation, and resistance to challenge by live bacteria were determined. In all treatments except the double deficient group, immunization significantly enhanced the intracellular production of superoxide anion subsequent to phagocytosis of *E. ictaluri*. The two selenium-deficient groups produced the lowest amounts of superoxide anion in both vaccinated and non-vaccinated groups. Extracellular superoxide anion in both vaccinated and non-vaccinated groups was lowest in the double deficient group. Glutathione peroxidase activity was lowest in the two selenium-deficient treatments, highest in the high selenium treatment, and unaffected by vaccination status. Fish fed the two vitamin E-deficient diets were more susceptible to red blood cell peroxidation than fish fed the remaining three diets. No significant differences were observed in the challenge studies.

Objective 5

Experimental design of this study involved five vaccinated treatments in duplicate (total of 10 groups, 20 fish/group): (1) control, (2) levamisole for 4 days prior to initial sampling, (3) cortisol (1700 mg/kg) 2 weeks prior to initial sampling, (4) cortisol + levamisole, and (5) Carrisyn. Immunologic assays were conducted 5 weeks after initiating the study. Mean plasma cortisol concentration of fish not receiving cortisol was 8.2 ng/mL, while that of fish receiving cortisol was 163.0 ng/mL. Pronounced immunosuppression was observed in fish receiving cortisol in that none of these vaccinated fish developed agglutinins during the study. In fact, of the cortisol treated fish, 4/10 fish in one replicate group and 7/10 in the other succumbed before the study was terminated.

The ameliorating effect of levamisole in counteracting the immunosuppressive effect of cortisol was not statistically significant ($P \leq 0.05$). The resistance enhancing effects of Carrisyn were further verified in that bactericidal and phagocytic assays were enhanced by a factor of 2-3 fold over control fish which had agglutination titers of 1:64-1:256. Eighty-two percent of the fish survived bacterial challenge. None of the fish which had received Carrisyn succumbed to challenge.

Objective 6

In an effort to ascertain the potential ameliorating effects of levamisole and Carrisyn upon stress-induced immunosuppression, the fish from one replicate of each treatment were stressed by placing in nets just below the surface of the water for 24 hours. Three fish in each tank were processed for: a) phagocytic assays, b) bacterial challenge, c) phage neutralization, and d) plasma cortisol assay prior to and immediately after imposing the 24-hour stress test. Across all treatments, stressed fish possessed a mean cortisol level of 93 ng/mL while unstressed fish possessed a mean cortisol level of 11 ng/mL. Phagocytic assays and other indicators of immune responsiveness were higher in levamisole treated, vaccinated stressed fish, than in those vaccinated stressed fish which had not received levamisole. However, there was no difference in resistance to challenge between stressed and non-stressed, levamisole-treated fish. Approximately 40% of the stressed fish which had been immunized ultimately succumbed to bacterial challenge whether or not they had received levamisole. The immunopotentiating effects of Carrisyn were not observed in stressed fish in that there appeared to be no difference in survival to challenge or other immuno-assays between fish receiving Carrisyn and those not receiving Carrisyn.

Impacts

The findings of this research project will be useful in designing diets to help promote immunity to *E. ictaluri*, designing diet/vaccination regimes for promoting immunity to *E. ictaluri*, and designing further studies to develop techniques to more efficiently immunize channel catfish against *E. ictaluri*.

Publications

Journal articles

Blazer, Vicki S. 1991. Piscine macrophage function and nutritional influences: A review. Journal of Aquatic Animal Health 3:77-86.

Wise, D. J., J. R. Tomasso, D. M. Gatlin III, S. C. Bai, and V. S. Blazer. 1993. Effects of dietary selenium and vitamin E on red blood cell peroxidation, glutathione peroxidase activity, and macrophage superoxide anion production in channel catfish. Journal of Aquatic Animal Health 5:177-182.

Wise, D. J., J. R. Tomasso, T. E. Schwedler, V. S. Blazer, and D. M. Gatlin III. 1993. Effect of vitamin E on the immune response of channel catfish to *Edwardsiella ictaluri*. Journal of Aquatic Animal Health 5:183-188.

Effect Of Nutrition On Body Composition And Subsequent Storage Quality Of Farm-Raised Channel Catfish

Termination Report for the Period May 2, 1989 to December 31, 1992

Funding level

Year 1	$275,000
Year 2	$275,000
Year 3	$275,000
Total	$825,000

Participants

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Project objectives

1. Determine effects of diet composition and feeding strategies (energy, protein, and type and amount of lipid) on yield, dressing percentage, body fat, subsequent frozen storage quality, and profitability of catfish grown to 0.5-1.0 kg (1-2 lb) sizes under conditions that reflect management practices used by most of the catfish industry.
2. Determine effects of finisher diets or alternative feeding rates and schedules on yield, dressing percentage, body fat, subsequent frozen storage quality, and profitability of catfish grown to 0.5-1.0 kg (1-2 lb) sizes under conditions that reflect management practices used by most of the catfish industry.
3. Determine effects of diet supplements on chemistry and sensory qualities of fat in fish flesh and stability of fish during subsequent frozen storage.
4. Develop procedures for disseminating these findings to appropriate clientele groups.

Principal accomplishments

Objective 1
Reducing protein in practical feeds to 24 to 26% (and thereby increasing energy/protein ratio) did not cause a reduction in growth, but caused a slight increase in body fat. This change in fat did not affect frozen storage quality.

Objective 2
Raising or lowering the protein content of finishing feeds (fed the last 4 weeks of growout period) did not affect growth or body composition of the fish.

Objective 3
Vitamin E fed at four times the dietary requirement protected the lipids in catfish muscle from autoxidation during abused (high temperature) frozen storage conditions. Adding various commercial antioxidants, lysine and carnitine, to the diet did not affect autoxidation of muscle lipids during storage.

Objective 4
An extension fact sheet, “Channel Catfish Production—Impacts of Diet Composition and Feeding Practices” (SRAC #187) has been prepared which describes major findings of this project in practical language. Many other publications in technical and trade journals have also been prepared.

Impacts

Results from this project have indicated to the catfish industry that protein, or protein/energy ratio, in catfish feeds can be decreased without reducing fish production and with no effect on frozen storage quality of the processed fish. This has allowed the protein percentage to be reduced in commercial feed from 32 to 28% which lowers the cost approximately $10 per ton. In 1992, many farmers changed to the lower protein feed. One large feed mill reported that 30% of the feed manufactured in 1992 was 28% protein as compared to less than 10% the previous year.

The study demonstrated to processors that large catfish have a thick layer of fat on the surface of the muscle and that removing this will enhance frozen storage quality. Processors have adjusted skinning machines to remove this layer of fat from the fish.

This funding has initiated research in various areas of catfish nutrition and processing at several institutions which has been continued with other funding. An example is the University of Georgia, Food Science Department, which was not previously involved in catfish research but has 16 publications on processing from this project and is continuing research in this area.

Publications

Journal Articles

Bai, S. C. and D. M. Gatlin, III. 1992. Dietary rutin has limited synergistic effects on vitamin C nutrition of fingerling channel catfish (*Ictalurus punctatus*). Fish Physiology and Biochemistry 10:183-188.

Bai, S. C. and D. M. Gatlin, III. 1993. Dietary vitamin E concentration and duration of feeding affect tissue a-tocopherol concentrations of channel catfish (*Ictalurus punctatus*). Aquaculture 113:129-135.

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Extension publications

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Lovell, R. T. and M. Li. 1991. Are fish farmers feeding too much protein to catfish. Highlights of Agricultural Research, Alabama Agricultural Experiment Station 38:6.

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Tidwell, J. H., C. D. Webster, and J. A. Clark. 1991. Proximate and fatty acid composition of channel catfish, (*Ictalurus punctatus*), during feeding, starvation, and refeeding. Annual Meeting of the World Aquaculture Society. (Abstract).

Webster, C. D., J. H. Tidwell, L. S. Goodgame, J. A. Clark, and D. H. Yancy. 1992. Effects of protein level and feeding frequency on growth and body composition of third year channel catfish reared in ponds. Annual meeting of the World Aquaculture Society. (Abstract).

Harvesting, Loading, And Grading Systems For Cultured Freshwater Finfishes And Crustaceans

Termination Report for the Period May 2, 1989 to April 30, 1993

Funding level

Year 1	$125,000
Year 2	$125,000
Year 3	$125,000
Total	$375,000

Participants

R. P. Romaire, T. B. Lawson, J. L. Avery, Louisiana State University
J. W. Jensen, J. M. Grizzle, L. L. Lovshin, R. K. Goodman, Auburn University
J. A. Collier, T. E. Schwedler, Clemson University
K. B. Davis, J. F. Payne, W. A. Simco, Memphis State University
M. J. Fuller, J. G. Dillard, M. W. Brunson, Mississippi State University
G. W. Lewis, J. Shelton, University of Georgia
J. V. Huner, University of Southwestern Louisiana

Administrative advisor

W. H. Brown, Associate Director
Louisiana Agricultural Experiment Station
Louisiana State University Agricultural Center
Baton Rouge, Louisiana

Project objectives

1. To develop and evaluate new methods, or modify and improve existing methods, to more efficiently concentrate and harvest channel catfish and crawfish with passive and/or active harvesting devices such as seines, nets, pumps, traps, or other appropriate techniques and equipment to improve harvesting efficiency and to increase profitability.
2. To develop and evaluate new methods, or modify and improve existing methods, of size-selective grading at harvest to more effectively handle catfish and crawfish to maintain or enhance product quality and to maintain or improve production and processing profitability.
3. To conduct comparative analyses of, and between, newly developed and conventional harvesting, loading, and grading equipment and procedures used in catfish and crawfish aquaculture. The standards for comparison should include direct and indirect costs associated with mortality, injury, and physiological stress.
4. To assist aquaculturists in utilizing the research findings by appropriate extension methods, such as regional workshops, on-farm demonstrations and consultations, field days, publications, and other educational materials.

Principal accomplishments

Catfish

The turbine fish pump loaded fish faster than either a vacuum pump or lift net, but it caused more injury. High pump speeds increased the severity of injury to fish. Trials should be conducted to determine the optimal speed of pumps. Injuries caused by the turbine pump did not reduce flesh quality and would not impact on fish harvested for processing. Fish harvested with pumps for restocking or for fee-fishing may have higher mortality than fish harvested with lift nets because of post-harvest disease outbreaks. A seine with a rubber-roller mud line was more effective for catching food-size channel catfish than was a seine with gathered-net mud line (commercial standard), particularly in ponds with deep mud bottoms. An electrified seine that was tested in eight ponds, 64 times with eight different voltage and electrical field configurations, did not improve catfish catch compared to conventional seines.

A mechanical grader size-graded fingerling catfish as rapidly and as accurately as commercial box graders. The mechanical grader was more effective in cold water than box graders. Catfish can be weighed as accurately by measuring displacement of water in transport tanks as by weighing with scales.

Stress in fingerling and food-size catfish, as measured by changes in serum cortisol, glucose, and electrolytes, was determined before, immediately after, and for several days after harvest. Stress response in catfish was similar among fish pumps and lift nets, and stress was less in cooler months. Stress recovery to pre-harvest levels occurred by the fifth day after harvest.

A comparative economic analysis of loading catfish from production ponds to live-haul trucks was made for an 8-inch turbine pump and a lift-net under conditions prevailing in the Mississippi Delta. The vacuum pump was not included because of low performance. Assumptions included custom harvesting with a boom and lift net capable of holding 1,800 pounds of fish, and a fish loading rate of 600-750 pounds per minute, a rate comparable to the turbine pump. There was little difference in cost of harvesting catfish by either method, with total harvesting cost estimated at $0.01905 per pound using a lift net, and $0.01955 using the turbine pump. Assuming a harvest of 25,000 pounds of fish, this cost difference amounts to only $12.50 per harvest from a typical pond.

Two major field days were held, one in Monterrey, Louisiana, and the other in Cohutta, Georgia, to showcase harvesting, grading, sorting, and transportation of catfish and other finfishes. Over 800 producers, vendors, researchers, and extension personnel from seven states attended the two exhibitions. Five fact sheets on harvesting, loading, and grading of finfish were published. A 30-minute video on harvesting, loading, and grading systems for catfish and other finfishes was developed and made available to state extension contacts in the Southern Region.

Crawfish

A pyramid trap increased trap catch 44% compared to a standard commercial stand-up trap. A pyramid trap density of 24 per acre produced optimum catch compared to 12, 36, and 48 per acre. Trapping frequency was evaluated to determine if the commercial practice of trapping 5 to 6 days per week could be reduced by using the pyramid trap. No reduction in yield occurred with trapping three days per week compared to 5 days per week. Trapping crawfish 3 days per week, every other week, reduced yield. A rotational trapping system, in which a portion of the pond (50% or 67%) was trapped for a week, followed by trapping the non-trapped portion the following week resulted in a yield comparable to 3 days per week and 5 days per week trapping. Harvested crawfish were smaller with rotational trapping.

A spiral crawfish grader was modified to fit in a harvesting boat and to separate harvested crawfish into four size grades. Roller spacings required to segregate crawfish into jumbo, large, medium, and peeler size groups (size grades requested by crawfish buyers were determined in laboratory trials). Commercial on-boat graders evaluated could not process large quantities of crawfish effectively because they easily jammed with debris and this required frequent stopping to clear the machine.

A trawl system, the USL crawfish skimmer, was developed for use on commercial crawfish harvesting boats. The skimmer, which eliminates the need for traps, was designed for use in late season when vegetation is minimal, crawfish are abundant, and prices are low. The skimmer was at least twice as effective as conventional traps when tested in late spring. For the trawl to be effective, trapping lanes with no vegetation must be made in ponds before flooding, and attractants must be placed in the lanes prior to harvest. The skimmer is most effective in late spring, and it is more selective for smaller crawfish than standard commercial traps.

Crawfish stress, as measured by changes in hemolymph concentrations of osmotic pressure, chloride, and sodium, were determined at different temperatures and salinities. Stress, due to salinity and temperature, was the same between red swamp crawfish and white river crawfish, and increased above and below 75°F. Both species acclimated to salinities from 10 to 30 ppt. Stress in red crawfish, during harvesting and storage in a cooler, was similar among males and females. Physiological stress indicators in crawfish were stable for up to 5 days in a cooler.

Impacts

Catfish

The turbine pump has potential in the catfish industry, especially if and when fish grading is required by farmers and processors. The fish pump is more suited to in-line size graders than lift nets. Presently, fish pumps are not accepted by the catfish industry, but pumps may be used in the future as catfish processors require, and are willing to pay for, size-graded fish. Lift nets provide more flexibility in loading transport tanks than do stationary fish pumps. Also, the fish pump does not permit determination of loaded fish weight with scales as does the lift net. Catfish loaded with a pump must be weighed by water displacement in the transport tank, a method not presently accepted by most catfish farmers.

The mechanical catfish fingerling grader is not used by fingerling producers because growers are not yet willing to pay a higher price for well-graded fingerlings compared to pond-graded fingerlings. Grading fingerlings with mechanical graders will become integrated into the industry when growers and processors demand a more uniform-sized fish. No mechanical grader tested was able to grade food-size fish with the speed required by industry. Grading food-size fish can be done, but it will increase the cost of producing catfish. Presently, processors will not pay more for uniform-sized catfish.

Some catfish farmers are using modified seines with the rubber-roller mud line, but acceptance is not yet widespread among farmers. More farm trials are needed to determine if rubber-roller mud line seines will replace the more commonly used gathered-net mud line seine. An electrified seine appears to have little potential.

Harvesting in cooler months should be done when possible because stress is reduced. If fish are to be handled a second time, a recovery period of at least 5 days is required. Examination of diseased fish during this project led to a better understanding of the tissue injury caused by traumatic injury and by pathogenic bacteria. The choice of harvesting equipment can be made on cost or facilities because all techniques evaluated resulted in similar degrees of stress to catfish.

Mechanical grading equipment and a new method of seine construction have potential to improve the industry. However, information gathered will not have much impact on the catfish farming industry until farmers are convinced that new technology is better than that presently used. Information is available to farmers about new harvesting, loading, and grading equipment. Comparisons have determined that presently employed loading equipment is as good as or better than new technology. Research findings on harvesting, loading, and grading systems for channel catfish and other cultivated warmwater finfishes have been extended to 800 commercial farmers, vendors, and extension and research personnel in the Southern Region through two regional workshops, on-farm demonstrations and consultations, field days, publications, and other educational materials. Educational materials developed have been distributed throughout the Southern Region as fact sheets and videos. These materials will be used in the development of Best Management Practices and Quality Assurance Program training.

Information from this research was used to develop an educational program for a major catfish processor, saving him over $100,000 in a 4-month period by training his personnel to reduce fish death from handling stress. Producers of minor aquaculture species such as red drum, hybrid striped bass, and gamefish benefited from information in this study. Funding for this project assisted in the training of three graduate students and numerous undergraduate student workers.

Crawfish

The crawfish harvesting research has had a significant positive economic impact on the crawfish industry. Aquaculture advisory agents with state cooperative extension services are recommending that producers use the pyramid trap design. If the pyramid trap is used, extension agents are also recommending that crawfish producers reduce their trapping effort from 5 or 6 days/week to 3 days/week, unless circumstances dictate otherwise. Although cost analyzes by economists are not yet complete, preliminary analyses indicate harvesting cost is reduced as much as 30% with pyramid traps and 3 days/week trapping. These findings have been communicated to crawfish producers at 12 crawfish production advisory meetings, and through extension newsletters. Contact with producers and extension agents indicate that 3 days/week trapping is being readily adopted. Producers are reporting that their catch has not been significantly reduced from previous years when trapping effort was higher, and they are realizing significant savings in bait and labor costs. If 20% of crawfish producers have adopted 3 days/week trapping with pyramid traps, a conservative estimate according to extension agents is that $2 million is being saved annually from reduced bait and labor costs. Rotational trapping needs further field evaluation.

The crawfish skimmer is a new concept and has been presented to a very conservative industry. Its adoption will be slow. The crawfish skimmer system has not yet had any direct effect on the crawfish aquaculture industry; however, it offers a new technology that eventually may complement conventional trapping. The outgrowth of this project was the organization of an ad hoc automated harvesting work group, which brought together four of the seven known groups developing propelled trawl-type systems for harvesting crawfish. The potential cost effectiveness of the trawling system has been simulated with a crawfish pond computer model, and the information has been disseminated at several public forums.

Although no new commercial in-boat graders have been developed as a result of this research, mechanical limitations in existing commercial graders have been identified and brought to the attention of fabricators so that the graders can be improved. Presently used methods of harvesting crawfish with traps, and storage in a cooler for up to 5 days, do not appear to place undue stress on crawfish. Salinity tolerance studies indicate that it may be possible to extend the culture of red swamp and white river crawfish into waters with moderate salinity.

Funds from SRAC for crawfish harvesting research have assisted the University of Southwestern Louisiana's (USL) Crawfish Center and Louisiana Agricultural Experiment Station in securing funding for gear development work and harvesting research from the following agencies: Gulf and South Atlantic Fisheries Development Foundation, U.S. Department of Agriculture, Crawfish Promotions and Research Board, and the Louisiana Board of Regents 8-G Competitive Grants Program. Four graduate students and six undergraduate student workers have been trained in this crawfish harvesting, loading, and grading project.

Publications

Journal articles

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Grizzle, J., J. Chen, J. C. Williams, and J. S. Spano. 1992. Skin injuries and serum enzyme activities of channel catfish, *Ictalurus punctatus*, harvested by fish pumps. Aquaculture 107:333-346.

Grizzle, J. and Y. Kiryu. 1993. Histopathology of gill, liver, and pancreas, and serum enzyme levels of channel catfish infected with *Aeromonas hydrophila* complex. Journal of Aquatic Animal Health 5:36-50.

Grizzle, J. and L. Lovshin. 1996. Injuries and serum enzyme activities of fingerling channel catfish (*Ictalurus punctatus*) harvested with a turbine pump. Aquacultural Engineering 15:349-357.

Lawson, Thomas B., A. de los Reyes, and G. Vidrine. 1995. Mechanical size-grading of post-harvest crawfish: Evaluation of a roller-type grader. Journal of Aquacultural Engineering 14:255-260.

Lawson, T. and R. Romaire. 1991. Evaluation of two new trap types and aerator-induced water currents for harvesting procambarid crawfish in ponds. Journal of Shellfish Research 10:349-354.

Lawson, T. and R. Romaire. 1991. Evaluation of two new trap types and aerator-induced water currents for harvesting procambarid crawfish in ponds. Journal of Shellfish Research 10:349-354.

Lovshin, L. and R. Phelps. 1993. Evaluation of a mechanical grader to separate fingerling channel catfish (*Ictalurus punctatus*) into length groups. Journal of Applied Aquaculture 3:285- 296.

Newsom, J. and K. Davis. 1994. Osmotic responses of haemolymph in white river crayfish (*Procambarus zonangulus*) and red swamp crayfish (*P. clarkii*) to changes in temperature and salinity. Aquaculture 126:373-381.

Newsom, J., S. Noblitt, and K. Davis. Osmotic responses of red swamp crayfish to trapping, sorting, and storage in a cooler. Journal of Aquatic Animal Health 6:183-185.

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Newsom, J. and K. Davis. 1991. Ionic responses of white-river crawfish (*Procambarus zonangulus*) and red swamp crawfish (*P. clarkii*) to changes in temperature and salinity. American Zoologist. 31:230.

Rode, R. A., L. L. Lovshin, and R. K. Goodman. 1991. Comparison of three fish-loading systems to harvest food-size channel catfish, *Ictalurus punctatus*. Aquacultural Engineering 10: 291-304.

Rode, R., L. Lovshin, and R. Goodman. 1991. Comparison of three fish-loading systems to harvest food-size channel catfish (*Ictalurus punctatus*). Aquacultural Engineering 10:291-304.

Romaire, R. 1989. Overview of harvest technology used in commercial crawfish aquaculture. Journal of Shellfish Research 8(1):281-286.

Steeby, J. and L. Lovshin. 1993. A comparison of seines equipped with rubber roller or gathered-netting mud lines for harvesting channel catfish in earthen ponds. The Progressive Fish-Culturist 55:133-136.

Extension publications

Baldridge, T. and D. Huffman. 1993. Crawfish production and harvesting economics in Louisiana. Louisiana Agricultural Experiment Station, Department of Agricultural Economics Research Report #695.

Huffman, D. and T. Baldridge. 1992. Crawfish harvesting economics. Louisiana Rural Economist 54:12-14.

Huner, J. and G. Faulkner. 1992. A brief description of the University of Southwestern Louisiana's crawfish trawl. Crawfish Center, University of Southwestern Louisiana, Lafayette, LA., June 1992, mimeographed. 4 pp.

Abstracts or Papers Presented

Collier, J. A. and T. Schwedler. 1992. Effects of an electric seine on harvest efficiency of channel catfish. Techniques for Modern Aquaculture, American Society of Agricultural Engineers, Spokane, WA.

Davis, K. B., J. Newsom, and B. Simco. 1992. Physiological stress in channel catfish, *Ictalurus punctatus*, harvested by lift net, vacuum pump, or turbine pump. Presented to Annual Meeting of the Catfish Farmers of America, February 1992, Orange Beach, AL.

Faulkner, G. and J. Huner. 1993. The University of Southwestern Louisiana's pond crawfish skimmer net. Annual Meeting, Louisiana Chapter, American Fisheries Society, Metairie, LA, February, 1993.

Lovshin, L. L. 1990. Comparison of three fish-loading systems to harvest food-size channel catfish. Catfish Farmers of America, Little Rock, AR.

Lovshin, L. L. 1992. Evaluation of a mechanical grader to separate fingerling channel catfish *(Ictalurus punctatus*) into length groups. Catfish Farmers of America, New Orleans, LA.

Newsom, J. E. and K. B. Davis. 1991. Ionic responses of white-river crawfish (*Procambarus zonangulus*) and red swamp crawfish (*P. clarkii*) to changes in temperature and salinity. Presented at the Annual Meeting of the American Society of Zoologists. December, Atlanta, GA. 31:230. (Abstract).

Steeby, J. 1991. A comparison of seines equipped with rubber roller or gathered-netting mud lines for harvesting channel catfish in earthen ponds. Alabama Fish Farmers Conference, Montgomery, AL.

Theses and Dissertations

Chen, J. 1991. Skin injuries and serum enzyme levels of channel catfish harvested with different loading equipment. M.S. Thesis. Auburn University.

Kiryu, Y. 1992. Bacterial diseases after harvesting channel catfish: Comparison of fish pumps to traditional methods and histopathology of fish infected with *Aeromonas hydrophila* complex. M.S. Thesis. Auburn University.

Newsom, James E. 1991. Osmotic responses of white river crayfish (*Procambarus zonangulus*) and red swamp crayfish (*P. clarkii*) to changes in temperature and salinity. M.S. Thesis. Memphis State University.

Rode, R. A. 1991. Comparison of three fish-loading systems for harvesting food-size channel catfish (*Ictalurus punctatus*). M.S. Thesis. Auburn University.

Preparation Of Extension Publications On Avian Predator Control In Aquaculture Facilities

Termination Report for the Period April 1, 1990 to December 31, 1992

Funding level

$15,000

Participants

J. T. Davis, Texas A&M University
M. W. Brunson, Mississippi State University
G. W. Lewis, University of Georgia
F. Boyd, Alabama APHIS/ADC/USDA
M. Hoy, Arkansas APHIS/ADC/USDA
W. F. Stevens, Louisiana APHIS/ADC/USDA
G. Littauer, Texas APHIS/ADC/USDA
A. Stickley, Jr, Mississippi S&T Field Station APHIS/ADC/USDA
United States Fish and Wildlife Service

Administrative advisor

Milo Shult, Vice President for Agriculture
University of Arkansas
Little Rock, Arkansas

Project objective

1. Produce a 20-minute educational video that describes the major avian predators, discusses their economic importance, effective control measures, and their cost/benefit ratio.
2. Develop fact sheets to supplement the video which describe bird identification procedures, probable damage, seasonality of occurrence, recommended control measures, and sources of technical assistance.
3. Ensure the widest possible distribution of these materials to producers, fish and game organizations, and ornithological societies.

Principal accomplishments

Fact sheets and a video were prepared and distributed throughout the U.S. Many organizations requested additional copies for use in their educational efforts. In addition, the information presented increased federal and state research agencies' endeavors to find better protection methods, and furnished a basis to give enforcement personnel a standard to judge the level of effort a producer was providing. In at least a few instances, the cost of avian depredations are now an integral part of budgets and cost analyses being prepared by aquaculture producers and financial institutions.

Impacts

Wide distribution of these educational materials has served to awaken organizations dedicated to the protection of avian predators, to the dilemma of the fish and shellfish producer. For many of these people, the documentation of depredations has served to change their attitude about the need to alleviate the problem. The compilation of the possible methods for control has helped producers make intelligent choices about what methods to use. Finally, many producers have been awakened to the fact that though some birds, such as the cattle egret, are a nuisance, they do very little actual crop damage.

Publications

A complete list of all SRAC publications, videos, and computer software is presented in the Appendix at the end of the project summaries for the Southern Regional Aquaculture Center.

National Extension Aquaculture Workshop

Termination Report for the Period October 1, 1991 to August 30, 1992

Participants

Carole Engle, Nathan Stone, University of Arkansas at Pine Bluff
James T. Davis, Texas Agriculture Extension Service

Principal accomplishments

Specialized training was provided for over 90 extension scientists from at least 43 states during the National Extension Aquaculture Workshop held in March 1992 at the 4-H Center in Ferndale, Arkansas. Featured were speakers from many agencies and organizations that impact on aquaculture production, marketing, and federal policy in the United States.

This training was jointly sponsored by the five Regional Aquaculture Centers and the states involved. In addition to speakers from within the group, representatives from several universities, the United States Department of Agriculture, Department of Commerce, Corps of Engineers, Food and Drug Administration, and Department of Interior presented papers and led discussions. "Networking with other Professionals," "Use of Therapeutants," "Aquaculture Waste Management," and "The National Aquaculture Information Center" occupied participants on the first day of the workshop. There was also time for other discussion groups and visits to a very well-stocked and maintained media center. Many participants got an opportunity to discuss points of view with experienced extension scientists throughout the workshop. In addition, they were exposed to many resource materials of immediate use and became acquainted with other extension specialists to contact for information when it is needed.

On the second day, the discussion switched to "Fish and Shellfish Inspections," "Marketing and Processing," "Production and Marketing Economics," and an excellent panel discussion on "You and the Law." Later a discussion of how to interface with extension efforts of the Fish and Wildlife Service alerted many of the participants to the possibilities of funds available and other joint efforts. Though this day was concerned with economics rather than biology, the learning opportunities were tremendous. "Time Management," which is a concern for all professionals, led off the discussion on the workshop's third day. This session was followed by a discussion of "Environmental Impacts, Endangered Species and Non-Tidal Wetlands." Facility design covered ponds, raceways, and recirculating systems in a manner that all non-engineers could easily follow. Next on the agenda was a session on "Improved Communications" with discussions ranging from electronic information networks to teleconferencing. Guidance on the use and misuse of these systems was particularly enlightening.

The final day was spent in group discussions on how the Regional Centers can better serve their clientele, followed by a tour of two premier aquaculture installations. One of these, Anderson Minnow Farms, is reputed to be the largest such installation in the world. The other, Keo Farms, allowed many to see first-hand some of the complexities of commercial spawning and rearing of hybrid striped bass and triploid grass carp. Over 60 of the participants stayed for an additional day to receive in-depth training in production and marketing of either hybrid striped bass or bait minnows. These sessions featured speakers from the commercial sector as well as researchers and extension specialists. Evaluations from the participants have been tabulated, and the workshop was rated 4 on a scale of 1 to 5. Two objectives of this workshop were for participants to receive training in current subject matter and to understand new information transfer capabilities. These received very high evaluation scores, as did an improved understanding of national initiatives and guidance in enhancing coordination with other agencies. When asked "What did you get from the program?", 41 of 42 respondents indicated that they got the names of other people to contact for help in difficult situations, and 40 said they received new, usable resource material. Over one-half stated that they received answers to questions, ideas that they could try immediately, and most indicated a better understanding of RAC programs. As this was the first of these ever held, these indicate that the participants received excellent training as well as taking part in a varied and intensive program.

At this time, no decision has been made about conducting workshops of this nature in the future. However, the participants rated the need for future workshops at 4.6 out of a possible 5.0 after considering the time and travel costs involved, and most preferred 2 or 3 years between workshops. A few of the participants commented that the program was too intense and too long, and indicated that if it was shortened and the format modified they would recommend the workshop to others.

Impacts

The total effect of this workshop will not be realized for 5 to 10 years. At this stage, it is known that there has been increased cooperation across regional lines in planning of projects and better communication between specialists. Knowing aquaculture specialists in other parts of the United States has enabled many specialists to secure assistance with specialized problems.

Educational Materials For Aquaculturists And Consumers

Termination Report for the Period May 1, 1991 to August 31, 1994

Funding level

Year 1	$39,642
Year 2	$59,000
Year 3	$34,500
Total	$133,142

Participants

J. T. Davis, G. Fipps, B. Higginbotham, K. Ladewig, B. Lesikar, J. T. Lock, S. Schaer, E. Smith, G. Clary, D. Logan, and D. Gatlin, Texas A&M University

F. E. Baker, L. de la Bretonne, M. Moody, D. Bankston, W. Lorio, J. Avery, and G. Lutz, Louisiana State University

M. Beem, Oklahoma State University

M. Brunson, C. Tucker, M. van der Ploeg, and D. Crosby, Mississippi State University

C. Cichra, F. Chapman, R. Francis-Floyd, R. Rottman, J. Shireman, and P. A. Reed, University of Florida

R. Durborow and W. Wurts, Kentucky State University

T. Hennessey and Ekk Will Tropical Fish Farm (Florida)

T. K. Hill, University of Tennessee

J. Hinshaw, T. Losordo, North Carolina State University

J. Jensen, M. Masser, Auburn University

G. Lewis, G. A. Schuler, R. Gilbert, R. Tyson, and P. T. Tybor, University of Georgia

J. Rakocy, University of the Virgin Islands

J. Whetstone, Clemson University

Administrative advisor

Milo J. Shult, Vice President for Agriculture
University of Arkansas
Little Rock, Arkansas

Project objectives

1. Prepare and distribute publications needed by production aquaculturists to keep abreast of the latest research and development information available.
2. Prepare and distribute processing and marketing information that will enhance the market for aquacultural commodities.
3. Prepare and distribute information for retailers and consumers which will enhance the sales of fish and shellfish products grown by aquaculturists.

Principal accomplishments

A total of 41 fact sheets and four videos were prepared, edited, reproduced, and distributed during the period of the project.

Impacts

The SRAC fact sheets have become the standard for practical aquaculture publications in the United States. They are also in wide demand throughout the world. This is a direct measure of their acceptability and usefulness to producers and scientists. The numbers that have been distributed exceed 200,000 to date.

List of impacts:

- County Extension Agents using SRAC educational materials in their offices can provide definitive answers to most production problems.
- Processors now have another ready reference on how to improve their product safety record. In addition, a consumer can secure information about the wholesomeness and safety of a product before purchase.
- Specialists report a marked decrease in their workload as they can now provide written information and spend their time more fruitfully working with actual producers who require immediate attention to specific problems.
- Many individuals request SRAC materials to obtain information relative to beginning some type of aquaculture endeavor. After learning about the challenges and work involved, at least 80% of the people requesting information decide not to pursue such an endeavor. Estimated average savings of expenses and lost investments are approximately $30,000 per inquiry.
- Aquaculture producers looking for an alternate crop have been able to assess the market and the requirements of another species. They can then decide, based on scientific studies, whether their installation would meet the animal's requirements.

Publications

A complete list of all SRAC publications, videos, and computer software is presented in the Appendix at the end of the project summaries for the Southern Regional Aquaculture Center.

Characterization Of Finfish And Shellfish Aquacultural Effluents

Progress Report for the Period May 1, 1991 to August 31, 1994

Funding level

Year 1	$145,000
Year 2	$169,000
Year 3	$141,500
Total	$455,500

Participants

J. V. Shireman, University of Florida
C. E. Boyd, Auburn University
D. E. Brune, Clemson University
R. P. Romaire and D. C. Huffman, Louisiana State University
C. Tucker and J. Waldrop, Mississippi State University
T. M. Losordo and J. M. Hinshaw, North Carolina State University
J. T. Davis, Texas A&M University System
C. Engle, University of Ark. at Pine Bluff
G. J. Burtle, University of Georgia
J. S. Hopkins, Waddell Mariculture Center

Administrative advisor

John T. Woeste, Dean
Florida Cooperative Extension Service
University of Florida
Gainesville, Florida

Project objectives

1. Characterize aquaculture effluents for finfish and shellfish aquaculture production systems.
2. Determine best management practices and investigate available and new treatment technologies to maintain high effluent water quality. These technologies will include water reuse, conservation, and recycling techniques.
3. Compare the economics of the management practices and treatment technologies in objective 2.
4. Develop and disseminate educational materials and conduct demonstration projects for producers and policy makers. This objective will be conducted throughout the duration of this project.

Principal accomplishments

Aquacultural effluents were characterized for catfish levee ponds by Mississippi State University, watershed ponds by Auburn University, hybrid striped bass by Waddell Mariculture Center, and crawfish effluents by Louisiana State University.

The water quality data generated by sampling 20 commercial catfish ponds for 2 years in Northwest Mississippi was combined with a hydrological model of pond overflow volume to describe the effects of water management practices on discharge of nutrients and organic matter from ponds. Water quality and pond overflow varied seasonally, with poorest water quality in the summer quarter and greatest pond overflow in the winter and spring quarters. Combining the modeled overflow volumes and measured concentrations of pond water quality variables revealed that seasonal changes in overflow volume are more important than seasonal changes in the composition of the pond effluent in determining the mass of nutrients and organic matter discharged from Mississippi catfish ponds. Specifically, predicted mass discharge (kg/ha of pond surface) was greatest in the winter when overflow volume was maximum and not in the summer when concentrations of nutrients and organic matter in the pond were highest.

The model also showed that managing pond water levels to maintain water storage capacity greatly reduces waste discharge. For example, maintaining a minimum 7.5-cm water storage capacity reduced average discharge of nitrogen, phosphorus, and organic matter in pond overflow by about 70% compared to ponds not managed to maintain storage potential. The greatest relative reduction in predicted mass discharge of nutrients and organic matter occurred in the summer quarter because the general nature of summer rainfall in Northwest Mississippi (sporadic events of relatively short duration associated with convectional storms) is such that maintenance of water storage potential is particularly effective at capturing rainfall and reducing overflow. In an average summer, managing pond water levels to maintain water storage potential reduced the predicted discharge of nitrogen by over 91%, phosphorus by over 88%, chemical oxygen demand by over 91%, and biochemical oxygen demand by over 92% compared to summertime discharge from ponds managed without storage potential. The model further indicated that no nitrogen, phosphorus, or organic matter will be discharged from ponds managed to maintain storage potential during exceptionally dry years. Using water storage capacity to reduce discharge in the summer, is particularly important because the quality of potential pond effluents is poorest and stream flows are at their annual minimum, resulting in low rates of dilution of any water discharged from ponds.

Reducing overflow volume by maintaining water storage potential in ponds, appears to be a simple, inexpensive, and highly effective technique for reducing nutrient and organic matter discharge. In addition to the environmental benefits accruing from reduced waste discharge, maintenance of storage potential in ponds helps conserve ground water resources by dramatically reducing the need for pumped water to maintain pond water levels during fish culture. If surplus water storage capacity is available, rainfall is captured in the pond rather than lost as overflow, and the stored water helps offset evaporative and infiltration losses.

Water samples were collected from 25 commercial catfish ponds in Central and West-Central Alabama. Ranges for variables were as follows: BOD5; 1.9-35.54 mg/L; settleable solids; 0-1.8 mg/L; suspended solids; 5.2-336.7 mg/L; volatile solids; 0.02-221.0 mg/L; total phosphorous; 0-1.85 mg/L; soluble reactive phosphorous; 0-0.74 mg/L; total Kjeldahl nitrogen; 0.58-14.04 mg/L; total ammonia nitrogen; 0.008-8.071 mg/L; nitrite-nitrogen; 0.001-1.410 mg/L; nitrate-nitrogen; 0-6.661 mg/L; dissolved oxygen; 0.8-16.8 mg/L; pH; 4.9-9.5. Concentrations of water quality variables were skewed toward the lower ends of these ranges. Concentrations of some variables were occasionally higher than those normally encountered in natural streams and exceeded recommended effluent concentration limits.

Watershed ponds with maximum depths of 3-5 m, which thermally stratify during warm months, are sometimes used for commercial aquaculture. Hypolimnetic water in six watershed ponds for aquaculture in Alabama were depleted of dissolved oxygen and had 2.4-43.2 mg/L of ferrous iron, 0.01-0.25 mg/L of total manganese, 0.24-3.59 mg/L of total ammonia nitrogen, and 0.07-1.29 mg/L of total sulfide. Concentrations of nitrite-N and 5-day BOD were no higher in hypolimnetic water than in normal surface water of aquaculture ponds. Volumes of oxygen-depleted water averaged 3.2 to 20% of total pond volumes, but much greater volumes of pond water contained less than 3 or 5 mg/L of dissolved oxygen. Discharge of hypolimnetic water from aquaculture ponds into natural waterways could have a negative impact on water quality.

Seventeen commercial and two experimental crawfish ponds in South Central and Southwest Louisiana were selected for effluent characterization. The ponds represented cultivation systems including rice-crawfish ponds (rice-crawfish rotation, rice double cropping); sorghum-sudan grasses or colonized by native terrestrial/native aquatic vegetation); and wooded ponds (native terrestrial/native aquatic vegetation and leaf litter). Macrophytic standing crop was estimated in each pond in mid-October, mid-January, and mid-April with quadrant sampling. Effluent samples were collected on 4 days in a 2-week period in November 1991 (late fall), February 1992 (winter), and late March - early April 1992 (spring). Summer samples were collected on at least 3 days during pond draining from late April through early July. Water was analyzed for parameters decided upon by the SRAC working group.

The Waddell Mariculture Center (WMC) assessed commercial striped bass hybrid (SBH) pond and effluent water quality. An attempt has been made to include commercial ponds with a wide range of production goals, including fingerling production ponds. Saltwater and freshwater ponds were included as well. Since pond dynamics can result in dramatic short-term fluctuations in many water quality parameters, more frequent sampling of SBH ponds at WMC was done. WMC ponds include intensive juvenile production ponds and growout ponds stocked at two densities. The lower stocking density and feeding rate was close to the average of commercial farms. The higher density was approached or slightly exceeded the limits of fish production in ponds without water exchange. All data sets were sent to North Carolina State University for inclusion into data bases.

Excluding fingerling production ponds, the fish biomass encountered in the commercial ponds ranged from 450 to 12,500 lbs/ac with an average of 3,700 lbs/ac. Feeding rates for production ponds ranged from 2 to 133 lbs/ac/day with an average of 50 lbs/ac/day. It is generally believed that digestion processes within the pond were capable of assimilating about 100 lbs/ac/day of feed if supplemental aeration is available to maintain dissolved oxygen. Thus, at the higher feeding rates being used by some commercial SBH farms, a degree of water exchange may be necessary to transfer part of the digestion process to the receiving stream or highland crops. Indeed the highest water exchange rates have been encountered in these ponds with the highest feeding rates.

In summary, the potential for adverse environmental impact from effluents of striped bass hybrid ponds is no greater than that of catfish ponds. Through thoughtful and well-informed farm design and operation, the potential for environmental impact can be virtually eliminated.

In order to determine the best management practices (BMP) for catfish effluents it was necessary to determine the timing and quantities of effluents discharged from commercial catfish farms in Mississippi. Data were collected from commercial catfish farms with 10,413 water surface acres devoted to food fish production, 1,261 water surface acres devoted to fingerling production and hatcheries that hatch more than 100 million catfish fry annually. These data indicated that the following conclusions are warranted:

- the cost of pumping (economics) tends to minimize the amount of water used on catfish farms;
- food fish and fingerling production results in only about 20 inches of water per acre per year being discharged into the environment;
- discharge of water from excess rainfall for food fish producers and management practices (draining ponds prior to stocking fry) for fingerling producers account for most of the water released;
- the time of discharge is such that the water in ponds contains the seasonally lowest concentration of potential pollutants when discharged;
- the time of discharge is such that the receiving streams are at, or near, their maximum flow;
- hatchery discharge water presents few, if any, potential problems;
- based on these data and analysis, the commercial catfish industry presents minimal environmental pollution problems from effluent discharge; and
- there is no apparent need to develop a plan for discharge water treatment at this time.

Researchers in Mississippi also found that the best time to release effluents from catfish ponds was during the winter when water quality was best. This coincided with the rainy season when most effluents were released. The study also found that water quality from ponds not drained each year had suitable water quality as in ponds where biological processes reduced nutrients and organic matter in excess of a single pond-volume discharge. This results in less effluents and reduced pumping costs.

Research at Auburn University indicated that of the nitrogen, phosphorous, and BOD discharged, 50% was discharged in the last 15-20% of the effluent discharged. Of the settleable solids discharged, 50% was released in the last 5% of the effluent discharged. These findings suggest that the best way to minimize the pollution potential of aquaculture pond effluents is to harvest ponds as quickly as possible, and either to not discharge water during the seining phase or to discharge this highly contaminated water into a settling basin or retention pond. It is feasible to allow effluents to flow untreated into the environment during the pre-seining phase of draining as concentrations of potential pollutants are low. Results from undrained and drained ponds were similar in the Alabama study. It was concluded that harvest without draining was a feasible technique for reducing the pollution potential of catfish farming.

A study utilizing fish effluents for crop irrigation was conducted in Georgia. Both soybean and wheat were grown. It was concluded that pond effluent can be applied as irrigation water to crops to satisfy a portion of the nitrogen requirement, but little phosphorous is provided. Pond water is generally not changed by flushing (25%), but effluent application to cropland could reduce the volume of effluent reaching natural systems. Pond flushing and refilling may reduce the amount of emergency aeration required for channel catfish ponds.

The influence of water recirculation in outdoor ponds coupled with the use of filter feeding fish was investigated as a BMP at a commercial catfish operation in Southeast Texas. They found that effluent parameters were within the ranges reported by other authors. This research suggests, although the pond systems were managed inconsistently, that production was significantly higher in the recirculated ponds versus static ponds with only a slight increase in levels of some of the measured water quality parameters. Investigators at North Carolina State University researched the use of water hyacinth-based treatment systems and wetland plant nurseries for effluent treatment. To look at the effectiveness of the water hyacinth-based treatment system, a system was set up at Tidewater Research Station, Plymouth, NC. Two different flow rates of 8000 L/d and 16000 L/d were chosen and studied. The system was found to be fairly efficient in removing suspended solids. The highest removal efficiency was about 90% while the average removal efficiency was 60%. The average removal efficiency, at the lower rate of 8000 L/day, was 67% while the average removal efficiency at the higher flow rate of 16000 L/day was 51%. Some reduction of phosphates was observed in the system. The average removal efficiency at the lower flow rate was 16.5% while the average removal efficiency at a higher flow rate was 9.5%. The average removal efficiency for nitrates at the lower flow rate was 38% while the average efficiency at the higher flow rate was 11%. The average TKN removal efficiency at the lower rate was 42% while the average removal efficiency at the higher flow rate was 31%. Thus, better efficiency was obtained at the higher detention time. Though a reduction in ammonia-N content of the effluent was observed in some cases, overall, the ammonia-N content of the effluent did not show any appreciable reduction and seemed to increase in quite a few cases which could have been caused by:

a) decomposition of organic matter into ammonia;
b) fixation of atmospheric dinitrogen by blue-green algae; and
c) nitrifying bacteria not having a chance to establish themselves.

The use of aquaculture effluents to grow nursery plants was also investigated. The three wetland plant species propagated during the 2-year study grew very well with no additional nutrient input. From this perspective, aquaculture pond water/effluent is a suitable (low-cost) irrigation and nutrient source for the propagation of those plants. From an aquaculture effluent control point of view, the only major benefit was suspended solids removal. From a farmer's perspective, this is an important finding. Through the application of this technology or by utilizing a constructed wetland, aquaculture pond farmers have an alternative to discharging untreated waters.

Researchers from the University of Arkansas at Pine Bluff (UAPB) conducted economic analyses on effluent management strategies for catfish and hybrid striped bass production in ponds and for trout production in raceways. Effluent management strategies analyzed for the warmwater pond systems included: using pond water to irrigate rice, use of constructed wetlands, and use of filter-feeding fish stocked in ponds. For the trout model, effluent management strategies analyzed included: constructed wetlands, filter-feeding fish stocked in ponds fed by the raceways, and the use of settlement basins. Results showed that effluent treatment with these technologies would increase production costs by $0.02 to $0.05 per pound for catfish and $0.28 to $0.31 per pound for hybrid striped bass.

The use of effluent for crop irrigation was shown to be the least expensive treatment option. The technique selected for effluent treatment, however, will be determined by regulations and impact on production. However, all treatments analyzed reduced net farm revenues due to either treatment cost, optimal farm size, or stocking rates. This study also showed that imposing effluent control will create barriers for new catfish farmers, particularly small-scale (less than 320 acres) farms.

An economic engineering approach was used to construct cost estimates for the parameters is shown below:

- three levels of production
  - small - 30,000 to 50,000 lbs/yr
  - medium - 100,000 lbs/yr
  - large - 230,000 lbs/yr
- two raceway sizes
  - 6’X 35’X 3'
  - 12’X 70’X 3'
- three flow rates
  - 500 gpm
  - 1500 gpm
  - 3000 gpm
- two management levels
  - new
  - experienced
- two final fish weights
  - 14 oz
  - 48 oz
- five effluent treatment methods
  - surface flow constructed wetlands
  - subsurface flow constructed wetlands
  - settlement basin
  - filter-feeding bighead carp
  - no treatment

Filter-feeding bighead carp had an ability to reduce the BOD level. Only when BOD levels were reduced to 2.5 mg/L did the constructed wetlands become feasible. The wetland system appeared to be more cost-effective at lower water flow rates and with smaller fish. Bighead carp production may not be practical in all trout producing regions. When bighead carp were removed from the model, net returns remained the same, but water flow rates (500 gpm) were reduced. When a tax of $60 per mg/L BOD discharged was levied, net returns were reduced. However, new producers or smaller farms in the model, paid the tax on effluent discharge rather than built constructed wetlands. Net returns were reduced by 1% to 2%.
A tremendous amount of work has been done by scores of researchers on the characterization of effluent from flow-through fish systems, particularly those from salmonid production. The enormous variation seen in the reported values illustrates the importance of factors such as mode of operation during measurement, stocking density, composition of feed and feed conversion efficiency, and the intensity of water use. Designers of systems for waste treatment options must consider very site-specific and management-specific parameters in planning to meet the myriad of environmental regulations applicable to flow-through systems in aquaculture.

Impacts

The data collected in this study characterized aquaculture effluents and should provide lawmakers and regulatory officials a rational basis for generating regulations regarding aquaculture pond effluents. The data also provides insights into methods for reducing the impact of discharge from aquaculture facilities and can be used to produce industry-wide, best management practices to address the effluent issue.

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Food Safety And Sanitation Of Aquacultural Products: Microbial

Progress Report for the Period April 1, 1992 to August 31, 1995

Funding level

Year 1	$85,000
Year 2	$225,000
Year 3	$260,000
Total	$570,000

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Project objectives

1a. Collect data that are available to define aquacultured food safety problems and to design a control program.
1b. Conduct a forum to assess all relevant data on food safety of aquacultured foods. This event will assemble all knowledgeable individuals that can bring their expertise to bear on this subject.
1c. Prepare and distribute a bibliography of the available publications, mimeographs, fact sheets, and videos relative to food safety and sanitation in the aquaculture industry.
1d. Evaluate data on microbiological quality in catfish, crawfish, and rainbow trout processing and distribution operations. Determine if there are critical control points which need attention.
1e. Do supplemental laboratory work to clarify areas of concern. This is designed to fill gaps in the database, not to conduct an industry-wide survey.
2. Investigate various methods to reduce and detect significant pathogenic and spoilage microorganisms on processed catfish, rainbow trout, and crawfish.
3. Conduct a food safety Hazard Analysis of Critical Control Points (HACCP) audit to determine if this approach would be cost-effective and result in increased product safety.
4. Produce new publications to complement existing publications on food safety and sanitation.

Principal accomplishments

Objective 1a

Efforts to assess the food safety of southern aquacultured products based on reported illnesses, literature reviews, and liaison with numerous related government programs reveal that cultured fish represent the safest source of muscle protein and related nutrients among all muscle foods produced in the United States. Cultured molluscan shellfish, which in the Southern Region are primarily hard clams, are more suspect for potential microbial foodborne illnesses, yet actual reported illnesses do not reflect any significant reporting of occurrences from cultured molluscan products. Likewise, shrimp and crawfish, as cooked, ready-to-eat items, are suspect, but not evidenced as problems. These conclusions are based on updated literature reviews per the previously identified sources through 1995 and supplements from pertinent agencies in regional, state, and federal programs.

The few reported illnesses associated with consumption of clams, *Mercenaria campechensis*, harvested in Florida involved encounters with the potential pathogen bacteria, *Vibrio vulnificus*. These limited illnesses typically involve “at-risk” consumers in that they have health conditions that compromise their immunity to infections introduced by consumption. These pathogens are indigenous to southern coastal waters approved for shellfish production, particularly during warmer months (April - October). In order to help reduce the levels of *Vibrio vulnificus* on oysters and related shellfish, the Interstate Shellfish Sanitation Conference (ISSC) introduced (August 1995) a new time-temperature harvesting scheme to retard growth of the bacteria on harvested products. The new harvesting guidelines, linked to growing water temperatures, are referenced in the Total Quality Assurance (TQA) and Hazard Analysis and Critical Control Point Manuals generated, in part, by this SRAC project.

Agency liaison through this project has matured into a number of collaborative projects to implement respective control measures for aquatic food product safety during processing. Meetings with the pertinent regional state agencies and their professional association, AFDOSS (Association of Food & Drug Officials of Southern States), have led to a formal partnership called the "Seafood HACCP Alliance for Education and Training." This partnership, formalized in June 1994 with support from the National Sea Grant Office, has developed a network of Cooperative Extension Services and related Sea Grant Advisory Programs working with representatives from the FDA Office of Seafoods, USDA, National Marine Fisheries Service, and all respective regional AFDO (Association of Food & Drug Officials) affiliates to design and deliver a uniform HACCP education and training program for all aquatic food processors. Aquatic foods include all seafoods (harvested, cultured, or imported).

SRAC's objective 1a provided the initial opportunity for the project personnel to draft and advance the "Alliance" concept in conjunction with the AFDOSS (AFDO of Southern States) organization. This educational "Alliance" will offer continuing controls for aquacultured product safety through the joint development of "core HACCP curriculum," establishment of a cadre of HACCP instructors, pilot-testing in processing firms, and maintenance of a "compendium" of approved processing methods and recommendations for HACCP monitoring and record keeping. To date the investigators have assisted in preparation of HACCP training modules for processing of molluscan shellfish and crawfish, which can include cultured products. This involvement assures a role for SRAC in providing essential HACCP training for aquacultured production and processing in Southern states.

Concurrent with the aforelisted activity, this project has helped foster regional collaboration in a joint USDA Extension Service project, "Implementation of TQA and HACCP Concepts for Processing Aquacultured Products." This project has been completed with the development and in-plant testing of HACCP programs for cultured molluscan shellfish (Steve Otwell, University of Florida with Mike Moody, Louisiana State University) and catfish (Anna Hood, Mississippi State University with George Flick, Virginia Tech). The second year of work will continue with cultured crawfish (Mike Moody, LSU with Steve Otwell, University of Florida) and trout (George Flick, Virginia Tech with Anna Hood, MSU). The SRAC project initiated this collaboration. Likewise, as a consequence of this work, the SRAC investigator has been asked to serve as the chairman of the Interstate Shellfish Sanitation Conference's HACCP Committee to investigate the integration of proposed HACCP concepts and regulations within the existing federal manuals which govern the production and processing of all cultured and natural harvested bivalves.

Two HACCP manuals for cultured shellfish have been generated by this work in conjunction with SRAC.

Objective 1b

An Aquaculture Safety Forum was held February 2-4, 1993, at the Auburn University Hotel and Conference Center. The 2 1/2-day forum brought approximately 45 industry, academic, and government agency representatives from 11 Southeastern states together to assess all the relevant data available on the safety of aquacultured foods. The plenary sessions provided opportunities for aquaculture researchers and Extension workers to update the group on recent research findings and other current topics. The breakout sessions afforded an opportunity for Forum participants to develop "white papers" about the present status and future needs of the chemical and microbial aspects of aquacultured foods in the Southeastern United States. All Forum participants received an evaluation form on which their perceptions of various aspects of the Forum could be rated on a scale of 1 (=Poor) to 5 (=Very Good). Forty percent of the evaluations were completed and returned. The following ratings are presented as means +/- standard deviations. The overall format of the Forum received a very favorable rating of

4.72 +/- 0.45. The attendees were also favorably impressed with the strength of the agenda (4.67 +/- 0.47) and the quality of the speakers (4.61 +/- 0.49). Evaluation respondents indicated that the degree to which the Forum addressed the issue of aquaculture products safety merited a rating of 4.56 +/- 0.60.

A 157-page "Proceedings of the Aquaculture Products Safety Forum" was produced. The Proceedings included transcripts of 20 formal presentations made during the plenary sessions, plus recommendations made by working groups regarding microbial and chemical safety of aquacultured food products. Nearly 275 copies of the Forum Proceedings were distributed to Extension workers, researchers, and government agency representatives in 33 states plus Puerto Rico and the Virgin Islands. While no formalized method was established to evaluate the Proceedings, informal comments were positive with regard to content, utility, layout, and design of the Proceedings.

A 60-minute live, interactive "Aquaculture Products Safety" satellite videoconference was produced which highlighted the objectives and recommendations developed during the Forum. Among the issues discussed were HACCP method of fishery product inspection, microbial aspects of aquaculture safety, and chemical residues and their relation to aquaculture safety. Another portion of the videoconference presented videotaped excerpts of interviews (conducted during the Forum) in which the interviewees discussed what they perceived as the greatest needs related to aquaculture safety, and what would be the most appropriate ways to address those needs. A final segment of the program was devoted to questions and answers, some of them phoned in from interested viewers.

Videoconference Evaluation Forms were sent to each Alabama Cooperative Extension Service County Office. Although relatively few of the forms were returned, those who did respond felt the videoconference was worthwhile. Ratings of the panelists' presentations ranged from "useful" to "very useful." The interactive segment of the program was deemed beneficial, with viewers feeling "somewhat involved." The technical quality of the production received ratings that ranged from good to excellent.

Objective 1c

Academic institutions, governmental agencies, and companies involved in processing aquacultured products were contacted. Requests were made to notify the researchers of any documentation within their groups that pertained to food safety and sanitation, as related to the aquaculture industry. Information to be included in the bibliography has been compiled and final corrections are being made. Copies of the bibliography will be made and distributed to those involved in freshwater aquaculture.

Objective 1d

Evaluation of microbial data (aerobic plate counts, total coliforms, and *E. coli*), representing five replicate samples from each stage of processing collected on five separate occasions over a 1.5-year period, has revealed a critical control point. The magnitude of the increase observed at the critical control point in processing is not significantly different between seasons. Process modifications evaluated to date have not significantly reduced the impact of the critical control point on the overall microbial load. Additional process modifications, such as chlorine sprays, are currently being evaluated by the processor as a means of reducing microbial loads after the critical point in processing.

Sampling of processing plants during different seasons demonstrated that aerobic plate, total coliform and *E. coli* counts are affected by season. During warmer months of spring and summer, all parameters measured increased 10- to 100-fold. The increase was associated with higher surface microbial loads of catfish entering the processing plant. Although pond water was not analyzed, a change in water quality during the warmer months is probably the origin of the increase, because the level of microorganisms on fish is usually a function of the microbial content of the water. Methods of controlling waste production, excess nutrients, and other parameters within the catfish ponds during this critical time period, should be evaluated to determine if initial surface microbial loads of catfish can be reduced prior to entering the processing plant.

A pathogen survey of fully-processed catfish fillets was conducted in conjunction with Virginia Polytechnic Institute and State University and Mississippi State University. Twenty fully-processed fillets from each of three processing plants were collected on a quarterly basis for 1 year. Investigators from Auburn University were assigned analyses for the following three pathogens: *Edwardsiella, Salmonella,* and *Shigella*. *Edwardsiella*, which is primarily a pathogen of fish, was isolated from 12.3% of 220 fillets sampled. *Salmonella* and *Shigella* were less common and were isolated from 2.3% and 1.8% of fillets sampled, respectively. A slight seasonal increase in the number of fillets harboring *Edwardsiella* was observed, with 63% of the positives occurring in the spring and summer. A similar trend was not observed for *Salmonella* and *Shigella,* which were isolated on rare occasions throughout the year. The incidence of *Salmonella* and *Shigella* on catfish is lower than reported incidences for most other raw meats.

Glycerol monolaurate (monolaurin) inhibition of *Listeria monocytogenes* was affected by pH and testing medium; monolaurin activity increased as pH decreased. Monolaurin interacted additively with citric acid and synergistically with acetic acid, benzoic acid, and lactic acid to inhibit *L. monocytogenes*. For growth prevention or destruction of *L. monocytogenes* in crawfish tail meat, 224 or 336 mM lactic acid was required, respectively. Destruction of the bacterium could be achieved with 224 mM lactic acid when 0.72 mM monolaurin was added.

Citric acid or potassium sorbate sprays applied to crawfish tail meat to a final concentration of 0.03 g/kg did not prevent growth of *L. monocytogenes* at 4°C. Potassium sorbate did, however, extend lag phase of the bacterium by 2 days. Thus, these treatments were not effective to control the bacterium.

Modified atmosphere packaging (MAP; 74.8% carbon dioxide, 10.4% oxygen, and 14.8% nitrogen) inhibited growth of *L. monocytogenes* in crawfish tail meat treated with 0 and 1% lactic acid (LA) and stored at 4°C when compared to air and vacuum packaging. No differences in effectiveness of the packaging atmospheres were observed with 2% LA. Addition of 200 micrograms/g glycerol monolaurate (ML) with 1% LA inactivated *L. monocytogenes* for 20 days at 4°C in each packaging atmosphere. This treatment reduced pH from 7.4 to 5.4.

Lactic acid addition to crawfish tail meat could increase the resistance of *L. monocytogenes* pasteurization temperatures. D60 values of *L. monocytogenes* in tail meat treated with 0, 0.5, 0.75, and 1% lactic acid were 4.68, 4.41, 3.46, and 2.49 minutes, respectively. Atmosphere surrounding the tail meat, whether air, oxygen, carbon dioxide, or nitrogen, had no apparent effect on heat resistance of the bacterium. *L. monocytogenes,* at levels naturally occurring, can be eliminated from crawfish tail meat by treatment with heat alone (60°C for 15 minutes) or heat (60°C for eight minutes) combined with 1% lactic acid. Crawfish tail meat dipped in 1% lactic acid were brighter in color (enhanced redness and whiteness), had reduced fishy odor, and were firmer than untreated controls. These character changes were not disliked by taste panelists.

The effects of monolaurin and lactic acid, singly or combined, on *L. monocytogenes* attached to catfish fillets revealed that monolaurin (up to 400 micrograms/ml) had no influence on counts. Conversely, lactic acid-treated fillets had reduced counts compared to controls. Dipping in 0.85, 1.70, or 2.55% lactic acid for 30 minutes reduced counts by 0.9, 1.4, or 1.3 logs, respectively. Extending dipping time to 60 minutes resulted in little additional decrease in counts. Combining monolaurin with lactic acid yielded results similar to lactic acid alone. Hence, population reduction ability of the two compounds resides with lactic acid and not monolaurin.

Planktonic and adherent cells of *L. monocytogenes* were subjected to heat, monolaurin, and acetic acid to evaluate biofilm removal from stainless steel. Planktonic cells were more sensitive than attached cells to the physical and chemical treatments. Effectiveness of 100 micrograms/ml monolaurin on destruction of biofilm cells was increased when combined with heat (60°C) or acetic acid (1%). Old biofilm cells (7 days) were more resistant than young biofilm cells (1 day) to the treatments. Cells in a rich nutrient environment were more resistant than those in a depleted nutrient environment. Results suggest that eradication of cells in biofilms is more easily accomplished when biofilms are young. Processors of aquacultured products should have frequent, routine cleaning and sanitation programs to minimize biofilm problems.

The growth and toxin production of *C. botulinum* type E in rainbow trout fillets held in vacuum skin packaging indicated no toxin was produced by *C. botulinum* type E in fillets stored at <3°C. This advanced packaging method improved the shelf-life and product appearance. The use of modified atmospheres at 10°C had little practical usefulness. Carbonic acid dips caused a slight reduction in microflora of trout (approximately a 1-log reduction); however, the effect was negated by the additional handling and cost involved in preparation of the dip. No *Salmonella* or *Listeria* were detected in any sample of rainbow trout during the study using FDA and USDA isolation and confirmation protocols.

Higher concentrations of carbon dioxide (60% and 100%) in modified atmospheres with no addition of oxygen extended shelf-life of trout fillets at least 7 days longer than trout packaged in atmospheres containing oxygen at 3°C. The presence of oxygen in atmospheres encouraged growth of aerobic bacteria, psychrotrophic bacteria, yeasts, aerobic sporeformers, coliforms, proteolytic, and lipolytic bacteria. The odor and appearance of fillets packaged in atmospheres containing oxygen were significantly less acceptable ($P \leq 0.05$). Proteolytic and lipolytic bacteria were extremely sensitive to high carbon dioxide atmospheres resulting in a 4 to 6 log difference after 10 -15 days of storage. The more rapid spoilage in trout packaged in oxygen containing atmospheres was probably due to breakdown of amino acids, fatty acid, and non-protein nitrogenous compounds by lipolytic and proteolytic bacteria.

Objective 1e

Data collected from each stage of processing from whole fish to finished product reveal that the overall microbial load on catfish increases by 100- to 1000-fold during processing. Three conditions are chiefly responsible for the increase: (1) contamination of flesh from equipment surfaces after the skin is removed; (2) growth of contaminating organisms during processing and holding; and (3) cross contamination of fillets during specific stages of processing. Analysis of farm-raised catfish fillets from several retail markets indicated an additional 100-fold increase in the microbial load during distribution and final marketing. Although differences in aerobic, total coliform, and *E. coli* counts were evident among retail markets, it is not possible from these data to determine the cause of the higher microbial loads. The incidence of three pathogens, *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* species on retail catfish fillets was also determined. *L. monocytogenes* was isolated from five fillets (5.4%), no *E. coli* O157:H7 was detected and a *Salmonella* species was isolated from one fillet (1.1%). Contrary to expected trends, no correlation existed between retail markets with high aerobic, total coliform, *E. coli* counts, and the incidence of the three pathogens. Data collected from these studies continues to build a database essential in determining the stages of processing, distribution, and marketing. This significantly impacts the microbial quality of farm-raised catfish and can be used to develop alternative processing and handling procedures to further improve the microbial quality and safety of catfish products.

Objective 2

Both indicative and pathogenic microbial flora of fresh aquacultured channel catfish fillets were sampled to evaluate the microbiological quality. Three catfish processors participated and were selected, with consent, to represent small through large processors. Products were screened during four different seasons (e.g., summer, fall, winter, and spring) and there were significant differences in the microbiological quality of fillets due to processing conditions and production seasons. All of the processors produced products that were acceptable from a microbiological safety and quality perspective. However, there was a problem with *E. coli* counts obtained from some of the products (*E. coli* counts in products were high during the summer season and decreased with relatively cooler weather). Some processors have purchase specifications based on *E. coli* counts for their products, and therefore meeting the specification could be difficult at times. It should be realized that not all *E. coli* cells are pathogenic.

Fresh catfish fillets were also screened for common and new and emerging pathogens. Products were obtained from the same processors and were analyzed at four different times of the year. In all, 120 samples were examined for each of the 11 pathogens, including *Campylobacter jejuni/coli*, *Escherichia coli* O157:H7, *Klebsiella pneumoniae* subspp. *pneumoniae*, *Plesiomonas shigelloides*, and *Vibrio cholerae*. *P. shigelloides*, an emerging pathogen, and *V. cholerae* were isolated from the fresh catfish fillets. Frequency of isolation of *P. shigelloides* was very low and the pathogen was isolated only when the weather was warm. The isolation rate for *V. cholerae* was higher, but was also isolated only during the warmer weather. Since these products are raw and are not consumed without cooking, the need for proper product handling after processing through the distribution system should be emphasized. Aquacultured products have a history of product safety and the combination of proper handling and cooking will prevent foodborne illness from these pathogens.

Fresh aquacultured catfish fillets were also surveyed for their antibiotic-resistant bacteria during the various seasons. There was a significant difference in the number of antibiotic resistant bacteria which was attributed to the production source.

To improve the quality of the fresh catfish products, several mechanical and chemical processes were evaluated. High pressure spray washing has been evaluated using water at low temperature. Chemical agents (lactic, propionic, and acetic acids) were also added to water to increase shelf-life of the products. Propionic acid was the best acidulant in reducing bacterial counts in broth cultures. However, during washing tests, all acids exhibited similar results by reducing bacterial counts by 1.0 log cycle.

L. monocytogenes does not appear to be very prevalent in aquaculture ponds or on whole catfish; contamination of fillets appears to occur post-harvest, and can be present on more than 30% of fillets.

Catfish fillets, prepared by harvesting and processing in an industrial setting at L & R Aqua Catfish Farms in Damon, TX, were treated by tumbling with 0.5, 1.0, 2.0, and 3.0% lactic acid for 1 or 3 minutes, followed by draining through placement of the fillet in a stainless steel, perforated tub for 1 minute. Each group of treated fillets was placed in individual 100-L plastic bags and held in refrigerated storage at 4°C with ice surrounding the bags.

In general, lactic acid treatments extended the shelf life of catfish fillets 2-3 days compared to the control. Higher concentration lactic acid treatments imparted a discoloration to the fillets, but no detectable off-odors were noted. Total volatile nitrogen values remained constant at around 22-24 mg/100 g for all treatments. Trimethylamine values similarly remained unchanged during the shelf-life study.

A procedure was developed and evaluated for a non-destructive method of sampling channel catfish for bacteriological analysis. The procedure is applicable for sampling of processed fish, fillets, frozen, and breaded products. Benefits of the procedure include: improved sensitivity of microbial detection, reduced time and cost of sample preparation for microbial analysis, and non-destructive, and easy accomplishment. The procedure has been standardized to maximize the detection of microbes on processed catfish, and data are being prepared to seek approval of the procedure as an official method of microbial sampling of processed catfish. The procedure was evaluated for the detection of bacterial pathogens. Studies have demonstrated that the rinse technique can consistently recover as few as five viable *E. coli* O157:H7, three viable *S. typhimurium*, and four viable *L. monocytogenes* inoculated per catfish fillet. Because the procedure involves sampling the entire surface of the processed fish and includes two pathogen enrichment steps, it can detect low numbers of pathogens on the surface of catfish.

A commercially-available, 1-day, rapid *E. coli* enumeration test (EC Petrifilm 3M Company) was evaluated and compared to a standardized FDA procedure. Data revealed that the rapid test provides accurate counts in 1/8 the amount of time as compared to the FDA procedure. The rapid test will allow processors to ensure the microbial quality of their product prior to shipment to the public.

Objective 3

A HACCP audit sheet was developed and evaluated by a commercial processor in Mississippi. The audit sheet will be available to those who are interested.

Objective 4

Reports and relevant information from research scientists as well as a literature review dealing with the food safety/microbial study issues have been obtained. Representatives of both processor and consumer groups were interviewed; both groups agreed that negative media coverage has resulted in consumers perceiving that numerous fishery products are unsafe, and that there is a need for written materials to bolster consumers' confidence in aquacultured products.

Approximately 70 published articles, fact sheets, videos, and oral presentations at technical and consumer meetings have been produced during the course of the project. Information on foodborne illnesses due to microbiological contamination, has been included in fact sheets and brochures. Valuable information including the purchasing, storage, handling, preparation, temperature control, spoilage, and processing of aquacultured products has been produced. Publications have been written on pesticides, residues, risks, chemicals, and regulators and their role in microbial food safety. All the information collected has produced substantial amounts of educational materials for the aquaculture industry and the general public.

Impacts

Objective 1a

Reviews of previous and current literature and data sources further substantiate the food safety status for southern aquacultured products. Project activity helped found a national "Seafood HACCP Alliance for Education and Training" which will lead to more uniform implementation of control measures for aquacultured product safety. Project activity also fostered cooperative projects funded by USDA to implement HACCP programs in actual aquaculture process settings for cultured bivalves, catfish, crawfish, and trout. Manuals for commercial guidance in implementation of TQA & HACCP programs are now available for cultured oysters and clams.

Objective 1b

Participant and viewer evaluation results were mentioned previously and demonstrate the very positive impacts that objective 1b had on three distinct audiences. Since the conclusion of the "Forum Project," many researchers, Extension workers, and government agency personnel have commented as to the "focusing" effect produced by the Forum. Many believe that the Aquaculture Products Safety Forum helped to reduce the amount of overlap and increase the complementary nature of subsequent aquacultured products research and Extension efforts in the Southeast.

Objective 1c

The bibliography on available information on food safety and sanitation as related to the freshwater aquaculture industry has provided a vital resource to those interested in these topics. The information is available both in the printed form and on computer discs.

Objective 1d

The aquaculture industry has benefitted directly from technical information generated in this project. Processing steps have been identified where microbial counts increase faster than at other processing sites, which makes these sites targets to control microbial proliferation, thereby improving quality and microbial safety of the processed product. The project has focused directly on conditions which promote quality and safety of marketed aquaculture products. The training of processing plant personnel during the collection of base line data and the feedback of data to the industry, will have a major impact on successful implementation of the HACCP concept and on the safety of aquaculture products.

Methods have been tested for the prevention of growth or destruction of the human pathogen *L.monocytogenes* on precooked ready-to-eat crawfish tail meat, and the inhibition of *C. botulinum* toxin in rainbow trout fillets. These methods can prevent costly foodborne outbreaks associated with these bacteria.

It has been demonstrated that the shelf-life of rainbow trout can be extended by 1 week at 3°C by packaging with 60% carbon dioxide and 40% nitrogen. Also, packaging of trout under 60% carbon dioxide/40% nitrogen did not significantly increase anaerobic spore counts during the 21-day storage period at 3°C.

Objective 1e

Substantial resources in terms of expertise and man-power have been provided to build a microbial database for processed catfish, which were previously not available to the aquaculture industry.

Objective 2

Catfish processors have continued to express an interest to initiate a total quality assurance program to meet present and future market demands for increased quality assurance of fresh and processed products. To assist the processors in this effort, a microbiological-based quality evaluation was performed on finished products. The microbiological-based quality evaluation program included a routine microbial evaluation of the processed products for selective indicative bacteria (aerobic, fecal coliform, and *E. coli* counts). Currently there are no standards in the United States for dressed fresh/frozen catfish products using indicative microbes as the criterion. However, there are general standards for fresh fish products developed by The International Commission on Microbiological Specifications for Foods. Wholesale distributors in the United States and Canada are stipulating purchase specifications for catfish products that processors must meet in order to prevent their rejection. These specifications are particularly directed toward *E. coli* counts even though all *E. coli* are not pathogenic. Unfortunately, the buyer's specifications that reflect high standards may not be consistently achieved during processing. The indicative microbiological quality control program could define the development of realistic and achievable standards. Processors could use these standards to market their products while assuring both product quality and safety.

To illustrate the benefit, if a buyer specifies a product count less than x cfu/g and the cfu/g are lower than the specifications by 10 fold, the processor may be able to request a premium price for the higher quality. Domestic consumers, however, are less quality conscious than the Canadian, European, and Japanese consumers. The European and Japanese consumers will pay premium prices for product quality. Processors have expressed a desire to expand their markets to Europe and Japan where, generally, the standards are higher. Also, these markets are desirable since they have the potential for greater profits. Thus, an indicative microbiological quality control program could be beneficial for the processor.

Listeria monocytogenes contamination of foods remains the focus of vigorous efforts by government and industry concerned with food safety, especially that related to ready-to-eat products. In a highly competitive market, as in the catfish industry, processes that could reduce initial numbers of bacteria on fillets or extend shelf-life and improve product safety, would result in a more stable economic environment for the catfish processor. Due to the decline of red meat consumption and steady increase in aquaculture fish consumption, this information may be vital to the industry by providing direction for future quality control programs.

Significant reductions in microbial populations could be obtained at processing facilities with alternative unit processing operations, such as the application of irradiation energy, the use of microbicidal chemicals in chilling waters, or temperature adjustments from harvest through product storage. The project has indicated that catfish products have pathogenic microbial profiles to prevent food borne illness in normal individuals. Also, pathogenic microbial profiles should permit international sales in most foreign countries.

The non-destructive procedure for sampling catfish is presently being used at the Fish Farming Center (Alabama Cooperative Extension Service) to evaluate catfish products from processing plants in Alabama. The rapid *E. coli* enumeration test will allow processors to ensure the microbial quality of their product prior to shipment to the general public and to demonstrate compliance with specific microbial specifications established by individual buyers in domestic and foreign markets.

Anti-listerial processing aids, such as Alta 2341, dehydroacetic acid, and nisin, can markedly lower levels of *L. monocytogenes* on catfish fillets. Ice impregnated with these antimicrobials reduces *L. monocytogenes* on catfish fillets and would incorporate easily into current retail practices.

Some improvement in shelf-life of catfish fillets can be obtained through lactic acid treatment at processing. However, the benefit of increase in shelf-life (2-3 days) would have to be weighed against increased processing costs to determine probable positive impacts of the treatment.

Objective 3

The HACCP audit will become a quality assurance tool for the aquaculture processing industry and result in increased economic benefits in the future. The audit form developed can be adopted or modified to fit individual needs.

Objective 4

Information gathered for this project will be a valuable tool for improving the microbiological safety of aquaculture products. This information will be given to state cooperative extension services to relay to the aquaculture industry because their knowledge of target industries and individuals within their state makes this method of education very effective. No other means are presently available to adequately relay this information than by state cooperative extension services.

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Theses or Dissertations

Bolton, L. F. 1993. Effects of antimicrobial agents and vacuum-skin packaging on shelf life of rainbow trout during refrigerated storage. M.S. Thesis. University of Georgia.
Denton, M. 1993. The occurrence and growth of *Salmonella* on rainbow trout fillets packaged in different atmospheres. M.S. Thesis. The University of Tennessee.
Dorsa, W. J. 1994. Effects of heat, lactic acid, and modified atmosphere packaging on *Listeria monocytogenes* on cooked crawfish tail meat. Ph.D. Dissertation. Louisiana State University.
Jackson, M. 1994. Microbial safety and quality of refrigerated trout using modified atmosphere packaging. M.S. Thesis. The University of Tennessee.
Liu, H. 1994. A rinse procedure for bacterial analysis of processed, farm-raised catfish products. Ph.D. Dissertation. Auburn University .
Pothuri, V. D. P. 1994. Combined effects of packaging atmospheres and chemical preservatives on the growth and survival *of Listeria monocytogenes* in crawfish tail meat. M.S. Thesis. Louisiana State University.

Aquaculture Food Safety: Residues

Termination Report for the Period September 11, 1992 to August 31, 1996

Funding level

Year 1	$100,000
Year 2	$155,000
Year 3	$101,000
Total	$356,000

Participants

G. Lewis, J. Shelton, P. Bush, C.R. Santerre, The University of Georgia
C. I. Wei, University of Florida
E. G. Alley, L. G. Lane, Mississippi State University
W. Rodgers, D. Xu, Auburn University
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D. G., J. T. Davis, Texas A&M University

Administrative advisor

Dr. Neal Thompson, Professor
University of Florida
Gainesville, Florida

Project objectives

1. Survey and review databases for pesticides, metal, and pharmaceutical residues in farm-raised catfish, crawfish, and rainbow trout.
2. Develop protocols and guidelines for a residue monitoring program at processing facilities.
3. Develop educational materials based on available literature on residues in farm-raised catfish, crawfish, and rainbow trout.
4. Develop a chemical application record-keeping system for producers.
5. Determine the fate of pesticides, metal, and pharmaceutical residues from the farm to the processing plant and finally to a product which would be prepared by the consumer.
6. Develop educational materials to make available information on residues in farm-raised channel catfish, crawfish, and rainbow trout so that consumers can more realistically assess issues related to consumer safety from scientific data.

Progress and principal accomplishments

Objective 1

A report was produced from data retrieved from FOODCONTAM, a national database consisting of state-generated information on pesticide and other toxic chemical residues in human foods. Information in this database are generated by state agriculture, food, and health protection agencies responsible for assuring the quality and safety of foods grown or imparted into their states. The data are presented in five separate computer-generated packets for FY 1986-1989, FY 1990-1992, FY 1993, FY 1994, and FY 1995.

Objective 2

Protocols and guidelines were developed and disseminated for a residue monitoring program. These protocols were used in collecting and analyzing residue data.

Objective 3

Development of educational materials was redirected to the SRAC Publications, Videos, and Computer Software Project.

Objective 4

A chemical application record system was developed in the third year of this project.

Objective 5

Work was concluded to determine the fate of residues during the processing of fish. Analytical methods for pharmaceutical compounds were developed at the University of Florida. Dosing of fish with antibiotics or pesticides and collection of fillets was completed at Auburn University. Processing of the dosed fish was done at the University of Georgia. Fish samples were analyzed at Florida and Auburn University for antibiotic residue analysis on raw and processed fillets. The University of Florida completed analysis of lipid and moisture concentration on samples. Two of six pesticides were analyzed at the University of Georgia

State University. Production studies involving residues from antibiotics applied during production were completed at Texas A&M University.

Objective 6

Progress for the first 3 years included: development of standard operating procedures for sample collection, sample preparation, pesticide analysis, metal analysis, and quality assurance. Producers' and processors' samples were submitted to the University of Georgia for sample preparation. Analysis of samples was completed at the University of Georgia and Mississippi State University. Residue data will be provided to cooperators in the eight states. Farm-raised channel catfish, rainbow trout, and red swamp crawfish were collected from commercial ponds and processing sites at intervals for the 2-year period. Locations for sample collection are as follows:

Location	Catfish Processors	Catfish Pond Sites	Rainbow Trout Pond Sites	Crawfish Production Sites
Mississippi	3	4	—	—
Alabama	2	3	—	—
Georgia	—	4	2	—
Louisiana	2	3	—	3
Tennessee	—	3	3	—
Florida	2	4	—	—
Texas	2	4	—	2
N. Carolina	—	—	20*	—
Total	11	25	25	5

*Samples were only submitted following a single collection from this location.

Pond sites for channel catfish, rainbow trout, and red swamp crawfish were selected to obtain diverse and representative sampling sites from each state. Catfish and rainbow trout were harvested from ponds within each state; fillets, including bellyflap, were collected and frozen. Crawfish were harvested; raw tail flesh was obtained and frozen. In addition, catfish, rainbow trout, and crawfish feed were collected for analyses when fish with elevated residues were found. Catfish fillets obtained from commercial processing facilities were collected and frozen. Frozen samples were shipped to the University of Georgia where a composite sample was coded, homogenized, frozen, and distributed to analytical facilities. Samples are being maintained below 0°C for 5 years for future retesting or additional residue analyses. Analyses for the following chemicals have been conducted:

Organochlorine
PCB's (1242, 1248, 1254, 1260)
Chlordane's
BHC's (Lindane, etc.)
Heptachlor
Heptachlor Epoxide
Dieldrin
Endosulfan I & II
Endosulfan sulfate
Endrin
o,p'- and p,p'-DDD, DDE, DDT
Methoxychlor
Toxaphene
Hexachlorobenzene
Mirex

Organophosphates
Chlorpyrifos
Diazinon
Malathion
methyl-Parathion
ethyl-Parathion

Pyrethroids
Cypermethrin
Fenvalerate

Metals
Copper
Cadmium
Lead
Mercury
Arsenic
Selenium
Chromium
Barium
Silver

Since pharmaceutical compounds are approved for use during production, samples of catfish are being maintained below 0°C until such time as the methods have been satisfactorily developed and additional funds become available. Multiples of all samples collected during this study will be maintained below 0°C for 5 years from collection date for subsequent residue determinations which may be of interest to the industry.

Quality assurance was conducted by the University of Georgia in a facility which is independent of sample analyses. Standard operating procedures have been developed to ensure the validity of the data generated during this study.

Anticipated benefits

Objective 1
The aquaculture industry now has a scientifically-generated residue database to support its contention that aquaculture products are safe for consumption.

Objective 2
Residues monitoring by producers will increase consumer confidence in the safety of aquaculture products.

Objective 3
Educational programs will also increase consumer confidence in the safety of aquaculture products.

Objective 4
Proper record-keeping will help insure that pesticides and animal drugs have been used in a safe manner.

Objective 5
Regulatory agencies are currently evaluating animal drugs for use in aquaculture systems. A major impact of this study will be to determine the fate of antibiotics from production through processing. It is not known whether prophylactic treatment with antibiotics will increase the residues in harvested catfish which have or have not been held for prescribed withdrawal times before harvest.

One of the important educational aspects which will result from this study will be a better understanding of the fate of antibiotics used in production. The information generated during this study will be communicated to production and processing segments of the industry to help avoid problems which may occur involving resistant organisms and residues in the processed fish.

Objective 6
The results generated during this objective are likely to have a major positive impact on the aquaculture industry. The data are expected to follow trends from other limited sampling experiments which demonstrate much lower residues in farm-raised products than in wild-caught fish. The results from this study will be used to find potential problems relating to elevated residues and solve these problems with the help of the producer or processor. Furthermore, this study will serve as a pilot study for the industry to develop a quality assurance program to routinely monitor for residues in aquaculture products.

Educational opportunities are also expected as a result of this project. First, producers and processors will be made more aware of the importance in reducing residues in aquaculture products. Second, there will be many analytical methods developed from this study which will be useful for people to conduct future testing. Third, undergraduate and graduate students as well as faculty participants, will become more aware of aquacultural products and practices and be better able to serve the industry.

Publications

Journal articles

Du, W. X., M. R. Marshall, W. B. Wheeler, M. Mathews, D. Gatlin, S. D. Rawles, D.-H Xu, W. A. Rodgers, and C. I. Wei. 1995. Oxytetracycline, sulfadimethoxine, and ormetoprim residues in catfish by HPLC. Journal of Food Science 60:1220-1224.

Xu, D., J. M. Grizzle, W. A. Rogers, and C. R. Santerre. 1997. Effect of cooking on residues of ormetoprim and sulfadimethoxine in the muscle of channel catfish. Food Research International 30: (In Press).

Abstracts or presented papers

Du, W. X., M. R. Marshall, W. B. Wheeler, and C. I. Wei. 1995. Determination of oxytetracycline, sulfadimethoxine, and ormetoprim residues in catfish using high performance liquid chromatography. Presented at the Institute of Food Technologists Annual Meeting at Anaheim, CA (Abstract 54E-13).

Du, W. X., M. R. Marshall, D.-H Xu, C. R. Santerre, and C. I. Wei. 1996. Effect of cooking on oxytetracycline residues in catfish. Presented at the American Chemical Society 212th Annual Meeting at Orlando, Florida in August (AGFD Abstract 48).

Theses or Dissertations

Du, W. X. 1994. Determination of oxytetracycline, sulfadimethoxine, and ormetoprim residues in catfish using high performance liquid chromatography. M.S. Thesis. University of Florida.

National Coordination For Aquaculture Investigational New Animal Drug (INAD) Applications

Progress Report for the Period September 1, 1992 to August 31, 1994

Funding level

SRAC funding (9/1/93-8/31/94)	$2,000
Total funding (9/1/92-8/31/94)	$35,180

Participants

R. K. Ringer and T. R. Batterson, Michigan State University

H. S. Parker, USDA/CSREES, Washington, DC

Project objectives

1. Ensure effective communications among groups involved with INAD applications, including liaison relations with Canada.
2. Serve as an information conduit between INAD applicants and the U.S. Food and Drug Administration/Center for Veterinary Medicine (FDA/CVM).
3. Champion preparation and submission of INAD applications by affected groups.
4. Seek opportunities for and encourage grouping of applications.
5. Function as an information source for INAD applications.
6. Coordinate educational efforts as appropriate.
7. Identify potential funding sources for INAD activities.

Progress and principal accomplishments

In September 1992, Dr. Robert K. Ringer, Professor Emeritus of Michigan State University, was hired on a part-time basis as National Coordinator for Aquaculture INAD Applications. Dr. Ringer served in that capacity through August 31, 1994. He also serves as the National Coordinator for USDA's National Research Support Project #7 (NRSP-7) for Minor Use Animal Drugs.

As National Coordinator for Aquaculture INADs he participated with FDA/CVM in educational workshops on INAD procedures and requirements. These workshops were conducted throughout the U.S. and attended by several hundred within the aquaculture community. This included workshops held in conjunction with the U.S. Trout Farmers Association, Boston Seafood Show, and Aquaculture Expo V in New Orleans. The workshop at the Boston Seafood Show was videotaped and is now available on cassette from the Northeastern Regional Aquaculture Center. In addition to the workshops, talks were presented on aquaculture drugs at the request of several organizations, including the World Aquaculture Society.

Dr. Ringer also helped in the preparation of a letter that FDA/CVM used in requesting disclosure information without such permission. As of September 1994, 70 disclosure permissions had been granted. A table containing information about these disclosures was recently made available to the general public. This included the names and addresses of the INAD holders as well as the drug and species of fish intended for use of the drug. It is intended that this table will be periodically updated after additional disclosure permissions have been obtained.

Every effort was made by the National Coordinator to encourage applicant grouping. The Coordinator also provided specific instructions on proper procedures and requirements for submitting applications to FDA to INAD applicants.

It was repeatedly stressed to the aquaculture community, that aquaculture INADs are merely a stop-gap measure and efforts must be undertaken to support approval of new animal drugs.

Anticipated benefits

Investigation and approval of safe, therapeutic drugs for use by the aquaculture industry is one of the highest priorities currently facing the industry. At present, only a few approved compounds are available to the industry and further development of the aquaculture industry is severely constrained by a lack of approved drugs essential for treating over 50 known aquaculture diseases. The FDA/CVM has afforded the aquaculture industry throughout the U.S. with a window of opportunity to seek approval of legal drugs to be used in their production practices. The need for additional drugs is great, but securing data necessary to satisfy the requirement of FDA/CVM for drug approval is time consuming, costly, and procedures are rigorous. The obtaining of drugs for legal use through the INAD process is one method by which the industry can provide FDA/CVM with data on efficacy and also aid the producer in their production practices.

Educating potential INAD applicants will save time and effort for both the industry and FDA/CVM. A National Coordinator for Aquaculture INADs would serve as a conduit between an INAD applicant and the FDA/CVM. The Coordinator would help to alleviate time-demands on FDA staff, thus allowing more time to process a greater number of applications, as well as increasing the breadth of research endeavors within the industry. The grouping of INAD applicants should help to alleviate redundancy, amalgamate efforts, and increase the amount of efficacy data, all of which should result in greater progress toward developing available, approved, therapeutic drugs.

Publications

Ringer, R. K. 1993. Workshop on INADs, NADAs, and the IR-4 Project. California Aquaculture Association, Oakland, October 11, 1993.

Ringer, R. K. 1993. INAD workshop: Proper drug and chemical use in aquaculture. 9th Annual Florida Aquaculture Association Conference, Fort Pierce, November 6, 1993.

Ringer, R. K. 1994. National INAD Coordinator's role in aquaculture. Aquaculture Expo VII/Annual World Aquaculture Society Meeting, New Orleans, January 13, 1994.

Ringer, R. K. 1994. State of current USDA regulations on drug, therapeutic, and chemical use. North Carolina Aquaculture Development Conference, New Bern, February 5, 1994.

Ringer, R. K. 1994. Investigational New Animal Drugs Workshop. Tropical and Subtropical Regional Aquaculture Center Industry Advisory Council Meeting, Honolulu, Hawaii, March 14, 1994.

Improving Production Efficiency Of Warmwater Aquaculture Species Through Nutrition

Progress Report for the Period September 1, 1992 to August 31, 1994

Funding level

Year 1	$280,310
Year 2	$249,485
Year 3	$234,705
Total	$764,500

Participants

D. M. Gatlin, W. H. Neill, J. T. Davis, L. V. DiMichele, and J. B. Cotner, Texas A&M University

E. H. Robinson, H. R. Robinette, and R. P. Wilson, Mississippi State University

R. T. Lovell and U. Hatch, Auburn University

R. T. Lochmann, University of Arkansas at Pine Bluff

R. C. Reigh, Louisiana State University

C. D. Webster and J. H. Tidwell, Kentucky State University

M. L. Gallagher, East Carolina University

G. J. Burtle and G. L. Newton, University of Georgia

K. B. Davis and B. A. Simco, University of Memphis

Administrative advisor

Dr. Greg Weidemann, Associate Dean/Director
Arkansas Agricultural Experiment Station
Fayetteville, Arkansas

Project objectives

1. Determine minimum effective levels of vitamin and protein/amino acid supplementation to maximize feed efficiency in commercial-scale channel catfish production.
2. Evaluate feeding strategies and their effects on commercial-scale channel catfish production. Of particular concern will be the effects of feeding time, frequency, and rate (satiation or restricted) on production efficiency. Feeding regimes to achieve maintenance of body weight and compensatory growth, will also be addressed.
3. Investigate nutritional aspects that are most limiting production of baitfish and hybrid striped bass. This will include determining digestible energy and availability of amino acids in practical feedstuffs for hybrid striped bass and the relative contribution of natural foods and prepared feeds to growth of baitfish under conditions of commercial production.

Progress and principal accomplishments

Objective 1

Several feeding trials in aquaria and experimental ponds have been completed and others are in progress to evaluate vitamin and protein/amino acid supplementation of practical diets for channel catfish. At the Mississippi State University Delta Research and Extension Center, two pond feeding trials (1994 and 1995) have been conducted to evaluate the need to supplement typical commercial catfish feeds with vitamin C, thiamin, riboflavin, niacin, pantothenic acid, and pyridoxine. Weight gain, feed conversion, feed consumption, survival, and hematocrit data indicated that supplemental thiamin, riboflavin, niacin, pantothenic acid, and pyridoxine may not be necessary in commercial catfish diets. Data from fish fed vitamin C test diets also indicated that the requirement appears to be less than 15 mg/kg which is much less than previously reported. Based on production data and liver vitamin analyses completed to date, it appears that supplemental riboflavin and pyridoxine are not needed in typical commercial catfish feeds used for growout. There also appears to be enough endogenous niacin in commercial catfish feeds to meet the requirement of channel catfish. Stress responses of catfish fed diets with various vitamin supplements were measured by associates at the University of Memphis. Data on plasma chloride, osmotic pressure, and cortisol levels during confinement, did not indicate any consistent difference due to reduced dietary vitamin levels. The stability of several B-complex vitamins in extruded catfish feeds also were determined. These vitamins are fairly to very stable during the extrusion process with relative retention of 65% for thiamin, 100% for riboflavin, 96% for niacin, 100% for pantothenic acid, and 70% for pyridoxine.

In 1996, additional evaluations of vitamin supplementation are continuing with the control diet being the same as used in 1994 and 1995. Diets without individual supplements of thiamin, riboflavin, niacin, pantothenic acid, or pyridoxine, or with 500 mg supplemental choline are being evaluated. The fish were stocked in May 1996 and have been fed the experimental diets once daily to satiation. Samples of 200 fish from each pond will be taken in October/November 1996 to evaluate treatment effects. Fish will be overwintered in the ponds and harvested in February 1997.

A similar investigation concerning vitamin E supplementation of diets for channel catfish was conducted in ponds at Texas A&M University in 1994-95. A typical commercial diet formulation (with approximately 10 mg vitamin E/kg provided endogenously) was supplemented with vitamin E acetate at either 0, 30, 60, or 240 mg/kg diet. No differences in growth, feed efficiency, and survival were observed over the 1-year trial; however, plasma tocopherol was directly correlated with vitamin E supplementation in the diet. Differences in liver tocopherol also were observed in fish fed the various diets with those fed the highest level of supplemental vitamin E having the highest liver tocopherol concentrations. Based on the lack of overt or histological signs of vitamin E deficiency in fish fed the basal diet, it appears that vitamin E supplementation of practical diets for growout of channel catfish can be reduced considerably.

In the Department of Biochemistry at Mississippi State University, laboratory experiments on the dietary riboflavin and niacin requirements of fingerling channel catfish have been completed. Fish were fed purified diets containing graded levels of dietary riboflavin or niacin for 8 and 12 weeks, respectively. The dietary riboflavin requirement of channel catfish for optimal growth was determined to be 6 mg/kg diet which is lower than the previously accepted value of 9 mg/kg diet. A riboflavin level of 4 mg/kg diet was sufficient to prevent the appearance of gross deficiency signs in the fish. The dietary niacin requirement for rapidly growing channel catfish was estimated to be 7.4 mg/kg diet which is about half the previously reported value of 14 mg/kg diet. In a separate experiment, it has been confirmed through direct evidence that dietary tryptophan does not serve as an efficient precursor of niacin in channel catfish. Based on the results of two previous niacin studies, a catfish liver NAD bioassay was developed to determine the bioavailability of niacin from feed ingredients commonly used in commercial catfish feeds. Menhaden fish meal (MFM), meat and bone/blood meal (MBM), wheat middlings (WML), cooked corn (CCO), uncooked corn (UCO), cottonseed meal (CSM), and soybean meal (SBM) were found to contain 105.3, 50.5, 153.3, 21.9, 12.8, 22.5, and 20.3 mg available niacin/kg, respectively. When compared to the total niacin content of each feed ingredient, niacin in animal feed ingredients (MFM, MBM) was found to be completely available to channel catfish. The availability of niacin in the cereals and cereal by-products, WML, CCO, and UCO, was 60, 44, and 28%, respectively. Niacin availability in the two oilseeds, CSM and SBM, was found to be 58 and 57%, respectively. It was concluded that supplementation of niacin may not be needed or can be significantly lowered in typical commercial catfish feeds because of the relatively high amount of available niacin found in the feed ingredients. Studies on the nutritional availability of vitamin E from the feed ingredients are currently being conducted.

An investigation to optimize dietary protein/amino acid supplementation of all plant, protein diets for channel catfish is nearing completion at Louisiana State University. Channel catfish have been produced continuously in sixteen, 0.08 ha (0.2 acre) ponds at the Aquaculture Research Facility in Baton Rouge, since the spring of 1994. Ponds have been top-harvested multiple times and restocked with fingerlings after top-harvests to maintain a density of 25,000 fish/hectare. Fish have been fed one of two, custom-formulated, 32% crude protein, extruded diets: one containing animal and plant protein (control) and one containing only plant protein (primarily from cottonseed and soybean meal with supplemental lysine). Each diet has been fed to fish in eight randomly-selected ponds, once per day. Diet allotments have been adjusted daily to provide as much food as the fish will eat. Since initiation of the experiment, 3,493 kg of fish have been harvested from ponds assigned the cotton seed/soybean (C/S) diet and 3,214 kg from ponds assigned the control diet. Live weights have averaged 0.7 kg and dressing percentages have averaged 59% for fish harvested to date in both treatment groups. Visceral fat content has averaged 1.5% in C/S-fed fish and 1.7% in the control group. A final (batch) harvest will occur in October-November 1996, at which time all ponds will be drained. Data analysis and report preparation will be completed in the spring of 1997.

At the University of Georgia, a study was conducted to evaluate replacement of menhaden fish meal in channel catfish diets with alcohol-extracted soy protein concentrate. Two experimental diets were formulated to contain either 8 or 16% soy protein concentrate to totally replace menhaden fish meal and some soybean meal which were included in the control diet at 8 and 43%, respectively. Dietary protein, energy, lysine, methionine, and phosphorus were the same among the three diets. Channel catfish in three size classes were stocked at 25,000 fingerlings/ha into 0.10-ha ponds. Partial harvests were made during the growing season of catfish that had reached market size for Georgia, > 0.2 kg/fish. After stocking in May, five partial harvests were completed and a final complete harvest was made in November when all remaining catfish were counted and weighed. Catfish production and conversion efficiency were similar for all three diets and resulted in total production of between 5,736 and 7,390 kg/ha for all ponds. Catfish survival ranged between 73% and 97% because of bird depredation and disease-related mortalities but was not significantly different among treatments. Thus, soy protein concentrate used in an all plant, protein diet allowed the omission of fish meal without negative effects on catfish production.

Objective 2

Several studies to investigate various feeding strategies in channel catfish production have been conducted and others are currently in progress. Laboratory experiments have been completed at Texas A&M University, in which effects of feed restriction and dissolved oxygen concentration on growth of channel catfish were investigated. Fish maintained at two levels of dissolved oxygen (60 and 92% of air saturation), were either not fed or fed at 1.5 or 3% of body weight per day for 1 month after which they were all fed to satiation for another month. Weight gain and feed efficiency of fish subjected to low dissolved oxygen were significantly reduced compared to those of fish maintained at the higher dissolved oxygen concentration. Compensatory gain of fish that were not fed or fed at the restricted rate for the first 4 weeks was limited. Another study is ongoing with channel catfish in ponds to evaluate restricted feeding regimes that may illicit compensatory growth responses. Catfish of two size classes were stocked at commercial densities and are either being fed to satiation every day, fed to satiation every third day, or not fed at all for a 3-week period, alternated with another 3-week period during which fish in all treatments are fed to satiation each day. The effects of these feeding regimes on growth, feed efficiency and body composition will be evaluated over a 6-month period.

At Auburn University, channel catfish of two sizes, year 1 (43 g) and year 2 (660 g), were stocked separately in 0.04-ha ponds in mid-November of 1994 and subjected to three overwinter management regimes: no feed, restricted feeding, and continuous feeding. Fish were challenged with *Edwardsiella ictaluri* the following spring. Among year 1 fish, those not fed during the 5-month overwintering period showed significantly higher mortality from *E. ictaluri* than fish fed continuously or restricted (not fed during December, January, and February). However, among year 1 fish, those not fed showed significantly lower mortality than fish fed continuously or restricted. In the spring of 1995, year 1 fish not fed during the previous winter (November 1 - April 20) lost 12% of their weight while year 1 fish fed during winter increased their weight by 99%.

Year 2 fish not fed during the winter lost 7% of body weight while year 2 fish that were fed increased their weight by 38%. During the following summer growing season, all fish in both size groups were fed to satiation. During the first 10 weeks of the summer period, specific growth rate and feed consumption of fish not fed during winter in both age groups were greater than those of fish fed during the previous winter. Feed conversion was the same among treatments in both age groups. At the end of the summer, there was no difference in body weight between the year 1 fish that had been fed during winter and year 1 fish that had not been fed during winter; however, the year 2 fish not fed the previous winter did not reach a similar weight as year 2 fish fed the previous winter. These data indicate that year 1 channel catfish can completely compensate and year 2 fish can partially compensate for previous winter feed deprivation if fed to satiety during the summer growing season.

In the Department of Wildlife and Fisheries at Mississippi State University, a study of the effects of size-class distribution and dietary protein level on protein utilization and feed conversion of channel catfish has been conducted. Fish averaging either 24.1 g or 392.1 g were stocked in earthen ponds as separate size classes and in a 50:50 ratio, in June 1994, and fed either a 28 or 32% protein diet daily to satiation. After 125 days, there were no significant differences in average harvest weight, weight gain, survival, feed conversion, or proximate composition of whole body, and fillets from fish fed either diet or stocked with only the same size versus mixed sizes. However, large fish stocked with small fish had higher percentage fillet, carcass and fat dressout (32.2, 51.5, and 2.3, respectively) than large fish stocked alone (29.7, 48.3, and 1.6, respectively). Small fish stocked alone had higher percentage carcass dressout (50.3) than small fish stocked with large fish (47.6). Additionally, small fish stocked in mixed-size ponds displayed significantly less average weight gain (148 g) than small fish stocked alone (264 g).

In December 1994, 26 g fingerlings were stocked with non-market size fish (remaining from the fall harvest of year 1 fish) to bring fish density to 24,719/ha in each of the 18 ponds used in year 1. Thus, the experimental design from year 1 (three fish size groups each fed 28 and 32% protein feeds) was continued into year 2 of the study. However, the treatment consisting of only large fish in year 1 became only small fish in year 2 because all fish reached market size in year 1. The two remaining size treatments were mixed size group ratios of large fish: small fish of approximately 1:2 (mixed-1) and 1:5 (mixed-2). Market size fish were removed by partial harvest in April, July, and October, 1995. Total number and weight of fish in each pond were determined by total harvest in November 1995. Average survival rate in mixed-1 ponds (74.5%) was significantly higher than in mixed-2 ponds (65.9%) or in small-only ponds (63.8%). The differential survival rate may influence interpretation of the following results. There was no interaction among the three size groups and two dietary protein levels, and there were no significant differences between diets for average market size and total production. However, across size groups at final harvest, small fish stocked alone (market size at harvest) displayed significantly greater average visceral fat (2.01%) than fish stocked as mixed-1 (1.4%) or mixed-2 (1.4%) groups. The small-only fish also had significantly greater average fillet yield (29.9%) when compared to fish in the mixed-1 treatment (28.6%).

Objective 3

Several studies have been completed or are in progress with baitfish and hybrid striped bass, to investigate various aspects of their nutrition and feeding. Investigators at the University of Arkansas at Pine Bluff (UAPB) have completed the following:

(1) The dietary protein requirement (29%) and optimal dietary energy:protein ratio (9.7 kcal/g) of golden shiners and goldfish in aquaria were established.

(2) A series of stable carbon isotope ratio studies has been completed with golden shiners. Isotope analysis of fish and plankton has been used to estimate the relative assimilation of natural and prepared feeds by golden shiners in ponds. Under the conditions of this study, fish obtained approximately 40-83% of their nourishment from the plankton, and the remainder from the prepared feeds. The percentage of plankton consumed by golden shiners was inversely related to the assimilation of the diets which varied with diet composition.

(3) A pond feeding trial performed jointly at UAPB and Texas A&M University was conducted to study the effect of different stocking densities (660,000 fish/ha at UAPB vs. 330,000/ha at TAMU) on the relative intake of natural and prepared feeds by golden shiners. Stable carbon isotopic analysis has been completed on samples from each site and final analysis of the data is in progress.

(4) Aquarium studies of the dietary lipid requirement of golden shiners and goldfish were completed. Golden shiners performed well when fed diets containing a wide range of lipid (7-19%), but performance showed a peak when the diet con tained around 12% lipid. Goldfish fed diets containing 4.5-7.0% lipid had the highest weight gain. However, survival of goldfish fed a diet with 4.5% lipid was significantly lower than that of fish fed diets containing 7.0-13.3% lipid.

(5) A pond trial comparing performance of golden shiners fed diets containing similar energy:protein ratios (10.3 kcal/g) and either high (31%) or low (24%) protein levels is in progress. Ponds will be harvested and final data will be obtained later this fall.

At East Carolina University, feeding trials have been completed to determine organic matter digestibility coefficients for dextrin, wheat starch, wheat flour, wheat middlings, potato starch, and corn starch in diets fed to original cross hybrid striped (palmetto) bass of two sizes (6g) and (95g). Digestibility coefficients for all carbohydrates were generally high (90.5-100%), indicating that simple and complex carbohydrates can be digested by these fish. However, potato starch was not well digested (66.0%) in either small or larger fish. Digestibility determinations also have been conducted at Texas A&M University with reciprocal cross hybrid striped (sunshine) bass. Apparent protein and organic matter digestibility coefficients have been determined for menhaden fish meal, anchovy meal, meat and bone meal, poultry byproduct meal, soybean meal, and cottonseed meal.

At Kentucky State University, experiments were conducted with hybrid striped bass to evaluate the effects of diet formulations on growth, body composition, and organoleptic qualities. In one experiment, sunshine bass in floating cages were determined to require a diet with 41% protein and a protein to energy ratio greater than 99mg protein/kcal when fish meal comprised 56% of the dietary protein. In another experiment, juvenile (20g) palmetto bass in cages were shown to require some fish meal in the diet to provide good growth; however, fish meal inclusion at 15% of diet produced similar growth as diets with higher levels of fish meal. Another study was conducted to determine frozen storage stability, fatty acid composition, and textural quality of sunshine bass. The n-3 fatty acids composed 1/3 of the total fatty acids in muscle, with eicosapentaenoic acid, 20:3(n-3), and docosahexaenoic acid, 22:6(n-3), being the most prevalent n-3 highly unsaturated fatty acids. Storage of muscle at -20°C for 6 months did not cause increases in lipid oxidation for skin-on fillets; however, skinless fillets exhibited marked increases from month 4 to 6. There was no charge in textural quality during 6 months of frozen storage. Flavor quality of sunshine bass fillets stored frozen (-10°C) with skin on for 0, 9, and 18 months also was evaluated by a trained taste-test panel. Diet had little effect on flavor quality, even after 18 months of frozen storage, except for a diet that had 35% fish meal and 10% menhaden fish oil which produced a "fishy" flavor. Frozen storage of sunshine bass fillets for up to 18 months did not greatly affect flavor quality.

Anticipated benefits

Based on results of the project to date, some modifications in diet formulations and feeding schedules for channel catfish can be made which will improve production efficiency and cost effectiveness of diet formulations. It appears, for example, that dietary supplementation of several vitamins may be reduced substantially. Advancements in these areas will significantly improve production economics by reducing diet costs and increasing the efficiency of feed utilization. The benefits obtained from these advancements will be substantial because over 1/2 of the variable production costs associated with channel catfish aquaculture relate to diets and feeding. Research concerning various feeding strategies also has identified means of improving health and increasing production efficiency of channel catfish. Significant advancements also have been made in obtaining specific information on the nutritional requirements of baitfish and hybrid striped bass, and how to meet those requirements most economically. The efficiency and profitability of baitfish production also should be improved by integrating information obtained in this project on nutritional requirements of these fish with pond management and feeding strategies to meet those requirements most economically. Additional information concerning nutritional requirements of hybrid striped bass and their utilization of feedstuffs also will facilitate the development of optimized formulations that will reduce diet costs and improve production efficiency of these fish.

Publications

Journal articles

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Kim, M. K. and R. T. Lovell. 1995. Effect of overwintering feeding regimen on body weight, body composition, and resistance to *Edwardsiella ictaluri* in channel catfish. Aquaculture 134:237-246.

Kim, M. K. and R. T. Lovell. 1995. Effect of restricted feeding regimens on compensatory weight gain and body tissue changes in channel catfish *Ictalurus punctatus* in ponds. Aquaculture 135:285-293.

Li, M., J. B. Rushing, and E. H. Robinson. 1996. Stabilities of B-complex vitamins in extruded catfish feeds. Journal of Applied Aquaculture 6(2):67-71.

Lochmann, R. T. and H. Phillips. 1994. Dietary protein requirement of juvenile golden shiners (*Notemigonus crysoleucas*) and goldfish *(Carassius auratus)* in aquaria. Aquaculture 128:277-285.

Lochmann, R. T. and H. Phillips. In review. Dietary lipid requirement of juvenile golden shiners *(Notemigonus crysoleucas*) fed diets differing in protein level in aquaria. Aquaculture.

Lochmann, R. T. and H. Phillips. 1995. Stable isotopic evaluation of the relative assimilation of natural and artificial foods by golden shiners *Notemigonus crysoleucas* in ponds. Journal of the World Aquaculture Society 27:168-177.

Ng, W. K., G. Serrini, Z. Zhang, and R. P. Wilson. In press. Niacin requirement and inability of tryptophan to act as a precursor of NAD in channel catfish, *Ictalurus punctatus*. Aquaculture.

Rawles, S. D., T. G. Gaylord, and D. M. Gatlin III. In preparation. Vitamin E supplementation of practical diets for channel catfish. Aquaculture.

Serrini, G., Z. Zhang, and R. P. Wilson. 1996. Dietary riboflavin requirement of fingerling channel catfish (*Ictalurus punctatus*). Aquaculture 139: 285-290.

Stahl, C. J., T. G. Gaylord, and D. M. Gatlin III. In preparation. Influence of feeding time and dissolved oxygen on growth performance and body composition of channel catfish. Fish Physiology and Biochemistry.

Webster, C. D., L. G. Tiu, J. H. Tidwell, P. Van Wyk, and R. D. Howerton. 1995. Effects of dietary protein and lipid levels on growth and body composition of sunshine bass (*Morone chrysops* X *M. saxatilis*) reared in cages. Aquaculture 131:291-301.

Webster, C. D., L. G. Tiu, J. H. Tidwell, P. Van Wyk, and R. D. Howerton. 1995. Effects of dietary protein and energy levels on growth and body composition of sunshine bass (*Morone chrysops* x *M. saxatilis*) reared in cages. Aquaculture 131:291-301.

Webster, C. D., L. G. Tiu, and J. H. Tidwell. In press. Effects of replacing fish meal in diets on growth and body composition of palmetto bass (*Morone saxatilis*) x *M. chrysops*) raised in cages. Journal of Applied Aquaculture 7(1).

Xiong, Y. L., E. A. Decker, S. P. Blanchard, A. D. Crum, N. C. Shantha, C. D. Webster, L. G. Tiu, and J. H. Tidwell. 1996. Dietary protein level has minimal effect on flesh quality of frozen stored sunshine bass (*Morone chrysops* x *M. saxatilis*). Journal of Applied Aquaculture 6(1):47-63.

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Gatlin, D. M., III. An overview of fish nutrition research supported by the Southern Regional Aquaculture Center. Aquaculture America, February 1996 (Abstract).

Ng, W. K., C. Keembiyehetty, and R. P. Wilson. In review. Bioavailability of niacin from feed ingredients commonly used for channel catfish, *Ictalurus punctatus*. Aquaculture: Proceedings of the 7th International Symposium on Nutrition and Feeding of Fish, College Station, TX, Aug. 11-15, 1996

Ng, W. K., C. Keembiyehetty, and R .P. Wilson. Bioavailability of niacin from feed ingredients commonly used for channel catfish , *Ictalurus punctatus*. 7th International Symposium on Nutrition and Feeding of Fish, August 1996. (Abstract).

Okwoche, V. O. and R. T. Lovell. Effects of winter feeding regimen on resistance to *Edwardsiella ictaluri* challenge by channel catfish, *Ictalurus punctatus*. 7th International Symposium on Nutrition and Feeding of Fish, August 1996. (Abstract).

Serrini, G., Z. Zhang, and R. P. Wilson. Dietary riboflavin requirements and lack of conversion of tryptophan to niacin in channel catfish. 24th Fish Feed and Nutrition Workshop, October 1995.

Stahl, C. J., W. H. Neill, and D. M. Gatlin III. Influence of feeding time and dissolved oxygen on the growth performance of fingerling channel catfish. Aquaculture America, February 1996 (Abstract).

Stahl, C. J., W. H. Neill, and D. M. Gatlin, III. Influence of feeding time and dissolved oxygen on the growth performance of fingerling channel catfish (*Ictalurus punctatus*). Aquaculture America, February 1996 (Abstract).

Unprasert, P. and H. R. Robinette. Influence of channel catfish, *Ictalurus punctatus*, size-class distribution on protein utilization and feed conversion. Aquaculture America, February 1996 (Abstract).

Unprasert, P. and H. R. Robinette. Influence of channel catfish, *Ictalurus punctatus*, size-class distribution on protein utilization and feed conversion. Aquaculture America, February 1996 (Abstract).

Webster, C. D., L. G. Tiu, and J. H. Tidwell. Effect of dietary regimen and frozen storage on lipid oxidation and textural quality of hybrid striped bass muscle. Aquaculture America, February 1996 (Abstract).

Webster, C. D., L. G. Tiu, and J. H. Tidwell. Effect of dietary regimen and frozen storage on lipid oxidation and textural quality of hybrid striped bass (*Morone chrysops* x *M. saxatilis*) muscle. Annual Meeting of the World Aquaculture Society, Bangkok, Thailand, January 1996 (Abstract).

Theses and dissertations

Keembiyehetty, C. N. 1995. Amino acid nutrition of sunshine bass (*Morone chrysops* x *M. saxatilis*). Ph.D. Dissertation. Texas A&M University.

Delineation And Evaluation Of Catfish And Baitfish Pond Culture Practices

Progress Report for the Period April 1, 1994 to August 31, 1996

Funding level

Year 1	$147,500
Year 2	$152,000
Year 3	$150,500
Total	$450,000

Participants

J. Crews, L. Lovshin, J. Jensen, M. Masser, C. Hyde, G. Whitis, D. Cline, and C. Reeves, Auburn University
C. G. Lutz, Louisiana State University
G. Clary and J. Lock, Texas A & M University
C. Engle, N. Stone, D. Heikes, and S. Killian, University of Arkansas at Pine Bluff
R. Gilbert, University of Georgia

Administrative advisor

Dr. David Foster, Associate Vice President for Agriculture-Extension
University of Arkansas
Little Rock, Arkansas

Project objectives

1. Develop catfish and baitfish standardized production and financial performance analysis (SPFPA-CC and SPFPA-BF) guidelines which include measures for evaluating the performance of commercial catfish and baitfish production systems.
2. Delineate and evaluate current commercial catfish and baitfish production practices (i.e., stocking, feeding, aeration, water exchange, pond size and configuration, harvesting, etc.) utilizing SPFPA-CC and SPFPA-BF guidelines.
3. Identify relationships between measures of production and financial performance, as calculated according to SPFPA-CC and SPFPA-BF guidelines.
4. Develop management tools to assist commercial catfish and baitfish producers, lenders, aquaculture specialists, and others in determining the efficacy of selected production practices.

Progress and principal accomplishments

Catfish

Objective 1

The Performance Evaluation Standards for Commercial Catfish Operations (PESCAT) is complete and is available for use by anyone interested in implementing the analysis. These guidelines establish standards for production and financial performance analysis of commercial catfish production systems. Producers have been heavily involved with the development of these guidelines. The guidelines are divided into two publications: one for fingerling production enterprises and one for food fish production enterprises. Standardized Production and Financial Performance Guidelines drafted previously are incorporated into all products described under objective 4.

Objective 2

Paper forms and software are available for use in collecting data to accomplish this objective. These analysis tools request the necessary data to describe commercial catfish production practices and farm characteristics for comparisons. Investigators are in the process of identifying cooperators and collecting data as they are available.

Objective 3

Relationships between production practices and measures of productivity and financial performance will be evaluated on an aggregate basis as soon as sufficient data are available. At this point, cooperators in each state have been identified and data collection is under way.

Objective 4

Significant efforts to date have been directed at accomplishing this objective. A PESCAT Handbook is available for fingerling and food fish operations. It contains 13 fact sheets which are detailed discussions on topics that needed further discussion beyond that contained in the Guidelines. It also contains sample reports, input forms for collecting necessary data, inventory maintenance forms, and software to facilitate calculating performance measures with a computer. Handbooks have been distributed to all participating faculty. An abbreviated version of the Handbook, called the PESCAT Toolkit, is being distributed as a promotional tool to generate interest in the program. It contains the vital fact sheets, input forms, sample reports, and software necessary to get started gathering data for analysis.

Baitfish

Standard Production and Financial Performance Analysis (SPFPA) guidelines and data forms have been developed, reviewed, and finalized for the four principal species of baitfish: golden shiner, goldfish, fathead minnows, and rosy red minnows. A codebook, database structure, and analysis worksheets have been developed and reviewed for data entry and analysis. The SPFPA-Golden Shiner guidelines, data forms, database structure, and worksheet analysis formulae were pilot tested in 1995 with a cooperating farmer and modifications made accordingly. The modified SPFPA guidelines and data forms were pilot tested this past year for goldfish, fathead minnows, and rosy red minnows, as well as with additional golden shiner cooperators. Cooperators for all these species were identified and interviewed to explain expectations and benefits of participation in the project. Enterprise budgets have been finalized for all species. However, we will wait to analyze another year's data to make further adjustments to the budgets before publishing them.

Anticipated benefits

Narrow margins between production costs and revenues result in challenges for managers of commercial catfish and baitfish production systems. Decisions must be made regarding resource allocation, optimal production alternatives, reinvestment, marketing strategies, use of credit, and many other issues. A standardized system to measure production and financial performance is necessary to monitor the impact that decisions have on the productivity, financial performance of entire farms, and more specifically on commercial catfish and baitfish enterprises.

Standardization lends itself to comparing performance of farms with different locations, management levels, production strategies, sizes, and other characteristics. This project proposes to delineate and evaluate current commercial practices by developing a standardized system of production and financial performance measures for catfish and baitfish operations independent of size, production methods, or marketing strategies. This standardized system will then be used to make an integrated evaluation of biological and financial risk, and the consequences of management decisions on productivity and profitability with a group of cooperating producers in five Southeastern states. The results will only be indicative of the cooperators and not necessarily the entire industry, but should begin to elucidate best management practices. At the conclusion of the project, the standardized system that has been developed will be available for producers to utilize throughout the nation.

Publications

The following three products are available from state faculty or from Greg Clary, P.O. Box 38, Overton, TX 75684; (903)834-6191; fax (903)834-7140; gclary@tamu.edu.

1. PESCAT Handbook (Fingerlings, Food Fish, or Combination) containing the following fact sheets:

- "What PESCAT Is and Is Not" (Clary);
- "What You Need to Get Started and Who Can Help" (Clary and Hnatt);
- "Errors in Estimating Fingerling Numbers and Value: The Black Hole Begins on Paper" (Lutz and Hymel);
- "Inventory and Other Data Worksheets for Financial Statement Accrual Adjustments" (Clary);

- "Depreciation of Broodfish for Tax Reporting - When and When Not to Depreciate" (Hnatt and Clary);
- "Developing, Reviewing, and Using the Depreciation Schedule" (McGrann, Lovell and Ewing);
- "Performance Based Borrowing" (Klinefelter);
- "Change in Owner's Equity" (McGrann);
- "Doing the Right Thing: Decision Making for Agricultural Families" (Doye);
- "Performance Evaluation Standards for Commercial Catfish Operations - Summary Analysis Worksheets (Foodfish/Fingerlings)" (Hnatt and Clary);
- "Suggested Methods for Allocating Overhead Costs" (Clary);
- "Chart of Accounts for Commercial Catfish Financial Records" (Clary);
- "Inventory Assessment Methods for Catfish Ponds (Van Wyk, Masser, Heikes, and Killian)" {in preparation}.

2. The PESCAT Toolkit contains selected fact sheets, input forms, sample reports, and a brochure describing the program and ordering additional resources.

3. PESCAT software is a program written in Access and compiled so anyone with at least Windows 3.1 can run it. It contains user-friendly forms for inputting data and reporting results. Considerable help is included within the program so the user can find information about PESCAT guidelines without referring to the Handbook. All data sheets and summary analysis forms are also available on Lotus™ spreadsheets.

All materials, including software which comes on four, small, high-density diskettes, is complementary as long as project funding is available to cover materials, reproduction, and mailing costs.

The following four products are available from the University of Arkansas at Pine Bluff Extension faculty at 1200 N. University Drive (or P.O. Box 4912), Pine Bluff, AR 71611; (501) 543-8537.

1. "Standardized Production and Financial Performance Analysis - Golden Shiners (SPFPA-GS)," published by project participants, UAPB, February 1995.

2. "Standardized Production and Financial Performance Analysis - Fathead Minnows (SPFPA-FM)," published by project participants, UAPB, February 1995.

3. "Standardized Production and Financial Performance Analysis - Goldfish (SPFPA-GF)," published by project participants, UAPB, February 1995.

4. "Standardized Production and Financial Performance Analysis - Rosy Red Minnows (SPFPA-RR)," published by project participants, UAPB, February 1995.

Two papers were presented at the Aquaculture '96 meeting of the U.S. Chapter of the World Aquaculture Society in Arlington, TX; February 1996:

"Common Errors in Estimating Catfish Fingerling Numbers and Value: The Black Hole Begins on Paper," by G. Lutz and T. Hymel.

"A Cost Analysis of Forced-Air Incubation of Catfish Eggs," by G. Lutz and T. Tiersch.

Publications, Videos, And Computer Software

Progress Report for the Period April 1, 1995 to August 31, 1996

Funding level

Year 1	$50,000
Year 2	$61,000
Total	$111,000

Participants

M. Brunson, L. R. D'Abramo, Mississippi State University
G. Lewis, R. Gilbert, J. Shelton, and T. R. Murphy, University of Georgia
R. Durborow, Kentucky State University
N. Stone, E. Park, and D. Archer, University of Arkansas at Pine Bluff
J. D. Bankston, Jr., S. Malone, W. Lorio, and F. Baker, Louisiana State University
C. Cichra and A. Lazur, University of Florida
M. Masser, Auburn University
K. F. Ladewig, M. Morat, R. Miget, K. Jefferson, and J. T. Davis, Texas A&M University

Administrative advisor

Dr. Dan Ezell, Interim Director
Clemson Cooperative Extension Service
Clemson, South Carolina

Project objectives

1. Review and revise, as necessary, all SRAC Extension printed and video publications.
2. Establish an ongoing project location to develop and distribute new SRAC educational publications and videos for Southern Region aquaculture industries. This project will be responsible for preparation, peer review, editing, reproduction, and distribution of all Extension and popular-type publications for all SRAC projects.
3. Place current, revised, and new publications in electronic format (e.g., Internet or compact disc) for more efficient use, duplication, and distribution.

Progress and principal accomplishments

Production of new and revised fact sheets is an ongoing process. Since the last annual report seven fact sheets have been completed and distributed. Fourteen fact sheets are in the writing and editing stage. The shooting script for the baitfish video was approved and completion is expected within the calendar year. Footage for the revised channel catfish video has been taken and the shooting script should be approved within the next 60 days. The new publications are the result of work conducted under other projects. As projects have been completed, Extension-type publications are prepared.

Work on placing all fact sheets into electronic format is progressing steadily. Problems with computer software and hardware have been overcome and distribution of compact discs is planned for early September 1996. These are being made available through normal SRAC channels and state Extension contacts in the region.

Anticipated benefits

The most direct benefit from this project to the aquaculture industry is the widespread and ready availability of detailed information on production and marketing constraints and ways to alleviate or manage those constraints. Such information is of particular assistance to those making decisions about entering the aquaculture business. Economics information is used by lending agencies as well as current producers in day-to-day decision making. Information on the use of therapeutants, pesticides, methods of calculating treatment rates, and possible alternative crops and marketing strategies is in constant demand by practicing aquaculturists. Videos that demonstrate techniques are a ready source of "how-to" information. Educational institutions at the elementary and high school level have recently started using extension materials to make students aware of aquaculture production and associated trades as a way of life for many people. Placing the information on the Internet and compact discs makes access easier, facilitates searching for needed information, and reduces storage space requirements for printed documents.

Producers will also benefit indirectly from the materials intended for use by consumers who buy the products, as well as from those materials that furnish background information on aquaculture. This information also helps in awareness and decision making when citizens are involved in regulating the industry. This is particularly important with the increased emphasis on possible environmental contamination resulting from agricultural practices. Information to date indicates a relatively minor impact (often of a positive nature) of aquaculture on the surrounding areas.

Publications

A complete list of all SRAC publications, videos, and computer software is presented in the Appendix at the end of the project summaries for the Southern Regional Aquaculture Center.

Management Of Environmentally-Derived Off-Flavors In Warmwater Fish Ponds

Progress Report for the Period June 1, 1996 to August 31, 1996

Funding level

Year 1	$261,000
Year 2 (projected)	$251,000
Year 3 (projected)	$171,000
Year 4 (projected)	$36,000
Year 5 (projected)	$41,000
Total	$760,000

Participants

T. K. Hill, University of Tennessee
P. Perschbacher, University of Arkansas, Pine Bluff
C. E. Boyd and R. T. Lovell, Auburn University
D. L. Park, Louisiana State University
H. L. Walker, Louisiana Tech University
K. T. Chung, E. Stevens, and T.Y. Wong, University of Memphis
D. K. Schlenk, University of Mississippi
D. J. Wise and E. H. Robinson, Mississippi State University
D. M. Gatlin and J. B. Cottner, Texas A&M University

Administrative advisor

Dr. Don Richardson, Dean
University of Tennessee
Tennessee Agricultural Experiment Station
Knoxville, Tennessee

Project objectives

1. Evaluate the feasibility of decreasing the incidence of fish off-flavors by reducing the amount of phosphorus available to support phytoplankton growth.
   a. Evaluate methods of reducing phosphorus input by diet modification, by determining the minimum phosphorus requirement for food-sized channel catfish and quantifying the reduction in waste phosphorus generation by food-sized catfish fed low-phosphorus feeds relative to presently available feeds.
   b. Evaluate methods of removing phosphorus from pond water by studying methods of enhancing rates of phosphorus removal from pond waters by pond bottom soils, and determining the feasibility of precipitating phosphorus from pond waters as sparingly soluble aluminum or calcium salts.
2. Evaluate the feasibility of reducing the incidence of fish off-flavors by manipulating pond phytoplankton biomass and taxonomic composition using biological and chemical control measures.
   a. Evaluate the effect of filter-feeding fishes on water quality and reduction or elimination of off-flavor in pond-raised channel catfish.
   b. Develop microbial pathogens to control blue-green algal abundance.

c. Determine whether plant phenolics (tannins) can control growth of microorganisms that produce odorous compounds in warmwater fish ponds.

d. Evaluate the effect of routine, low-level treatments of ponds with copper sulfate on phytoplankton communities, off-flavor incidence, and water quality in channel catfish ponds.

3. Determine the feasibility of managing fish off-flavors by reducing rates of MIB uptake by fish and/or enhancing rates of MIB elimination from fish.
4. Develop statistical models describing the within-pond variation in the degree of off-flavor in fish populations under various conditions.
5. Develop analytical techniques for assessing flavor qualities in fish.
6. Develop publications to educate producers and processors on the ecology of environmentally-derived off-flavors, off-flavor management, and the results of this project.

Progress and principal accomplishments

This project has a nominal starting date of June 1, 1996. As of the ending date of the reporting period for this report (August 31, 1996), the project has not been approved by USDA/CSREES. As such, no progress can be reported.

Anticipated benefits

Certain blue-green algae that are common in summertime plankton communities of warmwater fish ponds can produce earthy-smelling secondary metabolites. Those metabolites may be absorbed by fish, thereby conferring an earthy-musty flavor to the flesh. Off-flavored fish are not marketable and holding fish in inventory until flavor quality improves is a significant economic burden. It has been estimated that development of off-flavors in pond-raised channel catfish increases the per pound cost of production by 5 to 15%. This project proposes to evaluate practical management practices that may lessen the economic impact of environment-derived off-flavors. Two additional objectives of this proposal are to study the variability of the degree of off-flavor within a population of pond-reared fish and develop quantitative tools for determining the degree of off-flavor in fish. Information obtained from those studies may allow development of more effective sampling protocols to quantify the incidence and severity of off-flavor in pond-raised fish.

Appendix

Southern Regional Aquaculture Center Publications, Videos, and Computer Software
(effective October, 1996)

Publications

100	Site Selection of Levee-Type Fish Production Ponds
101	Construction of Levee-type Ponds for Fish Production
102	Watershed Fish Production Ponds—Site Selection and Construction
103	Calculating Area and Volume of Ponds and Tanks
120	Baitfish
121	Baitfish—Feeds and Feeding Practices
122	Baitfish Production—Enterprise Budget
140	Forage Species—Range, Description, and Life History
141	Forage Species—Production Techniques
142	Forage Species—Return on Investment
160	What is Cage Culture?
161	Cage Culture—Site Selection and Water Quality
162	Cage Culture—Cage Construction and Placement
163	Cage Culture—Species Suitable for Cage Culture
164	Cage Culture—Handling and Feeding Caged Fish
165	Cage Culture—Cage Culture Problems
166	Cage Culture—Harvesting and Economics
180	Channel Catfish—Life History and Biology
181	Feeding Intensively Cultured Catfish in Levee-Type Ponds
183	Processing Channel Catfish
184	Processed Catfish—Product Forms, Packaging, Yields, and Product Mix
185	Processed Catfish
186	Channel Catfish—Dietary Effects on Body Composition and Storage Quality
187	Channel Catfish Production—Impacts of Diet Composition and Feeding Practices
200	Largemouth Bass—Biology and Life History
201	Largemouth Bass
220	Trout Production—Handling Eggs and Fry
221	Budgets for Trout Production—Estimated Costs and Returns for Trout Farming in the South

222 Trout Farming—A Guide to Production and Inventory Management
223 Trout Production—Foods and Feeding Methods
224 Rainbow Trout
230 Alligator Production—An Introduction
231 Alligator Production—Breeding and Egg Incubation
232 Alligator Production—Growout and Harvest
240 Crawfish Culture—Site Selection, Pond Construction, and Water Quality
241 Crawfish Production Systems
242 Crawfish Production—Harvesting, Marketing, and Economics
243 Crawfish—A Healthy Choice!
260 Introduction of Exotic Shrimp—Quarantine and Disease Inspection
280 Pond Culture of Tilapia 281—Cage Culture of Tilapia
282 Tank Culture of Tilapia
300 Hybrid Striped Bass—Biology and Life History
301 Hybrid Striped Bass—Hatchery Phase
302 Hybrid Striped Bass—Pond Production of Fingerlings
303 Hybrid Striped Bass—Pond Production of Food Fish
320 Red Drum—Biology and Life History
321 Red Drum—Site Selection and Pond Construction
322 Red Drum—Production of Food Fish
323 Red Drum—Brood Stock and Hatchery Production
324 Red Drum—Production of Fingerlings and Stockers
340 4-H Aquatic Science Project—Guide to Raising Catfish in a Cage
341 4-H Aquatic Science—Catfish Cage Culture Record Keeping Project
350 Small-Scale Marketing of Aquaculture Products
360 Aquatic Weed Management—Control Methods
361 Aquatic Weed Management—Herbicides (Revised)
370 Pond Aeration
371 Pond Aeration—Types and Uses of Aeration Equipment
372 Selecting the Proper Pump
373 Piping Systems
374 Open Channel Flow in Aquaculture
375 Powering Aquaculture Equipment
380 Computer Software for Aquaculture—Descriptions and Evaluations
390 Transportation of Warmwater Fish—Equipment and Guidelines
391 Sorting and Grading Warmwater Fish
392 Transportation of Warmwater Fish—Procedures and Loading Rates
393 Transportation of Warmwater Fish—Loading Rates and Tips by Species
394 Harvesting Warmwater Fish
400 Avian Predators on Southern Aquaculture
401 Facilities
402 Control of Bird Predation at Aquaculture Facilities
410 Calculating Treatments for Ponds and Tanks
421 Introduction to Hormone-Induced Spawning of Fish
422 Capturing, Handling, Transporting, Injecting, and Holding Brood Fish for Induced Spawning
423 Determining Sexual Maturity of Broodstock for Induced Spawning of Fish
424 Hormonal Control of Reproduction in Fish for Induced Spawning
425 Hormone Preparation, Dosage Calculation, and Injection Techniques for Induced Spawning of Fish
426 Techniques for Taking and Fertilizing the Spawn of Fish
427 Induction and Verification of Triploidy in Fish
431 Testing Flavor Quality of Preharvest Channel Catfish
432 The Cultivation of American Oysters *(Crassostrea virginica)*
433 Biology and Culture of the Northern Quahog Clam *(Mercenaria mercenaria)*
434 Aquacultured Oyster Products
441 Aquaculture—Realities and Potentials When Getting Started
451 Recirculating Aquaculture Tank Production Systems—An Overview of Critical Considerations
452 Recirculating Aquaculture Tank Production Systems—Management of Recirculating Systems
453 Recirculating Aquaculture Tank Production Systems—Component Options
454 Recirculating Aquaculture Tank Production Systems—Integrating Fish and Plant Culture

461 Water Quantity & Quality Requirements for Channel Catfish Hatcheries
462 Nitrite in Fish Ponds
463 Ammonia in Fish Ponds
464 Interactions of pH, Carbon Dioxide, Alkalinity, and Hardness in Fish Ponds
465 Survey of Aquaculture Effluent Permitting and 1993 Standards in the South
466 Algae Blooms in Commercial Fish Production Ponds
467 Cost of Alternative Effluent Treatments for Catfish Production
468 Carbon Dioxide in Catfish Ponds
472 Submitting a Sample for Fish Kill Investigation
473 Use of Medicated Feed in Food Fish (Revised)
474 The Role of Stress in Fish Disease
479 Fee Fishing—An Introduction
480 Fee-Fishing Ponds—Management of Food Fish and Water Quality
481 Development and Management of Fishing Leases
482 Fee Fishing—Location, Site Development, and Other Considerations
483 Biology and Life History of Freshwater Prawns
484 Production of Freshwater Prawns in Ponds
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Western Regional Aquaculture Center Compendium Report *for the Period May 1, 1989 to August 31, 1996*

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The Nutritive Value of Alternate Protein Sources in the Diets of Cultured Salmonids

Termination Report for the period July 1, 1988 to March 31, 1993

Total funding level

$612,000 (July 1, 1988 to March 31, 1993)

Participants

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* *(funded participants)*

Project objectives

1. Develop methods of preparing high-quality fish meals from fisheries processing waste, and test the developed products.
2. Complete the studies evaluating wheat gluten as a potential replacement for fish meal in salmon and trout feeds, and studies comparing wheat gluten and corn gluten meal in specialized trout diet formulation.
3. Complete the studies of alternate fish meals containing biogenic amines by adding purified gizzerosine, found in fish meal acutely toxic to poultry, to rainbow trout diets.
4. Evaluate the maximum level of replacement of fish meal with rapeseed protein concentrate in salmonid feeds.
5. Determine the *in vitro* protein digestibility of diets containing soybean meal as a replacer for herring meal; effect of protease inhibitors on the activity of enzymes; profiles of pyloric ceca enzymes from different sources by SDS-PAGE; and amino acid profiles in various dietary protein samples.
6. Investigate the palatability and nutritional value of two different lactic acid silage co-dried products and determine the maximum substitution levels in salmonid feeds.
7. Quantitate the effects of heat-treating soybean meal on trypsin inhibitor levels, and on palatability and nutritional value of soybean-containing diets fed to coho salmon; define the maximum substitution levels of optimally heat-processed soybean meal in feeds for salmonids; determine *in vitro* protein digestibility of diets containing soybean meal as a replacer of herring meal.
8. Complete the detailed practical manual entitled "Methods of Evaluating Protein and Lipid Sources for Fish Feeds."

Anticipated benefits

The rapid growth of the aquaculture industry is creating the need for alternate protein sources of consistent high quality for practical feed formulations. The benefits anticipated for this research project are listed below.

1. The production and initial testing of high-quality, low-ash fish meal produced from fish filleting waste, which will be the subject of further study by the fish processing industry in Alaska. If successful, the product will make highest and best use of protein recovered from the Alaska fish processing industry and will help solve two environmental problems: ocean dumping of waste in Alaska, and excess phosphorus in salmon and trout hatchery effluents.
2. The demonstration that wheat gluten can replace more than half of the fish meal in commercial salmon and trout feeds, which will stimulate the wheat industry to expand the production of low-cost, animal grade wheat gluten.
3. The characterization of the toxicity of biogenic amines found in some fish meals, which will allow fish feed producers to explore limited use of such

products in fish feeds.
4. The positive results obtained by use of rapeseed protein concentrate in place of fish meal in salmon and trout feeds, which will encourage commercial production of rapeseed protein concentrate in Canada.
5. The development of the *in vitro* protein digestibility assay, which will give industry a new method of predicting the nutritional value of protein sources for aquaculture species without resorting to *in vivo* analyses.
6. The results of lactic acid silage research which show that palatability is a factor limiting potential use of lactic acid silage but that, through control of oxidation and co-drying with other protein sources, resulting products can be used to replace a significant portion of fish meal in trout and salmon diets.
7. The quantification of the effects of trypsin inhibitors and palatability factors in soybean meal, which shows that these factors are independent and additive in nature.
8. The publication of the WRAC manual will result in transfer of much of the information obtained by the project investigators during the 5 years of WRAC funding to industry and researchers throughout the region, the nation, and the world.

Progress and principal accomplishments

Objective 1

To determine the effects on the nutritional value of modified fish meals on juvenile chinook salmon, tests included the screening of dried whitefish meal before grinding and the deboning of whitefish processing waste prior to meal production.

Screening the dried carcass waste decreased the ash and increased the fat and protein contents of the resulting fish meal as compared to the non-screened product. These effects on proximate composition were further enhanced by mechanically deboning before meal processing, resulting in a meal comparable to high quality fish meal. In an 8-week feeding trial with chinook salmon, in which the modified meals completely replaced herring meal in the feeds, there were no significant differences in fish weight or feed conversion ratios between the modified meal test diets and the herring meal control diet.

Objective 2

A series of trials with post-juvenile salmonids confirmed the nutritional suitability of 50-60% replacement of fish meal with wheat gluten in feeds. Feeding trials with post-juvenile coho salmon in marine net-pens were conducted to test the feasibility of replacing up to 60% of fish meal with wheat gluten. All nutritional indices measured (body weight, body composition, specific growth rates, feed conversion ratios, protein retention values, and average mortality rates) indicated that feeds containing 60% replacement by wheat gluten resulted in high performance of the fish.

Wheat gluten was compared to corn gluten meal in a study in which about 2/3 of the fish meal in diet formulations used by the U.S. Fish and Wildlife Service in trout production hatcheries was replaced. The fish fed diets containing corn gluten meal grew well, but the diet resulted in fillets with a yellow color. Fish fed diets containing wheat gluten grew equally well, but the fillets were unpigmented and scored higher in sensory attributes than fillets from fish fed the corn gluten diets.

Objective 3

Feeding trials related to this objective were delayed this past year due to personnel and fish disease problems. The study will be completed during Fall 1993 at no charge to WRAC. This trial will complement previous studies with histamine, putrescine, and cadaverine supplementation of semi-purified salmonid diets that were performed during year 4.

Objective 4

Feeding trials were conducted to evaluate factors affecting the level of replacement of fish meal with rapeseed protein concentrate (RPC) in salmonid feeds. Results indicated that: (1) dietary zinc supplementation did not significantly affect growth of rainbow trout, regardless of dietary RPC level; (2) trout performance was not compromised when either undephytinized or dephytinized RPC comprised 39% of dietary protein; (3) improvements of the dephytinization procedure are necessary; (4) addition of the appetite stimulant betaine maintained normal feed intake at all levels of RPC replacement of fish meal; and (5) mineral metabolism of trout fed diets with dephytinized RPC appeared to be normal.

Objective 5

The percent degree of hydrolysis (%DH) of diets containing 25% soybean meal (SBM) protein for herring meal was lower than that for either herring meal alone or herring meal with 25% SBM protein concentrate. Linear regression analysis indicated high correlation ($r^2 = 0.86$-0.88) between %DH of the feeds and growth rate of coho salmon fed those diets. The effects of various protease inhibitors were quantitated. Based on the response of crude pyloric ceca enzyme preparations from trout and chinook salmon to specific biological and chemical inhibitors, it was concluded that salmonid pyloric ceca contain enzymes similar to trypsin and chymotrypsin, and some metalloproteases.

Characterization of pyloric ceca enzymes from coho and chinook salmon and from rainbow trout by SDS-PAGE revealed several components with alkaline proteolytic activity ranging in size from 22-73 K daltons. A low level of a 22 K dalton proteinase corresponding to trypsin was detected in all three preparations.

Although coho and chinook salmon appeared to have a 32.8 K dalton chymotrypsin and rainbow trout did not, results with the protease inhibitors confirmed the presence of chymotrypsin-like activity in rainbow trout.

The amino acid profiles (12 essential and 6 non-essential amino acids) of 27 protein feed samples were determined. A prediction equation for protein quality by multiple regression analysis will be formulated using data from the amino acid composition, *in vitro* digestibility, and feeding trial growth measurements.

Objective 6

The palatability of feeds containing lactic acid bacteria silage co-dried with either poultry by-product meal or herring meal was tested in a feeding trial with rainbow trout. Several dietary substitution levels (0, 15, 30, 45%) of lactic acid silage for herring meal protein were tested, as well as the effects of using either freeze-dried viscera or ensiled herring meal replacement of herring meal.

Palatability problems observed in previous trials were not apparent in this trial, and the ensiling process itself did not reduce performance of the diet. Based on the silage processing procedure used for this trial, it was concluded that 60% substitution of herring meal protein with lactic acid silage co-dried with either PBM or herring meal resulted in excellent growth of rainbow trout.

Objective 7

Two separate trials were conducted feeding SBM diets to rainbow trout. The first trial determined the effects of autoclaving SBM on trypsin inhibitor levels, then feeding diets containing heat-treated SBM to rainbow trout fry. A 10- to 20-minute autoclave treatment of SBM successfully reduced trypsin inhibitor levels to the low level found in Promoveal®. Diets containing a 10- to 20-minute heat-treated SBM substituted for 15% of herring meal protein resulted in performance parameters similar to those observed for the herring meal control group, even though all diets containing 0-, 5-, 10-, and 20-minute heated SBM performed well.

In the second feeding trial, which was designed to define the maximum suitable substitution level of 20-minute heated SBM for herring meal, it was determined that a 15% substitution resulted in performance equivalent to that of the herring meal control group. Levels of 20-25% heated SBM supported 80-90% of the growth of the control group. Even though the addition of 5% krill meal for herring meal protein masked the unpalatable character of a 25% unheated SBM diet, it did not improve the nutritional value over that observed for the corresponding non-krill diet.

Objective 8

The purpose of the WRAC manual is to compile all relevant information concerning evaluation of protein and lipid sources for aquaculture feeds. This subject is complicated and confusing, and the manual will describe and discuss appropriate methods for evaluating feed ingredients. The manual contains five chapters: (1) “Chemical Evaluation;” (2) “Biological Evaluation;” (3) “Microbial Evaluation;” (4) “Procedures for Conducting Nutritional Studies with Fish;” and a (5) “Self-study Section.” The target audience is the working professional and student of fish nutrition worldwide. To extend the relevance of the manual to a worldwide audience, the manual is not restricted to salmon and trout.

Usefulness of findings

The rapid growth of the aquaculture industry is creating the need for alternate protein sources of consistent high quality for practical feed formulations.

Year 5 Highlights

1) The production and initial testing of high-quality, low-ash fish meal produced from fish filleting waste, which will be the subject of further study by the fish processing industry in Alaska. If successful, the product will make highest and best use of protein recovered from the Alaska fish processing industry and will help solve two environmental problems: ocean dumping of waste in Alaska and excess phosphorous in salmon and trout hatchery effluents.
2) The demonstration that wheat gluten can replace more than half of the fish meal in commercial salmon and trout feeds, which will stimulate the wheat industry to expand the production of low-cost, animal grade wheat gluten.
3) The characterization of the toxicity of biogenic amines found in some fish meals, which will allow fish feed producers to explore limited use of such products in fish feeds.
4) The positive results obtained by use of rapeseed protein concentrate in place of fish meal in salmon and trout feeds, which will encourage commercial production of rapeseed protein concentrate in Canada.
5) The development of the *in vitro* protein digestibility assay, which will give industry a new method of predicting the nutritional value of protein sources for aquaculture species without resorting to *in vivo* analysis.
6) The results of lactic acid silage research which shows that palatability is a factor limiting potential use of lactic acid silage but that, through control of oxidation and co-drying with other protein sources, resulting products can be used to replace a significant portion of fish meal in trout and salmon diets.
7) The quantification of the effects of trypsin inhibitors and palatability factors in soybean meal, which shows that these factors are independent and additive in nature.
8) The publication of the WRAC manual will result in transfer of much of the information obtained by the project investigators during the five years of WRAC funding to industry and researchers throughout the region, the nation, and the world.

Work planned for next year

The results described were accomplished in the final year of funding for the project. Funding for this year of activity was 66% of the amount requested by the investigators, thus limiting the scope of research. Two activities will be completed in the next year at no additional cost to WRAC: determination of the effects of the addition of purified gizzerosine to semi-purified diets for juvenile rainbow trout, and the completion of the WRAC manual, “Methods of Evaluating Protein and Lipid Sources for Fish Feeds,” which is ready for review and revision prior to publication.

Impacts

Additional funding generated from other sources:

- $70,000 from the Washington Wheat Commission to evaluate wheat gluten as an alternate fish protein;
- $65,000 from USDA to evaluate alternate protein sources and plant proteins as replacement for fish meal in trout diets; and
- $20,000 from British Petroleum Nutrition to evaluate fish waste hydrolysates as a potential feed ingredient.

Support

		Miscellaneous support					
Year	**WRAC funding**	**University**	**Industry**	**Other Fed.**	**Other**	**Total Misc.**	**Total**
87-88	150,632						150,632
88-89	149,368						149,368
89-90	0						0
90-91	250,000						250,000
91-92	0						0
92-93	62,000						62,000
Total	**612,000**						**612,000**

(This section was not included in reports until 1993, so complete information is not available.)

Development of Methods for Control of Infectious Hematopoietic Necrosis Virus in Commercially Reared Salmonid Fishes (1)

Termination Report for the period July 1, 1987 to March 31, 1993

Total funding level

$863,000 (July 1, 1987 to March 31, 1993)

Participants

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* *(funded participants)*

Project objectives

During the final year of this initial 5-year project, the infectious hematopoietic necrosis virus (IHNV) work group maintained a high level of productivity, publishing seven papers in peer-reviewed journals and seven in technical literature. Production of extension products continued and, in year 5, research by the IHNV Work Group concentrated on:

1. Developing improved subunit or recombinant vaccines against IHNV;
2. Defining the antigenic nature of the IHNV glycoprotein and the resulting antibody response in the fish;
3. Understanding the aspects of non-specific immunity including interference by a non-pathogenic virus;
4. Improving our understanding about the biology of the virus, including the presence of a carrier state; and
5. Continued development of improved methods for rapid detection of the virus.

This work group's proposal for an additional 4 years of funding was subsequently approved.

Anticipated benefits

The development of an effective vaccine for protection of fish against IHNV remains an important goal for the control of this viral disease. Losses due to IHNV are estimated in the millions of dollars annually.

While much remains to be done in order to optimize the conditions for delivery of these preparations to fish, a license has been granted to a commercial company for development of the subunit vaccine. In addition, commercial interest has been expressed in several other products from this project, including the monoclonal antibodies, DNA probe, and primer set for the polymerase chain reaction.

These are powerful tools that will improve the speed and precision of IHNV detection and will be of enormous assistance in improving our understanding of the virus carrier state and providing important information about the antigenic and biochemical variation among strains of IHNV.

Results from the interferon protection and virus interference studies are encouraging in that they show the importance of non-specific immune mechanisms for controlling losses due to IHNV. These non-specific mechanisms may be suitable targets for selective breeding and genetic manipulation studies in future years. Research to define the antigenic epitopes on the glycop rotein of the virus will provide critical information about sites for the next generation of vaccines.

Progress and principal accomplishments

Means for inducing specific immunity in rainbow trout were tested, including methods to determine if immune adjuvants and immunomodulators would enhance protection to specific IHNV antigens. Synthetic peptides were used to define the neutralizing domains of the IHNV glycoprotein.

A single peptide of 20 amino acids from amino acid 321-340 was found to contain an epitope that induced neutralizing antibody to IHNV in trout. The anti-peptide antisera were tested in passive transfer trials in fish where they conferred immunity to rainbow trout fry against IHNV. The peptide was also tested in a competitive inhibition plaque titration assay.

Studies were begun to characterize the virulence determinants of the IHNV glycoprotein. Trials were carried out at virus doses of 10^3 and 10^5 $TCID_{50}$/mL using four monoclonal antibody-selected variants. All of the variants had reduced virulence at the low virus dose; however, at the higher dose, only RB-1 was attenuated.

The humoral response of rainbow trout was studied using western blots against IHNV antigens. The kinetics of appearance of the response to each viral protein was tested in a group of trout which had a single exposure to IHNV and from which a sample of 22 animals was taken at each time point. Initial analysis of the data revealed a predominating response to G glycoprotein and to the protein M1 in the majority of the fish at all time points; however, at 2 weeks post-challenge, the predominating response was to M1 protein.

Transmission electron microscopy of small rainbow trout and coho salmon challenged with IHNV was used to document differences with regard to the effects of the virus in the gills, esophagus, gastrointestinal tract, and skin as early as 1 hour post-challenge. In rainbow trout, there was marked edema in the esophagus 1 hour post-challenge, while in coho salmon the change was minimal. At 24 hours post-infection, rainbow trout esophagus exhibited severe intracellular edema with separation of the mucosal and glandular epithelium.

A spectrophotometric interferon assay was used to investigate the effects of several biological variables on interferon production. Both poly I:C and IHNV induced interferon production in a dose-dependent fashion, but responses to these two inducers were only weakly correlated.

Interferon production was greatly reduced by removal of adherent cells (largely macrophages and neutrophils). Non-adherent lymphocytes had little or no activity in the assay. The resistance of trout to challenge by IHNV increased with size as the fish grew from 0.7 to 4 or 8 g. Interferon production did not, however, differ between fish of different sizes.

Strains of IHNV were compared by cross-neutralization using hyperimmune sera from rainbow trout to the five electropherotypes of IHNV. The results indicated that trout do not readily distinguish among these viruses with respect to the development of neutralizing antibodies. Although the number of fish used to make antibodies and the number of strains of IHNV tested were limited, the results suggest that cross-protection between strains of IHNV should be possible following vaccination or prior to heterologous strains of the virus.

Stimulation of nonspecific host defenses to IHNV provided by prior exposures to an avirulent picorna-like virus (CTV) gave insights into the duration of the protection and the development of anti-IHNV serum antibodies. A 5 minute or 1 hour bath exposure to CTV provided up to 69% relative percent survival following IHNV challenge. This effect was present at 1, 2, and 4 weeks post-exposure to CTV, but was absent at 6 weeks.

The levels of IHNV antibodies were significantly higher ($P = 0.007$) among fish receiving prior exposures to CTV than among non-CTV treated fish challenged with IHNV. Initial investigations into the basis of the non-specific stimulation provided by CTV have as yet failed to demonstrate a substantial role for interferon.

Neutralization-resistant variants of IHNV were used to locate and characterize antigenic sites on the IHNV glycoprotein. Polymerase chain reaction (PCR), asymmetric PCR, and sequencing techniques were used to determine the location of the nucleotide sequence changes of the G gene of each neutralization escape mutant. Most of the neutralizing monoclonal antibodies were directed at epitopes that were near the center portion of the 508 amino acid protein.

Usefulness of findings

The development of an effective vaccine for protection of fish against IHNV remains an important goal for the control of this viral disease. Losses due to IHNV are estimated in the millions of dollars annually.

While much remains to be done in order to optimize the conditions for delivery of these preparations to fish, a license has been granted to a commercial company for development of the subunit vaccine. In addition, commercial interest has been expressed in several other products from this project, including the monoclonal antibodies, DNA probe, and primer set for the polymerase chain reaction.

These are powerful tools that will improve the speed and precision of IHNV detection and will be of enormous assistance in improving our understanding of the virus carrier state and providing important information about the antigenic and biochemical variation among strains of IHNV.

Results from the interferon protection and virus interference studies are encouraging in that they show the importance of non-specific immune mechanisms for controlling losses due to IHNV. These non-specific mechanisms may be suitable targets for selective breeding and genetic manipulation studies in future years. Research to define the antigenic epitopes on the glycoprotein of the virus will provide critical information about sites for the next generation of vaccines.

Work planned for next year

During the first year of the new 4-year project, research will focus on: (1) stimulation of specific immunity, where efforts will be placed on development of suitable adjuvants and delivery methods for enhancing the immune response to a peptide or subunit vaccine; (2) characterization of the structure of the viral glycoprotein to gain information about the number, location, and nature of the antigenic sites required for protection; (3) *in vitro* and *in vivo* screening of potential inducers of several forms of non-specific resistance; and (4) gaining a better understanding of the biology of IHNV including the carrier state in fish, replication in invertebrates, and the residual nature of the virus in sediments.

Impacts

This project was among the original set of proposals selected for funding by WRAC. In addition to fish disease researchers from federal and university laboratories in Washington, Oregon, California, Idaho, and Alaska, the IHN work group includes scientists from the commercial trout and fish vaccine industries, which provides important focus for the research and serves as a conduit for technical information transfer to and from these industries.

During the initial 5-year project, the Work Group established a high level of productivity, publishing many papers in peer-reviewed journals and the technical literature. The most significant accomplishments were:

1. the use of new techniques in molecular biology to develop and test a subunit vaccine against IHNV that has been commercially licensed;
2. the development of novel methods and new diagnostic reagents which have also received commercial interest (monoclonal antibodies, DNA probes, and polymerase chain reaction primers) that have improved the speed and precision of IHN diagnosis;
3. the application of state-of-the-art methods to obtain new information about the role of non-specific mechanisms of resistance to IHNV, including natural killer cells and interferon;
4. the establishment of long-term research to improve our understanding of the biology of IHNV (e.g., carrier state, reservoirs of infection, cellular receptors for IHNV, and genetic basis of resistance) and;
5. the initiation of research to determine the effectiveness of novel drugs and chemicals, nutritional changes, and genetic selection for reducing losses to the disease.

Support

Year	WRAC funding	Miscellaneous support					Total
		University	Industry	Other Fed.	Other	Total Misc.	
87-88	165,000						165,000
88-89	134,518						134,518
89-90	100,000						100,000
90-91	200,000						200,000
91-92	150,000						150,000
92-93	113,000						113,000
Total	**863,000**						**863,000**

(This section was not included in reports until 1993, so complete information is not available.)

Development and Improvement of Fish and Shellfish Broodstocks

Termination Report for the period July 1, 1988 to March 31, 1993

Total funding level

$524,100 (July 1, 1988 to March 31, 1993)

Participants

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* *(funded participants)*

Project objectives

The overall goal of this regional research project was to define breeding schemes to improve the performance of aquacultural stocks of fish and shellfish for growth, age/size at sexual maturity, gonadal development, and carcass quality traits. The specific objectives to achieve this goal were:

1. to estimate heritabilities, intraclass correlations, and net economic values for growth rate, age/size at sexual maturity, gonadal development, and carcass quality;
2. to determine phenotypic and genetic correlations between growth rate and age/size at sexual maturity, growth rate and carcass quality, growth rate and gonadal development, and gonadal development and age/size at sexual maturity;
3. establish ontogenetic patterns for the growth and reproductive traits;
4. establish protocols for the effective development of pedigreed populations of Pacific oysters; and
5. evaluate alternative breeding schemes based on the parameters estimated in objectives 1 and 2.

Anticipated benefits

Growth and maturation traits can be very important factors in the production potential of any aquaculture operation. Analyses of the results from this project have shown that genetically-based selection and breeding programs can have a positive effect on improving these traits in commercially produced aquaculture animals.

Thus, reliable information is now available to initiate a program for broodstock development and improvement in several of the more important species raised in the Pacific Northwest. Across species, most growth parameters have been shown to be amenable to change through the use of rather simple selection schemes.

On the other hand, lowering the incidence of precocious maturation in fish will require more exacting approaches, and response will, apparently, still be rather slow. Total age to normal maturation appears to be an easily altered trait through selection, but it has some genetic characteristics that may cause some difficulties.

The importance of experimental design and the consideration of environmental factors in the analysis of quantitative traits in Pacific oysters was also highlighted, and approaches to these problems were defined. Consequently, a strong base of information has been defined on which a broodstock development and improvement program can be established, along with the needed guidelines to conduct it on a commercial scale.

Progress and principal accomplishments

In the Western Region of the U.S., three major groups of species comprised the bulk of commercial aquaculture production when this study was initiated: Pacific salmon (*Oncorhynchus* spp.), rainbow trout (*O. mykiss*), and Pacific oysters (*Crassostrea gigas*). The project included a total of four species; chinook salmon (*O. tshawytscha*), coho salmon (*O. kisutch*), rainbow trout, and Pacific oysters.

All laboratories participating in the program agreed upon a common mating design (a hierarchical design) and standardized the culture conditions as much as possible. Further, the frequency and types of measurements and the procedures to be used for analysis of the data were defined based on the length of the reproductive cycle in each species. By coordinating these aspects of the program, the working group felt that interspecific comparison of the results would be enhanced and the statistical reliability of the analyses conducted would be maximized. The Pacific oyster component of this program was the most difficult to "fit" into some of the agreed upon criteria, but as much as possible it was conducted similar to the other species components.

Results to address objective1 revealed that among the fish species, the genetics of growth (measured by body weight) were very similar. The heritability (h^2) values for growth during the normal growout phases to market size were in the range of 0.15–0.20. These results indicate that improvements in growth could be made with a designed selection program. On the other hand, initial Pacific oyster results (based on wet meat weight) revealed h^2 values that ranged from 0.0 to 0.8, suggesting a large environmental component to growth. Refinements in the experimental protocol yielded an estimated h^2 value of 0.216, although it was found that the proportions of males and females in the sample had a significant effect on the estimates.

Estimates of the h^2 values for age at sexual maturity revealed some differences among the fish species; this value was not estimated for oysters. Precocious (early) male maturation in all three salmonid species exhibited a relatively low amount of genetic determination ($h^2 < 0.10$), suggesting the presence of other factors in the determination of this trait. In fact, a fairly strong statistical relationship between growth rate and early maturation was noted in all three species. With chinook salmon, significant positive correlation between the egg size of the dam and the proportion maturing at age 2 was noted. On the other hand, estimates of the genetics of the time to what is considered "normal" maturation age were relatively high in rainbow trout and coho salmon ($h^2 \geq 0.50$), provided the trait analyzed was the number of days from parent spawning to progeny spawning. Results with chinook salmon did not reveal such a high genetic determination for this trait ($h^2 \sim 0.15$), a difference that may be related to the longer reproductive cycle in this species (4–5 years) compared to the other two species (2 years).

Genetic analyses of gonadal development were largely inconclusive, primarily because of the limited number of samples that could be taken; sacrificing large numbers of fish was necessary for these. Results with coho salmon suggested that the simplest and most informative character to measure with respect to gonadal development would be Gonadosomatic Index (GSI), although it would not be very useful for ontogenetic assessment.

Carcass quality traits were not analyzed in the program, with the exception of meat weight in Pacific oysters. However, an economic model was developed to provide guidance for selection and breeding programs. This model yielded production costs that are partitioned into major items (e.g., labor and feed) and could be utilized with any aquaculture operation for which cost and sales data were available. Model output on the effects of decreases in production costs and/or increases in product quality would provide information to assist in determining what traits should receive emphasis in a selection and breeding program.

The results for objective 2 were very involved and proper interpretation required rather lengthy consideration. Therefore, only some of the more informative correlations will be mentioned as part of this report. In all species of fish studied there was a relatively strong negative relationship in males between growth and age at sexual maturity; that is, the most rapidly growing fish generally mature at a younger age. In rainbow trout, for example, precocious males were significantly heavier and grew more rapidly than normal males prior to sexual maturation. The conclusion from these results is that males with genotypes for early maturity also possessed genes for rapid growth rate. However, further analyses of results from the coho research suggested that growth at later ages is not related to precocity. Consequently, selection against precocity and for large adults may not be incompatible objectives for broodstock improvement.

The genetic and phenotypic relationships among other growth and reproductive traits were somewhat less conclusive. There were, however, positive genetic correlations between several growth parameters that can provide guidance for designing selection programs to improve broodstocks at different points in their life cycle.

Objective 3 was only partially addressed due to the paucity of data on gonadal development in most species studied. Initial studies on Pacific oysters revealed that data on very small (< 3 cm) individuals yielded conflicting results due to the influence of early rearing practices; thus, no further data were collected during early development.

Analyses of early growth data for the fish species revealed a major influence of maternal factors; this was most commonly expressed as a strong relationship between egg size and growth. Such influence became less important as the young fish developed, for the most part disappearing at about 7 months after hatching. The genetic influences on growth after this point were fairly similar among the fish species during the various stanzas of their reproductive cycle.

The results for objective 4 were met by: (1) adjusting breeding and growout protocols for the Pacific oysters used in this study to improve the analytical results and (2) developing a number of pedigreed lines of oysters that, in fact, provided the material for a WRAC-funded program on oyster broodstock development.

Only initial work on objective 5 was possible within the term of this program. A short-term selection program with coho salmon was undertaken to determine the potential of changing the proportion of precocious males by breeding. Results suggested that, in fact, some change could be realized, but the program would have to be carefully designed. Rainbow trout families were raised at different densities and growth was monitored between 129 and 251 days post-hatch. The results from this study demonstrated that while growth at high densities was somewhat less, the amount of environmental variation was significantly smaller in this group. These results suggest that estimation of breeding value on fish raised at higher densities yields a more accurate indicator of selection response and, thus, a better prediction of expected gain.

Usefulness of findings

The age at which a species reaches sexual maturity, the growth of the organisms to this point, and the genetic relationship between these traits are all economically important characteristics that need to be incorporated into the development and improvement of aquacultural broodstocks. Through the coordination of experimental approaches and the use of common breeding designs, results from research on the four species investigated in this program should provide reliable estimates of the needed genetic parameters.

Such information is essential to the design of effective breeding and selection plans. Reliable genetic information of the traits investigated will be available and can be incorporated into commercial programs.

Analyses of the data obtained on growth and sexual maturity in the four species investigated have, in general, reinforced the conclusion that precocious maturation is associated with early rapid growth and is a trait that will require special selection efforts to decrease its incidence in a population.

However, size at later ages does not seem to be strongly associated with early maturation and, thus, size of fish for later harvest could be improved without increasing the incidence of precocious maturation. The results obtained to date provide some important directions for planning broodstock development and improvement programs.

The confounding impacts of environmental variation on the genetic expression of, particularly, growth have also been highlighted in the results from some of the projects. Approaches to mitigate for these problems are being investigated through alteration of breeding design and/or modified husbandry techniques.

Results from these projects should yield methods by which (1) more reliable estimates of genetic parameters can be obtained and (2) culture conditions will be defined that will lead to an increase in selection response with a concomitant reduction in costs for such a program.

Further, the experimental techniques developed during the course of these investigations have led to model approaches that can be used for industry application. For example, the methods utilized for pedigreeing oyster populations and the rearing techniques developed for chinook salmon have elicited a strong interest from aquaculturists in the Western Region.

Work planned for next year

Two of the active programs (coho salmon and oyster projects) have completed the objectives set out in the original proposal and, thus, have finished the analyses planned. However, both will continue, to some degree, to enhance the genetic information already obtained; the oyster project as a new WRAC program on oyster broodstock, and the coho salmon project to maintain the lines selected on the basis of the incidence of precocious maturation.

The third, the chinook project, requires one more year to obtain the complete set of data, as originally intended, and it should be continued until March 1994. Data will be collected on the growth and reproductive traits of the fish that are part of this project, and the final analyses will be conducted to estimate the genetic parameters explaining the variation in these traits.

Impacts

1. Results from this program played a role in initiating the Oyster Broodstock Program, a federally-funded and industry supported program located in Newport, Oregon, to develop Pacific oyster broodstocks for the industry.
2. Rearing techniques developed for the chinook salmon component of the project have been utilized by aquaculturists in the Western Region.
3. The pedigreed lines of Pacific oysters formed the nucleus of a breeding program to investigate the efficacy of utilizing inbreeding/crossbreeding as an approach to development of improved broodstocks for the industry.
4. The relationship between early growth and precocious maturity in fish has called attention to the impacts of growth rate on early maturation rate in commercial production.

Support

Year	WRAC funding	Miscellaneous support					Total
		University	Industry	Other Fed.	Other	Total Misc.	
87-88	125,000						125,000
88-89	0						0
89-90	175,000						175,000
90-91	0						0
91-92	130,000						130,000
92-93	194,100						194,100
Total	**524,100**						**524,100**

(This section was not included in reports until 1993, so complete information is not available.)

Development and Improvement of Fish and Shellfish Broodstocks (Chinook salmon component)

Termination Report for the period April 1, 1993 to March 31, 1995

Total funding level

$29,900 (April 1, 1993 to March 31, 1995)

Participants

Anthony Gharrett,University of Alaska, Fairbanks, Alaska
William R. Heard,* University of Alaska/NMFS, Alaska
R. J. Heintz, National Marine Fisheries Service, Auke Bay, Alaska
William Hershberger (Chair),* University of Washington, Washington
John Joyce, National Marine Fisheries Service, Auke Bay, Alaska
William Smoker (TA),* University of Alaska, Fairbanks, Alaska

* *(funded participants)*

Project objectives

The objective of the chinook salmon component of the Broodstock Improvement Project is to gather and analyze basic genetic information on growth and reproduction in chinook salmon from a standardized experimental design. The standardized design was hierarchical mating of 50 females to 25 males. The estimation of genetic parameters is based on analysis of individual observations (PIT-tagged fish) of growth and maturation.

Anticipated benefits

Salmon aquaculturists (and salmon biologists and resource managers in general) will have a better understanding of the connection between growth and maturation of chinook salmon. The development of broodstock management procedures to maximize growth while minimizing early maturation remains an important goal in salmon aquaculture. Selection programs to improve growth performance will benefit from this project's estimates of genetic parameters concerning growth and maturation.

Progress and principal accomplishments

In August 1989, 100 chinook salmon were collected and spawned at the Little Port Walter Research station, a facility of the NMFS Auke Bay Laboratory. The laboratory has carried on research on chinook broodstock development for ocean ranching in Alaska since the mid-1970's, and this research on captive families was an extension of that basic research. Accomplishments assocated with this project were the development of a culture system that permitted tracking of individual growth and maturation in a large array of chinook salmon families.

In 1990, 48 families were ponded into single-family containers and cultured separately until the fish were large enough to tag with passive integrated transponders (PIT tags). Eight tagged fish from each family were placed into a 274.6 cubic meter seawater cage. The fish were observed (length, weight, maturity, and gender) semi-annually thereafter.

By July 1993, 50% of the fish had matured. The balance matured in 1994 at 5 years of age, and approximately 1,500 chinook salmon matured in total. The 48 family histories include records of precocity, growth during each year of life, and proportions maturing at ages 2, 3, and 5.

Analysis of the data demonstrates a significant inverse relationship between growth during the first year of life and the age of maturity in males ($P \leq 0.001$). While this relationship may not be surprising for early-maturing males (jacks), the early growth rates of males destined to mature at later ages also differed significantly. Characters, such as sire age, dam age, and egg size, also relate to growth during this same period, but the relationship between these characters and age at maturity provided contradictory results. Consequently, the relationship between growth and age at maturity does not seem to be mediated by parental characters.

Calculation of the heritability for growth and age at maturity among males revealed a large genetic component to variation in growth ($h^2 \sim 2.16$) and little genetic influence on age at maturity ($h^2 \sim 0.15$). This result, coupled with the relationship described above, suggests that changes of the average age at maturity in a population could result from selection for growth during the first year of life.

Usefulness of findings

Growth and maturation of salmon are traits important to the economic value of cultured salmon, ocean-ranched as well as farmed. Fast growth is desirable, but early maturation (jacking), which is associated with fast growth in chinook salmon males, is a detriment. Estimation of genetic parameters of growth and maturation for chinook salmon, along with comparable estimates for other species provided by other sub-projects, will form the basis for designing both effective broodstock management and effective gene conservation plans. These plans are important to both farmed and ocean-ranched broodstocks.

Recent attention has been drawn to declining body size and advancing age in ocean-going stocks of Pacific salmon. One hypothesis for explaining these trends considers the involvement of artificial selection as an underlying cause. The findings of this subproject and the project as a whole will be very useful in evaluating the hypothesis.

Work planned for next year

The project is at an end. There remains completion of the analysis of data and reporting of the analyses in the technical literature.

Impacts

Aquaculturists, particularly managers of salmonid broodstocks, have gained insight into the relationship between growth and unwanted maturation in cultured fish.

Support

		Miscellaneous support					
Year	**WRAC funding**	**University**	**Industry**	**Other Fed.**	**Other**	**Total Misc.**	**Total**
93-94	19,000	16,923	0	0	30,000	46,923	65,923
94-95	10,900	8,612			15,000	23,612	34,512
Total	**29,900**	**25,535**	**0**	**0**	**45,000**	**70,535**	**100,435**

Effect of Broodstock Nutrition on Maturation and Subsequent Reproductive Performance

Termination Report for the period July 1, 1988 to March 31, 1993

Total funding level

$334,747 (July 1, 1988 to March 31, 1993)

Participants

Randall K. Buddington,* Mississippi State University, Mississippi
Christopher Calvert,* University of California, Davis, California
Joseph Cloud,* University of Idaho, Idaho
Serge Doroshov (TA),* University of California, Davis, California
David A. Erdahl (Chair),* Fish Technology Center, Bozeman, Montana
Ron Hardy,* University of Washington/NMFS, Washington
David Hinton,* University of California, Davis, California
Carl B. Schreck,* Oregon State University, Oregon
Richard L. Swallow,* Coker College, South Carolina

* *(funded participants)*

Project objectives

1. Investigate the effects of husbandry and nutrition on reproductive development of domestic white sturgeon.
2. Investigate quantitative cellular changes in liver and ovary structure and function as a mirror of broodstock nutrition.
3. Determine the effect of dietary protein and lipid levels on the growth and reproductive performance of rainbow trout broodstock.

Anticipated benefits

Objective 1

The commercial sturgeon culture industry in California currently produces about one million pounds of sturgeon flesh for the food market yearly. This industry is currently dependent upon wildstock fish as a source of seedstock.

Future availability of wildstock fish is uncertain. Improved reproductive performance of domestic broodstock is critical to establishing an independent domestic seedstock supply for the growout industry. Elucidation of the impact of dietary components on reproductive performance of domestic stock is essential to the development of a domestic seedstock supply.

Objective 2

Two of the organs which must work in concert for optimal reproductive performance are the ovary and the liver. A better understanding of their structure and function will allow for the development of earlier indicators of suitability of a specific nutritional regime. This may also contribute to a better understanding of the cellular and tissue contributions, as well as permit the testing of additional regimens for their efficiency.

Objective 3

There is currently a growing awareness among broodstock managers that large fish yielding large eggs may not be as desirable as smaller fish yielding a higher relative fecundity. It is possible that by adjusting the protein and lipid levels in the diet of broodstocks, somatic growth could be limited while reproductive performance is maximized. Such a diet may also lead to a substantial reduction in feed cost, which is a major expense at virtually all fish-rearing facilities.

Progress and principal accomplishments

Objective 1

The goal of this work was to elucidate the nutrition and husbandry on the reproductive performance of domestic white sturgeon. Studies were conducted in collaboration with three commercial California sturgeon farms (Sierra Aquafarms, Sea Farms, and The Fishery).

Specific objectives during 1992-1993 were: (1) to establish a technique for the direct measurement of plasma yolk precursor protein vitellogenin (Vg); and (2) to evaluate correlations between plasma Vg, total calcium, and stage of ovarian maturity in female stocks from different farms. Samples of eggs and information on spawning performance was collected from domestic and wild-caught females during the spring 1993 spawning season. This material is currently being analyzed.

Work initiated the previous year on the establishment of an enzyme-linked immunosorbent assay (ELISA) for measurement of plasma vitellogenin (Vg) concentrations in domestic females was completed. Blood plasma and gonadal tissue was sampled from 72 females and 12 males of domestic stock raised under different environmental conditions. Fish were fed either Silver Cup (trout diet) or Ewos (modified salmon diet) feed. Ovarian development, egg diameter, total plasma calcium, and Vg were measured.

Objective 2

This study was integrated with an ongoing feeding rate study being conducted at the Fish Technology Center (FTC) in Bozeman, Montana. Eagle Lake rainbow trout were maintained on a daily feeding rate of either 0.4, 0.8, or 1.2% of body weight until the time of spawning (February 1993).

Male and female fish were included in the liver and gonad weights and the ratios of liver to body weight and gonad to body weight were determined for each. Blood was collected from each of the fish by venipuncture of the caudal venous sinus. Following this procedure, fixative (1/2 strength Karnovsky's fluid) was introduced via a tributary of the hepatic portal vein and sufficient fixative fluid was introduced to result in perfusion of liver and gonads.

After fixation, tissues were trimmed from individual fish and stored in fixative for return to UC Davis and subsequent work-up. Livers and ovaries were processed for routine (paraffin) and high resolution light microscopy (glycolmethacrylate) embedment. Sections were cut at 4-6 mm and strained by hematoxylin and eosin (H&E). Other liver pieces were processed for electron microscopy. In blood samples, serum vitellogenin was quantified using a microfiltration dot blot technique and a monoclonal antibody specific for trout vitellogenin.

Objective 3
Six experimental diets were formulated in a 2x3 factorial treatment arrangement. The main effects were protein and lipid level. There were two levels of protein, 32 and 42%, and three levels of lipid, 9.3, 12.3, and 15.3%. Each of the two protein levels was combined with each of the three lipid levels to formulate the diets. Each diet was randomly assigned to two tanks (12 tanks total), and each tank contained 55, 2 year-old rainbow trout.

At the time of spawning, each female was spawned individually and fish weight and egg weight recorded. Reproductive performance was evaluated by determining percent eye-up, egg size, relative fecundity, and fry size after initial feeding.

Weight gain with respect to treatment group was determined just prior to the time of spawning before the fish began to go off feed. The fish were evaluated over two complete reproductive cycles.

The fish were spawned for the first time during the months of October to December 1991 (6 months on feed) over an 11-week period, on five separate occasions. The fish were then spawned for the second time during October to December 1992, again on five separate occasions over an 11-week period.

Usefulness of findings

Objective 1
The commercial sturgeon culture industry in California currently produces about one million pounds of sturgeon flesh for the food market yearly. This industry is currently dependent upon wildstock fish as a source of seedstock. Future availability of wildstock fish is uncertain. Improved reproductive performance of domestic broodstock is critical to establishing an independent domestic seedstock supply for the growout industry. Elucidation of the impact of dietary components on reproductive performance of domestic stock is essential to the development of a domestic seedstock supply.

Objective 2
Two of the organs which must work in concert for optimal reproductive performance are the ovary and the liver. A better understanding of their structure and function will allow for the development of earlier indicators of suitability of a specific nutritional regime. This may also contribute to a better understanding of the cellular and tissue contributions, as well as permit the testing of additional regimens for their efficiency.

Objective 3
There is currently a growing awareness among broodstock managers that large fish yielding large eggs may not be as desirable as smaller fish yielding a higher relative fecundity. It is possible that by adjusting the protein and lipid levels in the diet of broodstocks, somatic growth could be limited while reproductive performance is maximized. Such a diet may also lead to a substantial reduction in feed cost, which is a major expense at virtually all fish rearing facilities.

Work planned for next year

Objective 1
Aspects of the work not completely finished are in final preparation stages. Similar work on other sturgeon species is planned utilizing other funding sources.

Objective 2
Aspects of the work not completely finished are in final preparation stages. Work is not continuing under WRAC support, but is being supported by alternative sources.

Objective 3
Aspects of the work not completely finished are in final preparations stages. Work is not continuing under WRAC support, but Bozeman FTC plans to continue dietary protein/lipid studies with other strains of rainbow trout.

Impacts

While results of these studies have not led to the development of any new products to date, data indicate that current broodstock diet formulations may contain higher than necessary levels of certain diet components. If further research verifies these data, it may very likely lead to the development of broodstock diets that are not only more efficient, but also function to reduce the level of certain hatchery effluent components.

Support

Year	WRAC funding	Miscellaneous support					Total
		University	Industry	Other Fed.	Other	Total Misc.	
93-94	19,000	16,923	0	0	30,000	46,923	65,923
94-95	10,900	8,612	0	0	15,000	23,612	34,512
Total	**29,900**	**25,535**	**0**	**0**	**45,000**	**70,535**	**100,435**

Improved Production of Salmonids Through Sex Control and Chromosome Set Manipulation

Termination Report or the period July 1, 1988 to March 31, 1994

Total funding level

$557, 500 (July 1, 1988 to March 31, 1994)

Participants

Christopher Calvert,* University of California, Davis, California
Joseph Cloud,* University of Idaho, Idaho
Serge Doroshov,* University of California, Davis, California
Anthony Gharrett (TA),* University of Alaska/NMFS, Auke Bay, Alaska
William Hershberger,* University of Washington, Washington
Orlay Johnson, National Marine Fisheries Service, Seattle, Washington
James Parsons, Clear Springs Foods, Incorporated, Idaho
Carl Schreck (Chair),* Oregon State University, Oregon
William Smoker,* University of Alaska, Alaska
Raymond Teplitz,* University of California, Davis, California
Gary Thorgaard,* Washington State University, Washington

(funded participants)

Project objectives

1. Improve existing and develop new methodologies for production of chromosomally and sex-manipulated salmonids.
2. Evaluate performance of salmonids produced by chromosome set manipulation.

Anticipated benefits

The availability of improved techniques and understanding of biological mechanisms determining performance will provide necessary methods and guidance to commercial producers for the culture of monosex and ploidy-manipulated salmonids. Together, these studies should increase the efficiency of producing sex-manipulated salmonids and give commercial farms a better knowledge of how these fish will perform.

Progress and principal accomplishments

This project has sought to improve the methodology for producing monosex and sterile salmonid fishes for aquaculture. This has involved producing, treating, and evaluating gynogenetic and triploid salmonids. The research has been carried out collaboratively through sub-projects in Alaska, California, Idaho, Oregon, and Washington (two sub-projects).

Optimizing the techniques for sex control is one major goal of the project. The sub-project at Washington State University has investigated variables in successful gynogenesis for salmonids. These studies are also relevant to the induction of triploidy and tetraploidy in salmonids. Research at Washington State University (WSU) showed that, for second polar body retention in rainbow trout, heat shock was most effective 10–40 minutes after fertilization, while pressure was more effective 40–60 minutes post-fertilization.

Heat shock treatments were not strongly dependent on incubation temperature, but pressure treatments were, with optimal treatments being applied later at lower incubation temperatures. Surprisingly, pressure treatments providing high yields of gynogenetic fry by polar body retention did not always result in high yields of triploids. This indicated that caution is needed about extrapolating from one method to the other.

The lack of an absolute correlation between gynogenesis and triploidy success may be because haploid individuals resulting from failed polar body retention die in gynogenesis experiments, while the diploids in the case of induced triploidy do not. First cleavage blockage studies involving rainbow trout at WSU showed that the blockage was temperature-dependent for both the heat and pressure treatments, with the optimal treatment times being delayed at lower temperatures.

Studies at WSU examined the possibility of using the proportion of eggs which turn white after heat shock as a screen for egg quality. One trial involving 18 females showed a high correlation (> 0.6) between the proportion of white eggs and subsequent mortality after fertilization. However, two later trials showed no correlation. If successful, this screen could provide a rapid, objective test of egg quality.

Research at the University of Washington (UW) sought to improve methods for producing tetraploid salmonids. Studies in rainbow trout at UW showed that heat is more effective than pressure for inducing tetraploidy. Tetraploid fry have been produced and reared. Such fish are useful for generating triploids by direct mating to diploids. The resulting triploids, generated without heat or pressure treatments, show improved growth and survival.

These studies have been extended to efforts to produce allotetraploid salmonids. A significant insight for induced tetraploidy has been that the time of first cleavage can be highly variable among individual eggs, and optimal times need to be defined in terms of mean time of first cleavage from an adequate sample of eggs.

Research at Oregon State University (OSU) has sought to test sex-reversal treatments for converting gynogenetic, female rainbow trout and chinook salmon into males. Combinations of immersion with 17-alpha methyltestosterone (MT) and feeding produced high percentages of masculinized fish in both species. Immersion in MT alone was successful but at lower rates. The optimal immersion time for both species appeared to be about 1 week after hatching.

Two other hormones tested (OHA; 11beta-hydroxyandrostenedione and NE; 19alpha norethisterone acetate) were less effective than MT. Males produced through immersion and feeding of MT generally did not develop sperm ducts, while animals immersed in MT tended to have functional sperm ducts. Cryopreserved semen from functional males produced 100% female populations.

Studies with chinook salmon tested the effectiveness of four additional compounds for sex reversal. Two aromatase inhibitors failed to reverse the sex of fish through either injection, immersion, or with a combination of immersion plus feeding. A synthetic androgen, mibiolerone, was effective with immersion treatments 1 week after hatch. This was the first demonstration of successful sex inversion in salmonids with mibiolerone. A steroidal inhibitor, 4-hydroxy-androstenedione, increased the percentage of precocious males.

Triploid salmonids have been evaluated at the University of California Davis (UCD) and in Alaska. Studies at UCD have focused on rainbow trout and have characterized the performance, cellular characteristics and reproductive development of diploids and triploids. Triploids and diploids were sampled at 8, 15, 20, 25, and 43 months of age. In these fish, the first cycle of gonadal maturation and spawning took place at 25 months, and the second cycle at 36 months.

Results were generally encouraging regarding the use of triploid female trout. Diploids and triploids were initially similar in fork length and carcass weight; by the final sampling, the triploid females showed advantages. The testes in triploid males were smaller than in diploid males and triploid females showed very little ovarian growth. Some extremely dilute sperm was produced by triploid males after GnRH hormone administration. Fertilizing normal eggs with this sperm resulted in a very low rate of surviving progeny with near-3n DNA contents.

Studies in Alaska focused on diploid and triploid hybrid Pacific salmon. Diploid and triploid hybrids among chinook, chum, and pink salmon were produced in 1989 and 1990, and their potential for use in aquaculture has been evaluated. Traits that have been monitored include: survival to hatching, seawater tolerance, fresh/saltwater growth, and early maturation and survival. All of the crosses had high survival except that between the chinook female and chum male.

In seawater challenges, hybrids that had a pink or chum salmon parent were most successful in the first year, while all lots showed high survival in the second year. The hybrids between pink and chinook salmon appear to be most promising for growth rate.

Evaluations of the various types of hybrids among the species continued in seawater net pens. Some pink-X-chinook and chinook-X-pink hybrids matured and were backcrossed to female pink salmon; allozymes of the parents and progeny are being examined. Pink-X-chinook and chinook-X-pink diploid and triploid hybrids were compared to diploid and triploid chinook controls for total lipid, total water, and pigmentation by the National Marine Fisheries Service (NMFS). Preliminary analysis indicated no differences in lipid or water content but higher carotenoid values in the chinook.

Selected triploids and hybrids produced in Alaska have been fixed in formalin and analyzed for ploidy at UCD. Ploidy variation and frequency of mosaicism were examined liver, kidney, spleen, and gonads. There was a consistency of ploidy in within-species diploids while, surprisingly, hybrids and triploids showed evidence of mosaicism. The significance of these results is still to be determined.

Studies at the University of Idaho (UI) have emphasized the study of satellite cells in trout. Satellite cells are myogenic stem cells which are derived from skeletal muscle. By analyzing their growth in culture, it may be possible to obtain insights into the genetic potential for skeletal muscle growth.

Comparisons of the growth of satellite cells from diploid and triploid rainbow trout muscle have been made at UI. Although no significant differences in proliferation or differentiation were detected between cells from diploid and triploid fish, higher numbers of mononucleated cells per gram of tissue were obtained from diploids than triploids. This may be because cells from the triploids were larger.

Studies of satellite cells isolated from young and old steelhead showed that the older fish had fewer cells which did not increase in number, fuse, or produce myosin in culture as effectively as those from young fish.

Usefulness of findings

The value and efficiency of salmonid aquaculture can be increased by culturing all-female and sterile populations. Methods for producing such populations have been used in research settings, but they need further refinement and communication to industry before wide implementation in commercial situations will be practical.

All-female culture has already been demonstrated to be valuable for salmonid aquaculture. All-female rainbow trout are being commercially reared in British Columbia. Such populations are desirable because females are more valuable as broodstock (fewer broodstock need to be held) and because of the value of eggs (e.g., with pink salmon for caviar). Females also show a lower rate of precocious sexual maturation and sexually-maturing fish are less valuable.

Where large, sterile fish are needed, triploid production may have merit. Female triploids appear to have growth and carcass quality advantages at and after sexual maturation. However, better information has been needed regarding triploid and diploid cellular and reproductive characteristics and performance in aquaculture situations. Methods for induction of triploidy have needed further optimization, and production of triploids using tetraploid broodstock is an attractive alternative which has been studied in this project.

Gynogenesis can be used to generate all-female salmonid populations. Such fish can then be treated with male hormone and, because they are XX in chromosome constitution, will be XX males. Improved methods for inducing gynogenesis have been developed in this project, and studies have sought to identify the most effective heat or pressure treatments for inducing retention of the second polar body.

The next phase in the process, sex-reversing XX salmonids into males, has been the focus of study at OSU. The XX males which are produced by sex reversal can be crossed to normal females to produce all-female populations. The goal has been to develop protocols that minimize the amount of hormone used, simplify the routes of exposure, and minimize the potential human toxicity of the hormones used. Protocols using immersion of embryos with MT or mibiolerone shortly after hatching simplify exposure treatments and minimize human exposure.

Studies of triploid and diploid rainbow trout at UCD showed that diploids and triploids trout grew comparably before sexual maturity, but that triploid females showed superior growth after sexual maturity. Triploid males produced no sperm. Without GnRH administration, the sperm they did produce resulted in a few near-3n progeny. Studies of satellite cell growth in culture at UI indicate similar growth of cells from triploid and diploid rainbow trout, a result consistent with the growth trials.

Triploid interspecific salmonid hybrids have previously been demonstrated to often be more viable than the corresponding diploid hybrids. The survival and performance of triploid and diploid hybrids involving the chinook, chum, and pink salmon has been investigated by the University of Alaska (UA) and the NMFS/Auke Bay Lab. The pink-X-chinook and chinook-X-pink hybrids are the most promising crosses identified in their studies. These fish may combine the meat quality of chinook salmon with the early seawater tolerance of the pink.

These studies have developed methods which should increase the ease and efficiency of producing all-female and sterile salmonids. We can also give the industry a better knowledge of how these fish will perform.

Work planned for next year

This was the final year of the project.

Impacts

Several commercial rainbow trout producers in the states of Idaho, California, and Washington are utilizing the procedures developed in this program to make triploid trout for production. In addition, some of the farms are utilizing the techniques for gynogenesis and sex reversal in broodstock development.

Besides the industry actively using these techniques, investigators that participated in the program have been communicating with farmers to relay recent findings and improvements in the techniques of sex control and chromosome set manipulation.

Support

Year	WRAC funding	Miscellaneous support: University	Industry	Other Fed.	Other	Total Misc.	Total
88-89	125,000	0	0	0	0	0	125,000
88-89	125,000	0	0	0	0	0	125,000
89-90	125,000	0	0	0	0	0	125,000
92-93	103,500	0	0	0	0	0	103,500
93-94	79,000	0	0	0	0	0	79,000
Total	**557,500**	**0**	**0**	**0**	**0**	**0**	**557,500**

(This section was not included in reports until 1993, so complete information is not available.)

Etiology and Control of Bacterial Kidney Disease in Cultured Salmon

Termination Report for the period April 1, 1990 to March 31, 1996

Total funding level

$272,700 (April 1, 1990 to March 31, 1996)

Participants

James Congleton (TA),* University of Idaho, Idaho
Diane Elliott, U.S. Fish and Wildlife Service, Washington
Stephen Kaattari (Chair),* Oregon State University, Oregon
Marsha Landolt,* University of Washington, Washington
Ron Pascho, U.S. Fish and Wildlife Service, Washington
Sandra Ristow,* Washington State University, Washington
John Rohovec,* Oregon State University, Oregon

* *(funded participants)*

Project objectives

1. Determine if *R. salmoninarum* secretes soluble factors which impair bactericidal activity of salmonid macrophages.
2. Determine if clinically-diseased salmonids express features of immune-complex related glomerulonephritis.
3. Develop a prototype vaccine for Bacterial Kidney Disease (BKD).
4. Develop monoclonal anti-T cell reagents, which may be used to determine the mechanisms of immunity and pathology to BKD.

Anticipated benefits

The precise mechanisms of pathogenesis of BKD remains an enigma. By taking the multi-faceted approach for this proposal, we hope to determine critical mechanisms of pathogenesis and apply this knowledge to the development of efficacious vaccines and diagnosis. Work at the University of Idaho is focused on the role *R. salmoninarum* has on effecting macrophage function. Understanding how this critical cell is apparently made dysfunctional will aid in preparing effective vaccines which do not include aggressins.

Research conducted by the U.S. Fish and Wildlife Service will reveal if certain immune responses may be pathological. Specifically, they are determining whether immune complexes may be forming within the kidney, which in turn would lead to a dysfunctional kidney and perhaps exacerbate the disease process. If antigens exist which induce such immune responses, it will be critical to identify them and remove them from any vaccine preparation.

The production of an easily applied oral vaccine of prophylactic value such as the one described herein, could have a tremendous positive impact upon the production of cultured salmonids in the Pacific Northwest as well as the Northeast and other regions of the globe. No one has yet produced anti-T cell reagents for the salmonid fish. As much evidence points to the importance of cell-mediated immunity in BKD, the development of these reagents should be of great benefit.

Progress and principal accomplishments

The University of Idaho's work indicates that cellular and soluble factors of *R. salmoninarum* do not appear to affect macrophage adherence or phagocytic activity. It also appears that *R. salmoninarum* cells increase adherence and phagocytosis of third party bacterial cells.

The U.S. Fish and Wildlife Service has found that, although severely infected fish from a live challenge do demonstrate signs of glomerulonephritis, the immuno-histochemical work indicates that it may not be of immune complex origin. If such an origin was the cause, there should be more deposition of complement fragments within the tissues. Such deposition was only seen in severely infected fish. Therefore, it would seem likely that although an immune complex form of immunopathology may occur, it would have to be secondary to other pathological processes.

Oregon State University has performed two term vaccination experiments which demonstrate the need for oral administration of the *R. salmoninarum* vaccine and that the whole cell vaccine must be prepared by heat-mediated induction of proteolytic cleavage of the p57 molecule. Enteric protection, although effective in some cases, may not be required for *R. salmoninarum*, as both enteric-coated and non-coated microspheres were capable of eliciting protection.

Washington State University has generated a library of monoclonal antibodies which recognize various cells and structures of the thymus. Among these mAbs are ones which are specific for Ig-lymphocytes. Other specificities were also found to occur such as general lymphocytic markers, B cell specific (Ig+), and one marker appears to be down-regulated during blastogenesis.

Usefulness of findings

Experiments at the University of Idaho provide confirmation of anecdotal information that *R. salmoninarum*, at times, appears to possess immuno-enhancing activity, particularly when encountering *A. salmonicida*. Although the most uniform *in vivo* observation has been the inundation of macrophages with *R. salmoninarum*, the observation of *in vitro* activation may provide the means of determining why such activity appears not to be prevalent *in vivo*.

The U.S. Fish and Wildlife Service has developed new techniques which will facilitate the examination of infected tissues for evidence of immune-related glomerulonephritis. The evidence thus far indicates that the precipitating disease conditions do not appear to immune complex-related. Therefore, it is likely that if immunopathology plays a role in this disease, it is a secondary role. The development of mAbs at Washington State University should facilitate the analysis of the mechanisms of immune reactivity, within the tissues, to BKD.

The successful demonstration of oral immunization should greatly facilitate the development and future use of this vaccine. Therefore, mucosal immunity may be critical for protection.

Work planned for next year

University of Idaho researchers plan to screen for possible factors which may interfere with macrophage activity. The National Fisheries Research Center will repeat a live challenge and use their improved technologies to screen for evidence of immune complex-mediated pathology. They will also use Washington State University's mAbs to determine if there is an obvious infiltration of, perhaps, T cells to sites of infection. Investigators plan to lend assistance to any other laboratory which may seek to perform field trials.

Support

		Miscellaneous support					
Year	**WRAC funding**	**University**	**Industry**	**Other Fed.**	**Other**	**Total Misc.**	**Total**
90-91	10,000	1,500	0	0	0	1,500	11,500
91-92	110,000	22,500	0	63,000	0	85,500	195,500
92-93	83,700	19,305	0	63,150	0	82,455	166,155
93-94	69,000	17,833	0	3,300	0	21,133	90,133
94-95	0	0	0	0	0	0	0
95-96	0	0	0	0	0	0	0
Total	**272,700**	**61,138**	**0**	**129,450**	**0**	**190,588**	**463,288**

Extension Aquaculture

Termination Report for the period July 1, 1987 to March 31, 1993

Total funding level

$293,896 (July 1, 1987 to March 31, 1993)

Participants

Ernest Brannon,* University of Idaho, Idaho
Harry Burcalow,* Washington State University, Washington
Fred Conte (Chair),* University of California, Davis, California
Steve Harbell, Washington State University/University of Washington, Washington
Al Lingg,* University of Idaho, Idaho

* *(funded participants)*

Project objectives

1. Build an interactive, computer-access system which can provide up-to-date information accessible from anywhere in the Western states. The primary intent is to provide Extension and commercial aquaculture personnel, and any other interested parties, rapid access to selected aquaculture information which exists in the formal literature, popular literature, and in "gray" literature.
2. Provide Extension personnel in the Western states with the necessary training, knowledge, and educational materials which will allow them to respond to basic aquaculture questions and disseminate basic information on fresh and saltwater aquaculture to clientele in their areas.
3. Develop a WRAC Extension publication packet containing information on: (a) specific required resources recommended for growing rainbow trout, salmon, oysters, and channel catfish in the Western Region; (b) general information on R&D and transitional R&D species; and (c) systems of interest.
4. Develop materials containing: (a) questions to be asked when making an assessment of a potential aquaculture site for investors and/or landowners; (b) information on a regional basis with regard to contemporary market pathways for rainbow trout, salmon, oysters, and channel catfish; and (c) sources and contacts for aquaculture regulatory information.

Anticipated benefits

The diverse climate and topographic conditions which exist in the Western states and allow the cultivation of a varied list of aquatic species is a challenge to both research and Extension resources. Limited resources, the difficulty of transfer of information due to vast geographic distance, and the size, range, and diversity of aquaculture in the region make the activities of aquaculture Extension difficult.

The development of a regional computer data system and communication network establishes the regional linkages between Extension specialists and advisors, and allows immediate access to aquaculture technology and information. This regional system complements and enhances the National Technical Information Center and allows immediate search capabilities so essential to local and regional Extension industry activities. Regional publications describing the required resources for the culture and methods of evaluating these existing resources for aquaculture development, are essential tools to Aquaculture Extension. Use of these tools for effective Extension work requires trained Extension personnel which, in turn, requires the development of training materials, periodic updating of information, and training workshops for Extension personnel.

Progress and principal accomplishments

The publication, "Evaluation of a Freshwater Site for Aquaculture Potential," was printed through the University of California, Davis (UCD) in September 1992, and over the next several months was distributed to the five Regional Aquaculture Centers and to the 12 states within the Western Region.

The original 5,000 copies of the publication were depleted by early January 1993, and an additional 3,000 copies were printed in February 1993. A second distribution was then made in Washington, Oregon, California, Arizona, and Wyoming. Additional copies for general use and for use in workshops are stored at

UCD.

Extension training manuals (salmon and oyster)
The two aquaculture training manuals, designed primarily to train county Extension personnel to more effectively work with existing and potential commercial growers, are being completed with original grant funds under a no-cost time extension.

These manuals are also designed to serve as reference and resource books for Extension personnel in providing information to university outreach programs. With the exception of selected charts and diagrams, most of the sections have been completed and are being field tested and reviewed.

Draft sections of the manuals have been provided to Extension personnel for use in state programs as a mechanism to move and extend the material, and to refine the sections before final printing.

Draft portions for 17 of the 21 chapter sections of the oyster manual have been completed, and several sections of the oyster manual are being updated with advancing technology and information. The addition of the salmon restoration section to the salmon manual draft is a valuable inclusion.

Extension and clientele training workshops
Training of county Extension personnel, producers, and general public using the evaluation of resources publication and draft training manuals, was accelerated in the past year. Draft sections of the training manuals are being used to extend information to farm workers and management personnel at oyster farms and salmon restoration facilities in California and Washington. Formal workshops on how to evaluate freshwater sites for aquaculture potential have been presented in 10 workshops in five states.

Usefulness of findings

Communication between participating Extension personnel, preparing the materials from the cooperating states, has led to a cross-flow of technical information and has increased the overall knowledge base within each state.

This has also resulted in a greater flow of technical knowledge being extended to segments of the commercial industry and other user groups. Completion of the evaluation of resources publication has resulted in a number of training workshops this past year, and plans are for continuation of workshops in the proceeding fiscal years.

Work planned for next year includes completion, review, and printing of the final sections of the oyster and salmon training manuals and conduction of training sessions using manual material and the evaluation of resource publications.

Impacts

None were reported.

Support

		Miscellaneous support					
Year	**WRAC funding**	**University**	**Industry**	**Other Fed.**	**Other**	**Total Misc.**	**Total**
87-88	60,000						60,000
88-89	65,982						65,982
89-90	66,500						66,500
90-91	0						0
91-92	54,000						54,000
92-93	47,414						47,414
Total	**293,896**						**293,896**

(This section was not included in reports until 1993, so complete information is not available.)

WRAC Shellfish Sanitation Forum: Proceedings Publication

Termination Report for the period April 1, 1987 to March 31, 1992

Total funding level

$25,000 (April 1, 1987 to March 31, 1992)

Participants

Fred Conte, * University of California-Davis, California
John Faudskar, Oregon State University, Oregon

* *(funded participant)*

Project objectives

1. Identify research priorities in shellfish sanitation.
2. Develop a problem statement for the WRAC Board of Directors to issue a call for proposals.
3. Stimulate and foster collaborative research among regional centers and other funding sources.
4. Provide a forum for industry and agency representatives to discuss and formulate plans to address issues of concern of both the industry and shellfish sanitation regulatory agencies.

Anticipated benefits

The forum would open dialogue between the shellfish industry, health service agencies, and the principal scientific community conducting research on national shellfish sanitation issues. It would focus the WRAC review process on potential research activities associated with shellfish sanitation and provide opportunity for collaborative, inter-regional research.

Progress and principal accomplishments

The forum was held in South San Francisco, California, with invited principal participants from the shellfish industry, university research community, and Western states' health service agencies. The 2-day conference produced three major principals and 15 minor principals that addressed key shellfish sanitation issues. The 18 issues were unanimously adopted by the industry, agency, and research participants. A problem statement identifying potential research areas was developed and submitted to WRAC.

The forum was conducted using $10,000 of the $20,000 total budget. The participants of the sanitation forum requested that Extension personnel who had organized the forum to use the remaining funds to produce a proceedings of the activities and the 18 principal issues that were adopted at the forum. A proposal was submitted to WRAC and the Extension organizers were asked to proceed. Notes and tapes originating from the forum were assimilated and transcribed into computer text. The process required a review of the varied positions taken by representatives from industry, agency, and science sections so that the positions to be presented represented the philosophies held by each group represented at the forum. The discussions surrounding the issues were consolidated to provide background on the issues and the reasoning behind the recommendations that were given by the section representatives in relation to the issues. Each issue was separated into a "chapter" to be reviewed by the forum's coordinating committee. During the development of the draft proceedings, invited presentations on the forum and its contents were presented to the 1989 meeting of the Pacific Coast Oyster Growers Association and both the 1989 and 1990 Pacific-Rim Shellfish Sanitation Conferences. The compilation of tapes and notes, including presentations at regional and national meetings, were conducted without the use of the remaining WRAC funds.

Usefulness of findings

The WRAC-sponsored Shellfish Sanitation Forum was regarded by the Forum participants as the most valuable shellfish sanitation meeting held in the Western Region. The development of the proceedings was viewed as an even greater potential contribution, as it will consolidate viewpoints in a written form that would be used as an educational tool in attempts to formulate meaningful policy with regard to regional and national shellfish sanitation issues, primarily the policy of importation of live shellfish. Usefulness of the publication was contingent upon either a unified industry position or agreement by the industry to support a publication that addresses a divided industry stance, with positions on both sides of the issues.

Work planned for next year

Apparent shifts in philosophy among industry growers and shellfish representatives resulted in cessation of the publication project.

Discussions by shellfish representatives in the California Interagency Committee for Aquaculture Development, the Federal Food and Drug Seafood Importation Conference in Washington, DC, and subsequent communications with members of the Pacific Coast Oyster Growers Association (PCOGA) demonstrated a departure by several major industry representatives from the original industry consensus on the 18 issues established at the San Francisco Forum. Although PCOGA administrative and grower representatives were major participants in the development of the 18 issues, other PCOGA members expressed disagreement with the positions taken on some of the 18 issues. Discussions designed to find a compromise position acceptable to the shellfish industry were held with PCOGA representatives at the 1991 Pac-Rim Shellfish Sanitation Conference in San Francisco, and continued at the September 1992 PCOGA meetings in Newport, Oregon. The industry's final position was to support WRAC's effort to produce a forum proceeding, but would not support the issues developed at the forum as being the representative position of the West Coast industry, and was opposed to a proceedings that described divided positions among the industry. Extension personnel made the decision to terminate the publication effort. The remaining $10,000 was untouched and returned to the funding source.

Impacts

The Shellfish Sanitation Forum focused attention among West Coast growers, sanitation regulators, and the scientific community on the key shellfish sanitation issues impacting the industry in Western states and nationally, identified areas of critical research for WRAC and established dialogues between industry and regulatory personnel that continued during the development and conduct of the Interstate Shellfish Sanitation Conference (ISSC) National Indicator Study (NIS). Since the NIS was funded at a multi-million dollar level, many of the research issues identified at the San Francisco Forum were addressed in the NIS by 70 percent of the representative scientists that attended the Shellfish Sanitation Forum. Most of the 18 issues developed at the forum were addressed in the NIS and the industry continues to be a participant in the ISSC process. The industry remains divided on the principal issues developed at the Forum.

Support

		Miscellaneous support					
Year	**WRAC funding**	**University**	**Industry**	**Other Fed.**	**Other**	**Total Misc.**	**Total**
87-88	25,000						25,000
88-89	0						0
89-90	0						0
90-91	0						0
91-92	0						0
Total	**25,000**						**25,000**

(This section was not included in reports until 1993, so complete information is not available.)

Western Region Aquaculture Industry Survey and Review of Constraints to Aquaculture Development and New Species

Termination Report for the period April 1, 1991 to March 31, 1995

Total funding level

$36,000 (April 1, 1991 to March 31, 1995)

Participants

Jim Bergeron, Oregon State University, Oregon
Rick Bottoms, University of Wyoming, Wyoming
Kevin Fitzsimmons (Chair),* University of Arizona, Arizona
Steve Flickinger, Colorado State University, Colorado
Gary Fornshell, University of Idaho, Idaho
Fred Gifford, University of Nevada Reno, Nevada
Steve Harbell, (TA) Washington State University, Washington
Terry Messmer, Utah State University, Utah
Ray RaLonde, University of Alaska, Alaska
Tony Vaught, Professional Aquaculture Services, California
Walter Zachritz, New Mexico State University, New Mexico

* *(funded participant)*

Project objectives

1. To give every industry member an opportunity to register an opinion on the constraints facing the industry and how the limited available funds should be spent.
2. To provide a contact person in each state who could compile the results of the survey, gain additional information from follow-up efforts, and bring that information to the attention of WRAC.
3. To familiarize many of the members of smaller industries with WRAC, especially in states which have only recently become active in WRAC.
4. To allow the person designated as the aquaculture extension contact for each state to become more familiar with their state's aquaculture needs and the interstate resources available.

Anticipated benefits

An important part of WRAC is its Industry Advisory Committee which, together with the Technical Committee, provides guidance to the WRAC Board of Directors for selecting research topics which have the greatest promise of benefiting the western aquaculture industry. In an effort to widen the base of industry input, WRAC requested that a survey of all aquaculture producers in the Western Region be conducted.

Progress and principal accomplishments

A work group was formed which included several state aquaculture extension specialists and industry leaders. This group developed a survey form which concentrated on constraints to aquaculture development in the region and the introduction of new species into commercial operations. Eventually, the work group was expanded to include a representative for each of the 12 Western Region states.

The work group of extension specialists and state association representatives prepared the survey form and attempted to deliver a copy of the survey to every aquaculture producer in each of the 12 Western states. State licenses, association membership rolls, and extension mailing lists were used to locate industry members. Each state representative in the work group was able to customize the survey to elicit additional information pertinent to individual states.

Some states distributed the survey exclusively through the mail and other states organized statewide meetings to cover multiple topics, including the survey. Over 1,000 survey forms were distributed to producers in the region; about 42% were returned. The survey queried the producers about constraints in the following categories:

- physical-sites, water availability, water quality, etc.;
- regulatory – permitting, laws, oversight boards, etc.;
- social – community interactions and conflicts;
- education, training, and Extension – availability of trained staff, continuing education, and extension support;

- processing and transportation - access to custom harvesters, haulers, and processing plants;
- marketing – access to marketing programs, interstate sales, generic product advertising, etc.;
- financial – state supported programs, access to investors, loan programs, or individual capital; and
- biological – supplies of fingerlings, disease, availability of drugs and therapeutants, nutrition, new species, etc.

Most survey participants completed all of the survey and offered additional detailed comments on various sections. Several of the states added additional topics and questions which were of special interest to their state. Portions of these comments are recounted in the text accompanying each state report. Several survey participants answered only the general questions and declined to fill out the rest of the survey, as they felt they were novices in the industry and not qualified to render detailed opinions.

The report of the survey results is divided into two sections, with a review of survey results concerning non-biological issues on a regional basis and on a state-by-state basis in the first part. The second part is a species-by-species breakdown using the sum of the species reports for each state. Some of the more pertinent biological issues are also reported for individual states within the text.

The survey itself focused on how often individual issues and groups of related issues were constraints, rather than on the severity of these constraints. This was done for two reasons. First, social scientists advised us that it would be difficult, if not impossible, to compare how severe a constraint was or to rank constraints from most to least severe. However, a survey could be effective to identify how widespread these constraints were.

Second, the goal of WRAC is to assist all industry members and not just those who have the greatest or most severe problems. By gathering information on which constraints are the most widespread, support can be directed to benefit the largest number of producers. Obviously, in most cases the most widespread constraints are also the most severe and this was demonstrated throughout the report.

Usefulness of findings

The non-biological issues most often cited were the financial and physical constraints. The social and community issues were the least cited constraints to industry development. The four most often cited constraints were: (1) availability of aquaculture sites; (2) the amount of state funds available to assist the industry through training, marketing, and other programs; (3) rules and regulations of state agencies responsible for aquaculture oversight; and (4) gaining agency approval for aquaculture sites.

In the second portion of the survey dealing with biological issues, several broad topics were addressed including: broodstocks, juveniles, genetics, nutrition, and diseases. For instance, survey respondents provided data on broodstock availability, costs, handling, and how often these were constraints. The availability of juvenile forms including eggs, spat, fry, and fingerlings as a constraint was reviewed. Survey participants were asked which disease or parasite was their greatest concern, and the responses were grouped by species for each state. These responses were then pooled to incorporate the responses of all the trout producers from the member states of the Western Region, all the catfish producers, the sturgeon producers, the oyster producers, etc. As an additional point of interest, all the responses were pooled to provide a broad idea of which biological issues seem to be the greatest constraint.

When all the species results were pooled, some trends become quite obvious. The constraint most consistently cited was restrictions on introduction of new species. This would include introductions of completely new species to the region, as well as movement of domestic stocks within the region. Considering the preponderance of established producers in the survey, their interest in working with additional species is notable.

The second most common concern for all species was introduction of diseases when stocking juveniles. This was a constraint recognized by almost all producers and points out the need for better rapid diagnostic techniques and better monitoring and certification of stocks of fish being sold to producers.

Work planned for next year

None

Impacts

By surveying all the producers in the West, we have a better understanding of which issues seem to be constraining aquaculture from providing more of the aquatic foods that are in such great demand. We expect that the results will assist WRAC and state agencies to guide their resources to focus on the issues which will provide the greatest benefit to the industry.

Aquaculture is the fastest growing segment of agriculture in the U.S. and the world. The number and severity of constraints and conflicts over water resources are bound to increase, and regulations covering all aspects of aquaculture are here to stay.

Efforts to maximize the income of farmers will come from utilizing more efficient technologies in aquaculture (producing more food with the same or fewer inputs). To maximize social benefits, it may be necessary to impose regulations on the industry to reduce external costs on other users of natural resources.

Industry members should recognize that they will be called upon to reduce the impacts they impose on others. Many natural resource use conflicts apply to this industry, and they are likely to become more common as we try to supply the growing demand for high quality seafood.

Support

		Miscellaneous support					
Year	**WRAC funding**	**University**	**Industry**	**Other Fed.**	**Other**	**Total Misc.**	**Total**
91-92	36,000						36,000
92-93	0						0
93-94	0						0
94-95	0						0
Total	**36,000**						**36,000**

(No other information was provided.)

Extension Information Retrieval System

Termination Report for the period April 1, 1993 to March 31, 1995

Total funding level

$35,840 (April 1, 1993 to March 31, 1995)

Participants

Abbas Ahmadi,* University of California, Davis California
Fred Conte,* University of California, Davis, California
John Faudskar, Oregon State University, Oregon
Gary Fornshell, University of Idaho, Idaho
Harry Guenthner (TA), University of Idaho, Idaho
Steve Harbell (Chair), Washington State University/ University of Washington, Washington
Terry Messmer, Utah State University, Utah
Raymond RaLonde, University of Alaska, Alaska
Jim Waldvogel, Oregon State University, Oregon

* *(funded participants)*

Project objectives

Two traditional tools used in Extension outreach include publications and fliers that contain subject matter information. In general, these materials are four or more pages in length and provide a strong mechanism for information distribution at the county and state level.

However, the time required in publication production and the difficulty in making revisions are major concerns, prompting interest in other, more current technologies. A computer-based information system could greatly improve the response to requests for aquaculture information, and could facilitate the development, storage, retrieval, and modification of Extension materials. The objectives of this project are as follows:

1. build a menu-driven, IBM-compatible computer program capable of listing, storing, retrieving, and printing Extension letters with accompanying flyers;
2. develop the capability of allowing the user to retrieve letters, make unlimited modifications, and printing on local letterhead;
3. develop the capability of allowing retrieval/ printing of authorized information fliers, while protecting the authored material from unscheduled modification;
4. utilize participating Extension and/or Sea Grant Specialists and Advisors as authors of five fliers per state, ensuring regional interest and/or application;
5. incorporate the letters and fliers into an information packet, with introductory cover letters; and
6. forward copies of the software program and accompanying information packet to Extension units within the Western Region, along with instructions for utilizing the system.

Anticipated benefits

This project will greatly improve the efficiency of response to requests for aquaculture information and will facilitate the development, storage, retrieval, and modification of Extension materials. Additional Extension fliers on aquaculture for the Western Region and a software program that facilitates the delivery of Extension materials will be created.

Progress and principal accomplishments

Outreach is a computer-based document management system that was developed at the University of California, Davis using WRAC funds. The program operates under Microsoft Windows version 3.1 or higher.

Outreach can store, sort, and retrieve all types of documents files. However, a major feature of the program is its ability to link protected documents with accompanying cover letters. In this function of the Outreach System, information can be incorporated in a series of document files and each document file can be linked to an accompanying cover letter which can be modified by the user, while the documents are protected from unauthorized modifications by users of the program. As a result, unaltered authored materials can be distributed to users of the program while accompanying letters that explain the material can be modified and personalized.

Outreach stores unlimited numbers of documents; storage is limited only by the size of the hard drive, CD-ROM, or network server. The program is specifically designed to handle not only copyright-protected documents such as Extension fliers and publications, but also to accommodate other applications within Extension, government agencies, and in the private sector. It also can be used to store and deliver information using digital spreadsheets, graphics, photographs, video, and voice.

Outreach is designed to view and print any document created under the Windows operating system. The files are font-independent, word processor-independent, and printer-independent. It facilitates the distribution of electronic documents, while maintaining all their visual characteristics, regardless of differences between the computer systems of the senders and receivers. Because of the unique file-locking system, protected authored files that are sent to another computer by fax or e-mail may be viewed on that computer or printed, but remain protected from unauthorized modification.

Outreach uses a platform-independent electronic file format and universal display language that captures all the information about text, fonts, layout, color, and graphics in a document. It thus provides all the information that is needed to reproduce a document that is an exact representation of the original.

Outreach works with popular document viewers, such as Common Ground™, Acrobat™, Envoy™, and Mosaic™.

Pre-distribution copies of the Outreach software program were distributed to Extension representatives at WRAC's October 1995 IAC/TC meeting. The software program is complete and fully-operational. The accompanying program manual was also distributed at the same meeting.

Usefulness of findings

None were reported.

Work planned for next year

In early 1996, the logistics for software sales and distribution will be established and the software will be marketed later in the year.

Impacts

None were reported.

Support

Year	WRAC funding	Miscellaneous support: University	Industry	Other Fed.	Other	Total Misc.	Total
93-94	15,920	27,300	0	0	0	0	43,220
94-95	19,920	0	0	0	0	0	19,920
Total	**35,840**	**27,300**	**0**	**0**	**0**	**0**	**63,140**

Development of Methods for Control of Infectious Hematopoietic Necrosis Virus (IHNV) in Commercially-Reared Salmonid Fishes (2)

Progress Report for the period April 1, 1995 to March 31, 1996

Total funding level

$386,100 (April 1, 1993 to March 31, 1996)

Participants

James Congleton,* University of Idaho, Idaho
Gary Fornshell, University of Idaho, Idaho
Ronald Hedrick (TA),* University of California, Davis, California
Marsha Landolt,* University of Washington, Washington
Scott LaPatra, Clear Springs Foods, Inc., Idaho
Jo-Ann Leong,* Oregon State University, Oregon
Jim Parsons, Blue Lakes Trout Company, Idaho
Ray RaLonde (Extension), University of Alaska, Alaska
Sandra Ristow,* Washington State University, Washington
John Rohovec,* Oregon State University, Oregon
James Winton (Chair), National Biological Service, Washington

* *(funded participants)*

Project objectives

1. Develop means for inducing specific immunity to IHNV in rainbow trout.
2. Develop means for inducing non-specific immunity to IHNV in rainbow trout.
3. Determine the genetic basis of resistance to IHNV among different stocks of fish.
4. Develop novel control methods using antiviral drugs and chemicals.
5. Understand the pathogenesis and virulence of IHNV in rainbow trout.

Anticipated benefits

The development of an effective method for protection of fish against IHNV remains an important goal for the control of this viral disease. Losses due to IHNV are estimated in the millions of dollars annually. While much remains to be done, a license has been granted to a commercial company for development of the subunit vaccine.

In addition, commercial interest has been expressed in several other products from this project including the monoclonal antibodies, the DNA probe, and the primer set for the polymerase chain reaction. These are powerful tools that will improve the speed and precision of IHNV detection. They and will also be of enormous assistance in improving our understanding of the virus carrier state, and providing important information about the antigenic and biochemical variation among strains of IHNV.

Research to define the antigenic epitopes on the glycoprotein of the virus will provide critical information about the sites for the next generation of vaccines for protection of fish. Results from the interferon protection and virus interference studies are encouraging in that they show the importance of non-specific immune mechanisms for controlling losses due to IHNV. These non-specific mechanisms may be suitable targets for selective breeding and genetic manipulation studies in future years.

Progress and principal accomplishments

During the third year of our renewal, the IHNV work group continued a high level of productivity publishing papers in peer-reviewed journals and the technical literature, conducted a highly successful extension workshop for the commercial trout growers of the Hagerman Valley of Idaho, and planned a second workshop on diagnostic methods to be held in Hagerman in October 1996. Research areas of emphasis included: 1) improving new generation vaccines against IHNV; 2) defining the antigenic nature of the IHNV glycoprotein and the resulting antibody response in the fish; 3) understanding aspects of non-specific immunity including interference by a non-pathogenic virus; and 4) improving our understanding about the biology of the virus.

Research continued in the development of effective vaccines for IHNV. One of the approaches to enhance the efficacy of vaccines for IHNV is concurrent administration of immunomodulators in an effort to identify trout cytokines as potential immunoadjuvants. Experiments were conducted with human recombinant interleukins IL-1, IL-2, IL-4, and IL-6 for their capacity to augment the immunization of fish with an anti-IHNV peptide vaccine. Fish receiving both the peptide and IL-2 were less susceptible to the lethal effects of IHNV challenge. When fish were immunized with a DNA vaccine in the presence of the hu-rIL, an increased immunoprotection was again observed.

Trout natural killer enhancement factor has been cloned and sequenced and its protein product has been expressed in CHSE-214 cells by transfection with the plasmid, pCMV-NKEF. The biological activity of the trout NKEF-like protein was assessed by measuring the cytolytic and apoptotic destruction of tissue culture cells expressing the NKEF by trout lymphocytes. There was a 3- to 4-fold increase in the cytolysis of the NKEF expressing cells as compared to those cells transfected with pCMV-luciferase or pCMV-galactosidase.

Persistence of IHNV in fish was assessed using PCR amplification and immunohistochemistry. Juvenile rainbow trout that survive IHNV infection continued to harbor virus, although infectious virus was not detectable from 46 days post-infection until maturation. Even at spawning, there was no detectable virus in fluids and the progeny did not come down with IHN. However, 1 and 2 year-old rainbow trout survivors were virus carriers and had extensive scarring in the kidney tissues and evidence for viral persistence. Truncated viral particles resembling the defective interfering virus particles of other rhabdoviruses were observed by immunogold electron microscopy in the tissues of survivors.

Studies were undertaken to determine the capacities of anterior kidney, skin, fins, and gills of rainbow trout for *in vitro* production of interferon-like cytokines (ILCs) in response to stimulation with IHN virus. Yields of infectious virus from the tissues were inversely related to ILC production and were greatest for gill tissue and smallest for kidney tissue. The ILC-producing capacity of skin of rainbow trout that survived an IHN virus challenge was enhanced relative to unchallenged control fish for up to 41 days post-challenge, but challenged and control fish did not differ at 52 days post-challenge. When skin samples were treated *in vitro* with ILCs (100 TCID50/mL) and subsequently challenged with IHN virus, the yield of virus was decreased by about 50%.

We examined the efficacy of immune-stimulating complexes (ISCOMS) to enhance IHNV immunization of rainbow trout. There was significant protection among fish treated with ISCOMS compared to untreated controls. These preliminary experiments indicated that even poorly-incorporated proteins were providing good protection to IHNV challenge.

In a second study, and after adjustments to ISCOMS formation protocols, trout were immunized and we anticipate even better protection. A cursory examination of the neutralizing antibody titers of fish injected with ISCOMS from the first trial indicated that significant titers were achieved following mmunization. The presence of these antibodies may underlie the protection afforded to ISCOMS-injected trout.

Investigation of host defenses that are stimulated by prior exposure to the avirulent chum salmon reovirus (CSV) continued. Exposure of rainbow trout to CSV provided enhanced survival over an 8-week period. Differences in humoral response in CSV-treated fish, suggests that other immune defense mechanisms may be involved. Histological specimens obtained from fish exposed to CSV, indicated that focal to multifocal hepatonecrosis had occurred. We were also able to detect CSV in fish 42 days post-exposure as previously reported. A non-lymphoid chronic acute phase response may account for the RPS results of fish previously exposed to CSV.

The central nervous system has been suggested as a potential site of IHNV infection because of similarities between IHNV and rabies virus. It has also been suggested that IHNV strains exist that have an altered tissue tropism differentially impacting fish life stage, clinical disease signs, and fish recovery. Results of tests done confirm a neurotropic potential.

A group of fish exhibiting behavioral abnormalities showed IHNV detectable in brain tissue only. Microscopic tissue changes were also evident in the brain, but not the anterior kidney. We demonstrated antigenic differences between virus isolates collected from brain tissue and from hematopoietic tissue. Virulence and pathogenicity differences were not evident *in vivo* when fish were challenged. These IHNV isolates had, however, been previously passed *in vitro*.

We have fractionated rainbow trout bile and have discovered that component(s) are present which have antiviral activity. At the beginning of the purification, the level of compound(s) required for achieving 99% viral inhibition began at approximately 10 mg/ml. After the final extraction into the ether, inhibition was achieved at a concentration in the tissue culture well between 10 to 100 µg/ml.

Several methods are now being investigated to further purify the active compound(s). Cutaneous and GI tract mucus were evaluated for immunological responses following exposure to IHNV. Antiviral activity in the GI tract was highest in mock infected fish and appeared to decrease in infected fish as infection progressed. Specific antibodies were not detected in cutaneous mucus or GI tract washings.

Virus was isolated from external and GI tract mucus in both challenged fish and showed significantly higher titers in fish challenged by the injection route. Histological examination of fish injected or waterborne-exposed to IHNV showed an inflammatory response in the mucosal epithelial layer of the skin with minor focal areas of cellular necrosis in severely infected fish. The active anti-IHNV substance in the mucus is now being extracted from large quantities of material and is being analyzed to see whether it is identical to the material in the bile.

Clones of rainbow trout were produced as aquatic models for biomedical research by the chromosome set manipulation. Within each set of clones, individuals were shown to be identical by DNA fingerprinting. cDNA was produced from spleens of individuals from each background and cloned into plasmids. When the sequences of the cytoplasmic and the Ig-like MHC II 2 domain were compared, they were highly conserved between individuals of the different isogenic clones.

Since MHC is implicated in antigen presentation and in disease resistance, the cloning of these segments is an important milestone. We developed long-term cell lines from inbred strains of rainbow trout which could be used to demonstrate true cytotoxic T cell activity *in vitro* among rainbow trout. The lines originated from the embryonic tissues of three androgenetically-derived lines of rainbow trout. Yields of IHNV are variable and the kinetics of virus replication are being determined. These lines will be useful for determining *in vitro* whether true T cell cytotoxicity against IHNV occurs by T-like effector cells from the rainbow trout.

Sequence analysis of glycoprotein genes of neutralization-resistant variants of IHNV was used to locate and characterize the antigenic sites on the viral glycoprotein. The majority of the neutralizing epitopes on the IHNV glycoprotein were located near the center of the 508 amino acid protein. A conformation-dependent epitope was located within antigenic site 1 at amino acids 230–231. Antigenic site 2 consisted of a major epitope (a portion of which was linear) at amino acids 272–276 that was conformationally linked to a minor epitope at amino acids 78–81.

We synthesized three peptides of 13 amino acids in length that included the antigenic sites. Serum from fish immunized with the peptides was assayed by plaque-neutralization and enzyme-linked immunosorbent assay. None of the groups of fish produced convincing neutralizing titers relative to controls recovering from natural infection, and the binding antibody titer was low and variable. The results suggested that these epitopes are either not sufficiently immunogenic due to length, antigenic mass, etc., or that the epitopes defined by our mouse monoclonal antibodies are different from those defined by the B cell repertoire of fish.

The glycoprotein gene of a neutralization-resistant variant of IHNV that was attenuated was sequenced and compared with the parental strain. Sequence comparisons of 12 glycoproteins from differing geographic areas was completed and revealed antigenic site 1 (amino acid 230–231) was highly conserved while antigenic site 2 (amino acids 273–276) was more variable than average. Analysis of isolates from Cedar River, Washington, over a 13-year period revealed little evolution or diversity of the isolates from a single geographic area. These data suggest that differing vaccine preparations might be required for different geographic areas; however, within a given area, the virus seems to be relatively stable.

Usefulness of findings

The development of effective vaccines for protection of fish against IHNV remains an important goal for the control of this viral disease. Losses at both commercial trout farms and mitigation hatcheries to IHNV are estimated in the millions of dollars annually. The subunit vaccine developed as a result of USDA and DOE (Bonneville Power Administration) funding has been patented and licensed non-exclusively to two companies, Microtek and DiagXotics.

Oregon State University has offered the license to any company that will exercise diligence in bringing the vaccine to market. These companies will have to determine what parameters are important in producing effective vaccine upon scale-up production of the recombinant bacteria. In addition, commercial interest has been expressed in several other products from this project including the monoclonal antibodies, the DNA probe, and the primer set for the polymerase chain reaction.

Because production of the subunit vaccine has been subject to variation in effectiveness in large scale preparations, researchers at Oregon State University have turned to genetic immunization. The laboratory trials have been very effective and DNA vaccination provides an important alternative. Initial indications are that this vaccine represents an attractive option for commercial development where it can be delivered by injection.

One method of increasing the effectiveness of vaccines is the concurrent administration of an immuno-stimulant. When the subunit vaccine was administered with immunostimulants such as muramyl dipeptide, b-glucan, or recombinant interlukin, there was enhanced protection against IHNV challenge. The results were dependent on the ratio of immunostimulant to vaccine administered to the young fish and the cost of the immunostimulant prompted a search for the fish genes that might act as immunostimulants. The isolation of the rainbow trout NKEF and Mx genes has made it possible to determine whether these genes might be useful in monitoring or increasing the immune response to IHNV upon vaccination.

Persistence of IHNV in survivors of IHN epizootics raises questions regarding the management of epizootic survivors. In the more than 2,000 sample tissues that were taken from surviving fish after 50 days, there has never been any evidence of infectious virus; however, there were definitely viral N and G proteins and N and G viral nucleic acid sequences. The apparent carrier prevalence from these studies is 17%.

There is also EM evidence for DI particles. In order for DI particles to exist, whole virus must be present. Yet no infectious virus has been recovered and the question of why there is no infectious virus remains unresolved. The finding is important because the prevalence of this carrier state in wild populations or populations in the field can now be determined.

Because fish lack the complex specific-immune responses seen in higher vertebrates, non-specific immunity plays a major role in combating infectious diseases. The avirulent Cutthroat Trout Virus (CTV) has the demonstrated ability to invoke at least one important nonspecific immune function (interferon induction) and perhaps others, as demonstrated by the enhanced IHNV neutralizing antibody titers found in trout exposed first to CTV then to IHNV compared to those fish receiving only IHNV. Further understanding these mechanisms may provide the means to enhance weak or poor immunogens used as vaccines for fish.

A library of monoclonals against PRP and an ELISA for PRP, once it is fully developed, will be useful not only in monitoring the response of animals being challenged or frankly infected by IHNV, but will probably be useful for monitoring populations undergoing stress or parasitic and bacterial infections. It is anticipated that local (mucosal) immunity exists in salmonids and that through the present studies we may learn to augment these defenses in trout. If a broad spectrum, anti-viral can be extracted from trout bile, it represents a socially useful and potentially lucrative product from a waste product of the industry.

A knowledge of methods for more efficacious delivery of viral proteins to stimulate protective immunity to IHNV infection are critical. While appropriate recombinant proteins may be produced, their appropriate recognition by the fish's immune response will be required for any successful vaccine. The studies with ISCOMS are directed towards this goal.

ISCOMS have the advantage of trapping and presenting selected viral proteins to the immune cells. They are stable and can be administered by oral routes as well. Our initial results suggest that trout respond by recognizing antigens incorporated into ISCOMS and that such recognition results in significant protection to IHNV challenge.

Studies showed that the gills and skin of salmonid fishes support replication of IHN virus soon after exposure to waterborne virus. Production of interferon by these tissues may be an important component of non-specific resistance to IHN. If resistance to *in vivo* challenge by IHNV is correlated with the capacity of target tissues for production of interferon, the interferon assay may be useful for identification of virus-resistant genetic groups.

Investigations of non-specific immune activity against IHNV are useful from the point of view that they are the immediate first line of defense which is available to the trout to defend against IHNV. Since the fish is likely to first contact the virus across the mucosal barrier, local immunity is extremely important. The compounds present in the bile are as important as a nonspecific defense for the fish, but there is an additional reason for studying these substances.

Currently there is an initiative by the NIH-NCI with respect to new, investigational drugs. It is highly speculative, but it is possible that the substances in trout bile may have anti-cancer or anti-AIDS efficacy.

Work ongoing in our laboratory on homozygous clones of trout (development of high- and low-resistant fish, sequencing of the MHC, and development of cell lines from the inbred fish) will yield tools for analyzing antigen presentation in commercial trout, which will ultimately be important in the design of future vaccines. Development of baculovirus expressed G protein will provide another subunit vaccine for testing in the final phases of this project.

An essential key to the development of vaccines is the understanding of the antibody response in fish and to determine the epitopes that are recognized by the fish immune system. The identification of specific peptides which might be used to induce immunity in fish against IHNV is important because it will enable us to define the different regions of the glycoprotein that are critical for inducing neutralizing antibody and reacting with specific host cell receptors. Identification of specific domains of the glycoprotein that might be responsible for different biological functions (e.g., different pathogenicity) may help to define the variation among strains of IHNV.

Delineation of the neutralizing epitopes on the viral glycoprotein is critical for development of improved vaccines. Further studies to determine the genetic diversity of virus strains at these important locations will be essential for understanding the variety of antigens that must be present in a successful vaccine and the ability of the virus to develop resistance to antibodies stimulated by vaccines. The identification of these potential binding sites will also provide a basis for investigating the relationship of these antigenic epitopes and virulence of the virus.

In addition to our research activities, we organized and participated in a highly successful extension workshop on IHN given in the Hagerman Valley of Idaho for the commercial trout growers. Over 20 participants met for a day to learn about progress from the IHNV work group and to share experiences and observations about the impact of the virus on the commercial trout industry. Planning was conducted for a second 1-day workshop on the newest diagnostic methods for IHNV (developed by this project) to be held in the Hagerman Valley in October 1996. This will be the prototype of a series of workshops that will be given in other areas of the Western U.S.

Work planned for next year

The promising protection seen with viral proteins incorporated into ISCOMS will be pursued by investigating immunization using more practical delivery routes (oral and immersion). Additional studies will examine the doses of viral proteins required to provide adequate protection. A large trial with CTV, in which several thousand fish are treated, is in the planning stages. This avirulent virus has been shown in previous studies to be a good stimulator of non-specific immunity.

We plan to undertake further studies that will attempt to define the mechanism by which aquareovirus-mediated resistance occurs. We also plan to further examine the effect of fish size and age, host anti-IHNV immune status, and the existence of IHNV strains with an altered tissue tropism as explanations for the occurrence of the “neurotropic” form of IHN.

We will continue to examine the potential of immunoadjuvants in enhancing the efficacy of IHNV vaccines. The trout NKEF will be expressed in *E. coli* and tested as an immunoadjuvant with a peptide vaccine against IHNV. In addition, we will determine whether vaccination of fish against IHNV alters the course of virus infection. The techniques that were developed to detect viral nucleic acid and viral protein in carrier fish will be used to monitor vaccinated and unvaccinated fish for persistent IHNV infection.

We will determine the ILC-producing capacity of various tissues of trout from families shown to have relatively high or low resistance to IHN virus challenge. Also, we will examine the ability of non-pathogenic viruses to induce resistance to IHN virus in lab and field studies.

We plan to chemically characterize the substances in the bile having antiviral activity and to identify and characterize the substance in gut mucus having anti-IHNV activity. It is planned to infect insect larvae with the baculovirus construct and purify the recombinant G proteins on nickel columns to yield enough protein for immunizing large trout by injection to examine the serum for the presence of serum neutralizing antibody.

Research will continue on characterizing the surface structure of the viral glycoprotein. This will provide essential information about the number, location, and nature of the antigenic sites on this protein responsible for protection. This information will be critical to the design of improved subunit vaccines.

Following the extension workshop hosted by the IHNV Work Group, industry input suggested that the use of low levels of iodine as a control strategy for IHNV is of renewed interest. Therefore, we will initiate research in cooperation with the trout industry on relative toxicity of iodine for trout and the effect of low levels of iodine on fish performance.

Impacts

None were reported.

Support

Year	WRAC funding	Miscellaneous support: University	Industry	Other Fed.	Other	Total Misc.	Total
93-94	130,000	88,200	146,000	428,800	0	663,000	793,000
94-95	130,000	41,575	126,000	518,654	0	686,229	816,229
95-96	126,100	93,075	131,000	728,941	0	953,016	1,079,116
Total	**386,100**	**222,850**	**403,000**	**1,676,395**	**0**	**2,302,245**	**2,688,345**

Development of Sturgeon Broodstocks

Progress Report for the period April 1, 1995 to March 31, 1996

Total funding level

$235,300 (April 1, 1993 to March 31, 1996)

Participants

Joseph Cloud,* University of Idaho, Idaho
Fred Conte (Extension), University of California, California
Walt Dickhoff (TA), University of Washington, Washington
Serge Doroshov,* (Chair) University of California, California
David Erdahl,* U.S. Fish and Wildlife Service, Montana
Gary Moberg,* University of California, California
Terry Patterson,* College of Southern Idaho, Idaho
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* *(funded participants)*

Project objectives

The main goal of this project is to develop sturgeon broodstocks and establish reliable production of domestic seedstock by the aquaculture industry. The specific objectives are to:

1. determine optimal pre-spawning temperature for captive sturgeon;
2. develop diagnostic techniques for broodstock management;
3. evaluate growth and reproduction of different stocks;
4. establish artificial reproduction of shovelnose;
5. develop cryopreservation of spermatozoa; and
6. conduct a sturgeon broodstock development workshop.

Anticipated benefits

Sturgeon is an aquaculture product of increasing importance, and a number of chondrostean species are threatened or endangered in the wild. This project is expected to support and enhance the developing sturgeon industry by establishing domestic broodstocks and a reliable supply of domestic fingerlings. Hatchery and diagnostic techniques, unique for sturgeon, will be used in broodstock management and for a captive breeding of endangered species.

Progress and principal accomplishments

Objective 1

Elevated temperature (above 15°C) negatively affects the post-vitellogenic phase of ovarian development in white sturgeon and leads to arrest of germinal vesicle migration and ovarian atresia. Holding gravid females at low temperature has become an accepted practice for normal sturgeon spawning in California, where rearing temperature often increases to 18–20°C during the pre-spawning season. Two methods are employed in different farms: transfer of fish into a temperature-controlled recirculation system at 12–14°C, with spawning during the spring; and transport of fish to a cold water trout facility (10–12°C), with spawning throughout the summer and early fall.

Objective 2

UC Davis, in collaboration with industry, continued on-site improvement of diagnostic techniques required for broodstock management. The surgical sexing technique implemented on California and Idaho farms was accurate in 94% of the cases. The technique is now used by sturgeon breeders for new broodstocks and caviar production fish.

An enzyme immunoassay for white sturgeon vitellogenin (VTG) was developed, and correlations between the plasma VTG, total calcium, and stage of ovarian maturity revealed that vitellogenic and pre-vitellogenic females can be accurately discriminated by total plasma calcium. However, a more direct technique of surgical gonad examination is currently employed.

Due to the biennial vitellogenic cycle in white sturgeon, maturing females can be segregated by three clearly recognizable ovarian stages: ripe (large black eggs); mid-vitellogenic (medium size eggs with moderate pigmentation); and pre- (or early) vitellogenic (small white or translucent eggs).

The detection of the pre-ovulatory ovarian stage is critical for sturgeon spawning, and the *in vitro* egg maturation bioassay was developed and introduced into farm practice. Females that exhibit a 70–100% germinal vesicle breakdown (GVBD) response in the assay are spawned in 2 to 4 weeks after sampling. During the spawning seasons of 1995 and 1996, UCD conducted ovulation induction trials with different hormonal treatments, including the mammalian luteinizing hormone-releasing hormone analog (LHRH-A), alone or in combination with dopamine antagonists pimozide or dopamine; common carp pituitary extracts (CPE), alone or in combination with a priming dose of LHRH-A. The results suggest that spawning performance of domestic sturgeon females can be considerably improved by the application of LHRH-A combined with a dopamine antagonist.

Objective 3

Data on growth, maturation, and reproductive performance of sturgeon broodstocks are continually collected in Idaho (Clear Springs Foods and College of Southern Idaho), and in California (The Fishery, Sierra AquaFarms, Stolt Sea Farm, and UC Davis Aquatic Center). Growth and reproductive development are monitored by repeated annual sampling of approximately 500 broodfish in California and 200 broodfish in Idaho, including 8-year classes (1982–1989) and three different stocks (from the Sacramento, Columbia, and Snake Rivers).

Rearing temperature is the main environmental factor affecting broodstock growth. Under similar husbandry, growth rates of the broodstocks raised at constant 15°C (Idaho) were approximately twice as low at the same age, compared to those raised at temperatures 18–22°C (California). Another factor influencing growth is broodstock origin. Observations on growth of three different broodstocks (from the wild parents in the Columbia, Snake, and Sacramento Rivers) revealed major differences in growth under the same husbandry and temperature, with the highest growth rate in the lower Columbia River stock (Clear Springs Foods, Idaho).

Under warmwater culture in California, white sturgeon undergo gonadal sex differentiation at age 18–22 months; males reach full sexual maturity at 4–5 years but the majority of females mature at age interval 6–10 years. Approximately 70 percent of females reach full sexual maturity at age 6–8 years and mean body weight 25–32 kg, and 30 percent delay sexual maturation to 9–11 years and body weight 35–40 kg. For sturgeon raised in Idaho, the proportions of mature males at the age 7–11 years ranged 20–50%, but only a few females (3 out of 46 sampled) had ovaries near the onset of vitellogenic growth. These observations suggest that rearing temperature is also a major factor influencing gonadal maturation in sturgeon.

Objective 4

Wild shovelnose broodstock have been captured from the Yellowstone and Missouri Rivers by drifting trammel gill nets and have been transported to either the Miles City State Fish Hatchery or the Bozeman Fish Technology Center (FTC). The effectiveness of LHRHa and the failure of CPE to induce spermiation and ovulation in shovelnose have been established. Practically every broodfish that has received an injection of 10–20 mg LHRHa/kg body weight has responded with ovulation, while females treated with CPE failed to ovulate. Males injected with CPE did not respond or produced only a small volume of milt (< 5ml), whereas those treated with LHRHa consistently produced 20–50 ml of milt.

Development of captive broodstock has been the major challenge, due to high mortalities of juveniles and difficulties in weaning larvae onto artificial diets during 1992–1995. However, during spring 1996, rearing of larval shovelnose was successful. Shovelnose were reared at the FTC (30 days, fed Biodiet, Biokyowa, and sturgeon starter SS9602) and at UC Davis (40 days, fed Biodiet, Silvercup, and SS9602).

At UC Davis, mean body size at 40 days was similar in all diet treatments, ranging from 332mg to 438mg; however, survival was significantly affected by diet. Shovelnose fed the SS9602 diet had the highest survival (65% and 80%), survival for fish fed Biodiet was 73% and 40% and the Silvercup treatment had the lowest survival (5% and 18%).

At the FTC, shovelnose fed the SS9602 had the highest survival (71% and 64%), fish fed Biokyowa had 16% and 0% survival and the Biodiet treatment had 3% and 9% survival. At 30 days, the fish fed Biokyowa were largest (300mg) and those fed Biodiet were the smallest (130mg).

Objective 5

Sperm storage and cryopreservation techniques are being developed for white sturgeon and shovelnose at the University of Idaho and the FTC in Montana. Using sperm motility as an endpoint, the most appropriate freezing solution is Hank's solution with 7.5% DMSO and 10% egg yolk, and the best cooling sequence is an initial cooling temperature of -9°C/min from 4° to -14°C, an intermediate cooling rate of 25°C/min from -14° to -80°C, followed by direct transfer into liquid nitrogen (-196°C). Post-thaw motility of sperm varied from 80% to 0%, and the most consistent source of variation was the male donor.

The cryopreservation technique was effective in terms of post-thaw motility, but the motility of cryopreserved sturgeon sperm does not predict fertility. In all cases, including highly motile cryopreserved sperm, the fertilization success (proportion of fertilized eggs that undergo first cleavage) was less than 4%.

This research has clearly identified the fact that the motility of cryopreserved sturgeon sperm is an unreliable indicator of fertility and that there are other physiological factors that contribute to sperm fertility. Future research will identify cellular parameters that can be used diagnostically to predict sperm fertility in a quantitative fashion. One approach will be to use transmission electron microscopy to identify the cellular changes that occur as a sperm cell becomes non-fertile during the freezing process. A second approach is to use cellular probes that have been recently developed to measure mammalian sperm fertility with flow cytometry.

Objective 6

The WRAC Sturgeon Broodstock Management Workshop is scheduled for September 27–28, 1996, at the College of Southern Idaho. The workshop agenda will include presentations from all the participants of this project and several other collaborators in sturgeon research (UC Davis; FTC; University of Idaho; Clear Springs Foods; College of Southern Idaho; and The Fishery, Inc.).

Usefulness of findings

The developing West Coast sturgeon aquaculture industry annually produces about 1 million pounds of food fish and recently started production of domestic sturgeon caviar. Until recently, the fate of this industry was dependent on unreliable seedstock supply from wild caught sturgeon, and the major goal of this project was to establish efficient domestic breeding of sturgeon.

The improvements of environmental regimes, spawning induction procedures, and diagnostics of reproductive conditions achieved in this project have directly contributed to commercial breeding of sturgeon. Recent trends in commercial production of F_2 fry from domestically-raised sturgeon indicate that the goal to develop broodstocks of white sturgeon and gain complete independence of the aquaculture industry from wild fish spawning has been achieved.

Hatchery technology has been established for wild shovelnose sturgeon, with major improvements in rearing juveniles achieved during this year. While the domestication and commercial culture of shovelnose are yet to be established, the availability of hatchery technology allows the use of this small and adaptable species as a unique laboratory model in applied and basic research for aquaculture (white sturgeon) and endangered chondrosteans (pallid sturgeon).

Studies on cryopreservation of sturgeon sperm revealed an impact of currently used cryopreservation techniques, successful in modern teleosts fish, on the ability of chondrostean spermatozoa to interact with eggs during fertilization. Understanding of structural and physiological changes caused by cryopreservation will allow for development of new methods and potential application of long-term gamete storage for breeding programs in the commercial industry and in wild stocks.

Work planned for next year

As a result of collaboration between researchers and commercial sturgeon breeders during 4 years of this project, the sturgeon industry has progressed from obtaining gametes from wild broodfish to spawning its own captive broodstock. This independence from wild stock was critical for the industry, but several unique aspects of sturgeon culture have transpired that must be examined.

We anticipate that future work will focus on management of the established broodstocks, including enhancement of reproductive performance, gamete cryopreservation, nutrition, prevention of vertical transmission of viral diseases, and establishment of a breeding program.

Impacts

Over 3 million larvae have been hatched from eggs of domestic fish during the last 2 years. Because of the greatly improved reproductive performance, fewer females were needed for spawning, and the first revenues from the production of domestic caviar began in 1995.

Support

		Miscellaneous support					
Year	**WRAC funding**	**University**	**Industry**	**Other Fed.**	**Other**	**Total Misc.**	**Total**
93–94	74,000	0	5,000	109,929	0	114,929	188,929
94–95	74,000	0	4,000	59,529	0	63,529	137,829
95–96	87,300	0	4,000	56,889	0	60,889	147,889
Total	**235,300**	**0**	**13,000**	**226,347**	**0**	**239,347**	**474,647**

Development of Pacific Oyster Broodstock

Progress Report for the period April 1, 1995 to March 31, 1996

Total funding level

$242,420 (April 1, 1993 to March 31, 1996)

Participants

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** (funded participants)*

Project objectives

1. Undertake a systematic inbreeding program for the Pacific oyster, using different methods of inbreeding.
2. Characterize the hatchery and growth performance and genetic markers of inbred lines.
3. Utilize crosses among inbred lines to estimate genetic components of variance in growth and sexual maturity.
4. Undertake interdisciplinary studies of the metabolism and performance of genetically manipulated stocks.
5. Make triploids by controlled two-way and three-way crosses among inbred lines and to test the growth performance of these triploids on commercial oyster beds.
6. Increase the participation of the region's research institutions and industry in breeding efforts.

Anticipated benefits

The first anticipated benefit of this project is basic information that will help commercial oyster breeders to decide between selection or crossbreeding as the best means for genetic improvement. Crossbreeding of inbred lines is the only way to improve triploids, which are produced by the industry but limited to food-rich grounds.

A second anticipated benefit is a demonstration to the industry that genetic differences in, and methods for improvement of, triploid performance exist. Finally, by extending our observations to the survival, growth, and metabolism of larvae and early juveniles, we hope to identify for industry breeders indicators of later growth performance that would permit early culling.

The Pacific oyster industry in the Western Region has moved several steps closer to initiating a commercial-scale selection program as a result of the information and methods provided by our WRAC research. In an earlier WRAC project, we showed that body size at harvest is heritable, implying that this trait can be improved by individual or family selection. A selection program to utilize heritable variation has now been initiated by the Molluscan Broodstock Program at the Hatfield Marine Science Center in Newport, Oregon.

Nevertheless, in the earlier WRAC project, we also detected a substantial non-heritable genetic component of growth variance, implying that improvement could be achieved by breeding superior hybrids. In the first 2 years of this project, we indeed demonstrated that crosses between pairs of inbred lines result in obvious hybrid vigor. Thus, the first anticipated benefit of this project is basic information, that will help commercial oyster breeders to decide between selection or cross-breeding as the best means for genetic improvement.

Crossbreeding of inbred lines will be the only way to improve triploids, which are produced by the industry but limited to food-rich grounds. A second anticipated benefit is a demonstration to the industry that genetic differences in, and methods for improvement of, triploid performance exist. Finally, by extending our observations to the survival, growth, and metabolism of larvae and early juveniles, we hope to identify for industry breeders indicators of later growth performance that would permit early culling of slow-growing oysters.

Progress and principal accomplishments

Systematic inbreeding (objective 1) was carried out at the participating laboratories at BML and OSU. At BML, an analysis of allozyme profiles in seven inbred lines derived by selfing of simultaneous hermaphrodite-founders (objective 2) was completed. Contamination of three families was revealed by more than two alleles in three lines; a total of five contaminants were excluded from analysis.

Fixation and segregation of allozyme markers in inbred oysters families deviated markedly from expectations of neutral inbreeding theory, most likely because of linkage disequilibria and strong selection within families. We detected two linkage groups of three loci each, one of which has been described for the American oyster.

Major portions of objectives 2, 3, and 4 were accomplished, as in year 1 and 2, by two 2x2 factorial crosses among inbred lines. Three generalizations have emerged from the experiments conducted to date (1993–1995): (1) viability and growth exhibit striking hybrid vigor in experimental crosses of inbred lines; (2) heterosis for viability or growth does not depend on life stage (larval vs. juvenile or adult) or nutritional level; and (3) heterosis for growth can be explained, in part, by differences in rates and efficiencies of protein turnover and the processes of maintenance metabolism; but may also be achieved by greater nutrient-uptake ability of hybrid relative to inbred oysters.

Thus, we have demonstrated that crosses among inbred lines permit estimates of genetic components of variance in growth and the physiology of growth, and that non-additive gene action is pervasive. These experiments make possible the mapping of genes causing hybrid vigor.

We continued systematic inbreeding of Pacific oysters (objective 1), using self-fertilization of females with cryopreserved sperm. Cryopreservation of sperm was continued at OSU in 1995 for use in self-fertilizing those that reverse sex in subsequent years. Sperm was obtained and cryopreserved in liquid nitrogen, as outlined in the 1994 annual report, from a total of 52 males. All males were individually marked and returned to local field sites so that, when these oysters mature as females later, their eggs can be self-fertilized.

From the work to date, we conclude that the inbreeding strategy of selfing sex-reversed oysters with cryopre-served sperm works but requires patience. Many of the existing inbred lines at BML were propagated by sib-mating, usually in the context of the crosses to be described below.

Part of objective 2, to characterize the genetic markers of inbred lines, continues to be done using allozymes. To these protein markers we have now added DNA markers, primarily the simple-sequence repeat-polymorphisms called microsatellites. Genomic DNA was extracted from a Pacific oyster, size-fractionated, and cloned into Bluescript. Clones were then probed with a mixture of oligonucleotides having all possible dinucleotide repeats; of the 110 clones initially identified as positive, only 66 were eventually developed as PCR-amplifiable markers. Together with allozymes and RAPD DNA markers, these microsatellite markers will become the framework for the first detailed map of the Pacific oyster genome.

Major portions of objectives 2, 3, and 4 were accomplished, as in previous years of WRAC support, by factorial crosses among inbred lines. In 1995, however, we increased the sizes of these experiments from crosses involving just two inbred lines at a time to simultaneous crosses of four inbred lines. These crosses were designed to increase our knowledge of the distribution of hybrid performance (survival, growth, and the physiological components of growth), relative to the performance of their inbred parents. Ultimately, we want to understand how hybrids compare to random-bred, commercial stocks.

In both of the experiments made in 1995, four inbred lines, each represented by just a single male and female, were crossed in all combinations. Progeny were stocked into 100-liter tanks, two for each inbred cross (n=4), and one for each hybrid cross (n=12), with the whole set of tanks replicated in two different rooms (42 larval cultures). Data on larval mortality, larval shell-length, and juvenile shell-height or live weight were analyzed by ANOVA to determine if hybrid vigor (heterosis) is expressed for any trait.

An operational definition of heterosis that may be applied to any cross of two inbred lines is provided by the absolute value of the ratio hp (potence) = $Q/L > 1.0$, where L is the difference between the two inbred parents for a given trait and Q is twice the deviation of the hybrid from the mid-parent value for the trait. When one of the inbred parents is not available for measurement because of high mortality, we simply test mean hybrid performance relative to the surviving parent and express heterosis by the ratio of hybrid to best parent.

The first cross (95X1), involving lines 93-23, 93-89-4, 93-89-5, and 93-89-6 was made on June 20, 1995. Larvae were sampled only on day 13, so no mortality data are available for this experiment; juveniles were counted and measured in December 1995. The second cross (95X2), involving lines 93-21, 93-89-6, 93-7, and 93-9 was made on July 18, 1995. Larvae were counted and measured on days 10 and 14 thereafter, allowing for an analysis of survival for this period. Measurements on juveniles were also made in December 1995 on some of the crosses.

Analyses of data from 95X1 is here restricted to the complete 3x3 factorial cross of lines 3, 4, and 5. The female from line 6 produced very few larvae and only a handful of juveniles. The ANOVA for shell length, with Sire (S), Dam (D), and Room (R) effects and interactions, explains 26% of total variance, and only the three way interaction (S *D *R) and the S *D interaction are significant sources of variance ($P=0.016$ and $P=0.012$, respectively). Indeed, the contrast of hybrid vs. inbred shows a highly significant average difference of 45 mm, on a grand mean shell length of 235 mm.

Of the six measures of potence made in this 3x3 cross, four hp values, ranging from 1.69 for 5x4 to 18.33 for 5x3, are significantly greater than 1.0, and two – both numerically greater than 1.0, 1.15 for 4x3 and 1.13 for 4x5 – are not significant. Heterosis for larval size at day 13 in this cross is strikingly obvious; for example, in the subset of crosses between lines 3 and 5, mean shell lengths are 220 and 227 mm for the 3x3 and 5x5 inbred larvae and 268 and 291 mm in the 3x5 and 5x3 hybrids. Note that the difference between the reciprocal hybrids, both of which show significant heterosis, is also significant, although the average reciprocal hybrid difference over the whole experiment, 4.5 mm, is not significant.

A total of only 165 juveniles were measured for the complete 3x3 of 95X1. Still, the ANOVA for shell height, with S, D, and S*D terms, accounts for 16% of variance, and S*D is the only significant source of variance (P=0.01). The contrast for hybrid vs. inbred juveniles reveals a highly significant average difference of 27 mm on a grand mean shell height of 40 mm. Five of six potence values are greater than one, three significantly so (5.17 for 3x4,7.06 for 4x3, and 5.38 for 4x5).

In the preceding years of this project we showed, over four separate experiments, that the relative performance of hybrid and inbred genotypes was consistent from the larval to the juvenile and adult stages. This finding not only falsifies a published hypothesis that the relative fitnesses of genotypes reverses from the larvae to the juveniles stages, but also lends support to our quest for indices of larval performance that are correlated with later juvenile and adult performance and might be used for early culling. The 95x1 data set allows a direct correlation of the larval and juvenile performances of nine genotypes; juvenile shell height at 6 months is positively though not quite significantly correlated with larval shell length at day 13. It will be of interest to examine this correlation in the 95x2 and 1996 experiments, in which sample sizes can be made larger.

Analyses of the 95x2 larval data are constrained by the complete mortality of larvae spawned by the line 6 female and the poor survival of larvae spawned by the line 7 female. Only one of eight hybrid-inbred comparisons of larval mortality (day 10 to day 14) was significant; the 1x7 hybrid had significantly greater mortality (-21.3% per day) than either the 7x1 or 1x1 larvae (-1.9% and -2.9% per day, respectively).

An ANOVA of a complete factorial cross of lines 1, 7, and 9 is possible only for shell length at day 10 and only for one room. The model with S and D main effects and S *D interaction, explains 22% of total variance, and only the S *D interaction is a significant source of variance (P=0.0001). The contrast of hybrid vs. inbred shows a highly significant (P=0.0001) average difference of 15 mm, on a grand mean shell length of 205 mm. In this experiment, however, only two of the six possible measures of potence – 7.03 for 1x7 and 3.22 for 9x7 – are significantly greater than 1.0; of the remaining non-significant potence values, two are less than 1.0 and two are greater than 1.0.

The diminished heterosis observed in this experiment can be attributed to the superior performance of inbred line 93-21. Despite being the most inbred line in this experiment, line 1 had the third best performance of all the nine families in this 3x3 cross, outperforming the 9x1, 7x9, 7x1, and 1x9 hybrids, in addition to the other inbreds. Such “heroes,” as Darwin termed them, are expected under the dominance theory of heterosis. Finally, the average reciprocal hybrid difference over the whole experiment, 10.1 mm, is significant (P=0.01).

We can summarize the accumulated evidence for heterosis in growth and survival of the Pacific oyster by examining the distributions of potence values or the ratios of hybrid to best parent, over all crosses performed to date. The majority of these measures of relative hybrid performance (45 of 53) are greater than 1.0. The data on larval mortality have very low statistical power because larval numbers are estimated with little accuracy or precision. Size is estimated more precisely, however, and of the 21 potence values for size that exceed 1.0, 18, or 86% are significant.

There can be no doubt that these experiments capture the phenomenon repeatedly. Also of interest is the frequency with which the reciprocal hybrids are significantly different from each other; this occurs 9 of 11 times for larval size, and 4 of 6 times for juvenile size. These differences between reciprocal hybrids, which are expected to have identical nuclear genomic content, on average, can be attributed either to nuclear-cytoplasmic interactions or possibly to non-genetic causes having to do with gamete quality.

We have continued our studies of the physiological changes that occur in faster-growing hybrid larvae compared to inbred larvae. In addition to ongoing studies of protein and respiration (see previous reports), we have expanded the physiological studies to include measurements of feeding rates and rates of ion transport.

Methods to study whether hybrid larvae were removing more algae from the culture containers were developed during Summer 1995. Preliminary data suggested that there might be a feeding effect (i.e., hybrid larvae may clear more algae than inbred larvae when both are fed at the same algal concentration); however, the variance of these first data sets were too high and further experiments are planned next season (using larger numbers of replicates in experiments to overcome variance) to address this important issue of possible feeding differences in larvae with different genotypes.

Another "energy saving" mechanism that is important in regulating animal metabolism is the cost of ion transport. In mammalian tissues this process, controlled by a single enzyme, Na+, K+,-ATPase, can account for ca. 30% of oxygen consumption. We discovered that hybrid larvae have lower amounts of this enzyme compared to inbred larvae, suggesting that the cost of ion transport is lower in the hybrid larvae. A lower maintenance cost for processes such as ion transport would allow more energy to be allocated to growth in hybrid larvae.

To summarize progress on objectives 3 and 4, we have expanded the sizes of crosses among inbred lines and continued to demonstrate that these experiments yield repeatable estimates of heterosis in growth and its physiological components. These estimates indicate that non-additive gene action is pervasive, causing hybrids to deviate significantly from mid-parent performance, usually to surpass the best parent. The practical importance of now distinguishing among the competing explanations of such hybrid vigor lies in choosing between two basic methods for genetic improvement of broodstocks – selection of individuals or families from within a randomly mating population or crossbreeding of inbred lines.

Crossbreeding is likely to surpass pure-line breeding only when a substantial portion of non-additive genetic variance is caused by over-dominance (heterozygote advantage); otherwise, the best genotype is a homozygous one that in principle could be produced by selection within a randomly mating line. Our experiments do not by themselves distinguish between these two alternatives. They do, however, make possible definitive experiments, intercrosses among the F_1 hybrids and backcrosses to parental inbred lines that will permit mapping of the genes causing hybrid vigor. These experiments were initiated in 1995 and are being carried out primarily with funding from the USDA's competitive grants program.

No progress on objective 5 has been reported by the University of Washington lab in 1995. We have been unable to produce enough experimental seed in the BML facilities to enable distribution to more than the Tomales Bay growout site; objective 6 will not be fully realized until it is possible to mass produce hybrid seed.

Usefulness of findings

The systematic inbreeding program that we have initiated and the inbred lines produced thus far represent the first, necessary step towards producing the three anticipated benefits of the project. We have demonstrated to the industry that inbred lines can be made and that their genetic integrity can be verified by genetic markers. We have developed experimental protocols for measuring the growth, survival, and metabolism of inbred and hybrid oysters.

Our experimental results reveal a substantial amount of heterosis for these important production traits. They also provide the means and the animals for further experiments that can definitively elucidate the genetic and physiological bases of heterosis. Finally, our results to date suggest that measures of larval performance correlate with later performance, which, if verified by further observations, would enable early culling practices in commercial breeding programs.

Work planned for next year

There are no significant departures anticipated from the original work plan. Objectives 5 and 6 have been delayed because of the limited numbers of inbred lines available for research, but this constraint is being steadily alleviated.

Impacts

There has not yet been time for this project to have direct impacts on the West Coast oyster industry, which has yet to develop breeding programs. At this stage, we are working with and educating the industry about the need and potential for sound breeding programs; presentations of project results were made at Taylor United, Inc., at the annual meeting of the Pacific Coast Oyster Growers Association, October 1–3, 1995, in Lynnwood, Washington, and at the annual meeting of the National Shellfisheries Association, April 14–18, 1996, in Baltimore, Maryland.

Support

		Miscellaneous support					
Year	WRAC funding	University	Industry	Other* Fed.	Other	Total Misc.	Total
93-94	74,000	0	500	84,800	0	85,300	159,300
94-95	85,000	0	500	69,800	0	70,300	155,300
95-96	83,420	0	500	99,800	0	100,300	183,720
Total	**242,420**	**0**	**1,500**	**254,400**	**0**	**255,900**	**498,320**

*Total of $254,400 received from USDA-NRICGP Agreement 92-37206-8003 for project entitled "The Genetic and Physiological Bases of Hybrid Vigor in Oysters," directed at the fundamental causes, not the applications, of hybrid vigor. This project complements the WRAC project, which is aimed at more practical objectives.

Pollutant Reduction in Salmonid Aquaculture by Diet Modification

Progress Report for the period April 1, 1995 to March 31, 1996

Total funding level

$298,329 (April 1, 1993 to March 31, 1996)

Participants

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Project objectives

1. Feed Ingredients/By-Products: Determine the levels and bioavailability of phosphorus in key feed ingredients and determine methods for reducing phosphorus levels and/or increasing phosphorus bioavailability in selected ingredients; develop an *in vitro* method for determining phosphorous bioavailability of feeds and ingredients that accurately reflects fish performance.
2. Phosphorus requirements of salmonids: Determine the minimum level of available phosphorus required to maintain adequate phosphorus status in rainbow trout.
3. Low-polluting Feeds: Develop and produce practical feeds for salmonids at various life history stages.

Anticipated benefits

This project will provide the essential information on phosphorous requirements of salmonids at various life stages, and the phosphorous bioavailability of major feed ingredients (both *in vivo* and *in vitro*). The methods necessary to produce this information will also be developed.

Information involving these two areas is needed to precisely formulate low-polluting feeds. The eventual development of low-pollution diets for the three major life history stages of salmonids will assist the aquaculture industry in meeting regulatory requirements and in producing a high-quality, cost-effective product.

Progress and principal accomplishments

Objective 1

Apparent digestibility coefficients of macro (Ca, K, P, Mg, Na) and micro (Cu, Fe, Mn, Sr, Zn) elements and protein in various feed ingredients were determined for coho salmon (initial mean body weight: 111 g), small rainbow trout and large rainbow trout (initial mean body weight: 43 g and 170 g, respectively) using yttrium oxide (Y_2O_3) as the inert marker and passive feces collection tanks. The feed ingredients tested were herring meal, menhaden meal, anchovy meal, deboned whitefish meal, poultry by-product meal, meat and bone meal, feather meal, soybean meal, corn gluten meal, wheat gluten meal, wheat middling, and wheat flour.

These ingredients were combined with a casein-gelatin, semi-purified diet at a ratio of 70% semi-purified diet to 30% test ingredient. Apparent digestibility of protein and the mineral elements in the semi-purified diet were also tested to provide an internal control and the values necessary to calculate apparent digestibility coefficients for the test ingredients.

The casein-gelatin semi-purified diet generally showed the highest digestibility or (net) absorption for all minerals and protein. Most ingredients except wheat gluten meal appeared to inhibit the absorption of trace minerals. Dietary calcium, phosphorus, and ash frequently showed inverse correlations with apparent digestibility coefficients of minerals and protein.

Large fluctuations of element digestibility or absorption were observed among days and among weeks. Feeding rate did not significantly correlate to the nutrient digestibility, whereas it did correlate to the net absorption in most nutrients except manganese, iron, and calcium. Fish size did not affect apparent digestibility coefficients of protein or minerals; however, some differences were found between rainbow trout and coho salmon.

An additional study was conducted to determine the effects of fish bone content on the apparent digestibility of macro (Ca, K, P, Mg, Na) and micro (Cu, Fe, Mn, Sr, Zn) elements in rainbow trout (initial mean body weight 110 g). Yttrium oxide (Y_2O_3) and chromium oxide (Cr_2O_3) were used as the inert markers and passive feces collection tanks. A standard salmonid diet was prepared using deboned fish meal, and the intrinsic fish bone was added back to produce experimental diets containing graded concentrations of fish bone. The apparent digestibility of phosphorus, calcium, magnesium, iron, zinc, and strontium in fish bone was significantly decreased ($P \leq 0.05$) as the fish bone increased in the diet.

The apparent digestibility of minerals in various common fish meals was determined as described above to investigate if significant difference existed among fish meals. In addition, several non-fishmeal animal ingredients were included for comparison. Ingredients tested were herring meal, capelin meal, anchovy meal, Peruvian FAQ anchovy meal, two menhaden meals (regular and low-temperature dried), whitefish deboned meal (two samples), whitefish whole meal, whitefish skin & bone meal, poultry by-product meal, feather meal (two samples), meat and bone meal, ring-dried blood meal, and casein-gelatin semi-purified diet. Apparent digestibility of phosphorus was lowered in a linear fashion as the concentration of calcium in the feed ingredients increased ($r = -0.93$; $P \leq 0.01$), suggesting that the level of bone in the ingredients was the primary factor governing apparent digestibility of phosphorus by the fish.

A number of feed supplements with the potential to increase the apparent digestibility of minerals were tested in fish diets. The 11 supplements tested were: citric acid; sodium citrate; potassium chloride; sodium chloride; histamine dihydrochloride; EDTA-disodium salt; sodium bicarbonate; a mixture of amino acids; ascorbic acid; a mixture of inositol and choline; and cholecalciferol. Apparent digestibility coefficients for macro (Ca, P, Mg, Na) and micro (Cu, Fe, Mn, Sr, Zn) elements in fish meal-based diets was determined using rainbow trout (initial mean body weight 232 g). Yttrium oxide (Y_2O_3) and chromium oxide (Cr_2O_3) were used as inert dietary markers.

Fecal samples were collected using passive collection tanks for 5 consecutive days followed by a 7-day acclimation period with the test diets. Apparent digestibility coefficients for phosphorus, calcium, iron, magnesium, manganese, and strontium were increased by citric acid supplementation. The apparent digestibility coefficient of manganese was increased by EDTA and sodium citrate, while apparent digestibility coefficients of copper and zinc were decreased by cholecalciferol and sodium chloride. The other supplements had no notable effects on apparent digestibility coefficients of the minerals.

In vitro Bioavailability Methods Development

The *in vitro* protein digestibility of five fish meal samples and casein were evaluated by pH-stat at 37ºC. Previously, we reported a goodness of fit (r^2) of 0.87 between apparent digestibility coefficient of protein (ADCP) and degree of protein hydrolysis by pH-stat (DH) at 15ºC for these samples. For assay at 37ºC with the 4-enzyme system, DH ranged from 28.4 ± 1.95% for casein to 17.02 ± 0.82 for BC herring meal. The DH range for samples assayed with trout pyloric caeca enzymes was 20.83 ± 1.22% for casein to 15.23 ± 1.61% for Chilean Negativo meal. The fit of DH vs. ADCP was r^2=0.91 for the 4-enzyme system and r^2=0.80 using trout pyloric caeca enzymes. The median assay time was reduced from ≈300 min to ≈40 min by increasing the assay temperature from 15ºC to 37ºC.

A method was developed to measure the bioavailability of phosphorous in feed samples. Initially, samples (10 mg N) were digested with a 4-enzyme cocktail for 2 h at 37ºC, pH 8 in 10 ml 0.20 M Tris-HCl; filtered through a 0.45 m filter; and the filtrate was ashed. The P content of the filtrate was determined after ashing using AOAC 965.17 for P in animal feed. The percentage of P that was solubilized differed for six samples analyzed and ranged from 98% (Casein) to 19.6% (Chilean negativo fish meal).

Problems with the method included slow filtration times and large filtrate volumes. Accordingly, the method was modified to use ashless filter paper rather than 0.45 m filtration and the P in the ashed residue was measured rather than that in the filtrate. Measurement of both soluble (filtrate) and insoluble (residue) P obtained with the modified method showed excellent recovery of total P when standard KH2PO4 was analyzed.

Pepsin was isolated from freeze dried stomachs of juvenile rainbow trout by either alkaline extraction of pepsinogen or acid extraction of pepsin using NaCl and 0.1% Triton X-100. The yield of pepsin activity recovered was approximately four times greater by acid extraction, indicating that most of the pepsinogen had been activated to pepsin prior to freeze drying. Assay of these extracts, with denatured hemoglobin as substrate, showed a pH optimum of 3.0 and a temperature optimum greater than 45ºC.

The main protein in the pepsin extract was shown to have a mass of ≈40 KDa by SDS-PAGE. A pH-stat method was developed to monitor pepsin digestion of protein at pH 3.0, 37ºC. Relatively large amounts of pepsin were required to catalyze the reaction at a reasonable rate. For example, 45 mg/ml porcine pepsin with 10 mg sample N required >3 h to complete the reaction. Also, more trout pepsin was required to achieve a similar rate of hydrolysis as porcine pepsin.

For fish meal samples, an unusually slow pH equilibration time (≈ 3 h) was required prior to addition of the pepsin. This was probably due to the acid-demineralization of bone material in the sample. Freeze dried, de-boned fish muscle rapidly equilibrated at pH 3.0. The degree of hydrolysis of fish muscle using this method was 2.5%, whereas that for casein was 5.6%. The DH of fish muscle or casein with the 4-enzyme system was similar with or without pepsin pre-digestion.

Objective 2

An experiment was conducted to determine the effects of reducing dietary phosphorus in rainbow trout diets below metabolic requirement (down to 0.15%) during three life periods (200–600 g, 300–600 g, and 400–600 g body weight) using survival, growth, and tissue mineralization as evaluation criteria. Other objectives include: determining the effect of reducing dietary phosphorus during these three life phases on final product quality; calculating the effect of using different depletion strategies on total phosphorus discharge in the effluent; comparing impacts of lowering dietary phosphorus on effluent composition and trout product quality; and designing a feeding program that will minimize environmental pollution without sacrificing fish production or marketing quality characteristics.

The trial involved 670 rainbow trout with an initial average weight of 200 g in a factorial design of seven diets, three depletion periods and three replicates of each treatment group. The study was conducted at the Hagerman Field Station utilizing 66, 130-l fiberglass tanks each supplied with 4 L/min 15ºC spring water. The diets were formulated to contain levels of phosphorus increasing in equal logarithmic increments from 0.15 to 1.20% (0.15, 0.21, 0.30, 0.42, 0.60, 0.85, and 1.20%) total phosphorus.

The remaining 45 tanks of fish received a standard commercial trout feed (Rangen Trout Production 5/32”). At the end of the trial, fish from each treatment were sacrificed and taken to a commercial fish processing plant (Clear Springs Foods) for evaluation of carcass quality during process processing.

There was an quadratic effect ($P \leq 0.05$) of diet on survival for the fish fed the diets starting at 200 and 300 g/f, but not for the fish starting at 400 g/f. Fish that received the experimental diets with a starting weight of 300 g/f had lower survival only when fed the lowest phosphorous diet (0.15% P). Fish in the 200 gram starting weight series showed decreased survival when consuming diets containing either 0.15% or 0.21% P.

There was no effect of diet on survival of the fish in the 400 gram starting weight series. The fish receiving the highest level of phosphorous supplementation had poor survival and weight gain, as compared to fish fed the next two lower levels of phosphorous. This effect of phosphorous supplementation has been observed in trials with rainbow trout broodstock and coho salmon fingerlings. Since this seems to be an effect of diet formulation rather than dietary phosphorous level, the fish in this treatment will not be considered when discussing the effect of phosphorous on fish performance.

Diet 6 contains 0.85% phosphorous, which is higher than reported to be required for rainbow trout fingerlings. Final weight was also affected by dietary phosphorous level for the fish in the 200 and 300 gram series, but not the fish in the 400 gram series. Dietary phosphorous can be reduced to 0.60% for fish started at 200 grams average weight without an adverse effect on weight gain.

For fish with an initial weight of 300 grams, dietary phosphorous can be reduced to 0.30% without affecting weight gain. Product yield, expressed as dressing percentage and finishing percentage, was not affected by dietary phosphorous level, regardless of the starting weight.

Product quality with regard to texture was judged by the quality assurance personnel at Clear Springs Foods to be good for fish fed all of the diets. There was no effect of a phosphorous deficiency on fillet texture. The protein, fat, moisture and ash content from a five-fish sample from each tank was determined.

There was also no effect of a phosphorus deficiency on body composition. There was an effect of dietary phosphorus ($P \leq 0.01$) on whole-body phosphorus levels. There was also an effect of initial starting weight on body phosphorus levels, but no interaction of the two main effects.

Low dietary phosphorus levels did reduce weight gain and body phosphorus levels, but did not reduce product quality or yield. Feeding a lower, but not extremely low, phosphorus diet reduced the overall amount of phosphorus fed without reducing fish performance. By feeding a low-phosphorus finishing diet, at the proper time, growth rate and product quality can be maintained and a reduction in effluent phosphorus levels will be observed.

When fish are fed a 1.2% phosphorus diet from hatch to 300 g, and then fed a 0.3% phosphorus diet until market, a reduction of 38% in effluent phosphorus is observed (compared to feeding 1.2% phosphorus diet during the entire production cycle). Different phosphorus levels in the finishing diet, and different starting weights, can be used dependent on feed ingredient prices at the time. This study demonstrated that dietary phosphorous levels can be reduced for finishing rainbow trout without affecting weight gain or product quality, which resulted in a significant reduction in phosphorus discharged.

Another study was conducted to determine the effects of reducing dietary phosphorus in rainbow trout broodstock diets below typical levels (down to 0.40%) using growth, feed consumption, tissue mineralization, and gamete viability as evaluation criteria and also to calculate the effect of feeding different phosphorous levels on total phosphorus discharge in the effluent.

This trial involved 360, 2 year-old domesticated rainbow trout. The study was conducted at the Ennis National Fish Hatchery, using the indoor facility. Fish were randomly assigned to one of 12, 525 gallon raceways. Each tank was supplied with 50 GPM of constant 54ºF spring water.

Four diets were fed to triplicate lots of 30 pre-selected, presumably female, fish. The open-formula brood diet (USFWS) was used as the control diet and contained ~1.16% phosphorous. The experimental diets contain either 0.4, 0.8, or 1.2% phosphorous. Fish meal, corn gluten meal, and wheat gluten contents of the diets were varied to result in the different phosphorous levels. The diets were produced by steam-pelleting at the Bozeman FTC, with ~2/3 of the fish oil being top-dressed onto the pellets.

There was an effect of diet ($P \leq 0.05$) on final fish weight. The fish fed the low phosphorous diet (0.4%) were smaller than the fish fed the medium (0.8%) or high (1.2%) phosphorous diets or the Standard Brood diet. There was no effect of diet on the number of eyed-eggs per female, the number of eyed-eggs per pound of fish, or in the percent eye-up. Eye-up ranged from 70–77%, values typical to that production hatchery.

There was also no effect of diet on fry survival or growth. There was an effect of diet on proximate composition of the brood fish. The fish fed the low phosphorus diet had a higher body fat content (10%) than the fish fed any of the other diets (avg 7.6%).

Feeding a low phosphorous diet (0.4%) did decrease weight gain and increase body fat relative to control diets, but egg production and quality criteria was unaffected. If a dietary phosphorus level was fed that did not decrease weight gain, then a safety margin with regards to egg production will be realized. Feeding a diet with 0.8% total phosphorus can be recommend for the first year of broodstock production.

Supplemental Dietary Vitamin D3 and Thyroid Hormone

The purpose of this study was to examine the effects in rainbow trout of supplemental dietary vitamin D3 and thyroid hormone in low and adequate phosphorus diets on whole body retention and specific tissue distribution of phosphorus, calcium, and magnesium. A 14-week feeding trial was conducted with triplicate tanks of fingerling rainbow trout (60 fish/tank) having an initial weight of 3.4 g. The test diets varied in concentrations of total phosphorus (0.15% or 0.5%), supplemental dietary D3 (0, 2,000, or 200,000 IU/kg), and dietary T3 (0 or 100 ppm). Diets were formulated to contain 45% protein and 16% lipid (dwb).

Blood, skin (with scales), intestine, vertebrae, and whole body samples were taken initially and then every fourth week to determine plasma and intestinal alkaline phosphatase activity, whole body lipid, phosphorus retention, and phosphorus, calcium, magnesium, and ash concentrations in plasma, skin, vertebrae, and whole body using the same methods described previously. Individual vertebrae were subjected to compression testing.

Body weight was significantly affected by T3 supplementation and dietary phosphorus level, but not by vitamin D3 supplementation. The addition of 100 ppm of T3 to the 2-MDT and 5-MDT diets dramatically increased fish weight gain for the first 4 weeks of the feeding study. As the study continued, T3-fed fish became anorexic and deformed, particularly those fed the low phosphorus diet (2-MDT), which also had high mortality (100% by week 14).

Although cumulative weight differences between the low and adequate phosphorus dietary treatments became significant by week 12, these differences were not extremely large; at the end of the 14-week feeding study when average fish weights had increased more than 6-fold, respective cumulative weights for the low phosphorus and adequate phosphorus treatments averaged 25.0 g and 28.0 g.

Dietary phosphorus concentration and T3 supplementation had a significant effect on mineral concentrations of skin with scales, whole bodies, and vertebrae of rainbow trout. No significant differences in mineral concentrations were observed between fish fed the 0.5% phosphorus diets and those fed the fish meal control diet. This is somewhat unexpected since results from last year's study indicated maximal mineral concentrations in fish fed up to 0.7%–0.95% dietary phosphorus.

The new results suggest that for fish of this size, 0.5% dietary phosphorus is adequate. In fish fed the low phosphorus diet for 14 weeks, phosphorus and magnesium concentrations in skin/scales from the low phosphorus dietary treatments had dropped to ~30% of their original values. Surprisingly, calcium concentrations were reduced even more dramatically, to approximately 15% of their initial values. The same trend in reduction of phosphorus and calcium was observed in whole body and vertebrae.

Of the tissues analyzed, the vertebral concentrations of phosphorus, calcium, and magnesium were affected the most by low levels of dietary phosphorus. By week 14, the respective phosphorus, calcium, and magnesium levels in vertebrae from the low phosphorus treatment had decreased to 17%, 15%, and 20% of their initial values.

Physical properties of the individual vertebrae were significantly affected by dietary phosphorus concentration. Although vertebrae from fish fed for 4 weeks were too small to compress using the Instron 1000, measurements with an ocular micrometer indicated that vertebral height was significantly less in fish fed the low phosphorus diets (2-MD was selected to represent the low phosphorus diets, and 5-MD represented the adequate phosphorus diets).

At week 4, there was a trend for the vertebrae from the fish fed the adequate phosphorus diet to have a larger area and volume compared to vertebrae from fish fed the low phosphorus diet. At week 12, compression testing showed that in comparison to the low phosphorus treatment, vertebrae from the adequate phosphorus treatment had significantly higher values for ultimate strain (%), ultimate force (g/mm^2), height, and densities. Retention of phosphorus, calcium, and magnesium was significantly affected by dietary phosphorus concentration and thyronine supplementation.

Fish fed the lower phosphorus diets had higher whole body phosphorus retention and lower calcium and magnesium retentions compared to those fed the adequate phosphorus diets. Compared to fish fed diets not supplemented with T3, fish fed the adequate phosphorus, T3-supplemented diet had significantly higher calcium retention and lower magnesium retention.

Surprisingly, phosphorus retention values for all fish fed the wheat gluten-based test diets were over 100%, as were the calcium retention values for fish fed the adequate phosphorus test diets. Mineral retentions >100% could be due to a number of factors, the most likely being that additional minerals, particularly calcium, were absorbed from the water supply. Although fish obtain the majority of their phosphorus from the diet, some phosphorus can also be absorbed from the water, an occurrence that might be stimulated especially in fish fed the phosphorus deficient diet.

Another possible reason for the higher than expected retention values might be the presence of phosphorus in undigested feed in the intestines of the 2 day fasted fish. However, fish fed the fish meal based control diets had very typical mineral retention values.

Usefulness of findings

The ultimate goal of this project is to formulate feeds that reduce nutrient levels in hatchery effluents, while maintaining good fish performance and product quality at a reasonable price. Both the digestibility and requirement data collected in the trials discussed in this report are required to formulate diets for the different life stages of salmonids.

Work planned for next year

Feeding trials will be conducted to determine the optimum level of citric acid supplementation to increase apparent digestibility of phosphorus, and to confirm, using apparent phosphorus retention in the body of trout, that the increase in the apparent digestibility coefficient for phosphorus was actually due to increased intestinal absorption of phosphorus, not conversion of phosphorus into a soluble form that was excreted in the feces but not collected by our feces collection procedures.

Additional studies will be conducted on low-ash fish meal produced from fish processing waste using a novel procedure to debone the fish processing waste prior to its conversion into fish meal.

The results obtained during this year will be used to formulate low-phosphorus feeds for trout using feed ingredients with high apparent digestibility coefficients for phosphorus and relatively low phosphorus levels. These feeds must be economically competitive with existing trout production feeds.

Because most of the feed used in the rearing cycle of trout production is used during the final growth stage, from 100–500 g, the low-polluting feeds will be tested using fish in this size range.

The two-step pH-stat *in vitro* protein digestibility method will be applied to feed protein samples of known ADCP to determine whether there is a better correlation than previously reported for the one-step method. Attempts will be made to obtain a higher yield of trout pepsin with higher specific activity by using the gastric mucosa from freshly-killed trout of market size.

Feed samples, stomachs and pyloric ceca from experiments at the University of Washington will be used to determine: (a) the relationship between *in vivo* P bioavailability and *in vitro* P solubility using one-step digestions (gastric and intestinal) and the two-step digestion and (b) influence of diets containing various amounts and forms of P on the performance of digestive enzymes in the *in vitro* assay.

A study is currently underway to examine how minerals are partitioned in the scales and skin of rainbow trout and coho salmon in response to dietary phosphorus intake. If the scales of salmonids are sensitive to phosphorus depletion, they would be a useful, non-invasive tool to monitor the phosphorus status of high value fish.

Practical finishing and broodstock diets will be formulated and fed in production settings. The finishing diet study will be conducted at a commercial hatchery in the Snake River Valley. The objectives of both of these studies is to further utilize information derived from previous studies in developing practical diets.

Impacts

None yet reported.

Support

		Miscellaneous support					
Year	**WRAC funding**	**University**	**Industry**	**Other Fed.**	**Other**	**Total Misc.**	**Total**
93-94	99,000	17,500	8,800	38,000	0	64,300	163,300
94-95	100,389	25,000	9,600	38,000	0	72,600	172,989
95-96	98,940	38,000	19,200	139,655	0	196,855	295,795
Total	**298,3298**	**80,500**	**37,600**	**215,655**	**0**	**333,755**	**632,084**

Improving the Quality of Intertidal Substrate for Oysters and Other Bivalves: A Strategy for Managing Burrowing Shrimp and Enhancing Habitat

Progress Report for the period April 1, 1995 to March 31, 1996

Total funding level

$212,996 (April 1, 1992 to March 31, 1996)

Participants

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Brett Dumbauld,* Washington Department of Fish and Wildlife, Washington
Kristine Feldman,* University of Washington, Washington
Steve Harbell (TA/Extension), Washington Sea Grant Coop. Extension, Washington
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* *(funded participants)*

Project objectives

1. Investigate whether oyster shell distributed on the intertidal as a thick “pavement” might stabilize and compact intertidal sediments and serve as a foundation for bottom culture of oysters as well as enhancing the habitat for predators of juvenile burrowing shrimp.
2. Examine predator-prey interactions that may reduce abundance of YOY burrowing shrimp in areas of oyster culture.
3. Examine settlement patterns of mud shrimp in field and laboratory experiments.

Anticipated benefits

The ghost shrimp *Neotrypaea californiensis* (formerly *Callianassa californiensi*) and the mud shrimp *Upogebia pugettensis* are common inhabitants of estuarine intertidal sediments from Southeast Alaska to Baja, California. At high densities, they can greatly influence the structure of benthic communities by excluding species that are unable to withstand the constant bioturbation associated with shrimp burrow construction and feeding activities.

Bivalve aquaculture along the Pacific Coast is adversely affected by burrowing shrimp which increase sedimentation rates and reduce the compaction of intertidal sediments. Oysters and other sessile bivalves often sink into the mud or are smothered by sediment deposition, particularly as settling larvae and spat. In Washington state, the insecticide carbaryl (brand name Sevin™) has been applied to oyster beds at low tide to control populations of burrowing shrimp since 1963. The chemical has been banned, however, in Oregon and California, and its use in aquatic habitats continues to raise environmental concerns in Washington due to its toxicity to non-target species and secondary effects on other estuarine resources.

The purpose of our study is to provide information on shrimp life history and interactions between shrimp and bivalve culture that can be incorporated into new or existing strategies to achieve more effective long-term management of shrimp populations. Whereas previous research has been directed primarily at pesticide application and impact analysis on adult shrimp and non-target species, this study places greater emphasis on understanding the nature of the shrimp larval recruitment process, given that this is perhaps the greatest obstacle in achieving long-term control.

Even if growers are able to eliminate juvenile and adult shrimp from their grounds, post-larvae enter estuaries annually from the near-shore coastal plankton to settle in intertidal sediments. Our research is directed at examining how ecological interplay between shrimp and bivalve culture, physical methods of disturbance, and predator-prey interactions may reduce the abundance of YOY shrimp on culture grounds.

Results of our study will contribute to the long-term stability and potential expansion of the oyster industry and other bivalve aquaculture along the Pacific Coast, allowing for responsible management and rational control of intertidal burrowing shrimp in bivalve culture areas.

Progress and principal accomplishments

Objective 1

Field experiments are in progress to examine whether the addition of oyster shell to carbaryl-treated and untreated mudflat reduces recruitment of YOY ghost and mud shrimp, while providing substrate suitable for oyster culture.

In July 1994, small plots (64 m^2) were established on mudflat dominated by ghost shrimp at Nahcotta in Willapa Bay, Washington. Experimental plots were arranged in a randomized block design with four replicates per treatment (16 plots total). Four plots were treated with carbaryl ("treated"), four plots were treated with carbaryl and then shelled ("treated + shell"), four plots were shelled without carbaryl application ("untreated + shell"), and four plots were left bare to serve as controls ("untreated").

The effect of carbaryl application was to eliminate existing shrimp from these treatments ("treated" and "treated + shell"), but retain shrimp on those treatments not sprayed ("untreated + shell" and "untreated"). We also constructed four unreplicated large plots (900 m^2) to examine any differences in trends in shell cover, shrimp density, crab density, and oyster seed density that might arise due to effects of spatial scale.

In July 1995, we initiated an identical field experiment on tideflats dominated by mud shrimp to examine the effects of carbaryl and shell treatments on YOY mud shrimp recruitment. Small plots were established along the Cedar River channel and large plots were established near the Nemah River channel in Willapa Bay. Percent epibenthic shell cover, YOY shrimp, and crab densities have been monitored at selected points in time from July 1995 through June 1996.

Epibenthic Shell Cover

On ghost shrimp dominated ground at Nahcotta, the average amount of epibenthic shell cover on small "untreated + shell" plots declined rapidly to 10% within 2 months after placement, whereas shell cover remained initially high on '"reated + shell" plots but then declined gradually to 30% 1 year after placement. In contrast, on mud shrimp ground at Cedar River, mean shell cover was still 90% on both "treated + shell" and "untreated + shell" plots 1 year after placement. Data from large plots reveal patterns similar to those on small plots, again illustrating the difference in the effect of ghost shrimp and mud shrimp on shell coverage.

These data suggest that, at our experimental locations, carbaryl application was necessary to retain epibenthic shell at the surface on ghost shrimp ground.

However, carbaryl did not appear necessary for shell retention on mud shrimp ground. Although site characteristics may account for some of the differences in percent epibenthic shell cover between treatments, we hypothesize that much of the variation can be attributed to differences in mobility, burrow stability, and general life history between the two species of shrimp.

Oyster Seed Measurements

Oyster seed was planted on ghost shrimp ground at Nahcotta in April 1995 and on mud shrimp grounds at Cedar River and Nemah in April 1996. On ghost shrimp ground, mean densities of seed declined to nearly zero within 5 months on both "untreated + shell" and "untreated" plots, as we observed for epibenthic shell. Seed densities on "treated" and "treated + shell" plots were similar over the first summer; however, by June 1996, seed densities were higher on "treated + shell" than "treated" plots.

Although only 5 months of data have been collected from experiments on mud shrimp ground, trends in seed densities are different when compared to the first 5 months of the same experiment on ghost shrimp ground. Specifically, on ghost shrimp ground, seed densities on "untreated" plots fell sharply in contrast to densities on "treated" plots; whereas, on mud shrimp ground seed densities initially declined on both "treated" and "untreated" plots but then leveled off at similar densities.

In addition to monitoring seed density, at all experimental sites we tagged individual shells covered with spat to measure spat density and growth over time. These data will be examined for differences in spat mortality and growth with respect to treatment.

YOY Shrimp, Manila Clam, and Crab Densities
Small and large plots were sampled in October 1995, and February and June 1996, to quantify ghost shrimp and mud shrimp recruitment into experimental plots. Results of these data will be included in the final report along with those on Manila clam recruitment and crab densities.

Crab Predation on Oyster Spat
In response to concerns that the increased abundance of shrimp predators associated with shell cover might also have a direct effect on juvenile oysters, we conducted predation experiments on oyster spat using J4 and J6 instar and 1+ Dungeness crabs. No oyster spat was eaten in any of the experimental trials, indicating that Dungeness crabs residing or foraging in epibenthic shell habitat will not likely be a source of spat mortality.

Manila Clam Experiment
Research conducted by Oregon State University and reported in last year's progress report and in Smith (1996), indicated that crab predation heavily impacts Manila clam survival in shrimp infested areas in Yaquina Bay and Tillamook Bay, Oregon. Addition of epibenthic shell and use of predator exclusion devices, particularly vexar cages, enhanced survival and proved to have potential for aquaculture, at least in areas dominated by mud shrimp.

We observed similar enhancement of clam survival in the oyster shell treatments at Nahcotta. Since carbaryl was used to remove shrimp in our treatments, we initiated an experiment in early July 1996 to distinguish the influence of predators from that of shrimp and shell on Manila clam survival. Results will be incorporated in the final report.

Objective 2
Prey Density Experiment
Predator-prey experiments between YOY Dungeness crabs and YOY mud shrimp were conducted to quantify prey consumption by predator as a function of shrimp density (see 1993–94 progress report for a similar experiment conducted with YOY ghost shrimp). Five shrimp densities of 1, 2, 3, 5, and 7 shrimp tank were used which represented the range of low to high densities of YOY shrimp sampled at Cedar River.

Although crabs preyed on mud shrimp, predation occurred in very few experimental trials. The shape of consumption rate and proportional mortality curves were suggestive of a density-dependent functional response; however, we could not detect a statistical difference with respect to shrimp density.

These data may help explain why mud shrimp survive better in shell than ghost shrimp. A principle benefit of shell with respect to oyster culture is the enhanced abundance of small YOY Dungeness crabs that prey on YOY shrimp. This is particularly true of ghost shrimp which have low abundance in shell.

Ghost shrimp recruit in late summer through fall into shell habitat occupied by large Dungeness instars (J4–J6) at fairly high densities. But mud shrimp settle into shell in May and June, either ahead of a new Dungeness year class or when crabs are still very small (J1–J3) and likely not significant predators of shrimp. In this sequence, mud shrimp have a better chance to escape crab predation at settlement and so are given an opportunity to burrow and grow before crabs reach a larger, more predatory size.

Prey-choice Experiment
Several prey were offered to J2, J4, and J6 instar Dungeness crabs to determine the relative ranking of preferences among prey types. The prey types offered included juvenile ghost shrimp, gammarid amphipods, bivalves, polychaetes, and J1 instar Dungeness crabs.

Results indicated that gammarid amphipods were eaten more often than any other prey type for all size crabs tested. However, ghost shrimp were the second most common prey eaten by J2 and J4 crabs, and in these trials, mean numbers of amphipods and shrimp eaten did not differ significantly (paired t-test, $P \leq 0.05$). In contrast, J6 predators ate the fewest ghost shrimp, preferring amphipods, clams, and polychaetes over shrimp.

Separate prey-choice experiments were also conducted with YOY mud and ghost shrimp to determine if J2 and J4 Dungeness crabs exhibited a preference for either species of shrimp. Results indicated that neither J2 nor J4 crabs exhibited a preference for one species over the other.

Potential-fish Predators

Gill nets were fished on the "treated + shell" and "untreated" large plots at Nahcotta during one low tide series each month from September 1995–August 1996 to characterize seasonal fish use patterns. Nets were also fished on the new shell plots at Nemah for comparison in June and August 1996. The most abundant fish caught in order of importance during the August–October ghost shrimp recruitment period were staghorn sculpin, shiner perch, and three-spine stickle-back.

Smelt and anchovy became important components of the catch during April–June when mud shrimp recruit to the benthos, but three-spine stickleback dominated the catch. Stickleback were not present at the Nemah site where juvenile chinook salmon were the most abundant fish caught in June.

Although preliminary results from August 1995 indicated that fish were significantly more abundant over the "treated + shell" plots, we did not find any significant trends in other months. The lack of effect, however, could be due to the small size of the plot relative to the intertidal area over which an individual fish forages. Fish caught during each species recruitment period were saved for stomach content analysis.

Objective 3

In contrast to recruitment studies on ghost shrimp, data collected thus far on mud shrimp suggest that recruitment may not be reduced by the presence of epibenthic shell deposits. We therefore examined differential settlement between mud and oyster shell treatments in the field during a peak settlement pulse in April 1995. Results will be included in the final progress report.

Laboratory experiments were performed as well to determine if mud shrimp exhibited a preference for oyster shell habitat or open mud under still water conditions. Although the mean proportion of post-larvae settling in shell was higher (mean = 62%) than in mud (mean = 38%) the difference was not statistically significant (paired t-test, P=0.20). In the laboratory experiment, mud shrimp post-larvae were capable of settling in both habitats with equal success.

Usefulness of findings

Burrowing shrimp threaten the profitability and, in some cases, the existence of shellfish aquaculture on the Pacific coast, which is an important component of resource-based economics for coastal communities. Our approach to shrimp control is based on an understanding of shrimp life history and their ecological relationships with other species in estuarine benthic communities.

The results of our studies will provide new directions for integrated pest management, which became the preferred alternative for shrimp control in the Supplemental Environmental Impact Statement. This resulted in an effort by state and county agencies to develop an integrated pest management plan (Burrowing Shrimp Control Committee 1992).

A program that considers the biology of the shrimp should provide a more effective and longer-term solution to shrimp control, and result in better management and protection of estuarine resources and habitats.

Work planned for next year

This project is now in its fourth and final year and is scheduled for completion March 31, 1997. With the exception of one sampling trip to Cedar River and Nemah in October 1996, we have accomplished the objectives set forth in our original proposal. We have made significant discoveries with respect to burrowing shrimp settlement patterns and predator-prey interactions in intertidal shell habitat that can be incorporated into future IPM plans.

Although shell habitat manipulation may not be suitable in some estuarine areas, we see great promise in the placement of excess shell back onto tideflats, beyond amounts presently used as cultch for seed.

The culmination of data collected from the Pacman shell mitigation site in Grays Harbor and from long-term experimental plots established at Nahcotta in Willapa Bay suggest that intertidal shell placement in combination with carbaryl can effectively reduce recruitment success of ghost shrimp and increase oyster seed survival compared to carbaryl application alone.

Data collected from Pacman and from experimental plots located on mud shrimp infested grounds at Cedar River and Nemah appear to differ greatly with those data collected on mudflats infested with ghost shrimp. While shell placement may prove to be a useful tool in combination with carbaryl to control ghost shrimp on some oyster beds, it is still too soon to draw any conclusions as to whether this technique would provide any benefit in areas inhabited by mud shrimp.

While we have collected 2 years of data from experimental plots at Nahcotta, we have only had the opportunity to collect 1 year of data on shell cover, shrimp recruitment, and seed densities from experimental plots at Cedar River and Nemah. What data we have collected, however, suggest that epibenthic shell may not reduce recruitment of mud shrimp.

Of equal importance are our preliminary findings that carbaryl application has not resulted in higher oyster seed (cultch) survival on “treated” plots compared to “untreated” plots. Although we could end our sample collections in October 1996, our conclusions about the effects of shell and carbaryl on mud shrimp recruitment and oyster seed survival would remain tentative.

By following these experimental plots for an additional year, we could assess longer-term changes in shell cover, shrimp recruitment, and seed survival and growth, and would know with greater certainty whether shell application is a worthwhile endeavor on mud shrimp grounds.

We have submitted a request for a 4-month extension of our project through July 1997, to continue sampling small and large field plots at Cedar River and Nemah through one more cycle of mud shrimp and crab recruitment, which occurs in late spring. We also are requesting a small amount of money to finance two field trips, sample processing, and data analyses. Samples would be collected in February and June 1997 at Cedar River and Nemah. Specifically, we would sample YOY shrimp and crab densities as we have been doing over the past year. Data on percent shell cover, oyster seed densities, and spat growth would also be collected. While February is within the 1996–1997 fiscal year, we would be unable to process and analyze those data prior to March 1997.

We strongly believe these additional data: (1) are necessary to compare results with those from ghost shrimp grounds and (2) would be beneficial in assessing long-term changes in experimental plots which would directly relate to the oyster growing cycle should intertidal shell placement be applied on a broad-scale to commercial oyster grounds inhabited by ghost shrimp and mud shrimp.

Impacts

We are optimistic that the results of our work on intertidal shell placement can be incorporated into strategies for integrated pest management that will benefit the oyster industry. An additional benefit of intertidal shell is that it provides refuge for juvenile stages of other commercial species such as Manila clams.

Placement of shell on bare mud flats has been a primary approach used in our studies of shrimp ecology and population dynamics. We have found that shell can act as a physical barrier to burrowing ghost shrimp just after settlement from the water column, but also can increase densities of predators of the shrimp as well such as Dungeness crabs. Shell “pavementing” may prove to be a useful tool in combination with carbaryl to control ghost shrimp on some oyster beds while providing a firmer substrate for oyster seed, as our data have shown.

It is too soon to draw any conclusions as to whether this technique would provide any benefit to areas inhabited by mud shrimp. Nevertheless, our findings highlight the need to consider each species of shrimp separately and to better integrate aspects of their unique life histories into culture practices and control operations.

Support

Year	WRAC funding	Miscellaneous support					Total
		University	Industry	Other Fed.	Other	Total Misc.	
92–93	6,457	0	0	0	0	0	6,457
93–94	39,000	0	1,000	6,000	1,975	8,975	47,975
94–95	89,610	0	3,000	0	3,160	6,160	95,770
95–96	77,929	0	3,000	0	0	3,000	80,929
Total	**212,996**	**0**	**7,000**	**0**	**5,135**	**18,135**	**231,131**

* Washington State Department of Fish and Wildlife.

Evaluation and Improvements of Solids Removal Systems Used in Aquaculture Production

Progress Report for the period April 1, 1995 to March 31, 1996

Total funding level

$70,141 (April 1, 1995 to March 31, 1996)

Participants

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Walter Zachritz (Chair),* New Mexico State University, Las Cruces, New Mexico

* *(funded participants)*

Project objectives

The overall goal of this project is to develop low-cost treatment technologies for the effective removal of suspended solids in aquaculture operations. The specific objectives are to:

1. develop a standard protocol and set of criteria for evaluating solids removal methods from aquaculture systems;
2. utilize the protocol to evaluate several solids removal systems in current use, or with potential for use, in the Western states;
3. propose and test modifications which will result in improvements in solids removal effectiveness; and
4. develop a model incorporating protocol data, economic data, and other factors to facilitate the selection of solids removal and control technologies.

Anticipated benefits

The need for reducing solids in aquaculture waters, be it for reuse or discharge, is one of the most pressing problems facing the aquaculture industry today. To achieve an effective solution, managers of aquacultural facilities need to address this problem on three fronts: feed and feeding practices, rearing unit design, and solids removal and disposal.

Work on feed and feeding practices has been supported by WRAC, among others. Rearing unit design, largely overlooked in the United States, has recently progressed as evidenced by the introduction of new water impoundment configurations. The work being carried out under this project will result in performance and economic characterizations of the wide variety of solids removal technologies being used in the industry today.

These characterizations will be of use in the design and operation of predictable and reliable solids removal systems that are appropriate for the economic and technical constraints of a given operation. In addition, modification in the design and management of existing solids removal systems will be proposed and tested, resulting in improved efficiencies of operation.

An important characteristic of this project is the breadth of water quality factors being considered. Solids removal systems do not only affect suspended solids concentrations, but a whole host of water quality parameters. The protocol developed, and the models being constructed are designed to account for the overall impact which solids removal systems have on water quality.

Progress and principal accomplishments

Objective 1

The protocol was developed and preliminary testing was carried out under a grant funded by the U.S. Department of Agriculture. Under the present grant, the protocol was tested intensively on two aquaculture production systems. The facilities tested utilized a clarifier/wetlands and two types of bead filters in recirculating, intensive tilapia production.

The clarifier/wetlands system was operated at New Mexico State University (NMSU) and the bead filters were operated at University of Arizona (UA). Two additional systems at commercial operations were analyzed under objective 2 of the project. Modifications were made to the protocol as test results were obtained and problems were identified.

The protocol parameters are organized under five categories: operational, solids removal system, water quality, solids characteristics, and economic considerations. Our protocol evaluation results showed that some parameters were difficult to measure – especially by inexperienced operators using portable analysis kits. Determination of particle sizes for solids using the method of sequential sieving as outlined in the protocol was very difficult. All sieve sizes clogged frequently, resulting in biased separations.

Alternative methods of particle size characterization based on instrumentation (e.g., Coulter ™ counter) were considered, but were not included since they could not be performed on the equipment available to most commercial operations or research laboratories. After review by the investigators, particle size distribution analysis was eliminated from the protocol until improved methods can be developed.

Chemical Oxygen Demand (COD), a measure of the carbonaceous oxygen demand of the water or sludge, was included in the testing procedure because it was easy to perform in the laboratory and because test results could be obtained quickly. This test was run on both filtered and unfiltered samples. At the low levels found in many of the samples (COD < 50 mg/L), the test lacks reproducibility since it is close to its detection limit.

Biochemical Oxygen Demand (BOD) is being considered as an alternate method for organic matter estimation. Although this analysis cannot be done in the field and results are typically obtained after 5 days (BOD), the test has better resolution at low concentrations than the COD test. In addition, BOD values are often used in discharge regulations, providing an additional incentive to switch from COD to BOD as estimates of organic matter concentration.

Suspended solids concentrations in many aquaculture production systems are typically very low. This is particularly true in salmonid raceway culture, where TSS values under 5 mg/L are common. Measurement of these low TSS are difficult and are subject to relatively large variability and experimental error. A possible strategy to reduce variability in the results is to use larger sample volumes, than those used to date, and to filter the samples unto pre-weighed filters in the field, rather than in the lab. These modifications are under review by the project team.

Analysis of the data collected from the various solids removal systems confirms our original assumptions about the need to consider multiple parameters in evaluating the performance of these systems. The systems tested differ not only in their solids removal efficiency but also in their impact on water quality parameters such as dissolved oxygen, phosphorus, and/or nitrogen concentrations.

Objective 2

Testing of several solids removal systems have been performed using the protocol. The application of the protocol to the solids removal systems in Idaho has lagged due to delays in installation of the solids removal equipment at the study farms in that state. The postponement of the full integration of our University of Idaho collaborator has also contributed to delays in progress of the on-farm work in Idaho.

We only have data from a few months on the fine-screen drum filter and an off-line settling basin. The fine-screen belt filter is not fully operational. That filter has been installed in an off-line settling basin as a final polishing procedure for effluent collected from solids settling areas in raceways.

Objective 3

This task was to start during the second year of the project as data on existing systems are collected and analyzed. We were able to start this task in the first year because matching funds became available to do a preliminary analysis of promising solids removal systems. The brush clarifier which utilizes simple brushes to remove solids particles could be used as both a stand alone system and as a retrofit to improve the performance of sedimentation or clarifier systems.

Preliminary testing was performed at the NMSU aquaculture facility examining three, bench-scale, clarifier configurations: conventional sedimentation, plate settler, and the brush clarifier systems. These preliminary tests indicated that the brush clarifier can provide good TSS removal at higher overflow rates than either the conventional system or a plate settler. Further work is needed to test this system at a larger scale and to locate potential operating commercial-scale facilities for prototype evaluation.

Objective 4

The models being developed under this objective include technical and economic aspects. The technical issues have to do with the overall water quality impact of the treatment systems, while the economic issues relate to the overall costs of the units including capital and operating components. Initial work under this objective has concentrated on the technical aspects. Models are being developed to quantify the water quality impact of various types of solids removal systems.

The models are being developed using EXTEND™, a modeling language available for Windows and Macintosh computers. The language allows the creation of “blocks” or subprograms for a particular process or treatment unit. These blocks can then be connected on the screen to other blocks representing components of an aquaculture system such as a water supply, holding and growout tanks, and aeration.

The models for the various treatment systems are being developed from theoretical analyses and using existing literature from the environmental engineering area. As our data collection progresses, the models will be tested and modified to improve the quality of their description of aquaculture solids removal. The solids removal blocks under development at this time are: 1) fine screen (drum) filter, 2) settling tank, and 3) granular media filter. Each model is described below.

Fine Screen (Drum) Filter

The microscreening operation is incorporated into a model which simulates a radial flow drum filter. A typical operating mode of such a filter is to keep the drum stationary and let the slurry flow into the drum and out through the screen.

As the screen begins to plug, the water level within the drum rises until a control device is activated, when a preset water level is reached. The drum filter then begins to rotate and spray bars at the top of the drum are activated to backflush the contaminants from the screen into a sludge tray which is suspended through the center of the drum.

The drum filter simulation incorporates three important aspects: (1) the ability to model particulate capture, (2) intermittent backwashing, and (3) the filter’s effect on the influent particle size distribution. An initial version of the drum filter assumed constant rotation of the drum and a fixed-thickness filter cake.

A second version simulates the changing removal efficiency of suspended solids due to gradual pore clogging and cake build up. The underlying model is based on volumetric and mass balances of the retained particles. The backwash event is triggered when the effective pore size of the screen has been reduced to a given fraction of the initial pore size.

Settling Tank

The settling tank model simulates the removal of suspended solids from water by sedimentation. The sedimentation rate is primarily a function of particle size, density, and shape. Assuming ideal flow and using Stoke’s Law, the terminal settling velocity of a spherical particle can be estimated.

This settling velocity is then compared to the overflow rate of the settling tank. If the settling velocity for a given particle size is greater than or equal to the critical velocity, then all such particles are assumed to be removed by settling. For particles with settling velocity lower than the critical velocity, the fraction of particles removed is approximately equal to the ratio between the two settling velocities.

Particle scouring and resuspension will be considered through empirical factors associated with certain characteristics of a particular settling tank design and operation. Water quality changes due to break down of settled particles are related to how long the solids are left in the settling tank, and will be incorporated into the model using empirical information.

Granular Media Filter
Although aquaculture wastewaters cause biofouling of filter media, properly designed and operated granular media filters can be a very effective solids removal operation, especially for recirculating systems.

A granular media filter model is being developed to simulate solids removal in gravity sand filters and in pressurized bead filters. The model is based on a steady state assumption, and uses trajectory analysis and mass balances to estimate particle removal. The current version of the filter model does not include filter ripening (the build-up of a filter cake over time), resulting in conservative estimates of filtration efficiency.

Several additional simplifying assumptions are made in the model at this time: particles being removed in the filter are spherical and uniform; the flow across the filter area is homogeneous; and the filter media consists of uniform and spherical particles.

Usefulness of findings

The results of this work can provide a basis on which to compare the reported performance of treatment units designed for aquaculture production systems. The protocol developed and initially tested under previous funding by USDA and extensively tested in this study should provide a standard by which regulators, commercial equipment manufacturers, and researchers can evaluate their suspended solids removal systems.

In addition, the examination of a wide variety of unit operations from differing production systems (recirculating and flow through) will provide a thorough, ongoing test of the protocol and sampling method. Results of the modeling work carried out to date have served to increase our understanding of the operation and performance of various types of solids removal systems. This understanding is useful in designing and conducting samplings, and in analyzing collected data. As data become available, models will continue to be refined, calibrated, and validated, increasing their usefulness to the industry.

Some regulatory agencies are altering the requirements that industries must meet for discharges and the degree to which they will utilize various technologies to manage these wastes. We hope to provide the industry with the information needed to make informed decisions about which solids removal systems are appropriate for their particular conditions.

Work planned for next year

The basic objectives of the project remain unchanged for the coming work year, except for the addition of the University of Idaho to the project team. The specific work plans for each project team member are outlined in the detail part of this report.

Impacts

None yet reported.

Support

Year	WRAC funding	Miscellaneous support					Total
		University	Industry	Other Fed.	Other*	Total Misc.	
95–96	70,141	0	0	98,000	40,000	138,000	110,141
Total	**70,141**	**0**	**0**	**98,000**	**40,000**	**138,000**	**110,141**

* In-kind support from NSF and WERC funded undergraduate summer research positions at NMSU ($10K) and UCD ($30K).