

ANNUAL PROGRESS REPORT

For the Period September 1, 1996 to August 31, 1997

January 1998

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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/subtropical 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 twelve 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 Katie Dalton, 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 ten 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), FY96 (Grant #96-38500-2631), and FY97 (#97-38500-3957) with monies totaling \$7,172,031. Currently, four grants are active (FY94-97); the first six grants (FY88-93) 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 vouchers for reimbursement. Thus, the Center staff serve as fiscal agent for both receiving and disbursing of funds in accordance with all terms and provisions of the grants.

Through August 31, 1997, the Center has funded or is funding 42 projects through 238 subcontracts from the first nine grants received. Funding for these Center supported projects is summarized in Table 1 below (pages 7-8).

During this reporting period, the Publications Office at ISU produced and distributed a number of publications including fact sheets, technical bulletins, videos, and the Center's newsletter. A complete list of all publications from this office is included in the Appendix under Extension.

Other areas of support by the Administrative Office during this reporting period included: monitoring research and extension activities and developing progress reports; developing liaisons with appropriate institutions, agencies and clientele groups; preparing, in coordination with the other RACs, both written and oral testimony for the U.S. House Appropriations subcommittee on Agriculture, Rural Development, Food and Drug Administration, and Related Agencies hearing in Washington, D.C.; participating in the NCC; numerous oral and written presentations to both professional and lay audiences; working with other fisheries and aquaculture programs throughout the North Central Region; and in conjunction with the Aquaculture Network Information Center (AquaNIC) maintaining a NCRAC web site (ag.ansc.purdue.edu/aquanic/ncrac).

PROJECT DEVELOPMENT

A joint Program Planning meeting of the BOD, IAC, and TC is held every year in the early winter. The IAC, with input from the TC, generates a list of priority areas for consideration by the BOD. Using their recommendation as guidelines, the BOD then selects priority areas for which project outlines will be developed. The BOD also specifies a maximum funding level for each priority area. Problem statements and objectives are then developed for each priority area by IAC and TC members at the Program Planning meeting. For projects with more than one objective, the IAC ranks the objectives by priority. The problem statement and objective(s) are then included in a workshop announcement that is broadly distributed throughout the North Central Region. The workshops are one-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 to 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 mostly for projects that were underway or completed during the period September 1, 1996 to August 31, 1997.

All publications, manuscripts, papers presented, or other outputs for all funded NCRAC project areas are listed in the Appendix.

Table 1.	North Central	Regional	Aquaculture	Center funded	projects.
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Project Area	Project Number	Proposed Duration Period	Funding Level	Grant Number
Extension	1	5/1/89-4/30/91	\$39,221 \$37,089	88-38500-3885 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 \$10,875	91-38500-5900
	5	9/1/93-0/31/97	\$10,875	92-38500-0910
			\$348,448	20200 1110
Economics and Marketing	1	5/1/89-12/31/91	\$127,338	88-38500-3885
	2	9/1/91-8/31/92	\$53,300 \$53,300	89-38300-4319 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 \$200,000	93-38500-8392
	5	9/1/95-0/51/97	\$704 785	95-58500-1410
Uribrid Stringd Dass	1	5/1/20 2/21/01	\$68,206	00 20500 2005
Hybrid Surped Bass	1	J/1/09-0/31/91	\$08,290 \$68,114	89-38500-3883
	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 \$150,000	89-38500-4319
	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
	4	7/1/70-7/31/78	\$655 556	90-38300-2031
Salmonide	1	6/1/00 8/21/02	\$0,000 \$0,000	80 38500 4210
Sannoinus	1	0/1/70-0/31/92	\$9,000 \$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	

Project Area	Project Number	Proposed Duration Period	Funding Level	Grant Number
NCR Aquaculture Conference	1	6/1/90-3/31/91	\$7,000	90-38500-5008
National Aquaculture Extension Workshop/Conference	1 2	10/1/91-9/30/92 12/1/96-11/30/97	\$3,005 \$3,700 \$6,7005	89-38500-4319 95-38500-1410
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 2	9/1/92-8/31/94 9/1/96-8/31/98	\$153,300 \$100,000 \$253,300	92-38500-6916 96-38500-2631
National Aquaculture INAD/NADA Coordinator	1	9/1/93-8/31/94 5/15/95-5/14/96 5/15/96-5/14/97 5/15/97-5/15/98	\$2,000 \$5,000 \$6,669 \$3,331 \$15,000 \$32,000	89-38500-4319 94-38500-0048 92-38500-6916 95-38500-1410 96-38500-2631
Tilapia	1	9/1/96-8/31/98	\$120,000	96-38500-2631
Aquaculture Drugs	1 2	7/1/96-6/30/97 12/1/96-11/30/97	\$27,000 \$5,000 \$32,000	95-38500-1410 95-38500-1410

PROJECT TERMINATION OR PROGRESS REPORTS

EXTENSION¹

Progress Report for the Period May 1, 1989 to August 31, 1997

NCRAC FUNDING LEVEL: \$348,448 (May 1, 1989 to August 31, 1997)

PARTICIPANTS:

Chiversity of wisconsin-winwadkee	vv iscolisili
Ohio State University	Ohio
Michigan State University	Michigan
University of Minnesota-Duluth	Minnesota
Kansas State University	Kansas
Ohio State University	Ohio
University of Minnesota	Minnesota
University of Nebraska-Lincoln	Nebraska
Michigan State University	Michigan
Southern Illinois University-Carbondale	Illinois
University of Minnesota	Minnesota
Kansas State University	Kansas
Iowa State University	Iowa
Kansas State University	Kansas
University of Missouri	Missouri
Southern Illinois University-Carbondale	Illinois
Purdue University	Indiana/Illinois
Purdue University	Indiana
	Ohio State University Michigan State University University of Minnesota-Duluth Kansas State University Ohio State University University of Minnesota University of Nebraska-Lincoln Michigan State University Southern Illinois University-Carbondale University of Minnesota Kansas State University Iowa State University Iowa State University University of Missouri Southern Illinois University-Carbondale Purdue University

PROJECT OBJECTIVES

- 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.

¹NCRAC has funded five Extension projects, the first three of which were chaired by Donald L. Garling. The fourth project was chaired by Fred P. Binkowski. A fifth 2-year project, which began September 1, 1995 and ended August 31, 1997, was chaired by Joseph E. Morris.

(6) Survey quarterly wholesale fish buyers in selected United States and Canadian cities with emphasis on the NCR.

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 NCRACfunded 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 and two National Aquaculture Extension Workshop/Conferences.

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. Many of these

requests have been met by providing fact sheets, technical bulletins, bibliographies, and detailed responses to specialized questions.

Prior to mid-1994 little coordination of international aquaculture information sharing existed. Materials from national and international agencies producing aquaculture 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. 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. It is the gateway to the world's electronic resources in aquaculture including the Regional Aquaculture Centers. It also serves as the home of NCRAC's web site (ag.ansc.purdue.edu/aquanic/ncrac).

Swann has coordinated the distribution of NCRAC Annual Progress Reports through AquaNIC. Currently, AquaNIC houses nine NCRAC extension Fact Sheets and nine NCRAC Technical Reports and 1991-1996 Annual Progress Reports. In addition, the May 1, 1991-August 31, 1996 Compendium Report has also been posted on AquaNIC. Other services provided on the NCRAC web site include a directory of administrative staff and various NCRAC committee members. extension contacts and the NCRAC newsletter. Other activities related to the AquaNIC and NCRAC web sites include the development of a World Wide Web 30 slide set for use in extension and Sea Grant Educator training.

AquaNIC has been recognized by various groups including:

- Bronze award from the 1996 National Agriculture Communicators in Education in the category of publication for the AquaNIC mouse pad.
- Certificate of appreciation from the USDA presented to Mark Einstein and LaDon Swann for leadership and service in creating and fostering the development of AquaNIC.
- Three star rating from McKinley Group's online editorial team (1996).
- Best of the non-commercial sites on the Internet by Progressive Farmer On-Line (1996).
- Exceptional agriculture-related web site by Ag View (1996).
- Editorial on the Success of the Aquaculture Network Information Center in *The Aquaculture News*, June 1996.

AquaNIC has now been expanded to include a beginner's section accessible from AquaNIC's Home Page. There are two areas currently available for the beginners. First, there are individual species pages for baitfish, channel catfish, crawfish, ornamental fish, striped bass, tilapia, trout/salmon, shellfish, and shrimp. On each of these pages electronic versions of key publications, lists of videos, photographs, frequently asked questions, and lists to extension/Sea Grant outreach contacts are provided. Similarly, automated discussion groups have been set up for each of the species. Each discussion group allows the user to post and reply to questions.

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; Gunderson has since assumed that responsibility. Two other individuals, who had served since the outset of the project as their state's aquaculture extension contact, were replaced in 1994. 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. Lee replaced Neils in Kansas in 1996. Hochheimer, who replaced Ebeling in Ohio, has now left Ohio State University.

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). Many of these individuals have, in turn, trained industry representatives in HACCP.

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, hybrid striped bass, walleye, and yellow perch culture, rainbow trout production, in-service training for high school vocational-agricultural teachers and polyploid induction in sunfish held in the region.

Three North Central Regional 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 and the third conference was held in Indianapolis, Indiana in February 1997. These regional meetings were attended by hundreds of individuals including persons from Canada. The next conference is scheduled for February 1999 in Columbia, Missouri.

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 five- to sevenminute 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 video tape from NCRAC's Publications Office as well as two other video tapes by the University of Nebraska-Lincoln that are reprises of the broadcast.

In support of extension activities being funded through research projects, i.e., hybrid striped bass and sunfish research projects, extension specialists have completed fact sheets/book chapters/videos. These extension materials arising from the combined efforts of both extension specialists and researchers will help to address many questions concerning aquaculture in the NCR.

In addition to the previously mentioned areas, several NCRAC extension contacts have been instrumental in fostering the continued growth of the aquaculture industry in the region. For example, Pierce has recently created the Cooperative Extension Aquaculture and Marketing Educational Program to facilitate the development and implementation of aquaculture educational programs in Missouri. Many of the NCRAC extension contacts have worked with industry and governmental representatives to produce state aquaculture plans and improved governmental regulations.

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.

OBJECTIVE 6

The quarterly survey of wholesale fish buyers was partially successful. One of the original investigators left their position and another took a leave of absence. As a result there were a limited number of reports from wholesale buyers in the region. However, an agreement was made with the Maryland Department of Agriculture to distribute their bi-weekly buyer's report to AquaNIC's web page. Purdue University receive faxed copies of the reports, which were re-entered, and converted for the web. Twenty-eight reports were distributed on AquaNIC.

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 landowner 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.

The wholesale buyer's survey has been popular among producers. However, to be successful the project needs at least one, half-time person who has contacts in the fish wholesaling industry. Therefore, the market

report either needs more support or to be terminated.

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.
- AquaNIC is rapidly becoming the entry point for people searching for aquaculture information on the web. AquaNIC's home page now averages more than 3,000 visits per month by people from more than 50 countries. The Illinois-Indiana Sea Grant Program has also created web pages for the Indiana Aquaculture Association, the Illinois Aquaculture Industry Association, NCRAC, and the World Aquaculture Society.

PUBLICATIONS, MANUSCRIPTS, WORKSHOPS, OR CONFERENCES

See the Appendix for a cumulative output for all NCRAC-funded Extension activities.

SUPPORT

	NCRAC-	OTHER SUPPORT					TOTAL
YEARS	USDA FUNDING	UNIVER- SITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	SUPPORT
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
1995-97	\$36,600	\$149,325	\$5,000	\$84,000		\$238,325	\$274,925
TOTAL	\$348,448	\$737,483	\$5,000	\$334,000	\$55,000	\$1,131,483	\$1,479,931

ECONOMICS AND MARKETING²

Project Termination Report for the Period September 1, 1993 to May 31, 1997

NCRAC FUNDING LEVEL: \$40,000 (September 1, 1993 to May 31, 1997)

PARTICIPANTS:

Susan T. 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
Daniel A. Selock	Southern Illinois University-Carbondale	Illinois
LaDon Swann	Purdue University	Indiana/Illinois

REASON FOR TERMINATION

The objective for this project was completed and funding was finally expended.

PROJECT OBJECTIVE

Develop cost of production budgets and expected revenues for the raising of foodsized walleye, yellow perch, and hybrid striped bass on farms in the North Central Region (NCR).

PRINCIPAL ACCOMPLISHMENTS *HYBRID STRIPED BASS*

Kohler compiled a mailing list of 56 producers of phase III hybrid striped bass (HSB) both within and outside the NCR. A mail survey was sent to these 56 producers to obtain data on production costs. The response rate was low due to a hesitation on the part of producers to reveal this information. Two large fish farms in Arkansas (Malone's and Keo) and one in Missouri (Osage Catfisheries) were visited to discuss HSB production and gather production information. In all three cases, other species in addition to HSB were produced, therefore, species-specific production figures were not available.

Kohler compiled an annotated bibliography on HSB production and production costs. This bibliography is available from Kohler for anyone needing the information. Kohler also summarized HSB cost of production estimates from six published reports on HSB production. Those estimated costs were presented at the North Central Regional Aquaculture Center (NCRAC) Hybrid Striped Bass Workshop in November 1995.

WALLEYE

Work has advanced on identifying and analyzing the cost of production for

²NCRAC has funded three Economics and Marketing projects. This termination report is for the third project that was chaired by Patrick D. O'Rourke.

advanced walleye fingerlings and food-sized walleye in intensive culture systems. O'Rourke and Illinois State University graduate students completed an extensive walleye production/culture literature review with the primary focus of finding 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. The literature reviews were reported in two Master's theses. The first thesis was finished in December 1994. It is an economic feasibility analysis of a tankbased intensive walleye fingerling production system. The second thesis, an economic feasibility analysis of a tank based intensive food-sized walleye system, was finished in August 1995.

Research experts and hatchery personnel familiar with walleye culture were surveyed using a modified Delphi approach for both the fingerling and food-sized studies. It was surprising that some "experts" were as reluctant to share research information as were some entrepreneurs/producers. This stage of the research was completed in 1995.

The two theses produced for this project contain the best economic feasibility data for any known/proposed production systems for commercial production of walleye fingerlings and food sized fish. Commercial production is considered to be potentially profitable but highly risky and uncertain due to lack of actual commercial production data for systems (especially for food-sized grow out), the difficulties in domestication of the fish and the potential market (price) impacts of commercial production.

YELLOW PERCH

Knowing the number of commercial producers of yellow perch to be very small, Riepe conducted a literature review in early 1993 to determine whether any data on the production requirements for yellow perch were available. Unfortunately, most research on yellow perch has been limited to attempts to spawn them out of season and successfully culture and harvest eggs, fry, and eventually fingerlings habituated to commercial diets. Riepe then rejected the historical method for developing enterprise budgets and used the economic engineering approach.

Riepe considered alternatives for obtaining the needed information, and came up with two methods. The first method used to collect production-related information upon which the budgets must be based was a record keeping procedure. Riepe developed a record keeping sheet for the non-funded collaborators involved in the yellow perch project who were testing the commercial scale feasibility of food-size yellow perch production systems. The record keeping sheets asked for the itemization of all costs and inputs into the production process used by the collaborators. The resulting information was of limited value.

The second method was a Delphi approach to obtaining the expert opinions of NCRAC researchers on the production relationships needed to underpin the yellow perch production cost budgets. Expert opinions were solicited from the researchers and extension persons involved in the NCRAC Yellow Perch Work Group during 1994. The opinion data were entered into a spreadsheet to average the responses and then re-submitted to the researchers. Also budget assumptions were clarified so that all researchers were thinking of production relationships relating to a similar set of assumptions. Expert opinions were solicited for several types of related production values (death loss, feed conversion, fingerling size, harvest size, etc.) for a producer with average skill under average conditions and then for minimum and maximum values representing above and below average skills and conditions.

It was not financially feasible to model all life stages in all production systems at all sizes of production. Researchers and the membership of the Indiana Aquaculture Association were queried to solicit their views on priorities for budgets modeled. The decision was made that the budgets to be modeled would be the life stage of advanced fingerling grow out; the production systems of cage, constructed fish pond, and recirculating tank; and two sizes, 2,268 kg (5,000 lb) and 22,680 kg (50,000 lb).

Sensitivity analysis was conducted to test the impact of alternative budget parameters (production values and individual cost items) on the overall break-even price. A Master's graduate student was assigned to conduct research in costs of producing perch in recirculating tank systems and a thesis was completed in 1995. Costs of growing out yellow perch in recirculating tank systems were analyzed for two sizes of operations; 1,588 kg (3,500 lb) and 2,268 kg (5,000 lb).

A technical bulletin and a fact sheet (NCRAC Extension Fact Sheet #111 and NCRAC Extension Technical Bulletin #111) detailed the costs of producing yellow perch in cages and ponds. These may contain the best economic feasibility study and data for any known/proposed production systems for commercial production of yellow perch in the NCR.

IMPACTS

Extension Liaison Garling hosted a Yellow Perch Workshop in June 1995. The results of Riepe's work on yellow perch production costs were presented at that workshop. Attendees indicated that they were considering the types of systems modeled by Riepe. O'Rourke presented the preliminary results of the work on walleve fingerling tank based system cost of production at the Minnesota Aquaculture Conference in February 1995. Kohler presented the results of the review of HSB production costs 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 those workshop presentations. As a result of this 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 publications may reduce the impacts of uninformed investment decisions by current and potential aquaculture entrepreneurs.

RECOMMENDED FOLLOW-UP ACTIVITIES

This project showed that good economics work could contribute to the knowledge base of species studies. Unfortunately the objective and the budget restricted the scope to fish that are not the most common produced commercially in the NCR. A new Economics and Marketing Work Group should be started and adequately funded for at least four years to continue the work of ascertaining the potential profitability of

various commercially adopted species and production systems for the NCR.

PUBLICATIONS MANUSCRIPTS, OR PAPERS PRESENTED

See the Appendix for a cumulative output for all NCRAC-funded Economics and Marketing activities.

SUPPORT

	NCRAC-	OTHER SUPPORT					TOTAL
YEARS	USDA FUNDING	UNIVER- SITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	SUPPORT
1993-97	\$40,000	\$59,683				\$59,683	\$99,683
TOTAL	\$40,000	\$59,683				\$59,683	\$99,683

YELLOW PERCH³

Progress Report for the Period September 1, 1993 to August 31, 1997

NCRAC FUNDING LEVEL: \$350,000 (September 1, 1993 to August 31, 1997)

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	s:	
Harlan Bradt, etc.	Coolwater Farms, LLC, Cambridge	Wisconsin
William Hahle	Pleasant Valley Fish Farm, McCook	Nebraska
John Hyink/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	Calamus State Fish Hatchery	Nebraska
Commission		
Forrest Williams	Bay Port Aquaculture, Inc., West Olive	Michigan

PROJECT OBJECTIVES

- 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 inpond 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.

³This progress report is for the fourth and fifth Yellow Perch projects funded by NCRAC. Both projects chaired by Jeffrey A. Malison. The fifth project continues and expands upon work undertaken in the fourth project. It is a 2-year study that began September 1, 1995 and concluded on August 31, 1997.

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, 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 the first year 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 eggs and fry. The research indicated that selection for larval fish cannot be made based on maternal size until the paternal influence on the trait was identified for the cross.

In 1997, MSU researchers studied the maternal and paternal contribution on larval yellow perch mouth gape and total length (TL). The study was designed to test the significance of the paternal contribution observed in the 1996 study. Based on the sire and dam components of the spawning stock used, estimates of heritability for fry TL and mouth gape were calculated. A true estimation of heritability was not calculated because the design of this study includes a fixed assignment of parental stock. However, the estimation of dominance or non-additive genetic variance can be calculated. The additive genetic variance for TL can be estimated using a crossclassification design. Two small-sized and two large-sized male yellow perch were mated to each of four females, two smallsized and two large-sized. This mating produced eight families sired by small males and eight sired by large males.

Data collected during 1996 and 1997 were used to develop an equation to predict mouth width (y) based on changes in TL (x):

y = -0.058461 + 0.062357x

($r^2 = 0.4227$; P = 0.0001). Measurements of gape height, gape width, and TL were taken using a dissecting microscope in conjunction

with the Optimas imaging system, $BioScan^{TM}$.

Studies at Purdue University (Purdue) were designed to quantify the dietary requirements for sulfur amino acids (methionine plus cyst(e)ine) and the dietary choline requirements, providing the framework for the legal use of betaine as a flavor additive in diets for yellow perch. To date, the dietary requirements for lysine, arginine, total sulfur amino acids, and the sparing effect of cyst(e)ine for methionine have been quantified in juvenile fish fed experimental diets. The dietary choline requirement study is underway.

Studies at Purdue were delayed during quantification of the dietary lysine requirement. It appears there is a biochemical antagonism between dietary lysine and arginine. That is, when dietary concentrations of one of these two essential amino acids is relatively high, it hinders absorption of the other. This is a common phenomenon in terrestrial animal nutrition, but is rare in fish. In fact, most people argue the antagonism simply does not exist in fish. The critical dietary levels of lysine and arginine will be explored this winter in juvenile fish. The dietary requirements for lysine and arginine were 1.5% and 1.4% of the dry diet, respectively.

The dietary total sulfur amino acid requirement for juvenile yellow perch is 1.0% of the diet and cyst(e)ine, a nonessential amino acid, can spare approximately 50% of the dietary requirement for the essential amino acid methionine.

In 1996, Ohio State University (OSU) researchers spawned yellow perch out-of season during September-October by shifting the photothermal condition (light hours and temperature) by six months. The natural spawning of yellow perch occurs in April-May at 12-14°C (53.6-57.2°F) and a 12 h photoperiod. The brood stock was maintained at higher temperature and longer photoperiod during September-February (18°C [64.4°F] and 13 h). The photothermal conditions were decreased gradually until June. The chill period (10°C [50.0°F] and 11 h light) was 60 days in duration (June-July) and was followed by gradually increased water temperature and longer day light (12°C [53.6°F] and 19 h). Following this period, 47% of the females were recorded as gravid and 24 were stripped or spawned naturally. The males spermiated during the entire shifted spawning period from August till September. The average relative weight of ovulated eggs as percentage of the female weight was $26.6 \pm$ 10.7%. Embryo survival through the eyedstage was $56 \pm 24\%$. Larval skeleton abnormalities $(45 \pm 15\%)$ and a low frequency of swim bladder inflation (44 \pm 34%) were observed.

Hatching occurred seven days after spawning incubation at 14°C (57.2°F). Just before hatching, the eggs were transferred to 20-L (5.3-gal) aquariums with continuous water flow at 20° C (68.0°F). Three days after hatching, fresh-water rotifers Brachionus calyciflorus and microalgae Dictyosphaerium chlorelloides were added three times a day to aquariums at an average concentration of 10 rotifers/mL (296/oz). Eighty percent of the larvae were found to have 1-4 rotifers in the gut at first feeding. Artemia nauplii were added six days after hatching. The combination of rotifers, algae, and Artemia was supplied until 14 days after hatching after which, only Artemia nauplii were offered to the larvae. Two different dry diets were tested for weaning 25 day old larvae, salmonid starter diet and experimental squid based diet. However, only 35 day old

juveniles were found to accept dry diets and were not weaned completely from *Artemia* until an age of 45 days.

Nine diets were tested as weaning diets, including two commercial (Zeigler trout starter and Biokyowa), one semi-commercial (F.T. Barrows, Fish Technology Center, Bozeman, Minnesota), and six experimental diets. Live food (Artemia nauplii) was used as a control. In addition, the semicommercial and one experimental diet ("walleye") were supplemented with 20% (initial fish biomass) Artemia. The commercial trout starter was coated with 5 or 10% (diet weight) krill hydrolysate as a feed attractant. One hundred fish (average wet weight 75.5 ± 5 mg; 0.0027 ± 0.0002 oz) were placed in each of 44 20-L (5.3-gal) aquariums. Fish were fed ad libitum, eight feedings per day. After 31 days, fish were sacrificed, counted, and sampled for length, wet weight, dry weight, and digestive tract enzyme activities. Percent survival to 31 days ranged from 35 ± 6.2 % (French diet based on freeze-dried liver and yeast extract, carboxymethylcellulose was used as a binder) to over 70% on a walleye diet (based on krill meal and herring meal as protein sources, included 2% krill hydrolysate, gelatin was used as a binder) or Barrows with 20% Artemia nauplii (manufactured by marumerization technique).

OBJECTIVE 2

Two years of trials have been completed by University of Wisconsin-Madison (UW-Madison) researchers at Coolwater Farms, LLC, to determine key parameters for producing yellow perch fingerlings habituated to formulated feed and reared in ponds for an entire growing season, and to compare the performance of two types of pond lighting and feeding systems. Ponds have recently been harvested and production data are being analyzed. Preliminary examination of the data indicates that rearing fingerlings in ponds for the entire first growing season can result in yields greater than 247,097 fish/ha (100,000 fish/acre), although variability in both pond productivity and fish size result in a wide range of production levels. Over the two years of data collection pond fingerling production ranged from 49,419 to 276,748 fish/ha (20,000 to 112,000 fish/acre), and averaged about 148,258 fish/ha (60,000 fish/acre). Autumn-harvested fingerlings ranged in size from 7.0-17.8 cm (2.8-7.0 in) TL (3-80 g; 0.1-2.8 oz total weight).

In 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. UNL was unable to provide a summary of their 1997 progress.

OBJECTIVE 3

During 1996, three yellow perch workshops were conducted. The University of Wisconsin Sea Grant Institute sponsored two workshops entitled: "Intensive Aquaculture of Yellow Perch in Conjunction with Recirculating Aquaculture Systems," 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 technology. UNL conducted a workshop in Nebraska.

In 1997, UW-Madison researchers sponsored an organizational meeting of producers of yellow perch that are using pond systems. The objectives of this meeting were to discuss common problems faced by these aquaculturists and determine specific topics to be addressed by an upcoming workshop(s). The group was unanimous in their identification of fingerling size uniformity and pond production variability as being the most critical problem areas. The group was also highly in favor of examining the potential of developing a cooperative association to purchase commodities (e.g., fish food) and market products (e.g., fingerlings and processed fillets).

WORK PLANNED

OBJECTIVE 1

MSU researchers will complete evaluations of parental age and size influences on larval size. The results will be used 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.

The dietary choline requirement and ability of betaine to spare the choline requirement will be completed winter 1997/1998 at Purdue.

Researchers will complete collection and analysis of the data and prepare manuscripts for publication.

OBJECTIVE 2

UW-Madison researchers will complete collation and analysis of the data and a manuscript describing fingerling production studies will be prepared for publication.

OBJECTIVE 3

One or more workshops demonstrating key facets of fingerling production and grow out will be scheduled by UW-Madison for the spring-summer of 1998.

IMPACTS

Quantifying critical nutritional requirements for targeted species reduces feed costs and allows variation in use of feed ingredients. The research completed at Purdue, MSU, and OSU are defining a yellow perch diet for use in the NCR.

If verified, the lysine/arginine antagonism will be the first record of this situation occurring in fish. Feed manufacturers can then limit both essential amino acids to the requirements. Total sulfur amino acid concentrations are typically the first limiting amino acid in diets that contain high levels of plant protein feedstuffs. Thus, the values quantified at Purdue are vital pieces of information for dietary formulation and provide the basis of equally important work on flavor additives.

The impacts of spawner size on larval survival and out-of-season spawning may significantly increase the annual production of yellow perch fingerlings. The procedure of shifting the spawning season has to be accompanied with indoor larvae rearing. The larvae rearing protocol developed in this project is based on a combination of microalgae and rotifer as the larvae first feed. Artemia nauplii is offered from six days after initiation of feeding. Weaning period started at 35 days and the fingerlings were completely weaned from Artemia to dry diet at the age of 45 days. Co-feeding of dry diets and Artemia as well as coating starter diet with krill hydrolysate significantly increased growth of yellow perch juveniles.

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 perch fingerlings. At the current market value of yellow perch fingerlings (\$0.02-\$0.04/cm; \$0.05-\$0.10/inch), the strategy of rearing fingerlings in production ponds for an entire growing season results in gross production revenues of \$14,826/ha (\$6,000/acre), with a range of \$7,413-\$23,474/ha (\$3,000-\$9,500/acre).

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.

Requests for information on yellow perch aquaculture continue to increase annually. Workshops done on yellow perch aquaculture in the NCR have enabled extension specialists and researchers to provide information on this species to established fish farmers, potential fish farmers, and the general public. The workshops have also provided a mechanism for yellow perch culturists to identify problem areas. For example, producers have identified the excessive variability in fingerling size and pond productivity as the critical problems currently faced by yellow perch fingerling producers. This provides valuable insight into future directions that are needed for yellow perch aquaculture research. Addressing these areas of concern expressed by current yellow perch producers will bridge the gap between research and solutions to real-world problems.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

See the Appendix for a cumulative output for all NCRAC-funded Yellow Perch activities.

SUPPORT

	NCRAC-		OTHER SUPPORT				
YEARS	USDA FUNDING	UNIVER- SITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	SUPPORT
1993-95	\$150,000	\$168,827	\$60,000	\$91,000 ^{abc}		\$319,827	\$469,827
1995-97	\$200,000	\$251,909	\$42,000	\$220,911 ^{ac}		\$514,820	\$714,820
TOTAL	\$350,000	\$420,736	\$102,000	\$311,911		\$834,647	\$1,184,647

^aSea Grant/USDC/NOAA ^bUSDI, Bureau of Indian Affairs

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HYBRID STRIPED BASS⁴

Progress Report for the Period September 1, 1995 to August 31, 1997

NCRAC FUNDING LEVEL: \$160,000 (September 1, 1995 to August 31, 1997)

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	5:	
Mike Freeze	Keo Fish Farm, Inc., Keo	Arkansas
Jerry Katt	Mid-Continental Fisheries	Illinois
Scott Lindell	AquaFuture, Turners Fall	Massachusetts
Robert Lyons	Lyons Enterprises, Morocco	Indiana
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PROJECT OBJECTIVES

- Examine fry (phase I) to fingerling (phase II) 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 II) to advanced fingerling (phase III) 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 the North Central Regional Aquaculture Center (NCRAC).

⁴NCRAC 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 was scheduled to end on August 31, 1997.

(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. 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 \$2.29 to \$3.45/kg (\$1.04 to \$1.57/lb). Those values were based on a stocking density of $100/m^3$ (2.8/ft³) 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.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

OBJECTIVES 1 AND 2 Southern Illinois University-Carbondale (SIUC)

Brood Stock Acquisition: In 1995/1996 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 h 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 h, 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 human chorionic gonadotropin (hCG) at a rate of 150 IU/kg (68.0 IU/lb) to induce ovulation. Males were injected at a rate of 100 IU/kg (45.4 IU/kg) to enhance semen production. Sunshine bass were made using extended striped bass semen obtained from Keo Fish Farm. Arkansas.

At least ten 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 h postfertilization.

Enumeration and Stocking of Larvae: At four days of age, the larvae were enumerated and subsequently stocked into ponds. Ponds used in this study are approximately 0.04 ha (0.10 acres). 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; incoming water was filtered using a 500- μ m mesh nylon sock. 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) four to five days prior to stocking. The inorganic fertilizer was applied at 25 kg/ha (22.3 lb/acre) twice weekly for five weeks. An additional application of cottonseed meal was administered once weekly at 25 kg/ha (22.3 lb/acre) starting in week four.

The third and final phase of strain evaluation of hybrid striped bass is currently being conducted. The hybrids being evaluated for aquaculture performance were produced from spawning three strains of white bass (Arkansas, Lake Erie, and South Dakota) with striped bass in May of 1996.

Ponds were stocked between October 25-30, 1996 with three different strains of hybrid striped bass obtained from phase II harvest; fish averaged 0.1 kg (0.22 lb) at time of stocking (stocking rate = 5,000 fish/ha; 2,024/acre). SIUC researchers are attempting to obtain a market size fish of 0.7 kg (1.5 lb) by the following fall.

Fifteen ponds averaging 0.04 ha (0.10 acre) were randomly assigned a treatment (N = 5per treatment). Fish in the ponds were fed until ice cover; feeding was resumed in the spring as the weather permitted. In the sampling (seining) done in April 1997 it was observed that there was no complete fish loss in any pond due to overwinter mortality. On the second week of April, twice a day feedings were initiated. Feeding at the surface increased around the middle of May as the water temperatures reached $16-18^{\circ}C$ (60.8-64.4°F).

Temperature and dissolved oxyen (DO) were monitored every morning beginning the third week of June. Around the middle of July as morning temperatures reached 30°C (86°F), emergency aeration with a PTO-driven paddlewheel was frequently used. Two separate replicates were lost on July 31, 1997 due to a combination of high water temperatures and complete overcast conditions for a 4-day period. To avoid excessive fish loss due to low DO problems, a limit on feeding commercial diets was placed at 56 kg/ha (50.0 lb/acre). Fish in most ponds generally fed well through the summer, especially when weather was steady for longer periods.

On October 5, 1997, five fish were sampled from each pond by hook and line and visually inspected for size and condition. All fish were in extremely good condition; most fish were beyond minimum marketable size.

Feeding of Phase I Fingerlings: Training the fish to accept commercial diets began 21 days poststocking. Fish were offered fry meal 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.

Harvesting Phase I Fingerlings: At 36-41 days of age phase I fingerlings were harvested by seining. Survival rate varied from pond to pond, but was generally poor. Fish survival in ponds ranged from 0-21%. Fish survival rates were markedly higher for hybrid striped bass ponds compared to white bass ponds, averaging 13% and 3%, 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).

Phase II Production: The harvested phase I fingerlings were restocked for phase II 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 II production was 25,000 fish/ha (10,118/acre). Fish were feed twice daily to satiation.

Harvesting Phase II Fingerlings: At the end of the growing season, phase II fingerlings were harvested by seining. Survival rates ranged from a low of 49% to a high of 86%. 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 57%. 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.

<u>SDSU</u>

Two groups of hybrid striped bass fingerlings (Arkansas and South Dakota hybrids) were transported from SIUC to SDSU to conduct strain comparison and density experiments (study began August 16, 1996). 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 (0.3 gal/min). Ammonia, nitrite, nitrate, pH, hardness, alkalinity, and carbon dioxide were measured every two days. Water temperature was maintained at 22°C (71.6°F) and DO was maintained near saturation by continuous aeration. A light/dark cycle of 12-h light/12-h dark was 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 2week period by feeding a #4 crumble ad libitum twice daily. 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 was done with belt feeders. The feeding rate was progressively reduced to 3% of body weight during the experiment to minimize overfeeding while maintaining a level approaching satiation. Also, pellet sizes fed were periodically increased with graded changes in body size. Group and individual measurements were made at weekly intervals; feed allotments were adjusted weekly. The same general protocol was applied to the density experiment. Four replicates each of five $(45/m^3; 1.3/ft^3)$, 15 (136/m³; 3.9/ft³), or 30 (273/m³; 7.7/ft³) South Dakota hybrids per 110-L (29.1-gal) aquaria were maintained. Performance characteristics (e.g., growth, conversion, condition, survival) were monitored in both experiments.

A strain comparison was conducted in a recirculating system of 38-L (10.0-gal)

aquaria September-December 1996. Proximate analysis will be conducted on the fish of this study in an attempt to identify differences between the strains.

Recirculating System: Two groups of hybrid striped bass fingerlings (Arkansas and South Dakota hybrids) were used to conduct strain comparison and density experiments in a recirculating system. South Dakota hybrids were used in the density experiment.

Fingerling sunshine bass (4.2 g \pm 0.3 SE) were randomly stocked at low ([45 fish/m³; 0.25 kg/m³] [1.3 fish/ft³; 0.02 lb/ft³]) medium ([136 fish/m³; 0.47 kg/m³] [3.9 fish/ft³; 0.03 lb/ft³]), and high ([273 fish/m³; 1.02 kg/m³] [7.8 fish/ft³; 0.06 lb/ft³]) replicated densities. Strain comparisons were conducted at the low density. All fish were conditioned to the recirculating system and fed for one week prior to beginning the experiment. Weekly backflushing of the sand filter and partial cleaning of biological filter media were done to maintain favorable water quality.

Feeding frequency was (by hand) three times per day until the seventh week when belt feeders were incorporated, then feeding occurred continuously over the 12-h day period. Feed used was a commercial hybrid striped bass diet (38% protein, 5% lipid; Southern States, Farmville, North Carolina). Fish were fed at levels nearing satiation by feeding 10% of total body weight for weeks one to three, 7.5% for weeks four to eight, and reduced to 5% for weeks nine to 14; rations were adjusted weekly. Feed size was fingerling crumble #4 for weeks one to eight and 3-mm(0.12 in) extruded pellets for weeks nine to14. Feed conversion was observed to decrease across all treatments when fish were switched to continuous belt feeding, as compared to three hand-feeding periods separated by 4-h intervals during the early portion of the study. Feed conversion

differed little among treatments until week 11 of the study. During weeks 11 through 14 the high density treatment showed significantly poorer feed conversion than low and medium treatments. Strain comparisons did not reveal any conversion differences.

Growth pattern differences among treatments began to emerge during the second week of the study. Sunshine bass held at the medium density produced the largest proportional weight gain by the end of the study. Condition (mean relative weight; W_r) at the conclusion of the experiment did not differ among treatments. The experimental fish were considered to be in good condition considering that a W_r value of 100 would be the 75th percentile of standard weights for hybrid striped bass. No differences were detected between the two strains for growth and condition.

The density index is used as a guideline to determining maximum rearing densities for safe fish production in flow-through raceway systems. This index relates fish density to fish length and is the proportion of the fish length used in determining kilograms of fish to be held per cubic meter of rearing space. Index calculations from this study indicate that 200-mm (7.9-in) sunshine bass can be safely reared at 8.8 kg/m³ (0.55 lb/ft³) (medium density index = 0.08) up to 14.85 kg/m³ (0.93 lb/ft³) (high density index = 0.14) in recirculating systems.

Because uniform size is an important processing and marketing aspect, the variability in individual weights over time were examined to determine whether feeding hierarchies might have been established within tanks. There were distinct differences that corresponded with densities; however, the medium culture density provided fairly uniform fish size. With the exception of ammonia, all monitored water chemistry was acceptable for the culture of hybrid striped bass. Mean total hardness, total alkalinity, and carbon dioxide were 377.5 ppm (SE = 10.7), 154.1 ppm (SE = 4.6), and 26.2 ppm (SE = 2.1), respectively, measured in the biological filter. Temperature and nitrate levels did not vary significantly among treatments. Nitrite concentrations increased linearly with density, but never approached toxic ranges. Dissolved oxygen levels were equivalent between low (7.3 ppm, SE = 0.4) and medium (7.3 ppm, SE = 0.4) density treatments, but slightly lower (6.8, SE = 0.4) in the high density treatment. Ammonia concentrations in the high density treatment were sporadically well above baseline levels; those measurements were associated with feeding and observed to decrease shortly thereafter. Unionized ammonia concentrations in the high density treatment were not determined to be potentially toxic, rarely exceeding 0.011 ppm as NH₃.

The highest mortality (22%) occurred in the medium density treatment followed by the high (9%) and low (0%) density treatments. However, the mortality that occurred in the medium density treatment was a single event and a direct result of a plugged water jet; therefore, losses were not directly attributable to density.

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-h haul. Cages were stocked at both field sites and were harvested in November 1996. The tank loading study was initiated in late-summer 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.

Studies at Purdue were designed to examine maximum density of hybrid striped bass raised in cages and tanks. Two separate field studies have been completed with two private producers in Indiana. In the first study, hybrid striped bass were grown through a production season and fed a standard diet. Initial densities ranged from 2.5-5.0 kg/m³ (0.16-0.31 lb/ft³) and final densities ranged from 6 to 31 kg/m³ (0.37 to 1.94 lb/ft³). There were no significant differences within or between sites. In the second field study, initial densities ranged from 4 to 18 kg/m³ (0.25 to 1.12 lb/ft^3) and final densities ranged from 26 to 45 kg/m³ $(1.62 \text{ to } 2.81 \text{ lb/ft}^3)$. There were no significant difference within sites, but significant differences between sites were identified. Eviscerated dress out percentages were not significantly different in either study and ranged 86-90%.

In the first tank culture study, densities ranged from 0.8 to 2.7 kg/m³ (0.05 to 0.17 lb/ft³) and final densities ranged from 8 to 28 kg/m³ (0.50 to 1.75 lb/ft³). Fish were fed a standard diet to satiation once each day and feed conversion ratios (FCR) ranged from 1.18 to 1.06. No significant differences were detected in weight gain, FCR, eviscerated dress out percentage, condition factor, or blood glucose or cortisol concentrations. A second laboratory study is underway with initial densities of 2.5 to 15 kg/m³ (0.16 to 0.94 lb/ft³).

University of Wisconsin (UW-Madison) Feed Training With Lights: In an attempt to feed-train hybrid striped bass fingerlings in ponds, in the spring/summer of 1997, 100,000 hybrid striped bass fry were stocked into a 0.4-ha (1.0-acre) pond at the UW-Madison's facilities at the Lake Mills State Fish Hatchery, Lake Mills, Wisconsin. The pond was equipped with a series of underwater lights and automatic feeders. Two weeks after the fry were stocked, the lights and feeders were turned on at night. Only a few fish were observed in the vicinity of the lights and feeders. The failure to observe many fish in the vicinity of the lights and feeders suggests that hybrid striped bass fingerlings are, or quickly become, photonegative or photo-neutral. The use of lights and automatic feeders to feed-train fingerlings in ponds does not appear to be an effective strategy for hybrid striped bass.

OBJECTIVE 3

Iowa State University (ISU) 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 (ISU), and LaDon Swann (Purdue). A hybrid striped bass fact sheet developed by Morris and Kohler is in review. An outline for the initial culture manual has been completed by ISU and SIUC researchers.

WORK PLANNED

Ponds at SIUC will be harvested on October 31, 1997. Strains will be compared by harvest data, i.e., mean length and weight and production. Proximate analysis and percent dress out will be performed on the fillets. Each pond will also be taste-tested for any off-flavor.

SIUC researchers are working with Mid-Continental Fisheries in Johnston City, Illinois on exploring how well hybrid striped bass can be marketed in Illinois. Prices obtained from selling fish live-on-ice and processed will be determined; supermarkets, restaurants, and hotel outlets will be addressed.

The second of the two tank culture studies will be completed at Purdue and continuing feed evaluations will be attempted with Rangen feeds, which recently began distributing feeds in the NCR.

IMPACTS

From the results of the previous year and the data collected this fall, it is hoped the best strain of hybrid striped bass under aquaculture conditions for this region will be determined. In addition, it will be determined if there are any differences in nutritional quality between each strain. High demand for a high quality, locally raised aquaculture product will hopefully spark interest in the farming of hybrid striped bass in the NCR.

In two separate series of studies conducted at Purdue, maximum densities of hybrid striped bass grown in cages or tanks have not been reached. Thus, the hybrid can tolerate relatively high density culture. The defining study on tolerance should be completed this winter.

A new producer began producing hybrid striped bass in cages in Indiana as a result of this project. He has established linkages with existing producers and they are jointly buying feed and fingerlings, and marketing fish. Two of the producers in Indiana will

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grow trout in their cages this winter, as a means of double cropping from the same production system. Mid-Continental Fisheries at Johnson City, Illinois plans to raise hybrid striped bass next year.

Several graduate and undergraduate students participated in this project. They will be

trained aquaculture production people when they finish their degrees.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

See the Appendix for a cumulative output for all NCRAC-funded Hybrid Striped Bass activities.

	NCRAC-		TOTAL				
YEARS	USDA FUNDING	UNIVER- SITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	SUPPORT
1995-97	\$160,000	\$150,744	\$55,019		\$50,000	\$255,763	\$415,763
TOTAL	\$160,000	\$150,744	\$55,019		\$50,000	\$255,763	\$415,763

SUPPORT

WALLEYE⁵

Project Component Termination Report for the Period June 1, 1990 to August 31, 1993

NCRAC FUNDING LEVEL: \$151,657 (June 1, 1990 to August 31, 1993)

PARTICIPANTS:

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Anne R. Kapuscinski	University of Minnesota	Minnesota
James E. Seeb	Southern Illinois University-Carbondale	Illinois
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Natural Resources		
(DNR)		
Kansas Department of	Milford State Fish Hatchery	Kansas
Wildlife and Parks		
Minnesota DNR	Devil's Track State Fish Hatchery	Minnesota
Ohio DNR	London State Fish Hatchery	Ohio
U.S. Fish & Wildlife	Garrison Dam National Fish Hatchery	North Dakota
Service		
U.S. Fish & Wildlife	Genoa National Fish Hatchery	Wisconsin
Service		

REASON FOR TERMINATION

The objectives for this work on Walleye were completed.

PROJECT OBJECTIVES

- (1) Genetically analyze selected walleye populations for potential use as brood stock.
- (a) Population genetics (baseline information on genetic composition of various walleye populations).
- (b) Quantitative genetics (comparison of phenotypic characteristics of progeny from selected walleye brood stock).

⁵NCRAC has funded six Walleye projects. This project component termination report covers all of the second Walleye project and Objective 2 of the fourth project. Objective 2 of the fourth project expanded upon the work of the second project. Robert C. Summerfelt chaired the second Walleye project, a 2-year study, and Jeffrey A. Malison chaired the fourth Walleye project, a 1-year study.

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(2) Measure genetic parameters required for efficient selection on fry and fingerling traits, using pedigreed families.

PRINCIPAL ACCOMPLISHMENTS *OBJECTIVE 1A*

Genetic analyses were conducted by Southern Illinois University-Carbondale (SIUC) investigators on four walleve populations that were potential candidates for providing brood stock from which fish could be taken for future selective breeding experiments. These populations came from: (1) the Mississippi River near Genoa, Wisconsin, (2) Spirit Lake, Iowa, (3) Milford, Kansas, and (4) London, Ohio. SIUC researchers developed methodologies to identify biochemical traits that would be useful for genetic analysis. Initially, a single population of walleye was screened for allozyme variation in a large number of enzyme systems using different tissues and buffer systems to maximize the number of loci, and identify polymorphic loci. A total of 39 enzyme systems were surveyed in 53 walleye fingerlings from Spirit Lake, Iowa. Six tissues were screened from each fish (eye, gill, heart, liver, kidney, muscle) and four buffer systems were used for starch gel electrophoresis. Seventy loci, of which 30 are polymorphic, were identified. Fourteen of the 21 polymorphic loci were significantly polymorphic, that is there was <95% frequency of the most common allele. Prior to this work, only 10 polymorphic loci were identified in walleye populations, of which only five were significantly polymorphic. Mean heterozygosity per locus for the Spirit Lake walleye was 0.070, somewhat higher than the value of 0.050 obtained when 39 loci were examined in a previous study of allozyme variation in walleye populations.

Fry (21-day-old) reared at Iowa State University (ISU) from eggs obtained from hatcheries at Milford, Kansas; Genoa, Wisconsin; and London, Ohio were examined, but the fry were too small to take specific tissues for allozyme analysis. Thus, it is recommended that fry not be used for future genetic analyses. Fingerling fish at least 50 mm (2.0 in) total length (TL) seem to be the earliest stage on which comprehensive walleye allozyme analyses can be conducted.

Total DNA samples were extracted from 40 fish, but not including fish from the Ohio stock, and processed for allozyme analysis to determine the extent of mitochondrial DNA (mtDNA) variation within and among stocks. Together, the mtDNA and allozyme data provide baseline information on genetic composition and variability of walleye populations that were being evaluated for potential use as brood stock. These data would be fundamental for development of any future selective breeding programs. The data showed that the Mississippi River walleye from Genoa, Wisconsin exhibited a high level of genetic diversity, which would make it useful for selective breeding. Therefore, this population was selected for development of pedigreed families in Objective 2.

OBJECTIVE 1B

Concurrent to the development of biochemical methods to distinguish fish stocks, SIUC and ISU were evaluating the cultural performance of progeny from several sources of walleye stocks from the North Central Region (NCR) to find stocks that have superior growth rates and improved feed efficiency to enhance the feasibility of commercial culture. The findings can also serve to identify stocks useful for brood stock selection. SIUC carried out evaluations using a tandem extensiveintensive (pond/tank) rearing system whereas ISU reared walleye from hatch in intensive (tank) culture conditions entirely on formulated feed.

SIUC obtained eyed-eggs from Genoa, Wisconsin (Mississippi River); London, Ohio (semi-domestic); Lake Wallenpaupack, Pennsylvania; and Lake Shishibogama in northern Wisconsin. After incubation, newly hatched fry were stocked into fertilized ponds near the SIUC campus. Fingerling walleye (30-50 mm [1.2-2.0 in] TL) were harvested from the ponds then placed in 1.8m (5.9-ft) diameter tanks and evaluated on their trainability to accept commercially prepared diets. In 1992, ISU obtained eyedeggs from six stocks in the NCR: (1) London State Fish Hatchery, Ohio (Ohio stock); (2) Milford State Fish Hatchery (Kansas stock): (3) Genoa National Fish Hatchery, near Genoa, Wisconsin (Mississippi River stock); (4) Spirit Lake Fish Hatchery (Iowa stock); (5) Garrison Dam National Fish Hatchery, Riverdale, North Dakota (North Dakota stock); and (6) Devil's Track State Fish Hatchery, Minnesota (Minnesota stock). The Ohio stock, considered the only domesticated stock of walleye, were progeny of a third generation captive stock that has been reared at the hatchery. The eggs were hatched and the fry reared at ISU in an intensive culture environment on formulated feed

Stock differences in performance of walleye were observed in both pond and tank culture environments. In the tandem pond/tank culture studies at SIUC, about 86% of the pond-reared fingerlings from the Mississippi River stock from Genoa, Wisconsin accepted commercially prepared diets, a higher feed acceptance than stocks from northern Wisconsin or Pennsylvania. Researchers from ISU observed substantial stock differences in regard to fish size at hatching, survival, and cannibalism of tankreared walleye. Cannibalism was highest in the Minnesota stock and lowest in a semidomesticated stock from the London Ohio hatchery. These findings suggest that the serious problem of cannibalism may be reduced by domestication.

OBJECTIVE 2

The study was a cooperative project between the University of Minnesota (UM) and ISU to prepare, culture, and evaluate pedigreed families of walleye. The families were founded from the same source population collected from the Mississippi River near Genoa, Wisconsin. A balanced, nested mating design of full- and half-sibling families was used, where each sire was mated to three dams. This mating design is desirable because: (1) data from half- and full-siblings allow precise estimation of necessary genetic parameters using data from only the parent generation of families, and (2) individual and family data allow application of combined selection on reproducing adults of this same parent generation. ISU raised 12 families and UM raised a different set of 12 families.

Trait heritabilities were estimated using 12 families of fry raised at ISU and growth related heritabilities of fingerlings raised at UM. Hatching length, gas bladder inflation, and cannibalism had sire heritabilities of 0.47 to 0.83, and growth related heritabilities ranged from 0.30 to 0.74 for length, and 0.41-0.93 for weight. Potential response to selection increases as heritability increases from 0 to 1. The finding suggests a strong heritability for growth, that cannibalism may be reduced through selective breeding, and that selection for faster growth should not increase cannibalism.

IMPACTS

OBJECTIVE 1A

Techniques were developed to discriminate walleye populations on the basis of

biochemical traits. The baseline information collected on genetic composition and variability of walleye stocks in the region can be used to recognize and maintain unique strains for aquaculture. However, it is only possible to conduct such programs with maximum efficiency, while minimizing the effects of inbreeding, if there are good baseline data on genetic variability, such as collected in this study. The Mississippi River walleye stock from near Genoa, Wisconsin exhibited a high level of genetic variability. Such data of genetic diversity has been used for identifying suitable stocks for a selective breeding program begun in Objective 2 of this project and carried forward in the fifth Walleye project. Because the genetic markers are passed from generation to generation, biochemical genetics is a powerful tool for recognition of differences among strains should they be developed by selective breeding.

OBJECTIVE 1B

The stock evaluation studies provided baseline information that would be needed to choose a source of brood stock for development of a captive domesticated brood stock for the NCR.

OBJECTIVE 2

Major elements of a selective breeding program were carried out: a pedigreed captive population was established by UM; UM and ISU collaborated to obtain estimates of heritability for important performance traits in indoor rearing conditions in both laboratory and production facilities; and plans and a written agreement for future collaboration between UM researchers and Aurora-Aqua, Inc., a Minnesota-based aquaculture company, were completed. Variations in performance were obtained between family groups of walleye from the same population. High heritabilities were calculated that indicate traits for fast growth and reduced cannibalism. Gains can be expected for many generations of continuous selection in the same direction on a given trait. The findings demonstrate that pedigreed family lines can be used to advantage in selective breeding programs for walleye. These findings suggest that efficient selection of walleye will successfully yield fish that have a low incidence of cannibalism and a faster growth rate, thus allowing commercial producers to rear fish to market size in a shorter time with reduced operating costs.

Walleye fingerlings reared at ISU were transferred to pond sites in northeast Iowa for use in a USDA-sponsored net pen research project carried out by a non-profit organization, Resource Conservation and Development for Northeast Iowa, Inc. of Postville, Iowa.

RECOMMENDED FOLLOW-UP ACTIVITIES

Additional biochemical genetics data needs to be generated to identify other stocks of walleye that could be suitable for a selective breeding program in the NCR.

Follow-up projects on brood stock selection and selective breeding should utilize stocks which have been shown to have higher performance characteristics. The work that has been done provides guidance on culture methods and measures of performance for future studies.

However, to undertake a successful selective walleye breeding program, will require a long-term funding commitment to carry out the work. It takes nearly three years for female walleye to reach sexual maturity and it will require many years of combined effort by a team with both genetic and fish culture expertise to carry out such a program.

PUBLICATIONS, MANUSCRIPTS, AND PAPERS PRESENTED

See the Appendix for a cumulative output for all NCRAC-funded Walleye activities.

SUPPORT

	NCRAC- USDA FUNDING		TOTAL				
YEARS		UNIVER- SITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	SUPPORT
1990-91	\$58,614	\$24,898				\$24,898	\$83,512
1991-92	\$53,043	\$25,931				\$25,931	\$78,974
1992-93	\$40,000	\$29,828				\$29,828	\$69,828
TOTAL	\$151,657	\$80,657				\$80,657	\$232,314

WALLEYE⁶

Project Component Termination Report for the Period September 1, 1993 to October 31, 1995

NCRAC FUNDING LEVEL: \$150,000 (September 1, 1993 to October 31, 1995)

PARTICIPANTS:

Terence B. Barry	University of Wisconsin-Madison	Wisconsin
Tom Harder	Max McGraw Wildlife Foundation	Illinois
Anne R. Kapuscinski	University of Minnesota	Minnesota
Terrence B. Kayes	University of Nebraska-Lincoln	Nebraska
Jeffrey A. Malison	University of Wisconsin-Madison	Wisconsin
Robert C. Summerfelt	Iowa State University	Iowa
Extension Liaison:		
Ronald E. Kinnunen	Michigan State University	Michigan
Non-funded Collaborators:		
Gene P. Hanson	Aurora-Aqua, Inc., Kandiyohi	Minnesota
Nebraska Game & Parks	North Platte State Fish Hatchery	Nebraska
Commission		
Ohio Department of Natural	London State Fish Hatchery	Ohio
Resources (DNR)		
Wisconsin DNR	Lake Mills State Fish Hatchery	Wisconsin

REASON FOR TERMINATION

The objectives for this work on Walleye were completed.

PROJECT OBJECTIVES

- Measure genetic parameters required for efficient combined selection of sub-adult and adult traits, using a pedigreed population of walleye.
- (2) Conduct field trials that compare effectiveness and costs of different pond and tank culture strategies for producing advanced fingerlings.

PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

Commercial production of walleye as a food fish has been constrained by a lack of genetically-selected, high-performance domesticated strains. When this study began, only one "domesticated" stock (London State Fish Hatchery, Ohio DNR) was available that was more than four generations removed from the wild. To overcome this deficiency, a comparative evaluation of walleye stocks from the North Central Region (NCR) and a performance evaluation of family groups, had begun in the second and fourth Walleye projects.

⁶NCRAC has funded six Walleye projects. This project component termination report covers two of the three objectives (Objectives 1 and 3) contained in the project outline for the fifth Walleye project. The third objective (Objective 2 in the project outline) was continued and completed in the sixth Walleye Project. The fifth Walleye project was a 2-year project that was chaired by Robert C. Summerfelt.

Those evaluations provided a foundation for this fifth Walleve project study of quantitative inheritance in this species by University of Minnesota (UM) investigators. Quantitative traits are described by measurements, such as length and weight. UM researchers founded a pedigreed population from gametes collected from a wild population, then obtained performance data of sub-adult and adult traits from the progeny of crosses from this population. The traits were length and weight at different ages, specific growth rate, survival rate, and incidence of deformities of fish belonging to 12 full-sibling families nested in four halfsibling families and a control group reared at the UM and at Aurora-Aqua, Inc., a commercial walleye aquaculture enterprise.

Sire and dam heritabilities were obtained for length and weight at 25 different ages (from day 1 to day 375). Heritability values, which can range from 0 to 1, were sufficiently high that selective breeding should produce a good response in the next generation: 0.41 for day-47 weight, and 0.93 for day-247 weight. Crosses between adults which had high weights when they were 47-days old would yield progeny with increased weights at 47 days of age. Such results and projections suggest selective breeding of walleye can improve this species' performance in captive environments for the potential benefit of commercial aquaculture.

OBJECTIVE 2

Field trials were conducted in 1993-1995 at sites in: (1) northern Illinois by personnel of the Max McGraw Wildlife Foundation in collaboration with researchers from Iowa State University (ISU); (2) west-central Nebraska (North Platte State Fish Hatchery) by personnel of the University of Nebraska-Lincoln (UNL); and (3) south-central Wisconsin at the Lake Mills State Fish Hatchery by University of Wisconsin-Madison (UW-Madison) investigators.

Fish culturists at the Max McGraw Wildlife Foundation raised walleye from fry to fingerlings in ponds and by intensive methods, and they habituated pond-reared fry to formulated feed for rearing to advanced fingerlings. A commercial-scale facility was developed to intensively raise walleye from hatch to advanced fingerlings (150 mm; 5.9 in). This culture system was single-pass (i.e., not reuse or recycle), with three 650-L (171.7-gal), and three 1,200-L (317.0-gal) tanks. The same facility was used in both 1994 and 1995 culture seasons for fry culture and for habituating pond-raised fingerlings to formulated feed.

An artificial turbid-water culture system was used to raise fry on formulated feed. This system was effective in reducing the clinging of fry to the sidewalls of tanks. Fry grew from 7.7 mm (0.3 in) long at day 2 posthatch to 13 mm (0.5 in) by day 11, when 95% of the fry had food in their stomachs and 94% had fully inflated gas bladders. Problems occurred with bacterial disease in 1994 and gas supersaturation in 1995. But the technology transfer of the fry culture system seems to have been successful, inasmuch as the problem with bacterial disease is commonplace with walleye culture everywhere. The mean length of intensively cultured fish that survived to day 49 were similar to mean lengths of pond-reared fingerlings of about the same age.

Pond culture of walleye at the Max McGraw Wildlife Foundation was done in two 0.4 ha (1.0 acre) ponds, stocked with 2-4 day-old fry at a rate of 275,000/ha (111,293/acre). When zooplankton populations declined, a light-harvesting technique was used to harvest fish for the tank feed habituation and rearing study. In 1994, 41% of the fingerlings in the ponds were captured with the light-harvesting technique. In 1995, with additional experience and some improvements in the technique, more than 80% of the fingerlings in the two ponds were captured by light-harvesting.

Some of the pond-reared fingerlings at the Max McGraw Wildlife Foundation were transferred to 1.2- and 1.5-m (3.9- and 4.9ft) diameter cylindrical tanks in the hatchery building for habituation to formulated feed and then raised to a target size of 150 mm (5.9 in). Two experimental conditions were examined: tank size (650 and 1,200 L; 171.7 and 317.0 gal) and tank color (light blue and black painted tanks). In both 1994 and 1995, food conversion was lower, growth faster, and percentage of fingerlings that reached the 150-mm (5.9-in) target size higher for fish raised in black tanks than fish raised in blue tanks.

In 1995, total variable costs (labor, feed, chemicals, fertilizer, pumping costs) to produce fingerlings by the tandem production method, averaged \$0.42 for 127.8 mm (5.0 in) fingerlings reared in blue tanks and \$0.46 for 140.5 mm (5.5 in) fingerlings reared in black tanks. Tank size was less important, but results were better for the smaller tank — perhaps because a single feeder in both large and small tanks provided fish easier access to feed in the smaller (one feeder per 1.2 m^2 [12.9 ft²] of tank surface) than the larger (one feeder per 1.8 m^2 ; 19.4 ft²) tank.

Field trials conducted by UNL investigators evaluated pond aeration, fertilization, and fry stocking density in ponds at the North Platte State Fish Hatchery. In one seven week study, employing 17 0.4 ha \times 1-m-deep (1.0 acre \times 3.3-ft-deep) ponds, the effects of no aeration and continuous "Quad-Air" diffuser aeration were compared, as well as two different fertilization rates (150 or 225 kg/ha [133.8 or 200.7 lb/acre] per week of alfalfa pellets, supplemented with liquid phosphoric acid), and stocking rates of 405,000 and 607,000 fry/ha (163,904 and 245,653 fry/acre) rather than a normal stocking rate of 250,000 fry/ha (101,175 fry/acre). This study revealed consistently high levels of dissolved oxygen (DO) in all ponds and no appreciable aeration, fertilization, or stocking-rate effects on survival (71-81%) or on the size (37-44 mm [1.5-1.7 in] total length [TL]) of fish harvested.

In a second UNL study, the effects of stocking rate and continuous aeration on walleye fingerling production in 18 heavily fertilized 0.4-ha \times 1-m-deep (1.0 acre \times 3.3ft-deep) ponds were investigated. Walleye fry were stocked at rates of 405,000, 607,500, and 800,600 fry/ha (163,904, 245,855, and 324,003 fry/acre). All ponds were fertilized at a similar rate (340 kg/ha [303.3 lb/acre] per week of alfalfa pellets, supplemented with liquid phosphoric acid). During the sixe and one-half week study, DO levels typically remained near saturation levels and rarely went below 5 mg/L (ppm), irrespective of aeration. At harvest, fish stocked at 405,000/ha (163,904/acre) were significantly longer (40 mm [1.6 in] TL) and heavier (0.47 g; 0.017 oz) than those stocked at either 607,500/ha (245,855/acre) (35 mm [1.4 in] TL, 0.31 g [0.011 oz]), or 800,600 fry/ha (324,003/acre) (34 mm [1.3 in] TL, 0.28 g [0.010 oz]). The size at harvest of fish stocked at the two higher densities did not differ significantly. Neither stocking rate nor aeration had a significant effect on survival (69-79%), or the total biomass of fish harvested per pond (126-166 kg/ha; 112.4-148.1 lb/acre).

Collectively, these results suggest that walleye culture ponds in the NCR are being operated at far below their production potential in terms of harvestable numbers of fingerlings, and that at the tested rates of fertilization, which were comparatively high, the need for supplemental aeration in the Great Plains states of the NCR, except for emergency applications, may be minimal because of the climatically normal windy conditions.

In conjunction with the field trials to evaluate the effects of stocking and fertilization rates on the number of walleye fingerlings that can be produced per unit of pond surface area, UNL investigators also developed and tested various large-scale, low-stress harvesting systems employing lights arranged and operated in a variety of ways to attract fish into specially designed passive-capture gear (i.e., two types of modified, open-topped fyke nets). These trials resulted in 1994 in the repeated capture of 20,000-60,000 fingerling (20-30 mm [0.8-1.2 in] TL) walleye in a 15-20 min interval, from heavily stocked production ponds (400,000 fry/ha; 161,880 fry/acre). Together the results of these field trials and harvesting studies demonstrate that (using appropriate pond stocking, fertilization, and harvesting strategies) the number of walleye fingerlings that can be produced per unit of pond surface area can be increased by 160-320% above presently established standards, with no significant detrimental effect on survival and only a comparatively small reduction in fish size. The success of this approach depends on harvesting before depletion of the forage base and before high summer water temperatures become a problem. Experience has demonstrated that walleye of 34-44 mm (1.3-1.7 in) TL and smaller can be readily harvested and habituated in tanks to conventional starter diets.

In 1995, catastrophic losses of walleye fry occurred in production ponds across Nebraska because of extremely cold spring and early summer weather. Consequently, the numbers of fish captured per 15-20 min trial run using light-harvesting techniques were significantly lower (1,210-31,900 fish) than in 1994, and highly variable from run to run with each of the systems tested. But despite the production problems in 1995, a sufficient number of trials were done to indicate that the geometries of the different harvesting systems had little significant effect on the numbers of fish captured and that, accordingly, under commercial conditions, operators should employ the system that is least expensive to build and operate.

As a consequence of the catastrophic losses of walleye fry and subsequent stocking needs of the Nebraska Game and Parks Commission in 1995, a field trial to compare the merits of rearing small walleye fingerlings to advanced-fingerling size intensively in tanks versus extensively in ponds was not practicable. Only a small number of pondreared advanced-fingerling walleye were produced.

UW-Madison researchers completed field trials at the Lake Mills State Fish Hatchery in south-central Wisconsin to identify the minimum size at which pond-reared walleye fingerlings can be successfully habituated to conventional formulated diets in tanks. In these trials about 65% survived the transition from ponds to tanks when fingerlings were harvested at 30 mm (1.2 in) TL, but only 40% survived when harvested from ponds at 20 mm (0.8 in) TL. The increased mortality of the smaller walleye resulted primarily from increased levels of stress and mechanical abrasions during pond harvest.

In addition to length, condition factor (i.e., the ratio of weight to length) of walleye fingerlings is a critical determinant to successful habituation to formulated feeds and intensive culture conditions. Fingerlings with a high condition factor tolerated harvest stress better and habituated more successfully than thin fish. The condition factor of pond-reared fingerlings is directly related to the amount of forage available in ponds prior to harvest. Accordingly, if the amount of forage in a pond declines significantly, it is probably advantageous to harvest ponds early (i.e., harvest relatively short fish with a high condition factor, rather than delaying harvest until the fish are longer but thinner).

Another important consideration in the tandem pond/tank method of culturing fingerlings is that the number of fingerlings which can be produced in a pond declines as harvest size increases. Thus, although the success rate at habituating small fingerlings to formulated feeds may be reduced, harvesting fish at a small size will typically result in higher numbers of fingerlings harvested from ponds and, therefore, may result in more fish surviving the habituation period.

IMPACTS

OBJECTIVE 1

Researchers from UM obtained high heritability estimates of desirable production traits from a pedigreed population. Their findings strongly support a recommendation for a systematic selective breeding program.

OBJECTIVE 2

Field trials conducted at the Max McGraw Wildlife Foundation validated the advantages of turbid water culture technology — the procedure definitely benefits intensive fry rearing of walleye to 30 days of age. A major benefit of the night-lighting harvest technique was that it eliminated the harvesting of tadpoles and larval salamanders, which had been a chronic problem at the Max McGraw Wildlife Foundation. These studies also demonstrated that survival and growth was improved when fingerlings harvested from ponds were cultured in small black tanks as opposed to large blue tanks, and that tank color was more important than tank size.

Collectively, the UNL findings suggest that walleye culture ponds in the NCR are being operated at far below their production potential in terms of harvestable numbers of fish. The same is probably true for the pond production of other important varieties of fish, such as yellow perch and hybrid striped bass, which NCRAC has identified, along with walleye, as having significant potential for commercial aquaculture development.

The immediate direct impact of the UNL component of the project on the production of walleye fingerlings for stocking by the State of Nebraska can be readily documented. The average estimated number of 25-50 mm (1-2 in) TL walleye fingerlings produced by the Nebraska Game and Parks Commission at the North Platte State Fish Hatchery and statewide for the three-year period 1990 to 1992 was 1,343,316/year (the North Platte hatchery was essentially the state's sole producer of walleye fingerlings). In 1994, the estimated numbers of the same size walleye fingerlings produced at the North Platte hatchery and statewide were 3,403,261 and 4,261,520, respectively. The increased production of walleye fingerlings (by a factor of 2.5), or an increase of about 2,059,945 fish) in 1994 at the North Platte hatchery was directly attributable to the UNL research efforts there. Of the statewide overall increase in walleye fingerling production in 1994, compared to the 1990-1992 average, about 71% was attributable to the UNL research efforts at the North Platte hatchery; about 29% was attributable to walleye fingerling production at the Calamus State Fish Hatchery, which began operating in 1991. Walleye production at the North

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Platte hatchery was increased by an even larger margin in 1995.

In economic terms (assuming an average market value of \$0.05/fingerling), according to existing production statistics, the North Platte hatchery, using traditional methods, has annually reared walleye fingerlings with a total direct market value of about \$67,200 or \$9,765/ha (\$3,952/acre). UNL research efforts increased these amounts to \$107,160 (total production) and \$24,710/ha (\$10,000/acre), respectively. If the "best" methods developed by the project were fully implemented at the North Platte hatchery, as they nearly were in 1995, these figures could potentially reach \$204,150 (total production) and \$29,652/ha (\$12,000/acre). Present estimates suggest the enhanced walleye stockings of 1994 and 1995 may have increased the size and economic value of Nebraska's walleye fishery by as much as 50%. Based on such indicators, the Nebraska Game and Parks Commission has increased hatchery-pond stocking rates for walleye fry from 247,100/ha (100,000/acre) to 308,871/ha (125,000/acre).

Studies conducted at UW-Madison showed that the tandem pond/tank method of producing walleye fingerlings habituated to formulated feeds and intensive culture conditions can be expected to produce 65,000-195,000 habituated fingerlings/ha (26,306-78,917/acre) [100,000-300,000 fingerlings/ha \times 65% habituation]. Once the habituation period has been completed, relatively few losses should normally be incurred during grow out to an advanced fingerling size (150-200 mm [5.9-7.9 in] TL). These and other production data are now available for economists for comparisons of the effectiveness and costs of different pond and tank culture strategies for producing advanced walleye fingerlings.

RECOMMENDED FOLLOW-UP ACTIVITIES *OBJECTIVE 1*

A long-term commitment is required to commence development of domesticated brood stock and carry out genetic selection. A major reason for large-scale highly efficient commercial production of poultry has been investment by universities. government, and commercial interests in selective breeding. No matter what species is cultured, aquaculture enterprises in the NCR can benefit from genetic improvement of stocks. Because walleye require nearly three years for female fish to reach sexual maturity, it will require many years of combined effort by a team with both genetic and fish-culture expertise to carry out the long-term rearing that is needed. However, based on what has been achieved with the culture of Atlantic salmon, substantial benefits may be gained in only two generations of purposeful selective breeding of carefully chosen founder stocks.

OBJECTIVE 2

Field trials provide opportunities for extending laboratory findings to commercialscale aquaculture. It may be the most effective way to extend research findings to the industry, as well as develop a cadre of producers who can pass on effective technologies to others. Studies on intensive fry culture indicate that bacterial gill disease (BGD) and columnaris disease are critical factors affecting success of this type of walleye fingerling production. BGD severely constrains intensive fry culture, with or without turbid water: and columnaris disease is invariably a cause of mortality in handling pond-reared fish and in tandem pond/tank culture. The environmental correlates to BGD are not well known, although the incidence of columnaris disease is known to be related to handling damage and stress from high temperatures. There is a critical

need for studies of ways to prevent and control these diseases.

Further studies are needed to determine the number of walleye fingerlings that can be produced in ponds when the fish are harvested at different sizes, ranging from 20-30 mm (0.8-1.2 in) TL. Also, research is needed to compare the habituation to formulated feeds and intensive culture conditions of pond-reared walleye fingerlings harvested at different fish condition factors. This information is needed to more closely define the optimum production parameters and the most cost effective use of the tandem pond/tank method of producing walleye fingerlings.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

See the Appendix for a cumulative output for all NCRAC-funded Walleye activities.

SUPPORT¹

	NCRAC-		TOTAL				
YEARS	USDA FUNDING	UNIVER- SITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	SUPPORT
1993-94	\$72,725	\$111,029	\$11,250 ^a	\$11,000 ^b	\$57,420°	\$190,699	\$263,424
1994-95	\$77,275	\$44,773	\$13,080ª	\$11,000 ^b	\$32,350 ^d	\$101,203	\$178,478
TOTAL	\$150,000	\$155,802	\$24,330	\$22,000	\$89,770	\$291,902	\$441,902

¹This is funding for all three objectives of the fifth Walleye project.

^aAurora-Aqua, Inc.

^bWisconsin Sea Grant/USDC/NOAA

°1993-94: Max McGraw Wildlife Foundation (\$14,900), Minnesota Department of Natural Resources (\$820), Nebraska Game and Parks Department (\$41,700)

^d1994-95: Max McGraw Wildlife Foundation (\$14,900) and Nebraska Game and Parks Department (\$17,450)

WALLEYE⁷

Project Component Termination Report for the Period September 1, 1993 to August 31 1997

NCRAC FUNDING LEVEL: \$175,000 (September 1, 1995 to August 31, 1997)

PARTICIPANTS:

University of Minnesota-Duluth	Minnesota
University of Nebraska-Lincoln	Nebraska
Michigan State University	Michigan
North Dakota State University	North Dakota
University of Wisconsin-Madison	Wisconsin
Purdue University	Indiana
Illinois State University	Illinois
Purdue University	Purdue
Iowa State University	Iowa
Illinois Natural History Survey	Illinois
Michigan State University	Michigan
Spirit Lake State Fish Hatchery	Iowa
Dundee	Illinois
Calamus State Fish Hatchery	Nebraska
London State Fish Hatchery and	Ohio
Senecaville State Fish Hatchery	
Lake Mills State Fish Hatchery	Wisconsin
Genoa National Fish Hatchery	Wisconsin
	University of Minnesota-Duluth University of Nebraska-Lincoln Michigan State University North Dakota State University University of Wisconsin-Madison Purdue University Illinois State University Purdue University Illinois Natural History Survey Michigan State University Spirit Lake State Fish Hatchery Dundee Calamus State Fish Hatchery London State Fish Hatchery and Senecaville State Fish Hatchery Lake Mills State Fish Hatchery Genoa National Fish Hatchery

REASON FOR TERMINATION

The objectives for this work on Walleye were completed.

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.

⁷NCRAC has funded six Walleye projects. This project component termination report covers Objective 2 of the fifth project and all of the sixth project. Objective 2 of the fifth project was continued in the sixth project (Objective 4) where is it was completed. Both the fifth and sixth projects were 2-year projects. The fifth project began September 1, 1993; the sixth project began September 1, 1995. Robert C. Summerfelt chaired the fifth project and the last three months of the sixth project; Terrence B. Kayes chaired the first 21 months of the sixth project.

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- (2) Characterize the economics and institutional aspects of the domestic market for walleye as food fish, fingerlings, and other intermediate products.
- (3) Offer 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) Compare the performance (survival, growth, and feed conversion) and carcass characteristics (fillet yield, proximate analysis, and organoleptic properties) of walleye × sauger hybrids produced from different parental stocks reared under intensive and tandem extensive-intensive culture systems.

PRINCIPAL ACCOMPLISHMENTS *OBJECTIVE 1*

Research on this objective was conducted by investigators from Iowa State University (ISU), the Illinois Natural History Survey (ILNHS), University of Nebraska-Lincoln (UNL), and University of Wisconsin-Madison (UW-Madison). Some aspects of the project by ISU and ILNHS, and by UNL and UW-Madison were interdependent. ISU collected data on fish growth, feed efficiency, and measures of digestible energy of walleye reared under laboratory conditions. These experiments included a comparison of fish growth in tanks at two temperatures, and several feeding rates. ISU provided ILNHS with data on fish size, feeding rates, water temperatures, and samples of fish, fish feces, fish feeds, and an analysis of the feeds for development and testing of a bioenergetics model.

Because feed is a major variable cost in the production of fish and other animals,

determination of appropriate feeding rates, expressed as percentage of the body weight fed per day (%BW/d), is essential to optimize feed efficiency and growth. A producer must decide on a feeding rate related to fish size and water temperature. Empirical methods, growth models, nutritional energetics, and bioenergetics modeling provide options for determining feeding rates. ISU evaluated methods based on prior experience, growth models, and dietary energy; and then provided ILNHS with data for bioenergetics modeling.

ISU determined digestibility of the WG-9206 walleye grower diet, compared fish growth and feed efficiency at 20° and 25°C (68.0° and 77.0°F), and conducted five trials to evaluate feeding rates (%BW/d). Digestibility of the WG-9206 diet (52.1% protein, 15.0% fat, 66.2% moisture, 0.7% fiber, 9.6% ash) was 86.2% for protein and 70.4% for dry matter. The digestible energy of the diet was estimated to be 3,572 kcal/kg (1,620 kcal/lb). The protein-energy ratio (digestible protein ÷ digestible energy) was 30.4 mg (0.001 oz) of protein per kJ energy, a higher value than average for finfish, which suggests that for walleye of this size, energy levels in the WG-9206 diet were insufficient. in which case fish had to catabolize some protein for energy, which may have reduced their growth rates. In the temperature trial, growth rates and feed efficiency were higher at 25°C (77.0°F) than 20°C (68.0°F). To avoid epizootics of bacterial diseases common at the higher temperature, a temperature of 23°C (73.4°F) is recommended. In the feeding-rate trials, higher feeding rates were required at higher temperatures, and feeding rates decreased with increasing fish size, poor health (i.e., disease), and stress. Feeding rates may have been affected by feed quality, especially energy content. Although the multiplicity of factors affecting feeding of fish make it

difficult to define feeding rates for walleye across the entire spectrum of fish size and temperature ranges at which culture may be done, the ISU findings suggest that for fish habituated to formulated feeds, a 2%BW/d rate is suitable at 20°C (68.0°F) and 3%BW/d at 25°C (77.0°F) for fish of 170 mm (6.7 in) total length (TL); and 1.2 %BW/d at 20°C (68.0°F) and 1.5 %BW/d at 25°C (77.0°F) for fish of 330 mm (13.0 in) TL.

ILNHS modified and calibrated a bioenergetics model to simulate walleye growth under different temperature and dietary regimes. ILNHS used the model to make predictions on the basis of empirical data provided by ISU. The bioenergetics model predicts either growth or consumption under specific environmental conditions. The simulations resulted in different growth rates for particular feed types and temperatures. Maximum growth occurred in simulations at 21.5°C (70.7°F) and with BioDry 1000 pelleted feed. Under these conditions, walleye could reach food size faster, depending upon the proportion of maximum food consumption (35 days at 50%, 24 days at 75%, and 20 days at 100%). Comparisons of these simulations to tank experiments performed by ISU show predicted growth was much higher than observed, given the experimental feeding regime. Likewise, predicted feed consumption based on observed growth was substantially lower than the amounts of pelleted feed added to the tanks. Walleye in the ISU experiments did not grow as fast as observed in previous experiments, suggesting problems with environmental conditions, health of the walleye, feeding rates, or model predictions. ILNHS will also test the model predictions using data from research by ISU and UW-Madison. After further testing, correction, and validation, the model can be applied to various aquaculture settings to

make recommendations on feeding rates in relation to fish size and water temperature, to maximize growth rates of walleye to food size.

The main focus of UNL investigators in the first year of the sixth Walleye project was to raise a large number of Age-0 juvenile walleve in ponds for use in the second year 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 (221.9-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 starterseries diet (Nelson and Sons, Inc., Murray, 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 $13.3 \degree C (55.9 \degree F)$ well water, and turned over to personnel of the Calamus State Fish Hatchery for overwintering. Between October 1996 and January 1997, over 50% of the remaining walleye succumbed for unknown reasons, and the project was terminated.

The decision to end the Nebraska component of Objective 1 of this project was based on poor performance as noted above and the 1996-1997 effort exhausted not only all of the North Central Regional Aquaculture Center (NCRAC) funds allotted for the UNL component of the walleye project, but also well over \$20,000 in UNL and state funds.

The effort made in these abortive attempts at the Calamus hatchery suggests that various site-specific factors, yet to be identified, may be essential to culturing walleye on a large scale. Such factors may include differences in genetic stock, water chemistry, watertemperature patterns, and/or basic facilities design or operation.

UW-Madison researchers compared growth rates and feed efficiencies of walleye reared in ponds and in tanks of different sizes, and measured physiological indicators of stress in walleye reared using different culture techniques, to identify least-stress culture methods. UW-Madison researchers also completed experiments that measured changes in serum concentrations of cortisol, glucose, and chloride following acute-stress challenge tests in walleye at different temperatures. The data reveals that the cortisol response of walleye to an acute stressor is faster than in many teleosts, but peak levels are comparable. Walleye held at 15°C (59.0°F) had reduced peak cortisol

levels but increased time for return to baseline compared to walleye at 21°C (69.8°F). Water temperatures above optimal (25°C; 77.0°F) accelerated the initial rise to peak levels and delayed the return to baseline, suggesting a stronger and more prolonged stress response.

UW-Madison investigators found no difference in growth rates of walleye raised to food size in 750-L (198.1-gal) and 12,000-L (3,170.1-gal) tanks. Similarly, a holistic health-assessment index showed no differences in the overall health or condition factor of fish reared in the two tank sizes. Walleye reared in the larger tanks, however, displayed a markedly different activity level than those in the smaller tanks. Fish in the 12,000-L (3,170.1-gal) tanks remained actively swimming high in the water column, exhibited obvious schooling behavior, and an aggressive response to food. Walleye in the 750-L (198.1-gal) tanks generally remained sedentary on or near the bottom of the tanks.

At the time of this report, UW-Madison researchers have been unable to conduct the pond studies proposed under this objective due to significant delays in the construction of experimental pond facilities.

OBJECTIVE 2

The economics team, comprised of investigators from Purdue University (Purdue), Illinois State University, and North Dakota State University (NDSU), characterized the economics and institutional aspects of the domestic markets for walleye as food fish, fingerlings, and other intermediate products. Reliable market information on wild-caught supplies is essential for commercial growers to plan their production, financing, and marketing strategies. An understanding of marketing channels and institutional structures will provide aquaculturists with insights into the impact that farm-raised walleye products could have on domestic markets for this species.

Production information was collected from research/extension experts, public and private suppliers, and producers of walleye fingerlings by telephone interviews and mailed survey instruments. Phase I supermarket and restaurant surveys (different survey forms for restaurants and supermarkets) asked for general information on purchases and sales of fish/seafood and questions regarding the firm. The initial mailing was completed during the last week of August and the first week of September 1996.

The collection and analysis of published and secondary data on walleye exports from Canada to the United States is near completion by NDSU. A thesis on this work should be finished in December 1997 and is anticipated to result in other publications in 1998.

During the first year of the walleye marketing survey project (September 1, 1995-August 31, 1996), Purdue investigators focused on literature review, survey design, mailing list development, survey instrument development, and initial survey mailings. The literature review covered previously completed seafood marketing surveys as well as trade literature of the firm types to be surveyed. Survey design encompassed making determinations of precise definitions of firm types to be surveyed, scope of the survey, methodology employed to conduct the survey and analyze the results, and the time line in which to complete the survey process followed by data analysis and report writing. Survey instruments were developed for three firm types or groupings. Separate instruments were developed for restaurants and supermarkets. Two separate

instruments, to be used in sequence, were generated for each of these firm types. A single survey instrument was produced for surveying the firm type grouping "wholesalers," which included seafood wholesalers, seafood retailers, food service distributors, grocery wholesalers, and fish brokers. A random representative sample of businesses in each firm type was purchased from a private mailing list company. Purdue investigators discovered that there was a group on campus that contracted with inhouse and outside groups for survey services. Assistance from this group was obtained for finalizing survey instrument development, survey design, mailing list management, and mailings. The mailings of the initial surveys of restaurants and supermarkets were completed at the end of the first year of the project.

In the second year of the project (September 1, 1996-August 31, 1997), the focus was on conducting the mail survey, completing survey instrument development, data entry, data analysis, and report writing. The survey services group at Purdue disbanded, leaving Riepe to manage the mailing list, conduct survey mailings, enter the survey data, and analyze the data largely on her own. This greatly expanded the previously anticipated time line. Follow-up survey mailings were completed by March 1997. However, data entry and analysis were well underway and completed in May 1997. The first draft of the first manuscript was completed and reviewed in July and early August. Revisions were nearing completion by the end of the second year of the project.

OBJECTIVE 3

Three workshops were held in the NCR in the spring and early summer of 1996 and 1997 to demonstrate technology for walleye aquaculture. The topics were: (1) intensive culture of walleye from fry to fingerlings on

formulated feed, May 7, 1996, at the Max McGraw Wildlife Foundation, Dundee, Illinois (co-sponsored by Max McGraw Wildlife Foundation); (2) production of advanced fingerling walleye raised on minnows in ponds and then trained to formulated feed in intensive culture systems, June 18, 1996, at Spirit Lake State Fish Hatchery, Spirit Lake, Iowa (co-sponsored by the Iowa DNR); and (3) spawning walleye (collection, transportation, and stripping of brood fish and incubation of eggs), April 17-18, 1997, at Spirit Lake State Fish Hatchery, Spirit Lake, Iowa (cosponsored by the Iowa DNR). Workshop participants were from Illinois, Iowa, Michigan, Minnesota, Nebraska, Pennsylvania, Wisconsin, and the Canadian provinces of Ontario and Manitoba.

Over four hours of video tape recordings were taken by UNL of the first workshop at the Max McGraw Wildlife Foundation for production of a video on the intensive culture of walleye fry. Given the large amount of video tape shot under marginal lighting conditions, some technical difficulties associated with editing a quality video on intensive fry culture have been identified and need to be addressed. Because of the cost and time requirements to do this, final release of the finished video is not anticipated until mid-1999.

In each workshop, participants were able to observe an on-going activity related to the workshop topic. For example, in the April 1997 workshop at Spirit Lake, participants were able to view gill netting from a boat, observe sorting fish by ripeness, preparation of extended semen, spawning, egg enumeration, and incubation. Some participants were able to strip eggs and semen from ripe fish. The value of such live demonstrations and "hands-on" activities, as made available through these workshops, cannot be overestimated.

OBJECTIVE 4

ISU and UW-Madison investigators undertook comparison of the performance of purebred and hybrid walleyes (male sauger \times female walleye) produced from gametes obtained from several geographically distinct stocks of walleye and sauger from the midwest. Brood stock were obtained with the assistance of several state (Iowa, Ohio, South Dakota, and Wisconsin) resource management agencies and the U.S. Fish and Wildlife Service's Genoa National Fish Hatchery. In 1994, hybrids were produced from three stocks of female walleye and a single stock of sauger — male sauger collected from the Mississippi River near Genoa, Wisconsin and female walleye collected from the same site, and from Spirit Lake, Iowa, and Rock Lake, Jefferson County, Wisconsin. Growth rates of the hybrids were compared with pure stock walleye from Rock Lake, Wisconsin, which were half-siblings of the hybrids. Culture was carried out at ISU from hatch to 74days posthatch.

In both 1994 and 1995, the length at hatching of pure stock walleye was greater than that of any of the hybrids, but as early as 28-30 days, hybrid walleye were longer than pure stock walleye. In 1995, hybrids were produced by crossing female Spirit Lake walleye with saugers collected from the Ohio River, Mississippi River, and Missouri River. When fry were 28-days old, the Missouri River hybrids were longer than any other group; by 83 days, the Mississippi River hybrids and Ohio River hybrids were similar in size, and both were longer and heavier than the Missouri River hybrids and the pure stock walleye. In both 1994 and 1995, most, though not all, hybrid crosses grew faster than the parental stock (pure walleye) through the first 74-83 days posthatch. The source of the dam (walleye) was more important than the source of the sire (sauger). In both years, hybrids produced by crossing Spirit Lake walleye and Mississippi River sauger grew faster through the first 74-83 days posthatch than any other hybrid cross.

Some of the fish raised at ISU were transferred to UW-Madison investigators, who raised these purebred and hybrid walleye to food size. Their studies clearly showed that, compared to purebred walleye, hybrid walleye have markedly superior growth and performance characteristics when grown to food size by intensive culture systems. One hybrid cross, the Spirit Lake (Iowa) walleye female × Mississippi River sauger male, consistently outperformed all other purebreds and hybrids in terms of survival, growth, and feed conversion. A particularly important finding was that the improved growth of hybrids continued as fish approached market size.

Another important result of these studies was that sexually related dimorphic growth becomes apparent in all groups of purebreds and hybrids once the fish reach about 150 g (5.3 oz), i.e., well before market size. As the fish approached market size, hybrid females were growing nearly twice as fast as hybrid males, and more than six times faster than purebred males.

Organoleptic and carcass composition studies revealed no difference between purebreds and hybrids. Taste panels expressed a high degree of consumer preference for both, describing them as firm, flaky, and tender with an absence of any off flavors. Proximate analysis indicated that fillets were very low in fat (1.1-1.7%).

IMPACTS

OBJECTIVE 1

ISU researchers determined the protein and energy digestibility of the WG-9206 diet, which is presently the standard diet for grow out of feed-trained walleye fingerlings. Comparison of walleye growth at 20°C (68.0°F) and 25°C (77.0°F) demonstrated a higher growth rate at 25°C (77.0°F), but to reduce the incidence of disease a temperature of 23°C (73.4°F) is recommended as an optimum temperature for commercial culture. A multiplicity of factors affecting feeding of fish make it difficult to define feeding rates for walleye across the entire spectrum of fish sizes, temperatures, and energy content of feeds, but recommendations have been developed for walleye from 170 mm (6.7 in) to 330 mm (13.0 in) TL at 20°C (68.0°F) and 25°C (77.0°F).

After further testing, corrections, and validation, model simulations conducted by ILNHS may prove useful in recommending optimal energy levels in feeds, feeding rates, and temperatures for walleye growth in commercial aquaculture.

Field trials done in Nebraska over the past six years have shown that the number of walleye fingerlings produced per surface area of ponds by extensive culture methods can be increased by 160-320% (to well over 600,000 harvested fingerlings/ha; 242,820 fingerlings/acre) by appropriate pond fertilization, stocking, and harvesting techniques. However, survival of the pondreared fingerlings under intensive culture conditions by Nebraska investigators was unsuccessful. Because of this experience, the Nebraska Game and Parks Commission has decided to discontinue further efforts in this regard for the foreseeable future.

The failure in one-year trial done in Nebraska for intensive culture of walleve fingerlings suggest that potential investors in commercial walleye aquaculture, before starting a major venture, should have a thorough knowledge of the known technologies for culturing this species, have or gain considerable practical experience in the application of these technologies, and be prepared to spend a number of years conducting pilot projects to resolve sitespecific problems. Without this level of investment and know-how, the likelihood of long-term financial success appears slight, based on present knowledge, particularly with respect to the production of food-size walleye. Without far more research, commercial walleye aquaculture remains an extremely risky enterprise.

To help reduce such risk, the research done by UW-Madison investigators to characterize the performance and stress responses of walleye grown to food size under different conditions provides information needed to determine which culture techniques can be used to rear this species in a time frame and manner conducive to commercialization. Also, information generated by ILNHS and ISU researchers will help in preparation of guidelines and tables for predicting growth and determining appropriate feeding rates of juvenile to food-size walleye under different conditions.

OBJECTIVE 2

Publications from the marketing survey component of the project will benefit persons currently involved in or planning to produce walleye in aquaculture systems for the foodfish market by providing factual data on which to ascertain and evaluate marketing options and on which to base marketing plans, marketing strategies, enterprise budgets, and production decisions. These plans will be useful as business tools for individual aquaculturists and to help obtain outside capital. Survey data will also be useful to aquaculture research, extension, and marketing professionals in industry, government, and academia in terms of enhancing their understanding of food-fish markets for walleye and identifying and planning appropriate avenues for future activities and investigations. Lenders will also find the survey data useful for evaluating walleye aquaculture loan proposals.

OBJECTIVE 3

Regional workshops were held in conjunction with real-time, walleye aquaculture production activities. Such workshops provided excellent opportunities for training because participants directly observed techniques and potentially benefited from "hands-on" opportunities to learn from experienced practitioners. Workshops that involved direct observation of cultural practices proved useful for both extension educators, as well as students and fish culturists wanting to learn new techniques. Workshops also provide a unique opportunity to capture voice and video recordings of workshop speakers and fish culture activities that have been arranged for the participants. Finally, workshops provide opportunities for participants to get acquainted with experienced professionals and share experiences with other people involved in similar activities — thereby providing beneficial contacts.

OBJECTIVE 4

One major constraint to the development of a commercial walleye food-fish industry is the relatively slow growth of walleye when reared from advanced fingerlings to foodsize fish under typical aquaculture conditions. The studies under this objective identified a hybrid cross which has markedly superior growth and performance characteristics compared to purebred walleye. Organoleptic and proximate analyses found no important differences between purebred and hybrid walleyes. The commercial use of walleye hybrids and of monosex female populations should substantially reduce the time and costs required to produce food-size walleye.

RECOMMENDED FOLLOW-UP ACTIVITIES

OBJECTIVE 1

Based on studies done at the Max McGraw Wildlife Foundation and by ISU and Iowa DNR investigators, more research is needed on strategies of feeding walleye, including such considerations as feeding frequency, feeding rate, feeding for compensatory growth, automatic versus demand feeders, time of day, and light levels. Bioenergetics models, such as those examined by ILNHS researchers, should be refined and validated to gain full benefits of their potential to predict growth and food consumption rates by walleye in various aquaculture settings. Additional experimental work with different feeding rates and diets of known digestible energy are needed to further test such models. Once a model is validated for a diversity of cultural conditions, it could then be used to predict optimum thermal regimes, amounts of feeds required, and growth rates for cultured walleye.

The field trials conducted in Nebraska failed because of high mortality in habituating pond-reared walleye to formulated feed to produce food-size walleye by intensive culture practices that earlier had proven to be effective in culturing advanced walleye fingerlings by many other investigators in Ohio, New York, South Dakota, and Iowa. In Nebraska trials, early walleye fingerlings produced by extensive pond culture appeared to habituate rapidly to artificial diets and extensive culture conditions. But after habituation, these fish failed to thrive and were gradually lost, despite major investments in planning, fish, facilities, equipment, supplies, and technician time. The loss of fish in these field trials was not obviously attributable to major problems with disease, cannibalism, feed consumption, or swim-bladder inflation. A possible flaw in the design of production-tank drain structures appears to be one likely cause of fish losses.

Nebraska Game and Parks Commission personnel have noted suspected waterchemistry problems at the Calamus State Fish Hatchery, where both the reservoir water and well water are soft. No specific water-chemistry problems have been identified after extensive testing. However, experienced Commission fish culturists have had difficulties at the Calamus hatchery with the intensive production of a number of species, including walleye. Whether due to site-specific factors or differences in the level of experience or attentiveness of personnel, the failure of the Nebraska field trials to produce advanced fingerlings underscores the need to fully understand factors affecting the success of this cultural technology. However, such efforts are essential at the regional level if the intensive culture of walleye to food size is ever to be proven commercially feasible.

Researchers at UW-Madison are committed to undertake studies on the growth and stress responses of walleye reared to food size in ponds — aspects of walleye aquaculture that have not yet been examined. Studies will be initiated upon completion of pond-construction project currently underway at the Lake Mills State Fish Hatchery. These trials will be done using funds from alternative sources, and the findings made available to the public through NCRAC.

OBJECTIVE 2

Publications will continue to be produced on walleye fingerling markets, the institutional aspects of the Canadian wild-caught walleye fishery, and purchase and sales information from firms in walleye marketing channels. A technical bulletin is planned that will contain detailed survey data on firms purchasing walleye and the specifics of their purchases. Following this, a fact sheet will be developed on the restaurant market for seafood. If time and circumstances allow, other fact sheets will be produced based on various aspects of survey data, such as the supermarket market for seafood, and wholesale firms as seafood markets.

OBJECTIVE 3

Walleye Work Group members have expertise on the status of walleye culture for presentations at state and regional meetings. Such presentations at meetings heavily attended by practicing or potential commercial walleye aquaculturists should be encouraged and actively supported by NCRAC. Significant findings and procedures developed by recent Walleye Work Group research should be summarized into fact sheets, videos, and other extension products, to provide commercial walleye culturists with up-to-date information. Future workshops should be held using extension materials and other information that has or will be developed to demonstrate

the latest technology for culturing walleye and walleye hybrids.

OBJECTIVE 4

Before the benefits of selective breeding become available, the identification of a superior strain of walleye hybrid and the finding that females outgrow males offer great potential benefits for the commercial culture of walleye as a food fish. Certain stocks of walleye and hybrid walleye are obviously superior for intensive culture than others. Work along several lines is still needed, however, to facilitate the commercial use of these findings. First, a cooperative effort between NCRAC, commercial producers, and fisheries management agencies is needed to make the fast-growing fish stocks available to the private sector. Second, methods for producing monosex female walleve hybrids need to be developed. Third, unlike some interspecific hybrids, hybrids of walleye and sauger are known to be fertile, and their escape into the wild from commercial aquaculture facilities may be viewed as a threat to native walleye populations by the fisheries management agencies of some states. Accordingly, methods for producing sterile hybrids need to be developed.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

See the Appendix for a cumulative output for all NCRAC-funded Walleye activities.

SUPPORT¹

	=						
	NCRAC-		TOTAL				
YEARS	USDA FUNDING	UNIVER- SITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	SUPPORT
1995-96	\$117,897	\$143,355		\$50,000ª		\$193,355	\$311,252
1996-97	\$57,103	\$89,841				\$89,841	\$146,944
TOTAL	\$175,000	\$233,196		\$50,000		\$283,196	\$458,196

¹This is funding for only the sixth Walleye project. It does not include the funds for Objective 2 of the fifth Walleye project which was continued and completed in the sixth project (Objective 4). ^aIllinois-Indiana Sea Grant Program

Annual Progress Report 1996-97

SUNFISH⁸

Progress Report for the Period September 1, 1994 to August 31, 1997

NCRAC FUNDING LEVEL: \$287,590 (September 1, 1994 - August 31, 1997)

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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	Missouri
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Jeffrey A. Malison	University of Wisconsin-Madison	Wisconsin
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Robert J. Sheehan	Southern Illinois University-Carbondale	Illinois
Robert C. Summerfelt	Iowa State University	Iowa
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Industry Advisory Council 1	Liaison:	
Charlie Stevens		Iowa
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	Little Grassy State Fish Hatchery	Illinois
Conservation		
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Ron Johnson	Spruce Creek Fish Farm	Minnesota
Myron Kloubec	Kloubec Fish Farms, Amana	Iowa
Missouri Department of		Missouri
Conservation		
Tribal Council	Red Lake Band Chippewa	Wisconsin
National Biological	Midwest Science Center (formerly USFWS	Missouri
Service	National Fisheries Contaminant Research	
	Laboratory)	

⁸NCRAC has funded four Sunfish projects. This progress report is for the third and fourth projects. These projects continue and build upon the first two projects. Donald L. Garling chaired the third project and Robert J. Sheehan chairs the fourth project. Charlie Stevens was appointed to serve as the Industry Advisory Council Liaison for the fourth project.

NORTH CENTRAL REGIONAL AQUACULTURE CENTER

PROJECT OBJECTIVES

- 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.
- (4) Compare feeding trials for grow out of locally available 5.1-10.2 cm (2-4 in) black crappie (*Pomoxis nigromaculatus*) and female green sunfish (*Lepomis cyanellus*) with a male bluegill (*L. macrochirus*) hybrids in:
 - (a) ponds at dissimilar latitudes in the region, and
 - (b) recirculating systems using compensatory feeding strategies.
- (5) Establish baseline physiological measures for small 2.5-7.6 cm (1-3 in) black crappie subjected to handling stressors and to test the effect of salt and temperature on stress reduction.

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 (production manual and accompanying video tape), as a high priority need for demonstrating the commercial feasibility of centrarchid sunfish aquaculture in the region. Such activities will provide research-based materials for information transfer to commercial producers and individuals interested in production of sunfish to meet these market demands.

Defining the critical nutritional requirements for targeted sunfish will enable development of diets that meet, but not exceed, their requirements; such findings will help to minimize feed costs, the largest annual variable cost in aquaculture. 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. To date, most of the research and commercial production of sunfish has focused on utilizing pond systems (extensive 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.

Based on previous studies, aquaculturists who wish to produce sunfish as food fish need to use either black crappie or the male bluegill \times female green sunfish F₁ hybrid (BG \times GS hybrid). There is a need for information that addresses the relative use of either fish for food fish production in this region. Black crappie and BG \times GS hybrid side-by-side pond production studies offer the best approach for providing this needed information. The fourth sunfish project will also provide information on whether stocking density and the inclusion of a period of habituation to commercial feeds in the production cycle affects the performance of the two taxa.

The Purdue University (Purdue) study will identify appropriate diets for $BG \times GS$ hybrids that can be purchased immediately. Further, growth rates, food conversion rates (FCRs), survival, and final weight at the end of the growing season at two different latitudes will be available from the Purdue and Southern Illinois University-Carbondale (SIUC) studies.

Recent evidence from the University of Missouri's (UM) compensatory growth studies with BG \times GS hybrids indicates that a capacity exists for substantially reducing grow out times for fish in aquaculture. Other possible benefits associated with compensatory growth include increased growth efficiency, influences on proximate composition of flesh, and delayed maturation. Compensatory growth induction (i.e., better growth with less food) is an easily-applied low-cost/cost-saving technique that both increases growth rates and improves food conversion efficiency in these fishes.

Understanding the impact of compensatory growth on production in recirculating systems is very important to commercial producers. Improved growth efficiency and reduced feed costs are of obvious value. Although reduced feed wastage is critical to optimize recirculating systems, fluctuations in ammonia levels from feeding and nonfeeding periods may result in performance problems by creating instabilities in the bacterial communities in biofilters. The application of process control technology in compensatory growth feeding trials at Pittsburg State University (PSU) will demonstrate the importance of continuous management on optimization of these production systems.

Clarifying the factors that influence the transition of pond-harvested crappies to intensive tank culture will provide a foundation for industry efforts toward intensive food fish production. Practical strategies of habituating large numbers of fingerlings to commercial diets and high rearing densities require that this transition be achieved cost effectively. Either intensive rearing from egg or the habituation of pond reared crappies to commercial feed could prove to be viable strategies. The University of Wisconsin-Milwaukee (UW-Milwaukee) will explore the hurdles that need to be overcome and determine the comparative costs of both methods; information needed for improved food fish production.

Researchers at Iowa State University (ISU) already have demonstrated out-of-season spawning and production of fingerling sunfish raised in intensive culture. Research begun on intensive culture of BG \times GS hybrids on formulated feed in a recycle system will demonstrate the feasibility and methodologies for using this technology for raising BG \times GS hybrids to food size. Compensatory growth feeding strategies feeding frequency, percent body weight per day, fasting intervals — are important to water quality, feed utilization, and fish growth and will also be analyzed by ISU researchers.

Initial experiments are required to establish the physiological response of black crappie to a standardized handling stressor; this has not been previously documented. Investigators at the University of South Dakota (USD) and the University of Wisconsin-Madison (UW-Madison) are determining in preliminary studies whether use of salt additions or temperature reductions may be useful for mitigating this stress response and reducing handling-related mortality.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

During the 1994-1996 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. ISU personnel have since taken over the development and subsequent production of a video addressing various production areas of $BG \times GS$ hybrids. Footage for the upcoming video related to sunfish reproduction has been completed. The video will depict methods for determining the sex of brood stock, species identification, and out-ofseason spawning techniques. Michigan State University (MSU) and ISU personnel have completed drafts of the new sunfish Culture Guide; it is scheduled for completion by summer 1998.

OBJECTIVE 2

Researchers at MSU have empirically determined the optimal energy level for growth and protein retention in 125 mm (4.9 in) BG \times GS hybrids 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 between the experimental diets and the control diet; hence the semi-purified diet is suitable for the remaining phases of these trials.

Studies at Purdue were designed to quantify the dietary requirement for phosphorus (P) and the optimum lipid to carbohydrate ratio. Through four separate evaluations with four basal diet formulations, it appears the dietary requirement of $BG \times GS$ hybrids for P is $\leq 0.5\%$ of the dry diet. In defining the optimum ratio of lipid to carbohydrate, researchers at Purdue compared $BG \times GS$ hybrids to pure bluegill. Pure bluegill grew significantly better than the $BG \times GS$ hybrid, a finding verified in a previous study. Based on weight gain, feed conversion, muscle lipid concentrations, and intraperitoneal (abdominal cavity lining) fat concentrations, both pure bluegill and $BG \times GS$ hybrids grow best when fed diets containing no less than 10% dietary lipid in the form of fish oil. Neither genetic group tended to accumulate intraperitoneal fat with values ranging from 3-5% of body weight. Similarly, fillet lipid concentrations were relatively uniform, ranging from 3-9% of the dry matter, lower values than for many other fish species.

OBJECTIVE 3

One component of this objective was for ISU researchers to spawn sunfish out-of-season through temperature and photoperiod manipulation under laboratory settings (bluegill and $BG \times GS$ hybrids). ISU researchers stocked adult fish at a ratio of two males to four females (170 g; 6.0 oz) per 640-L (169.1-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 six month period (December 1994 - May 1995); 40 spawns averaging 20,000 larvae each were obtained from 24 females. BG \times GS hybrids were successfully produced fall 1997.

A second component of this objective that was done by ISU was to develop a
procedure for tank-rearing larval bluegill and larval BG × GS hybrids. Results indicate that the protocol for tank-rearing larval bluegill and larval BG × GS hybrids should include using brine shrimp prior to using a commercial diet. It appeared that larval BG × GS hybrids could digest the commercial diet at the onset of exogenous feeding. However, without brine shrimp nauplii (BSN) (*Artemia franciscana*) much lower survival rates resulted. Survival rates of about 25 and 37% can be expected for bluegill and BG × GS hybrids, respectively, by following this protocol.

The primary goal of the 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-ofthe-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 three days and were habituated to commercial starter feed within 14 days. Survival 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, BSN 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.

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 a commercial formulated diet within 14 days of arrival and have been maintained on a rearing regime that is intended to promote gonadal development.

Researchers at UM have examined the potential to increase growth rates of BG \times GS hybrids during grow out by using feeding schedules that bring out these fishes' compensatory growth response (increased growth following a period of fasting). BG \times GS hybrids were held individually in experimental enclosures submerged in larger water-recirculation tanks. Water temperature was maintained at 24°C $(75.2^{\circ}F)$ 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 $BG \times GS$ hybrids 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.

In the third sunfish project, SIUC researchers used practical diets containing crude protein levels of 32, 36, 40, and 44% and compared their ability to promote growth of BG \times GS hybrids in two culture systems: recirculating culture system and culture ponds.

Recirculating Culture System

The indoor culture phase has been completed. Year 1 adult $BG \times GS$ hybrids (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 98 to 100% and did not differ significantly between treatments. Weight increase and feed conversion efficiency were highest for the 44% crude protein diet and were significantly greater than the 32 and 36% diets. In addition, fish fed 32% protein diets had the lowest weight increase percentage as well as feed conversion efficiency. Except for ether extractables (lipids), proximate analysis of fish whole bodies revealed no significant differences; fish fed 32% protein diets had the lowest levels of ether extractables. These data indicate that optimal crude protein levels are likely to be in excess of 40% for BG \times GS hybrids in recirculating culture systems. 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.

Pond Culture

The pond production phase has been completed in terms of the feeding trial, harvest, and dress out determination. Dress out data dealing with length, weight, sex, gutted weight, headless weight, and fillet has been collected. Juvenile $BG \times GS$ hybrids (mean weight = 12 g; 0.4 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 with 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 day 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 (86.0°F). Resulting data was of limited use due to natural recruitment of F₂ offspring.

Year 1 adult BG × GS hybrids (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 with four replicates per treatment). Stocking density was 13,875 fish/ha (5,615 fish/acre). All ponds were limed and fertilized two 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 (ppm) was applied with a tractor driven paddle wheel.

Percent survival ranged 78-83% and harvest weight ranged 1,260-1,515 kg/ha (1,124.2-

1,351.7 lb/acre); no significant differences were evident. Mean harvest weight ranged 117.8-142.8 g (4.2-5.0 oz), with fish fed 32% protein with significantly lower weights than the other three diets. Percent size increase ranged 19-25%, with fish fed 32% being significantly lower than the other three diets; fish fed 44% had the greatest increase. Feed conversion ratios ranged from 1.42-1.57.

Under intensive conditions where the formulated diet supplies virtually all the nutrition for $BG \times GS$ hybrids, a dietary crude protein level of 40% appears required to support maximal growth, and minimal feed conversion ratio. Whole body proximate analysis shows no significant trends relative to dietary crude protein under these conditions. When the diet is offered as a supplement to natural foods, such as in a pond situation, a dietary crude protein level of 36% is adequate for promoting the maximum mean harvest weight. Feed conversion ratios are minimal at the same dietary crude protein level. Pond harvest data do not seem to provide helpful information due to the high variability in terms of survival.

OBJECTIVE 4

SIUC initiated a pond study on April 11, 1997 with BG × GS hybrids (mean length = 72 mm [2.8 in]; mean weight = 6.4 g [0.2 oz]), obtained from Fountain Bluff Fish Farm, Gorham, Illinois, and black crappie (mean length = 107 mm [4.2 in]; mean weight = 14.2 g [0.5 oz]), obtained from Van Winkle's Fish Farm, Birdseye, Indiana. The two taxa were stocked individually in ponds at two stocking densities, 9,000 and 14,000 fish/ha (3,644 and 5,666 fish/acre) (five replicates per treatment, total = 20 ponds). Feeding with 3.5 mm (0.1 in) , 40% protein SilvercupTM floating trout feed was initiated immediately after stocking, and feeding rates were increased as needed during the study. Feeding was not evident in the crappie ponds at first, whereas the BG \times GS hybrids fed well. Crappie were observed feeding for the first time in late May. The first year of this pond study will be completed in November 1997.

At Purdue, $BG \times GS$ hybrids were to be fed practical diets ranging in protein concentrations in ponds during 1997. However, pond construction was not completed until fall 1997. Therefore, the evaluation will take place in 1998. In addition, overwinter management and feeding strategies will be evaluated during winter 1997-1998.

A 117-day compensatory growth (CG) study with BG \times GS hybrids fed a commercial diet in a recirculating system was completed at UM in 1997. An objective was to determine which CG feeding schedule produced the best growth when a commercial diet was fed. A second objective was to determined whether the growth improvement from CG feeding schedules (relative to daily fed controls) differed when a commercial diet was used versus a natural food (mealworm), as in a previous study.

A control and four treatment groups each consisted of four replicates of 10 (approximately 3 g; 0.1 oz) BG × GS hybrids. Replicate groups were held in opentopped containers (25 L [6.6 gal] water volume) that were ½ submerged within 950-L (251.0-gal) recirculation tanks. Water temperature was 24 °C (75.2 °F) and photoperiod was 15-h light/9-h dark. The commercial feed was RangenTM Trout Diet. Treatment groups were deprived of food for either 2, 8, 14, or 20 days, then hand-fed to satiation every day (three feedings per day) for as many days as hyperphagia (daily consumption exceeding control group) persisted. Once hyperphagia ceased, food deprivation was begun again for the appropriate number of days, then refeeding would again follow, etc. Food consumption was monitored daily and fish weights were determined weekly for each replicate.

Although none of the CG treatment groups outgrew the controls, they all reached final mean weights that were not significantly different than the controls (P > 0.05). Periods of hyperphagia followed all food deprivation periods as in the earlier UM study, and increased in duration with longer deprivation periods. However, the magnitude of the hyperphagia was substantially less than in the previous study. UM's findings suggest that the CG response of BG × GS hybrids differs according to the type of food used.

UW-Milwaukee researchers continue to maintain a 1994 year class of black crappie captive brood stock, which will be used to produce the larvae for feeding trials from the onset of first-feeding. Using temperature and photoperiod as environmental conditioners for gonadal maturation they have been successful in producing a limited number of gametes. However fertility was low, probably due to poor gamete quality. They speculate that the fish were too young to spawn successfully. They anticipate the fish will spawn in spring 1998.

As a back-up to their efforts to produce YOY from laboratory and wild spawns, UW-Milwaukee researchers obtained 2,741 pondspawned 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 six days after the beginning of the trial, the general population consumed mainly 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 flowthrough 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.

ISU researchers spawned green sunfish females and male bluegill (July 1, 1997) to produce hybrids. The progeny were first fed live and frozen brine shrimp two to three times daily, then weaned to a commercial fry feed (Fry Feed KyowaTM B-250). These tank-reared fingerlings were transferred from the spawning facility to the rearing facility on August 14, 1997, at 10-15 mm total length (0.4-0.6 in). The water for these fish was recycled through a newly developed recycle system which consisted of a microscreen drum filter to remove large solids, a high pressure sand filter to remove additional solids, a downflow, forced-draft packed column used for nitrification, and ultraviolet lamps for disinfection. Temperature was maintained at 24° C (75.2° F).

On July 18, adult fish from the same stock (i.e., not the identical brood stock used to obtain the first group of hybrids, but from the same group of brood stock) were transferred from ISU and stocked in ponds at Kloubec Fish Farms, Amana, Iowa, to produce a second group of hybrids from natural spawning. On August 22, 15 to 25 mm (0.6-1.0 in) pond-reared fingerlings produced at Kloubec Fish Farms were transferred to ISU. They were held in a quarantine facility with a single-pass water supply to avoid contaminating the recycle system with infectious microbial organisms. During the 14-day quarantine interval, the fish were habituated to formulated feed. The pond-reared fish readily converted to Fry Feed KyowaTM, using a mixture of B-400 and B-700. The habituation process was exceptionally successful, with less than 0.2% mortality.

Both the tank-spawned fish and the pondreared fish were raised in the recycle system until mid-October when mean sizes of both were >50 mm (2.0 in). The experiment was then begun to compare growth of control fish fed a standard feeding regime (three times daily, fed to satiation on the last daily feeding) to a CG feeding regime group. The group fed the CG strategy will be fasted two days, then fed daily until the percent daily feeding rate is not different from the control group, then two days of fasting will follow, and then the cycle will be repeated.

PSU modified and improved their recirculating system by adding a backup power supply and installing process control technology. The data logger from Campbell Scientific Inc. continuously monitors water quality parameters (temperature, dissolved oxygen, pH, and air temperature), alarms under loss of power, notifies responsible parties of problems, and allows dial-up status reports. The logger also controls photoperiod, waste removal water flow, and water pump operation.

Initial feed-acceptance training trials were completed at PSU with 75-100 mm (3.0-3.9 in) black crappie, purchased from Osage Catfisheries, Osage Beach, Missouri. Fifty fish were stocked into one of two rearing tanks (4.0 kg/m³; 0.25 lb/ft³) and trained from BSN to 2.4 mm (0.1 in) ARCATTM floating pellets. At the end of a 71-day training trial, there were 27 non-feeders in one tank and two in the other. Average length increases were 0.6 mm (0.02 in) in both tanks, with no change in weight in one tank and a 0.6 g (0.02 oz) loss in the other. All of the fish died shortly after the trial due to an ammonia poisoning from a newly purchased, used pump.

A small recirculating system consisting of 12, 16-L (4.2-gal) aquaria was developed at PSU to run individual feeding trials. Fish were larger in these trials, 150-200 mm (5.9-7.9 in) and had been previously trained to the ARCATTM pellet in another project. In the individual setting, these fish refused ARCATTM and had to be retrained to a 2.5 mm (0.1 in) semi-moist pellet from BiodietTM. Initial trials comparing starvation periods of two, three, and four days compared to daily feeding suggested the 2-day starvation period yielded the best results.

OBJECTIVE 5

Juvenile black crappie were reared extensively at the Gavins Point National Fish Hatchery in Yankton, South Dakota. The fish were collected by hatchery staff and transported directly to USD in late summer (pond temperature 21 °C [69.8 °F]). About 540 black crappie (59 mm, 2.6 g; 2.3 in, 0.09 oz) were held for \ge 1 week for acclimation in approximately equal lots for duplicate experiments in two circular tanks, each containing 330 L (87.2 gal) of recirculating, continuously aerated, treated city water at 20 °C (68.0 °F).

On the day of each experiment, a sample of 45-60 fish was removed from one test tank to provide initial pre-stress measures of blood characteristics; this sample size was necessary as the small size fish required several to be pooled to provide enough plasma for physiological analysis. The remaining fish in the tank were then subjected to aerial emersion in a dip net for 30 sec and placed for recovery in individual lots of 35-45 fish, into buckets containing 30 L (7.9 gal) of aerated water and held in separate tanks to maintain constant temperature. Fish were sampled at 0.5, 1, 3, and 6 h and after one day by removing a recovery bucket containing a separate sample at each time; this approach eliminated the possibility of stressing remaining fish by serially sampling from a single tank. An additional bucket containing 20-30 fish for each duplicate experiment was not sampled; it was checked to assess delayed mortality at 24-h intervals for 72 h.

Fish removed for blood sampling were immersed immediately into a lethal solution (400 mg/L; 0.05 oz/gal) of tricaine methane sulfonate; fish were completely immobilized within 60 sec. Blood was drawn from each fish using a capillary tube after severing the caudal peduncle with a razor blade. Blood removal from each fish was completed within 12-15 min. Because of the very small and variable volumes of blood obtained, samples were pooled into about 10-12 tubes per time point using about four to eight samples per tube. Plasma from tubes containing pooled samples was frozen for later analysis.

Duplicate experiments were conducted on consecutive days and samples were shipped on dry ice to UW-Madison for analysis. No delayed handling mortality was evident in either of the recovery tanks after three days.

WORK PLANNED

The Sunfish Culture Guide and associated video are scheduled for completion in 1998. Final analysis of the nutritional data for sunfish will be analyzed fall 1997 and final report completed in 1998. Proximate composition of fillets and statistical analysis of remaining data associated with SIUC's study in the third sunfish project will be completed by mid-January.

Ponds will be harvested in early November in the SIUC Year 1 study in the fourth sunfish project. Production, survival, mean length and weight, dress out, feed conversion efficiencies, and liver and gonadal somatic indices will be determined and compared among treatments. Black crappie preliminary feed-training trials will also begin in early November, in preparation for Year 2 studies. The grow out study examining diets with varying crude protein concentrations at Purdue will be conducted in 1998. Further. Purdue researchers will examine overwinter feeding and management strategies. UW-Milwaukee researchers will evaluate and compare the habituation to formulated diets of intensively reared larvae from the onset of first-feeding and pond produced fingerling black crappie. Additional experiments are planned at UM to isolate the factor or factors that determine whether CG for BG \times GS hybrids results in growth exceeding that of controls versus only catchup growth. While both outcomes hold potentially important implications, knowledge of regulatory mechanisms are of importance.

Feeding trials for grow out of $BG \times GS$ hybrids will be conducted at ISU in a culture facility with a recirculating system to compare daily feeding with a CG feeding strategy involving a 2-day fasting interval each one-week feeding cycle. The experiment will continue until fish are raised to 227-340 g (0.5-0.75 lb), and the results of the two feeding strategies will be compared. Additional monitors for flow and water level will be installed in the recirculating system at PSU. New fish will be purchased, stocked, and trained to pellets. Once trained, feeding trials at 4 kg/m³ (0.25 lb/ft³) will be initiated again. Additional trials testing the 2-day through 4-day starvation trials will be conducted in the small recirculating system to reinforce earlier findings at PSU.

Plasma samples will be analyzed by USD and UW-Madison researchers for cortisol in late 1997-early 1998. Because of the small sample volumes, it is likely that only plasma cortisol will be measured as a first priority. Also, insufficient blood was available to conduct differential blood cell counts. In August or September 1998, additional experiments are planned to evaluate the use of altered water temperature and addition of salt to recovery media as a means of mitigating the physiological stress response and to enhance survivorship.

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 BG × GS hybrids 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 aquacultural 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.

UM's findings suggest that the CG response differs according to the type of food used. Hybrids fed mealworms on certain CG schedules outgrew daily fed controls in their previous study but did no more than catch up to controls when a commercial diet was provided. Considering both studies together, an exciting potential is indicated for using CG feeding schedules with sub-maximal feeds (e.g., mealworms), to achieve BG \times GS hybrid growth rates approaching those

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obtained with commercial feeds. Comparing between the present and previous study, daily fed control fish (similar to standard feeding in aquaculture) grew 1.5 times faster on the commercial diet than on mealworms. Growth rates of hybrids fed a sub-maximal feed on a CG schedule outgrew fish fed a commercial diet. The implication is that lower quality feeds in conjunction with CG feeding methods could provide the same or better yields in commercial settings, while at the same time reducing production costs, due to lower feed costs. Further, two fish farmers in Iowa, one in Minnesota, one in Wisconsin, and another outside of the North Central Region are now using information on tank spawning of BG × GS hybrids developed by ISU. The commercial collaborator, Myron Kloubec (Kloubec Fish Farms, Amana, Iowa), is developing facilities for producing hybrid bluegill out-of-season.

PUBLICATIONS, MANUSCRIPTS, AND PAPERS PRESENTED

See the Appendix for a cumulative output for all NCRAC-funded Sunfish activities.

	NCRAC-		TOTAL				
YEARS	USDA FUNDING	UNIVER- SITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	TOTAL SUPPORT
1994-96	\$174,999	\$177,300	\$12,012 ^a			\$189,312	\$364,311
1996-97	\$112,591	\$146,368				\$146,368	\$258,959
TOTAL	\$287,590	\$323,668	\$12,012			\$335,680	\$623,270

^aFarmland Industries, Inc.

SUPPORT

SALMONIDS⁹

Project Component Termination Report for the Period June 1, 1990 to August 31, 1996

NCRAC FUNDING LEVEL: \$479,796 (June 1, 1990 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
Extension Liaison:		
Ronald E. Kinnunen	Michigan State University	Michigan
Non-funded Collaborators	s:	
Hugo Kettula	Seven Pines Trout Hatchery, Lewis	Wisconsin
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I. Navarro	University of Barcelona	Spain
Nebraska Game & Parks	Calamus State Fish Hatchery	Nebraska
Commission		
K. Warner	National Center for Agricultural Utilization	Illinois
	ARS, USDA, Peoria	
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

REASON FOR TERMINATION

The objectives for this work on Salmonids were completed.

PROJECT OBJECTIVES

(1) Develop less-polluting diets. Develop practical rainbow trout diets using

regionally available feed ingredients, including fish meal analogs.

(2) Determine the practical limits on rearing density of juvenile rainbow trout by examining the effects of selected high rearing densities on trout stress

⁹NCRAC has funded three Salmonid projects with the first beginning June 1, 1990. The first project initiated work on developing less-polluting diets. That work continued in the second and third projects. Work on using high rearing densities began in the second project and continued in the third. Paul B. Brown chaired the first and second projects; Ronald R. Rosati chaired the third project until he left Illinois State University after which Terence B. Barry became chair.

responses, survival and growth. Use stress and performance responses in trout to evaluate culture system design and operation under practical conditions. 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).

PRINCIPAL ACCOMPLISHMENTS *OBJECTIVE 1*

Investigators at Michigan State University constructed a nutritional phosphorus (P) mass balance model for coho and chinook salmon as a method of estimating P losses from the Platte River Anadromous State Fish Hatchery. For the production period of January 1993 through May 1994, the P mass balance model indicated that 37.7% of P fed was retained by fish, 21.0% was discharged in the feces, and 41.3% was discharged in dissolved form. Without any raceway solids removal, a maximum of 2.8 kg/metric ton (MT; 5.6 lb P/ton) of fish produced was discharged into the hatchery's stabilization pond. This loss rate was the lowest reported for a salmonid hatchery. Efficient removal of raceway solids could have reduced hatchery P losses to 1.8 kg P/MT (3.6 lb P/ton) of fish produced.

Work was completed on improving the digestibility and utilization of nutrients in feeds which should also reduce nutrients in aquaculture waste water. Rainbow trout were fed diets containing untreated or dephytinized soybean meal and corn gluten meal with or without supplemental zinc (Zn) to determine if fish dietary phytate impairs Zn bioavailability in fish. Fish fed diets containing untreated soybean meal and corn gluten meal without supplemental Zn (basal diet) were not Zn deficient after 170 days based on growth, whole fish Zn, P and protein content, total bone Zn, and the

activity of alkaline phosphatase and carboxypeptidase B. Although bone Zn concentrations were reduced in fish fed the basal diet, total bone Zn increased in all fish regardless of dietary treatment. The basal diet contained enough available Zn to offset any negative effects of phytate on Zn bioavailability. Future assessments of Zn status in fish should be based on changes in Zn-dependent metabolism or total bone Zn rather than bone Zn concentration.

Researchers at Ohio State University compared the growth rates of rainbow trout fed five different diets in which fish meal protein was replaced by an animal byproduct 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. Analysis of P levels in fecal samples, however, indicated that diets containing animal by-products were not less-polluting.

Research at Purdue University (Purdue) first defined the methods for precisely determining available P from feedstuffs. Fecal collection methods were evaluated, and then a new indicator of nutrient availability was developed. After defining the appropriate methods, available P was determined from single ingredients in diets, as well as multiple ingredients fed to both rainbow trout and Atlantic salmon.

Purdue researchers found that a fish mealfree 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. 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 filets from fish fed a commercial diet. Two graduate students were trained during this project.

OBJECTIVE 2

University of Wisconsin-Madison (UW-Madison) investigators conducted laboratory studies to compare the performance of juvenile rainbow trout stocked at density indices (pounds of fish/[cubic feet water × average fish length in inches]) of 0.45 (generally recommended maximum rearing density), 0.90 and 1.35 at loading rates ranging from 491.3 to 982.6 g/(L·min) [4.1 to 8.2 lb/(gal·min)]. Fish were reared at $15^{\circ}C$ (59.0°F) for four weeks at the end of which time the fish were weighed, measured, and subjected to an acute stress challenge test to evaluate treatment effects on fish growth and specific physiological stress responses. High mortality occurred at a loading rate of 982.6 g/(L·min) [8.2 lb/(gal·min)], probably due to elevated unionized ammonia levels. Fish reared at densities of 5.68 and 8.51 g/($L\cdot cm$) [0.90 and 1.35 $lb/(ft^3 \cdot in)$] grew significantly less than fish reared at 2.84 g/(L·cm) $[0.45 \text{ lb/(ft}^3 \cdot \text{in})]$ at loading rates of either 491.3 to 719.0 $g/(L \cdot min)$ [4.1 and 6.0 lb/(gal·min)]. Fish reared at densities of 5.68 and 8.51 g/($L\cdot cm$) [0.90 and 1.35 lb/(ft³·in)] gained 78% and 62% of the weight of the fish reared at 2.84 $g/(L \cdot cm)$ [0.45 lb/(ft³·in)], respectively. These results contrast with earlier studies conducted at UW-Madison showing that similar high rearing densities had no effect on the growth or feed conversion of juvenile rainbow trout reared at 9.8-11.9°C (49.6-53.4°F) at a loading of 299.6 g/(L·min) [2.5 lb/(gal·min)]. Cortisol levels were higher at 24 h post-stress in fish reared at low density

compared to fish at medium and high density. This difference was attributed to the establishment of stressful social hierarchies in the low-density tanks (which break down under crowded conditions). Fish reared at high density had higher baseline cortisol levels and longer (though not higher) increases in cortisol following an acute stressor relative to fish reared at low density with corresponding changes in glucose and chloride levels. The data indicate that high rearing density can have a negative impact on fish growth and food conversion if loading rates are between 299.6 and 491.3 $g/(L \cdot min)$ [2.5 and 4.1 lb/(gal·min)] and/or rearing temperature is greater than approximately $12^{\circ}C$ (53.6°F). The negative consequences of high density/loading appear to be at least partially mediated by physiological stress responses.

A 10-week production-scale field trail was performed at the Calamus State Fish Hatchery by University of Nebraska-Lincoln 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 oxygensupplemented cylindrical tanks. Six of the latter were each equipped with a sealed packed column supplied with oxygen, and assigned fingerling trout at a rearing density of 2.84 and 5.68 g/(L·cm) [0.45 and 0.90 $lb/(ft^3 \cdot in)$] (three tanks per treatment). Six raceways equipped with conventional packed columns were also each assigned fish at a density of 2.84 and 5.68 g/(L·cm) [0.45 and $0.90 \text{ lb/(ft}^3 \cdot \text{in})$] (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

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challenge test and Goede health assessment were performed. The results indicated that juvenile rainbow trout could be reared at twice the generally recommended density with no negative impact on growth, food conversion or health. Similar results were found in both tanks and raceways.

Investigators at UW-Madison identified a physiological measure of stress that was well correlated with growth in rainbow trout serum cortisol levels 3 h following an acute handling stressor. Individual fish that consistently showed low 3 h post-stress cortisol levels (i.e., fish that recovered rapidly from stress, defined as "low" fish) had a mean specific growth rate (SGR; % weight gain per day) of 0.54, compared to a mean SGR of 0.41 in unselected fish. Fish with consistently high cortisol levels at 3 h post-stress and a low SGR ("high" fish) were also identified. A selection process was initiated in December 1995 to identify "low" and "high" fish for subsequent breeding. Starting with 160 two-year-old fish obtained from Seven Pines Trout Hatchery, five female and five male "low" fish, and nine female and five male "high" fish were selected. The fish were bred in the autumn of 1996 but there was poor fertilization success and very low larval survival due to a system-wide water quality problem. The selected fish were rebred in the autumn of 1997. Sperm from each selected "low" male was used to fertilize a subgroup of eggs from each selected "low" female, and likewise for the "high" fish and randomly selected controls. Groups of larval fish from each population ("low," "high," and control) are currently being reared for subsequent performance evaluations.

IMPACTS

 The less-polluting diets developed during this project will benefit aquaculturists facing strict regulatory pressures to reduce waste nutrients in effluents.

- The use of regionally available plant and animal by-product protein sources as substitutes for fish meal will reduce the cost of trout feed manufacture by at least 15% by reducing both ingredient and transportation costs. Diets formulated with fish meal analogs were found to have little or no impact on fish growth, feed conversion or flesh quality, and were also less polluting.
- The results demonstrated that fish farmers may be able to at least double rainbow trout production from their *existing* facilities if loading rates are kept low by supplemental oxygenation and/or increased water flow rates. These findings are particularly important to trout farmers in the NCR who are generally constrained by limitations in water and rearing space.
- 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 physiological indicator of stress resistance and superior growth identified in this project will be invaluable for developing fast-growing, stress-resistant trout strains for use under the distinctive aquaculture conditions found in the NCR (i.e., relatively small-sized farms, low water flows, and variable water temperatures).

RECOMMENDED FOLLOW-UP ACTIVITIES

 Factors that impact available P have not been determined. The results indicate that mineral interactions have the most profound effect on available P in diets fed to fish. Of the 20-24 essential minerals required by vertebrates, specific levels and forms of minerals that impact available P in fish have not been determined.

- Further explore available animal byproduct utilization as replacement of fish meal in combination with plant proteins available within (canola, soybean) or outside (cottonseed) of the region.
- Evaluate brood stock diets prepared with fish meal analogs.
- Evaluate the effects of diet formulations devoid of fish meal on fillet quality.
- Evaluate diets prepared with fish meal analogs with other salmonid species.
- Evaluate the stress responses of the offspring of "low" and "high" rainbow trout to determine the heritability of the stress response. Evaluate the performance of the offspring of selected "low" fish under commercial aquaculture conditions. Develop a stress-resistant, fast-growing strain of rainbow trout for the NCR using the stress response as a selection criterion. Determine if the stress hyperresponsive "high" fish identified in this study (which have stress

and growth characteristics typical of "wild" trout) will have advantages for stocking recreational fisheries.

- Conduct integrative research to evaluate the combined effects of the improved feeds, rearing strategies (e.g., high density), and fish strains identified or developed during this project on the salmonid aquaculture industry in the NCR.
- Extend the insights and accomplishments of the completed salmonid project to other aquaculture species in the region, including yellow perch and walleye. It may be possible, for example, to develop less-polluting yellow perch diets made with animal by-product or plant protein sources, or produce a fast-growing, "domesticated" strain of yellow perch by selecting for and breeding stress-resistant fish.

PUBLICATIONS, MANUSCRIPTS, AND PAPERS PRESENTED

See Appendix for a cumulative output for all NCRAC-funded Salmonid activities.

YEARS	NCRAC- USDA FUNDING		TOTAL					
		UNIVER- SITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	SUPPORT	
1990-92	\$129,799	\$184,843				\$184,843	\$314,642	
1992-94	\$149,997	\$237,493			\$66,700ª	\$304,193	\$454,190	
1994-96	\$200,000	\$208,083		\$18,590 ^b	\$15,000°	\$241,673	\$441,673	
TOTAL	\$479,796	\$630,419		\$18,590	\$81,700	\$730,709	\$1,210,505	

TOTAL SUPPORT FOR THE THREE PROJECTS

^aNebraska Game and Parks Commission

^bUniversity of Wisconsin Sea Grant

^cInternational Collaborative Program for OSU to work jointly with the National Fisheries University of Pusan, Korea

NATIONAL AQUACULTURE EXTENSION CONFERENCE¹⁰

Project Termination Report for the Period October 1, 1996 to August 31, 1997

NCRAC FUNDING LEVEL: \$3,700 (October 1, 1996 to August 31, 1997)

PARTICIPANTS:

Donald W. Webster	University of Maryland	Maryland
National Steering Committ	tee:	
Fred S. Conte	University of California Davis	California
David P. Crisostomo	University of Guam	Guam
James T. Davis	Texas A&M University	Texas
John W. Ewart	University of Delaware	Delaware
Steve C. Harbell	Washington State University	Washington
Joseph E. Morris	Iowa State University	Iowa
Nathan M. Stone	University of Arkansas-Pine Bluffs	Arkansas
National Advisory Commit	tee:	
Ted R. Batterson	Michigan State University	Michigan
Paul B. Brown	Purdue University	Indiana
Richard A. Croft	Center for Tropical and Subtropical	Micronesia
	Aquaculture Industry Advisory Council	
William DuPaul	Virginia Sea Grant Advisory Program	Virginia
Kevin M. Fitzsimmons	University of Arizona	Arizona
Joseph A. Hankins	Freshwater Institute	West Virginia
Ronald G. Hodson	North Carolina Sea Grant Program	North Carolina
Kevin D. Hopkins	University of Hawaii	Hawaii
Gary L. Jensen	USDA Cooperative State Research, Education, and Extension Service	Washington, D.C.
James P. McVey	NOAA National Sea Grant College Program	Maryland
Raul H. Piedrahita	University of California	California
Leo Ray	Western Regional Aquaculture Center (RAC) Industry Advisory Council	Utah
Robert Rheault	Northeastern RAC Industry Advisory Council	Rhode Island
Gerald Williamson	Southern RAC Industry Advisory Council	Arkansas
J. Larry Wilson	University of Tennessee	Tennessee

¹⁰NCRAC has provided funding along with the four other Regional Aquaculture Centers for two national aquaculture extension meetings; the first was called a National Aquaculture Extension Workshop whereas the second was called a National Aquaculture Extension Conference. This termination report is for the second meeting which was held April 8-12, 1997 in Annapolis, Maryland.

NORTH CENTRAL REGIONAL AQUACULTURE CENTER

REASON FOR TERMINATION

Objectives of the project were completed.

PROJECT OBJECTIVES

- (1) Learn successful approaches to problemsolving through case studies that can be replicated in other states.
- (2) Demonstrate and conduct hands-on experience with state-of-the-art computer applications for improving delivery of extension programs.
- (3) Identify national extension priorities and critical issues with development of corresponding action plans for implementation.
- (4) Identify potential interregional extension projects, such as curriculum development or national decision-support databases.
- (5) Share educational materials and programs in addition to expertise.
- (6) Strengthen regional and national communication networks to improve services to customers.
- (7) Examine successful extension components and outcomes to RAC research projects and develop approaches to improve integration across RACs nationwide.
- (8) Develop collective strategy to define extension's role in measuring impacts of RAC projects and collaboration with others in academia and private sector.
- (9) Strengthen communication networks to leverage resources and talent sharing.
- (10) Improve business management skills related to aquaculture and enhance

knowledge concerning marketing of aquatic products.

(11) Develop a method to evaluate the impact and accomplishments associated with conference after one year (1998).

PRINCIPAL ACCOMPLISHMENTS

The National Aquaculture Extension Conference was organized by a National Steering Committee comprised of members appointed by the five RAC Directors. It served to organize the conference, plan the major topics for inclusion, and act as the main management body for the meeting. Each member served as the contact for all other extension and industry members within their respective regions. There was also a National Advisory Committee which provided input from the aquaculture industry, aquaculture researchers, RACs and Sea Grant Directors, and Advisory Service leaders.

Extension and Sea Grant agents from across the United States and its territories met in Annapolis, Maryland, April 8-12, 1997 for the second National Aquaculture Extension Conference. The meeting was sponsored by USDA's Cooperative State Research, Education, and Extension Service (CSREES) RACs and NOAA's National Sea Grant College Program. This was the first time since 1992 that aquaculture outreach professionals had gathered to share ideas and expertise and discuss ways to improve educational programs for the aquaculture industry.

The meeting included a two-day conference where topics covered new methods of providing educational programs and information. Sessions included methods of using the Internet and the World Wide Web sites to deliver information and how to work with clientele to help them use these computer resources. The five RACs figured prominently in the program with the major part of the first day devoted to coverage of recently completed projects and directions for the future. Educational programs included business planning and finance for aquaculture projects, methods of dealing with information requests, and ways for extension professionals to deal with unproven technology. A session on offshore aquaculture featured both a synopsis of a conference held in Portland, Maine in 1996 and subsequent industry developments.

There was also a poster session where extension agents and specialists from 33 different states displayed their presentations. As in the 1992 meeting, a resource room provided an opportunity to display publications, software, and related products available for support of aquaculture programming. A computer room, equipped with six state-of-the-art machines, provided an opportunity for attendees to try software programs for the design and management of aquaculture businesses.

Following the first two days of the conference, a series of five intensive short courses provided an opportunity to hone skills in a variety of areas. The University of Maryland's Horn Point Environmental Laboratory in Cambridge, Maryland provided the location for "Shellfish Aquaculture Techniques" and "Striped Bass and Hybrid Production." The Biological Resources Engineering Department at the University of Maryland College Park organized and hosted a program on "Recirculating Aquaculture Systems" and the Columbus Center in Baltimore was the site for "Biotechnology in Aquaculture" and "Internet and Web Page Construction." The final day included tours designed to highlight

various aquaculture businesses and research facilities in the Northeast region.

Exit questionnaires rated the conference, including the short courses and tours, very highly. The extension specialists also noted that they will be able to use a great deal of the information provided.

IMPACTS

The National Aquaculture Extension Conference provided:

- A national forum for agents and specialists to meet and discuss critical issues affecting the aquaculture industry.
- A means to learn about technology that can increase the effectiveness and timeliness of program delivery.
- A joint meeting for Land Grant and Sea Grant supported personnel to discuss similarities and differences in their respective programs as well as their potential for future interaction.
- A way to increase communications among the outreach personnel through the use of electronic mail, the Internet, and the World Wide Web.
- Training in topic specific subject matter that can be directly applicable to agents' and specialists' programs in their respective states and regions.
- An opportunity to participate in a nationally organized program of aquaculture education technology and share program developments with peers.
- Hands-on training in the production of several important species.
- On site visits and discussions with operating personnel at several commercial production and aquaculture research facilities in the Northeast region.
- Personalized instruction in various aspects of computer program technology for the enhancement of aquaculture production management.

NORTH CENTRAL REGIONAL AQUACULTURE CENTER

- Publication of all papers, posters, and other important presentations in a nationally distributed conference summary document.
- An assessment for use of new technology through timely updating of conference information and on-line registration for attendees through a web site at the University of Maryland.
- Broad availability of conference proceedings through posting of the summary document on the World Wide Web at the University of Maryland.
- A forum for new projects to take form leading to the development of interregional and national projects.
- A method for assessing the impacts of this educational program and measuring the need for future conferences and training events on the national level.

RECOMMENDED FOLLOW-UP ACTIVITIES

The National Steering Committee will hold a final meeting to assess the written evaluations from the conference and to plan for a follow up survey. This will serve to provide information on how much gained from the conference was utilized by the agents and specialists in their respective programs as well as how beneficial the education effort was for them. It will also serve as a way to find out the extent of support within the network for future national conference and educational programs and to see what projects and programs developed out of the meeting that can be applied on a regional and national level.

The effectiveness of the World Wide Web was demonstrated during the development of the conference through the timely posting and updating of information on a web site. This included on-line registration which was utilized by almost one quarter of the registrants. This was also enhanced by the posting of the conference summary with all of the papers and posters on a web site with links from the table of contents to the participants' papers. Counts of the number of contacts accessing the site will be maintained to assess the effectiveness of this means of communications.

In there are any future conferences, it would be very beneficial if the funds could be issued from only one source, such as directly from CSREES, rather than the five individual RACs.

PUBLICATIONS, MANUSCRIPTS, AND PAPERS PRESENTED

See the Appendix for a cumulative output for all NCRAC-funded National Aquaculture Extension Workshop/Conferences.

YEARS NCRAC- USDA FUNDING	NCRAC-		TOTAL				
	USDA FUNDING	UNIVER- SITY	INDUSTRY	OTHER FEDERAL	OTHER ^a	TOTAL	SUPPORT
1996-97	\$3,700	\$14,950		\$20,000	\$14,800	\$49,750	\$53,450
TOTAL	\$3,700	\$14,950		\$20,000	\$14,800	\$49,750	\$53,450

^aEach of the four other Regional Aquaculture Centers contributed \$3,700 for the conference

SUPPORT

WASTES/EFFLUENTS¹¹

Progress Report for the Period September 1, 1996 to August 31, 1997

NCRAC FUNDING LEVEL: \$41,704 (September 1, 1996 to August 31, 1997)

PARTICIPANTS:

Ira R. Adelman	University of Minnesota	Minnesota
Fred P. Binkowski	University of Wisconsin-Milwaukee	Wisconsin
Christopher C. Kohler	Southern Illinois University-Carbondale	Illinois
Industry Advisory Council Lie	aison:	
Harry Westers		Michigan
Extension Liaison:		
LaDon Swann	Purdue University	Illinois/Indiana
Non-Funded Collaborators:		
Antony Grabowski	Milwaukee County House of Correction Fish Hatchery	Wisconsin
John Hyink	Glacial Hills, Inc./Alpine Farms	Wisconsin
Jeffrey A. Malison	University of Wisconsin-Madison	Wisconsin
John Wolf	Glacial Hills, Inc./Alpine Farms	Minnesota

PROJECT OBJECTIVES

- (1) Study and evaluate solid waste management by:
 - (a) describing the relevant physical characteristics of fecal material from fish fed commonly used commercial feeds,
 - (b) developing diets to maximize integrity of fecal pellets without loss of fish performance and compare the physical characteristics of these pellets to those in subobjective a, and
 - (c) developing operational and engineering solutions to minimize destruction of larger particles and to remove all particulates.

(2) Develop a report that:

- (a) Describes the potential benefits of aquacultural by-products (effluents and solids) in the context of Integrated Resource Management and Sustainable Development,
- (b) Characterizes the differences between the aquacultural discharges and other agricultural and industrial discharges, and
- (c) Identify case studies of previous controversies highlighting real versus perceived impacts of aquaculture.

¹¹NCRAC has funded two Wastes/Effluents Projects. This progress report is for the second project which is a 2-year study that began September 1, 1996 and is chaired by Fred P. Binkowski.

ANTICIPATED BENEFITS

Characterization of the possible differences in fecal waste properties of important regional alternative species will assist in the engineering design and operation of rearing systems for waste removal. This project will provide needed information for engineers and producers attempting to make cost/ benefit analyses before employing removal technologies. In addition, data obtained will be reviewed with engineering collaborators to suggest possible engineering solutions to waste management.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1A

University of Wisconsin-Milwaukee Researchers investigated the physical properties of freshly deposited feces generated through intensive tank culture of vellow perch fed commercial feeds at various phases of the production cycle at commercial rearing densities and grow out temperature (18-23°C; 64.4-73.4°F). The phases examined to date include: (1) perch approaching market size (approximately 100-150 mm [3.9-5.9 in] total length) and fed Zeigler Bros. trout grower, (2) mature perch at marketable sizes (>150 mm; >5.9 in) fed Ziegler Bros. trout grower, (3) advanced fingerlings (50-100 mm; 2.0-3.9 in) fed Ziegler Bros. salmon starter, and (4) young fingerlings (25-75 mm; 1.0-3.0 in) habituated to feed exclusively on Biodiet #2 starter feed.

Initial attempts at individually isolating or anesthetizing fish to collect freshly egested fecal material were found to be unreliable. Therefore, a low-head, side-stream siphoning device was constructed and installed on circular rearing tanks so that freshly settled fecal materials and uneaten food could be collected. The collecting basin (18.9-L [5.0gal] pail) for the siphoned material was

raised so that the water surface in the collection basin was just below that of the rearing tank. For 2.44-m (8.0-ft) diameter tanks, a smooth semi-flexible clear vinyl tube (approximately 28-mm [1.1-in] diameter) and a side-stream flow of around 4 to 7 L/min (1.1 to 1.8 gal/min) was used to siphon waste. In this way the water velocity within the siphon was low enough (adjustable in the range of <1 to 15 cm/sec; <0.4 to 5.9 in/sec) to collect the fecal material with minimal handling and disturbance. The clear tube allowed visual monitoring of the condition of the waste during collection. To insure freshly deposited material the rearing tank was cleaned at the start of each collection period. By arranging for the inflow to the tank to rotate the water, settled material would rapidly settle and collect in the sump at the base of the central standpipe of the circular rearing tanks. By attaching the siphon tube to collars mounted on the outer diameter of the standpipe and holding the collecting end at a fixed height above the bottom in the central collecting sump, settled solids could be continuously removed from the rearing tank. For perch larger than approximately 100 mm (3.9 in) in size this collection device could be left unattended even over night without fish entering the waste collection stream. Small fingerlings tended to enter the waste collector even though the opening was positioned very close to the tank bottom and feces from these fish were collected by hand-directing the low-head siphon hose.

In a tank of adult perch at a rearing density of 84 kg/m³ (5.2 lb/ft³) fed a ration of 2.4% (approximately 2 kg; 4.4 lb) this low-head siphon device would collect 4 to 5 kg (8.8 to 11.0 lb) of sludge that was 8 to 10% solid on a dry weight basis (320 to 500 g; 0.7 to 1.1 lb). This represents a recovery of 17-26% of the food solids entering the tank on a dry weight basis. The literature reports that for salmonids, 25-30% of the dried weight of food is converted to feces on a dry weight basis. Therefore, between 69% to nearly 100% of the excreted solids produced in the rearing tank were removed by this simple device that concentrates the recovered waste in a side-stream of 4 to 5% of the tank's water inflow. In effect, with only a few pieces of inexpensive hardware, the rearing tank itself acts like a "swirl concentrator." With improvement this device could be incorporated as an inexpensive initial clarifier component, prior to further fine solids removal, in recirculating aquaculture systems (RAS). Fecal waste and uneaten food particles are removed directly from the rearing tank relatively intact before they are further broken up by more turbulent components of RAS.

Overall, freshly deposited feces (N = 887) ranged from 0.4 to 6.2 mm (0.02 to 0.24 in) in diameter and 0.6 to 23 mm (0.02 to 0.91 in) in length. Median fecal pellet diameters were 0.7 mm (0.03 in) (N = 344), 1.6 mm (0.06 in) (N = 240), 2.6 mm (0.10 in) (N =182), and 3.2 mm (0.13 in) (*N* = 121) for perch in the size categories of 25-75 mm (1.0-3.0 in), 50-100 mm (2.0-3.9 in), 100-150 mm (3.9-5.9 in), and >150 mm (>5.9 in) total length, respectively. The corresponding median lengths of intact fecal particles for these size categories were 4.8 mm (0.19 in), 4.0 mm (0.16), 6.7 mm (0.26 in), and 5.4 mm (0.21 in), respectively. Fingerling perch on the Biodiet feeds tended to have longer feces in relation to their diameter and the feces tended to lack the multifolded rough character of the pellets of larger sized perch fed the Ziegler feeds.

Settling velocities of individual fecal and food particles were determined in a 180 cm (70.9 in) high settling column (10-cm [3.9in] diameter). As anticipated by Stoke's law, settling velocities increased with increasing

particle size and density. Settling velocities for feces increased gradually over a range of 0.4 to 5.0 cm/sec (0.16 to 1.97 in/sec) (N =204) with increasing fish size. The settling velocities of the intact food granules and pellets were higher (5.0-16.0 cm/sec; 1.97-6.30 in/sec) than settling velocities of feces of similar diameter, except for the smaller granules of Biodiet #2 starter and feces of the fingerling perch with diameters <1 mm (<0.004 in) and settling velocities from 0.7-3.2 cm/sec (0.28-1.26 in/sec) and 0.4-1.8 cm/sec (0.16-0.71 in/sec), respectively. This difference mainly reflects the higher density of the pelletized food compared to the less dense fecal material after passage through the digestive tract.

Specific gravity measurements of freshly collected fecal solids were made before and after uniformly compressing the water from approximately 50 mL (1.7 oz) of collected sludge by centrifugation for 5 min at 2,500 rpm pouring off the water, determining the resulting volume and weight of solid material and comparing it to the weight of an equal volume of deionized water and correcting for temperature. The overall mean specific gravity by this method was 1.055 (SD = 0.019; N = 36) after centrifugation and 1.029 (SD = 0.013 N = 24) without centrifugation. Differences in specific gravity of feces based on the type of food used were not detectable.

Initial attempts to examine the friability of feces of larger perch fed Zeigler grower diets using direct observation of small numbers of fecal particles in water in Erlenmeyer flasks were unsuccessful due to the heterogeneous nature of the fecal particles. Some of the larger fecal pellets appeared to consist of fine solid material approximately the size of the finely milled material in the formulated diet, encased in an outer more durable mucus-like shell that either was a solid smooth-surfaced

stream of material or became folded on itself and compressed into a larger diameter rough surface pellet. In some fecal pellets the proportion and durability of the outer casing appeared to vary in thickness and a relatively small proportion of fine material was inside this casing. These fecal pellets were extremely compressed and durable and tended to have a gravish to white color compared to the less durable brownish type encasing large amounts of finely milled material. Some fecal pellets tapered along their length from wide easily friable character to the more durable gravish cast. This complicated the visual determination of when the pellet was completely broken down. For this reason it seemed better to use a larger (45-70 mL; 1.5-2.4 oz), more representative sample of fecal material to quantify fecal friability. Samples of intact settled fecal material collected by low-head siphoning and an approximate settled volume of 45-70 mL (1.5-2.4 oz) of material was scooped into a 150 mL (5.1 oz) graduated beaker, the contents of this beaker were poured into a 250 mL (8.5 oz) Erlenmeyer flask capped with parafilm and subjected to mechanical agitation of 0, 5, 15, 30, 60, 120, and 240 sec duration at 300 rpm on an orbital rotary shaker. The contents of the flasks were then poured into Imhoff cones and the settleable solids determined along with the volume of intact particles by visually determining the boundary between the fine broken settled solids (less than the diameter of intact fecal material) and the more rapidly settled mainly intact fecal material. The difference in settled volume of the intact feces versus the fines as a percent of the settled solids in the cone was used to express the degree of breakdown. Four or five repeated samples at each time duration were used to express the breakdown of fecal material over time.

Using this technique it was found that the feces of the larger perch fed Ziegler grower

and salmon starter diets rapidly decreases from 60 to 80% intact material to around only 20% of the durable type of intact fecal material after only 5-60 sec of agitation at 300 rpm. While fecal material of fingerling perch fed Biodiet #2 starter also started out 70-80% intact tended to remain around >60% intact even after 240 sec at 300 rpm.

Southern Illinois University-Carbondale To date most of what has been done on this project involves building the fecal collectors and experimental system as well as developing a procedure that readily works with the materials at hand. The same systems with modest modifications should be applicable to use with the other species (hybrid striped bass, yellow perch, rainbow trout, and possibly largemouth bass). Tilapia and hybrid striped bass are in hand and ready for actual trials to commence. Trials should be underway by the end of October and completed for tilapia by the end of December 1997. Hybrid striped bass should be completed approximately eight weeks later.

The experimental recirculating system is equipped with a biofilter (1,000-L; 264.2gal), a sump/particulate filter (110-L; 29.1gal), and five aquaria (110-L; 29.1-gal) which serve as experimental units. Residence time for the recirculating system is approximately 48 h. Residence time for the individual aquaria is 15 min. Temperature is maintained against the ambient using emersible heaters and a chiller.

The experimental units are in the form of 110-L (29.1-gal) all-glass aquaria equipped with plexiglass fecal collectors. The tilapia must be at least 25 g (0.9 oz), preferably 50 g (1.8 oz), to confine the animals to the area over the fecal collectors without causing damage to fish when escaping around barriers. Stocking density needs to be at least 12 individuals per experimental unit to

insure fecal samples of adequate mass/volume (approximately 3.0 g [0.1 oz]/sample [wet weight]) and to control aggression associated with lower stocking densities.

The fish, prior to use, are held in a holding system composed of a separate 1,000-L (264.2-gal) recirculating system and fed for maintenance using the 32% crude protein catfish diet. Environmental conditions are maintained to be the same as those in the experimental system.

The experiments are to be divided into three trials where replication is done through time using a Latin square design. A random sample of 60 fish from approximately 300 is pulled from the holding tank and divided into five groups of 12 each. Each group is placed into an experimental unit and given a 7-day acclimation period. During the acclimation period fish are fed a ration of the conditioner diet to be approximately 2.5% of their body weight divided into three feedings per day at 8:00, 12:00, and 16:00. During the experiment the fish are confined over the collector using a removable barrier except during feeding. Immediately prior to feeding, the fecal reservoirs are removed and cleaned. The barrier is then removed to allow the fish to approach the front of the tank for feeding. Food is applied at the fastest rate the fish will eat without allowing the pellets to hit the bottom. Upon completion of a feeding bout, the fish are gently driven back over the fecal collector, where the barrier is replaced. The tank is then siphoned of any waste feed or extraneous feces and the fecal reservoir is then replaced. At the end of the acclimation period the same protocol is followed with the feeding trial except the five treatments are randomly assigned to the five experimental units. Beginning on the second day of feeding, the feces within the fecal

collector reservoir are isolated for further analysis prior to the 12:00 and 16:00 hour feedings.

Fecal sampling entails depositing the contents of the fecal collector onto a 100- μ m mesh Nitex screen within a Buchner funnel. The sample is then subjected to aspiration for 30 sec. The sample is then carefully moved to a pre-weighed aluminum pan for weight determination. The sample is then subdivided to yield an approximately 1.5 g (0.05 oz)sub-sample to be placed into a 50 mL (1.7 oz) Erlenmeyer flask with 15 mL (0.5 oz) of experimental system water. The balance of the sample is stored for proximate analysis. Samples are then rated on a scale of 1 to 5 (5 indicates the fecal sample is nearly intact, 4 indicates approximately 75% intact, 3 indicates approximately 50%, 2 indicates approximately 25% intact, and 1 indicates form is lost). The samples are then exposed to shaking at a rate of 100 rpm with an orbit diameter of approximately 2.5 cm (1.0 in). Samples are rated again after five and 15 minutes. Treatments are compared while taking into account day within a trial (2-7). Rating data is then analyzed as ordinal data using non-parametric statistics. Proximate analysis data is to be analyzed using ANOVA and Duncan's multiple range test where significant differences are found.

OBJECTIVES 1A AND 1C

University of Minnesota

A break in the production cycle at the University of Minnesota facility occurred while adult tilapia, which became infected with *Aeromonas hydrophila*, were treated, held for a withdrawl period, and marketed. The stress from this disease limited the ability of the fish to tolerate decreased water quality and thus the degree to which the tanks could be loaded. During this time the fish were fed a maintenance ration. This lessened the nutrient input and resulted in better water quality and a lower suspended solids load than would normally be experienced in a production system. The disease problems necessitated a complete shutdown of the production system tanks. The shutdown afforded an opportunity to rework system configurations and make repairs and improvements. In preliminary work with the tilapia production systems before the disease outbreak, total suspended solids levels in the three system types ranged from approximately 10 to 45 ppm. These values correspond well to average values reported in the literature.

OBJECTIVE 1C

Southern Illinois University-Carbondale A trial has been attempted using 20 g (0.7)oz) fish obtained from Aquamanna, Inc. in Indiana. The trial was initiated August 5, 1997. The trial reached week six before a system failure (de-chlorinator malfunction) resulted in loss of experimental animals. The loss was detrimental in terms of life and time lost but the experimental animals were of questionable quality for the experiment being done. The fish appeared stunted. Smaller, high quality juveniles are going to be of size by the end of October to mid-November when the biofilter of the production system is back on line. The feeding trial should be completed approximately 14 weeks later (early March).

OBJECTIVES 2A-C

Using the reference list from the previous North Central Regional Aqauculture Center (NCRAC) Wastes/Effluents project as a base, a library search was conducted to identify and gather published literature (to date approximately 400 periodical articles, theses, and reference texts) relevant to the preparation of the report on the beneficial use of aquaculture waste by-products and alternative effluent treatment procedures applicable to the industry in the North Central Region.

In relation to subobjectives 2a and 2b, reference materials concerned with the fertilizing properties of aquaculture solids and sludges and the comparable properties and uses of agricultural manures and municipal sludges and their uses for land application and container media for vegetable and nursery stock have been identified and collected. Composting of these types of wastes to improve their utility has been another focus of the literature search. The coupling of aquaculture with hydroponics to reduce dissolved nutrient discharge and the use of composted products to suppress fungal disease are also a focus of the literature search. Information is also being gathered on the use of constructed wetlands and vegetative buffer strips as a possible means of reducing solids and nutrient discharge in aquaculture effluents.

These references also include information on nutrient transport and availability on forested land, agricultural field crops, wild fields, cultivated grass and forage crops, and wetlands in relation to the general utilization of sewage sludge and manure that can provide a contrast and comparison to aquacultural effluents and solids.

WORK PLANNED

OBJECTIVE 1B

<u>University of Wisconsin-Milwaukee</u> Researchers plan to test the influence of an experimental diet incorporating binding agents on the physical properties of yellow perch feces. Evaluation of fecal physical properties would use the techniques from work on Objective 1a. At the completion of these investigations, a manuscript will be prepared for publication concerning the physical characteristics of yellow perch wastes through the production cycle and in relation to diet.

OBJECTIVE 1C

University of Minnesota

The tilapia production system tanks at the university have been stocked with fish and the biofilters are being acclimated to production levels. When the suspended solids loads in the production tanks reach the target level, the growth trial will begin and sampling for waste characterization will continue. An additional collaborator has been identified in the region who is utilizing a commercial diet which has gained popularity with local producers raising fish in recirculating systems. This addition will allow us to make some comparisons between similar tilapia production facilities using different commercial feeds.

OBJECTIVES 1A-B

Southern Illinois University-Carbondale All work is underway as described above. <u>University of Wisconsin-Milwaukee</u> Researchers will continue to gather and review literature to prepare the report. They will attempt to identify case examples for subobjective 2c and will complete the report by the end of the project period.

IMPACTS

- Provide a broad base of information with regard to alternative species for rearing system design.
- System design of settling basins and clarifiers will be improved through the use of data gathered during this project.
- Literature review will allow knowledge-based decisions to be made regarding best management practices for the removal and treatment of aquaculture effluent.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

See the Appendix for a cumulative output for all NCRAC-funded Wastes/Effluents activities.

YEARS	NCRAC-		TOTAL				
	USDA FUNDING	UNIVER- SITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	SUPPORT
1996-97	\$41,704	\$54,132				\$54,132	\$95,836
TOTAL	\$41,704	\$54,132				\$54,132	\$95,836

OBJECTIVES 2A-C

SUPPORT

NATIONAL AQUACULTURE INAD/NADA COORDINATOR¹²

Progress Report for the Period September 1, 1992 to August 31, 1997

NCRAC FUNDING LEVEL: \$32,000 (September 1, 1993 to May 14, 1998)

PARTICIPANTS:

Robert K. Ringer	Michigan State University	Michigan
Rosalie A. Schnick	Michigan State University	Wisconsin

PROJECT OBJECTIVES

- 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 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.
- 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

¹²Ted R. Batterson serves as the facilitator for this multi-year project interacting with a steering committee in overseeing the Coordinator's activities.

than 50 known aquaculture diseases. CVM has afforded the aquaculture industry throughout the United States 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 New Animal Drug Applications (National NADA Coordinator) serves as a conduit between an INAD/NADA applicant and CVM. The National NADA 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 United States 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 cassettes 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 and the position has remained full time in Year 3 (May 15, 1997 to May 14, 1998).

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, United Kingdom, 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 (external microbicide, INAD #9671), luteinizing-hormone releasing hormone (spawning aid, INAD #9318), common carp pituitary (spawning aid, INAD #9728), Aqui-S (anesthetic, INAD #9731), another sponsor for amoxicillin (oral antibacterial, INAD #9853), EarthTec® (external microbicide, INAD #9996), copper sulfate (external microbicide, INAD #10-046), Ovaprim® (spawning aid, INAD #10-040), and fumagillin (microsporidiosis control, INAD #10-106). Three sponsors renewed their commitment to their INAD/NADA process for formalin, chloramine-T. and oxytetracycline.

PROGRESS ON 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]). A meeting was held with CVM on October 30, 1996 to gain clarification on the design of the protocols for conducting pivotal efficacy studies on aquaculture drugs (especially chloramine-T) that are used in water treatments. That meeting was followed by a meeting on November 7-8, 1996 with INAD holders of chloramine-T to coordinate efforts on draft label claims, design protocols for pivotal clinical field trials, and identify pivotal study sites for chloramine-T. Several large, active compassionate INADs are held by public aquaculture agencies and

organizations. Several of these INAD holders (e.g., U.S. Fish and Wildlife Service [USFWS]) are conducting pivotal efficacy studies for several potential label claims of chloramine-T. Akzo Nobel Chemicals, Inc. (Dobbs Ferry, New York) submitted a letter to their existing INAD (INAD #8086) file on July 21, 1997 committing to the development of a NADA on chloramine-T for aquaculture use. Akzo Nobel Chemicals, Inc. has also indicated that the company will fund genotoxicity studies required by CVM.

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. Phelps Dodge Refining Corporation (El Paso, Texas) submitted an application for an INAD/NADA (INAD #10-046) on April 3, 1997 and is actively pursuing the development of product chemistry data. Efficacy data and the environmental assessment have been submitted to CVM by the Stuttgart National Aquaculture Research Center and the target animal safety technical section is in preparation.

On October 18, 1996, CVM accepted data on formalin to be used in support of a NADA for control of certain fungi on the eggs of all finfish and certain external protozoa and monogenetic trematodes on all finfish. When CVM accepts the amended or new NADA from a sponsor for formalin, the NADA approval will be complete and there will be no need for INADs on formalin for these claims. A data call-in was issued on July 11, 1997 to all INAD holders of formalin by the IAFWA Project for the claim that formalin controls or prevents mortalities related to external infections of saprolegniasis on all fish. CVM has issued a contract to an outside expert who will review the called-in data and write a final report.

The National NADA Coordinator met with the potential sponsor of fumagillin, Sanofi Sante Nutrition Animale (Libourne Cedex, France) on April 19, 1996 in Paris, France to discuss cooperative efforts and the potential for development of a NADA in the United States. Sanofi committed to an INAD/NADA on fumagillin in June 1997. The NADA Coordinator is determining the potential of fumagillin to control or prevent hamburger gill disease in catfish and whirling and proliferative kidney diseases in salmonids. Contact has been made with several potential researchers.

A meeting was held April 12, 1997 with CVM to discuss the data requirements for hydrogen peroxide as an external microbicide and how to obtain the data so that an approval can be achieved for all the uses for which the drug seems to be efficacious.

A meeting was held on April 11, 1997 with CVM to discuss remaining data requirements to obtain full approval for oxytetracycline. CVM indicated that additional data would be required to expand the NADA for oxytetracycline as an oral antibacterial at temperatures below 9°C (48.2°F) and at doses above the current label and that at least one pivotal efficacy study would be required for these uses to be added to the label.

Abbott Laboratories (North Chicago, Illinois) was in the process of preparing the last portion of a technical section to complete the data requirements for NADA

approval of sarafloxacin when concern for development of disease resistant pathogens in humans with the use of fluoroquinolones in animals was raised as an issue by the Centers for Disease Control and Prevention. The Catfish Farmers of America (CFA) sent a letter written by the National NADA Coordinator to the U.S. Food and Drug Administration commenting on the impending ruling regarding the prohibition of extra-label use of fluoroquinolones. The CFA was concerned that this regulation (1) establishes that these drugs, when used in the catfish industry, "are capable of increasing the level of drug resistant zoonotic pathogens (pathogens that are infective to humans) in treated animals at the time of slaughter" and (2) will negatively impact or stop the approval of a fluoroquinolone, sarafloxacin, and other fluoroquinolones for the catfish industry. The catfish industry and researchers have agreed to consider developing a risk assessment on the use of sarafloxacin in catfish to control enteric septicemia to alleviate concerns of disease resistant pathogens developing in humans from the use of this fluoroquinolone.

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.

UMSC has completed an efficacy and safety evaluation of Aqui-S in two size ranges of

six representative freshwater fish species. The report was sent by the National NADA Coordinator on July 18, 1997 to all Federal-State partnership stakeholders/cooperators for their decision on which anesthetic, benzocaine or Aqui-S, should be the IAFWA Project drug. Twenty-four votes were cast for Aqui-S and no votes for benzocaine. Detailed assessments will be made of what data requirements will be addressed by the sponsor and what data requirements will be addressed by the IAFWA Project.

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®.

The National NADA Coordinator and Dr. David Erdahl (USFWS) met with Syndel International Inc. (Canada) in Seattle, Washington on February 23, 1997 to discuss the development of Ovaprim® in the United States. Another meeting was held with CVM on April 11, 1997 to discuss the strategy for development and the data requirements to gain an approval in food fish. Syndel International Inc. (Canada) recently obtained an INAD (#10-040) for its gonadotropin releasing hormone analog product, Ovaprim®. USFWS and other INAD holders are working with Syndel to develop the technical sections of the NADA package.

Schnick worked with CVM. Auburn University, Rangen, Inc. and tilapia producers to develop INAD #9647 on 17αmethyltestosterone (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 \$27,000 to Southern Illinois University-Carbondale and the University of Wisconsin-Madison to conduct a target animal safety study on MT with walleye and provided \$5,000 for Auburn University to conduct a literature review of the environmental data on MT for NADA submission to CVM. The human food safety portion of the NADA submission on MT was recently submitted by Auburn University to CVM for review, and CVM has accepted MT as safe.

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.

A Drug Approval Oversight Subcommittee was formed to aid the IAFWA Project to achieve its goal of obtaining drug approvals for United States public aquaculture. The first meeting was held May 5, 1997 in Hot Springs, Arkansas.

MEETINGS AND SPECIAL ACTIVITIES As National NADA Coordinator, Schnick 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.

CVM held a Joint Canadian-United States Workshop on Jurisdiction of Sea Lice Treatment and Control in September 1996 that will impact aquaculture drug approvals. One of the action items resulting from the workshop is the strategies and mechanics to institute forums for harmonization activities, i.e., the establishment of a joint Canada and United States Aquaculture Working Group. This means that data could be shared and certain requirements for all drugs could be harmonized so that there could be joint submissions leading to approvals being granted simultaneously in both countries. The National NADA Coordinator met on October 30, 1996 in Rockville, Maryland with Dr. Meg Oeller, CVM Liaison to NRSP-7, and Dr. William Gingerich (UMSC) to discuss coordination of the mutual projects that NRSP-7 and the IAFWA Project have in common. Both projects are working on chloramine-T, copper sulfate, hydrogen peroxide oxytetracycline, potassium permanganate, and sarafloxacin. Schnick also discussed coordination of the other NRSP-7 projects on common carp pituitary, erythromycin, and amoxicillin.

CVM held a meeting on February 13, 1997 with several representatives from the aquaculture community to discuss the effects on aquaculture of two recent laws, the Animal Medicinal Drug Use Clarification Act and the Animal Drug Availability Act. CVM also released a document on April 30, 1997 that further summarizes the two laws and the associated regulations.

The National NADA Coordinator chaired a special session on partnerships for aquaculture drug approvals at World Aquaculture '97 held in Seattle, Washington on February 22, 1997.

The National NADA Coordinator helped to coordinate the International Harmonization Workshop for Aquaculture Drugs/Biologics held in Seattle, Washington on February 24, 1997. The purpose of the workshop was to create an educational forum to exchange information and identify issues between public and private sectors and international organizations with the goal of initiating follow-up strategies to advance harmonization of drug maximum residue levels, aquaculture drug approval standards, and biological licensure. Several committees were set up to advance the harmonization of aquaculture drugs and biologics. The

National NADA Coordinator is the chairman of the committee to identify approved drugs worldwide for aquaculture and which drugs are being pursued for approval.

To attract more pharmaceutical companies to aquaculture, the National NADA Coordinator is working on gaining information on the market for aquaculture drugs both in the United States and worldwide. She gave a seminar to the Pfizer Animal Health Group on May 5, 1997 to encourage the company's interest in developing its products for aquaculture.

The National NADA Coordinator presented a seminar on aquaculture and its drug needs to representatives of Schering-Plough Animal Health on August 26, 1997. She encouraged them to consider developing their oral antibacterial, florfenicol, for the United States market.

Starting February 1997, the National NADA Coordinator was elected to a two-year term on the Board of Directors of the U.S. Chapter of the World Aquaculture Society.

The National NADA Coordinator wrote a draft letter on July 30, 1997 about regulatory options that would encourage animal drug approvals for minor species and for minor use. The options included: (1) criteria for the determination of a minor species or a minor use, (2) creating additional statutory authority, (3) administrative and regulatory changes, (4) creating incentives, and (5) extending existing authority.

The National NADA Coordinator wrote a letter on May 29, 1997 in support of the efforts by the Office of New Animal Drug Evaluation (ONADE) to the Director of that office, Dr. Robert Livingston, because the progress that the aquaculture industry is making toward approvals has been helped by the ONADE.

The National NADA Coordinator wrote a letter on June 4, 1997 to Dr. Gary Edwards, Assistant Director-Fisheries, USFWS, in support of having the Bozeman National INAD Office expanded in its scope to include other entities under their INAD exemptions. Currently, USFWS is pursuing the mechanism that would allow other public agencies and private producers to be cooperators under USFWS INADs.

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) continue to seek the sponsorship of other oral antibacterials; (3) 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; (4) help determine the potential for approval of two anesthetics, benzocaine and Aqui-S; (5) try to help the industry overcome negative attitudes about fluoroquinolones and in particular, sarafloxacin, so that sarafloxacin can be approved to control enteric septicemia in channel catfish; (6) identify potential funding sources for INAD/NADA activities; and (7) 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.

The National NADA Coordinator is organizing and chairing a follow-up

workshop and round table to the International Harmonization Workshop for Aquaculture Drugs and Biologics that will be held in Edinburgh, Scotland on September 17, 1997 to identify approved drugs worldwide for aquaculture, identify those drugs that are being pursued for approval, and determine where cooperative efforts can begin. Schnick will report on the progress made at the workshop and round table to another follow-up session to the International Harmonization Workshop for Aquaculture Drugs and Biologics on February 15, 1998 at Aquaculture '98.

The National NADA Coordinator is organizing and chairing a producers' session on compassionate INADs at Aquaculture '98, Las Vegas, Nevada, February 15-19, 1998. Schnick will also give a presentation at a special session on aquaculture drug approvals at the same conference.

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 18 of the 19 high priority aquaculture drugs and activities on seven new drugs of interest to aquaculture. Ten new INAD/NADA sponsors have initiated new INADs and progress has been made toward unified efforts on existing and new INADs/NADAs. Three sponsors have renewed their commitment to the INAD/NADA process on their drug products.

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 one of the three current NADA sponsors of formalin.

PUBLICATIONS, MANUSCRIPTS, PAPERS PRESENTED, AND REPORTS See Appendix.

NATIONAL AQUACULTURE INAD/NADA COORDINATOR

SUPPORT

YEARS US FUN	NCRAC-		TOTAL				
	USDA FUNDING	UNIVER- SITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	SUPPORT
1992-93				\$17,000ª		\$17,000	\$17,000
1993-94	\$2,000			\$12,180 ^b	\$4,000°	\$16,180	\$18,180
1995-96	\$5,000		\$22,750 ^d	\$70,000	\$11,000 ^f	\$103,750	\$108,750
1996-97	\$10,000		\$29,000 ^g	\$46,920 ^h	\$26,000 ⁱ	\$101,920	\$111,920
1997-98	\$15,000		\$42,000 ^j	\$54,631 ^k	\$11,000 ¹	\$107,631	\$122,631
TOTAL	\$32,000		\$93,750	\$200,731	\$52,000	\$346,481	\$378,481

^aUSDA funding through a Cooperative Agreement with NCRAC

^bUSDA funding through a Cooperative Agreement with NCRAC (\$8,500) and FDA's Office of Seafood Safety (\$3,680) ^cNortheastern Regional Aquaculture Center (\$2,000) and Southern Regional Aquaculture Center (\$2,000)

^dAmerican Pet Products Manufacturers Association (\$7,500), American Veterinary Medical Association (\$10,000), Catfish Farmers of America (\$2,000), Florida Tropical Fish Farm Association, Inc. (\$500), Natchez Animal Supply (\$1,000), National Aquaculture Council (\$1,000), Striped Bass and Hybrid Producers Association (\$500), and American Tilapia Association (\$250)

^eUSDA 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 Center for Tropical and Subtropical Regional Aquaculture (\$5,000), Fish Health Section of the American Fisheries Society (\$1,000), and Northeastern Regional Aquaculture Center (\$5,000)

^gAmerican Pet Products Manufacturers Association (\$1,000), American Veterinary Medical Association (\$10,000), Catfish Farmers of America (\$10,000), 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)

^hCVM (\$18,400) and USDI/NBS International Association of Fish and Wildlife Agencies Project (\$28,520) ⁱCenter for Tropical and Subtropical Aquaculture (\$10,000), Fish Health Section of the American Fisheries Society (\$1,000), Northeastern Regional Aquaculture Center (\$10,000), and Western Regional Aquaculture Center (\$5,000) ^jAmerican Pet Products Manufacturers Association (\$1,000), American Veterinary Medical Association (\$10,000), AquaCenter, Inc. (\$2,500), Aqui-S New Zealand Ltd. (\$2,500), Catfish Farmers of America (\$10,000), Earth Science Laboratories, Inc. (\$2,500), Florida Tropical Fish Farms Association, Inc. (\$1,500), Gurvey & Berry, Inc. (\$5,000), National Aquaculture Association (\$2,000), Simaron Fresh Water Fish, Inc. (\$2,500), Striped Bass & Hybrid Producers Association (\$1,500), and Western Chemical, Inc. (\$1,000)

^kCVM (\$18,519) and USDI/BRD International Association of Fish and Wildlife Agencies Project (\$36,112) ¹ Center for Tropical and Subtropical Regional Aquaculture (\$10,000) and Fish Health Section of the American Fisheries Society (\$1,000)
TILAPIA¹³

Progress Report for the Period September 1, 1996 to August 31, 1997

NCRAC FUNDING LEVEL: \$86,500 (September 1, 1996 to August 31, 1997)

PARTICIPANTS:

Paul B. Brown	Purdue University	Indiana			
Konrad Dabrowski	Ohio State University	Ohio			
Paul A. Fuerst	Ohio State University	Ohio			
Donald L. Garling	Michigan State University	Michigan			
Christopher C. Kohler	Southern Illinois University-Carbondale	Illinois			
Kerry W. Tudor	Illinois State University	Illinois			
Industry Advisory Council Liaison:					
Curt Stutzman		Iowa			
Extension Liaison:					
Donald L. Garling	Michigan State University	Michigan			
Non-Funded Collaborator:					
Dr. Victor Wu	National Center for Agricultural Utilization, ARS, USDA, Peoria	Illinois			

PROJECT OBJECTIVES

- (1a) Develop and/or identify cost-effective feeds for tilapia culture in recirculating systems that minimize waste generation.
- (1b) Compare and evaluate economically important traits of current commercial tilapia strains in the North Central Region with other strains cultured in recirculating systems.

ANTICIPATED BENEFITS

Producers throughout the North Central Region (NCR) are raising tilapia. However, the combination of species and culture systems are not operating at peak efficiency. Diets fed to tilapia are most often modified

catfish diets. Those same diets are thought to cause increased muscle lipid concentrations in catfish. If the same problem exists in tilapia during the grow out phase of production, then the same problems will occur as in catfish. Fish containing relatively high concentrations of lipid in the muscle are subject to more rapid uptake of off-flavor compounds from the water. Further, shelf life of the product can be impaired because of the higher degree of lipid oxidation that can occur. Higher lipid concentrations in fillets is often the result of an imbalanced protein to energy ratio. Thus, the benefits of this line of research are continued improvement of diets fed to tilapia in recirculating systems, continued development of all-plant diets, enzymatic feedstuff enhancement, and use of animal

¹³NCRAC has funded only one Tilapia project to date. This is a 2-year project that began September 1, 1996 and is chaired by Donald L. Garling.

agriculture co-products that can be easily manufactured in this region, and continued improvement in product quality for the consumer.

The ability to evaluate genetic differences within and between strains, and to determine the degree of hybrid mixture within some strains, will assist the design of future work to select strains which are better adapted to culture conditions which will be utilized in the northern United States, and to assist in the evaluation of genetic schemes such as the production of YY male lines, which can be used to improve aquacultural production. Gene markers for hypervariable neutral polymorphisms have been shown to be able to discriminate among populations and species with better resolution than morphometric traits. These gene markers also have the potential for application in aquaculture, including identification of individuals, families, and species, and labeling of brood stocks. They can also be of importance in the identification of hybridization between stocks and species and in the monitoring of inbreeding rates in managed stocks for proper fisheries management.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Nile tilapia (*Oreochromis niloticus*) from the same genetic stock have been used for all nutritional experiments conducted at sites participating in research on Objective 1a. This stock has also been included in genetic research on Objective 1b.

This project has provided seed monies that have been supplemented by industry and the institutions involved. The ratio of other support to North Central Regional Aquaculture Center (NCRAC) funds has been almost 2:1.

OBJECTIVE 1A

Studies at Purdue University (Purdue) were designed to quantify the optimum protein to energy ratio in all-plant diets fed to tilapia raised in tank culture situations. The first phase of the project was funded by a private company and the second phase was funded by NCRAC. In the first project, the optimal dietary crude protein concentration for grow out of tilapia in tank culture situations and fed an all-plant diet was 28%. The second study is underway. Preliminary results indicate tilapia grow maximally when fed lipid concentrations of 4% and lower.

Researchers at Illinois State University (ISU) have extruded two feeds from formulations provided by Purdue: (1) 28% crude protein and (2) 34% crude protein. The feeds were determined to be the most effective of seven experimental all plant-protein diets tested in aquaria at Purdue. One group of fish were fed the 28% percent protein diet for eight weeks in the nursery. Three hundred seventy-five fish from that group with an average weight of 14.7 g (0.5 oz) were then transferred to a grow out tank where they will be fed for six weeks. A second group of fish were fed the 34% percent diet for eight weeks in the nursery. Three hundred twenty-nine fish from that group with an average weight of 11.5 g (0.4 oz) were then transferred to a second grow out tank where they will also be fed for six weeks. Due to the commercial production schedule at the ISU aquaculture facility, feeding of the two diets will be replicated over time. There will be at least three replicates for each of the experimental diets and one control diet. The experiment will be concluded prior to August 31, 1998.

Researchers at Ohio State University (OSU) have raised two groups of Nile tilapia fry, one at high temperature (HT) at 35°C (95.0°F) and the control group (C) at 22°C

 $(71.6^{\circ}F)$ for 60 days in recirculation systems. Juveniles at initial size of 12 g (0.4 oz) were divided into triplicate groups and offered five different artificial diets. Fish meal protein was gradually replaced in these feeds with animal by-product mixture (blood meal, feather meal, meat-and-bone meal, poultry by-product meal) at the level of 0, 25, 50, 75, and 100%. Feeding experiments with both HT and C groups were carried out at 25°C (77.0°F). HT fish fed more vigorously and grew significantly better compared to the C fish group; the average weights reached in HT males and females over the period of six months were 129.3 ± 28.7 and 84.1 ± 20.3 g $(4.6 \pm 1.0 \text{ oz and } 3.0 \pm 0.7 \text{ oz})$, respectively. There were no significant differences in growth among fish fed on fish meal-free or animal by-product based diets. There were significant differences in some chemical elements (e.g., copper and iron) but not in nitrogen and phosphorus in fish body among HT and C groups of tilapia.

Researchers at Michigan State University (MSU) are evaluating the effect of phytic acid, contained in many oil seed meals, on protein digestion and availability and the use of the enzyme phytase to ameliorate these effects. They have completed preliminary experiments that indicate feeding Nile tilapia to satiation three times per day improved growth and feed utilization compared to fish feed one, two, or five times per day. Two studies to determine the rate of feed and fecal movement through the intestine to determine an appropriate procedure for digestibility trials and phytate binding studies have also been completed. They are currently in the process of completing evaluation of the samples and data analysis from this experiment. The data suggests the current accepted model for describing food passage in tilapia is inadequate and a new model is in order. Feeding trials to compare growth, feed efficiency, and digestibility in

fish fed similar soybean-based diets, with and without phytase pretreatment, are being conducted. This trial is designed to examine the effects of graded levels of solvent extracted soybean meal, with and without phytase pretreatment, on fish growth and performance. This trial is scheduled to be completed before the middle of December 1997.

Researchers at Southern Illinois University-Carbondale (SIUC) will evaluate *Yucca shidigera* extract as a feed additive to reduce fecal ammonia. The experimental culture system that will be used for this evaluation is up and running. Diets have been prepared and are in cold storage. Fish to be used are now in the fry stage and are in the process of being reared to the 25 g (0.9 oz) starting weight for the study.

OBJECTIVE 1B

Researchers at OSU have isolated a series of short tandem repeat microsatellite loci from the species Astatoreochromis alluaudi. These are being tested on a series of tilapine species to verify their utility and genetic variability. In addition, a set of microsatellite markers which were isolated by Thomas Kocher of the University of New Hampshire are being tested to determine their variation in populations of O. niloticus. The O. niloticus populations being examined include an aquacultural stock being maintained at OSU Piketon aquaculture facilities to be used for growth and sex reversal studies, a stock recently isolated from the wild, and a set of natural populations from East Africa in the Lake Victoria basin and other lakes of Uganda. A set of studies have been completed on the use of randomly amplified polymorphic DNA applied to O. niloticus populations. These will be compared to the aquaculture strains in the next year. The evaluation of genetic variability will allow a better assessment of the variability between

strains which is expected to be observed in ongoing growth and sex-reversal experiments being carried on by Dabrowski at OSU. In addition, the OSU genetics lab has become strongly connected with the USDA tilapia genome effort coordinated by Thomas Kocher. OSU researchers participated in the USDA workshop on genome mapping for aquacultural species in May 1997, and are now an active participant in development of a regional project for genome mapping of aquaculture systems.

Researchers at SIUC have obtained two stocks each of Nile, red, and white tilapia. One strain of Nile tilapia fingerlings were provided by Purdue and another strain was purchased from Aquamanna, Inc. in Indiana. Florida red tilapia fingerlings were provided gratis by Ray DeWandel of Ocean Rich in California. Happy Knight red tilapia brood stock were purchased from International Strategies, Inc. in Arizona, and Rocky Mountain white tilapia were purchased from Rocky Mountain White Tilapia Co. in Colorado. The second white strain was obtained in-house from a backcross of an Oreochromis niloticus male with an O. aureus $\times O$. niloticus female. Five recirculating holding systems are being utilized for spawning and grow out of the six strains. An experimental unit has been constructed consisting of a stainless steel rack with 18, 118-L (31.2-gal) glass aquaria placed side by side, providing three replicate tanks per strain. The aquaria will be equipped with self-leveling siphons that drain onto the rack and down into the biofilter sump. After filtration, the water will be pumped back up to a head tank on the rack and flow by gravity to the individual aquaria.

WORK PLANNED *OBJECTIVE 1A*

grow out study examining diets with varying protein to energy ratios will be completed this winter at Purdue.

ISU will complete commercial production scale evaluations of the two all plant diet formulations provided by Purdue. The experiment will be concluded prior to August 31, 1998. There will be at least three replicates for each of the experimental diets and one control diet replicated over time.

MSU researchers will evaluate three additional soybean products with and without phytase treatment. In addition they are preparing to run a concurrent study to evaluate *in vivo* protein and indispensable amino acid digestibility using the same graded levels of ingredient incorporation. Digestibility trials will be run concurrently with all of the feeding trials. They will also isolate and identify intestinal compounds containing phytic acid joined to protein components; and evaluate the role phytic acid has in decreasing the ability of fish to break down proteins for utilization.

SIUC will complete their evaluation of *Yucca shidigera* extract as a feed additive to reduce fecal ammonia.

OBJECTIVE 1B

OSU researchers will continue the evaluation of the microsatellite loci in both natural and aquacultural strains. In addition, these markers will be evaluated on a standard set of tilapia families to assist in adding genetic markers for the development of a tilapia genome map.

Researchers at SIUC will begin strain evaluations as soon as 60 fingerlings of each strain reach a weight of approximately 25 g (0.9 oz).

IMPACTS

Research at Purdue is designed to develop all-plant diets for tilapia grown in tank culture systems. The minimal dietary crude protein concentrations have been quantified and the optimal protein to energy ratio study is underway. These results will help maintain or lower feed costs for producers in the NCR, plus provide formulae that can be manufactured in this region. These feeds are being tested at a commercial scale at ISU.

OSU has provided strong evidence that a diet devoid of fish meal with animal byproducts can be used to produce marketable size tilapia and the nitrogen (protein) level was not affected.

MSU researchers have demonstrated that feeding Nile tilapia three times per day

improves growth and feed utilization compared to more or less frequent feedings.

The development of genetic markers and assessment of genetic differences between strains will help to assess expected differences in response to growth conditions and differences to conditions inducing sex-reversal for different aquacultural strains. In addition, development of new markers will have a significant contribution to the effort to develop a genome map for tilapia which can be used to direct future selective breeding for improved aquacultural production.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

See Appendix.

YEARS NCRAC- USDA FUNDING	NCRAC-		TOTAL				
	UNIVER- SITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	SUPPORT	
1996-97	\$86,500	\$94,937	\$56,566			\$151,503	\$238,003
TOTAL	\$86,500	\$94,937	\$56,566			\$151,503	\$238,003

SUPPORT

SAFETY OF 17 α -METHYLTESTOSTERONE FOR INDUCTION OF SEX INVERSION IN WALLEYE¹⁴

Progress Report for the Period July 1, 1996 to August 31, 1997

NCRAC FUNDING LEVEL: \$27,000 (July 1, 1996 to August 31, 1997)

PARTICIPANTS:

Christopher C. Kohler	Southern Illinois University-Carbondale	Illinois
Jeffrey A. Malison	University of Wisconsin-Madison	Wisconsin
Industry Advisory Council Liais	on:	
Rosalie A. Schnick		Wisconsin
Extension Liaison:		
Joseph E. Morris	Iowa State University	Iowa

PROJECT OBJECTIVES

- To conduct a Target Animal Safety Study under Good Laboratory Practice (GLP) compliance for walleye fingerlings fed 17α-methyltestosterone for induction of sex inversion to produce genotypic females that produce viable sperm.
- (2) To evaluate selected dosages of 17αmethyltestosterone for efficacy at inducing sex inversion in walleye.

ANTICIPATED BENEFITS

Sexual dimorphism exists in walleye (*Stizostedion vitreum*), with the female demonstrating faster growth and reaching a much larger size than males. Therefore, the development of monosex female populations of walleye would be beneficial to commercial walleye production by increasing production efficiency. The development of all female populations would, therefore, benefit management techniques by developing a trophy fishery since only female have trophy size potential, and would benefit culturists due to the rapid growth of females which would increase production efficiency.

One method of producing all female populations is to treat juvenile fish with androgens to induce phenotypic sex inversion of genetic females and then utilizing the sperm from these masculinized females to fertilize normal eggs, resulting in 100% female offspring. Moreover, the fish destined for human consumption are not treated with hormones. This technique has been successfully applied to various aquaculture species including the grass carp (Ctenopharyngodon idella), chinook salmon (Oncorhynchus tshawytscha), tilapia (Oreochromis niloticus), yellow perch (Perca flavescens), and most recently walleye and sauger (Stizostedion canadense).

Fertilizing eggs with the sperm of partially sex-inverted genotypic females has produced all female populations of walleye. The sexinverted females were produced by feeding 50-mm (2.0-in) total length (TL) walleye a

¹⁴NCRAC has funded several projects on "Aquaculture Drugs," this being one of them. This project is chaired by Christopher C. Kohler.

diet containing 17α -methyltestosterone (MT) at a rate of 15 mg/kg (0.00024 oz/lb) of food for 60 consecutive days. However, this concentration and timing of MT was only about 50% effective in inducing partial or complete sex inversion. In yellow perch, sex inversion at greater than 90% was accomplished utilizing MT at concentrations of 1.5 to 60 mg/kg (0.000024 to 0.00010 oz/lb) in the diet, when fed to perch initially 20-35 mm (0.8-12.4 in) TL. A concentration of 15 mg/kg (0.00024 oz/lb) for 60 days is speculated to produce 100% sex-inverted females in walleye if treatment is begun in fish at 35-40 mm (1.4-1.6 in) TL.

Results of this study could become part of the data package that would be required by the Food and Drug Administration (FDA) for approval of a New Animal Drug Application (NADA) for use of MT for sex inversion in percids.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

At Southern Illinois University-Carbondale (SIUC), the initial dosage regime of 0, 15.0, 45.0, and 75.0 mg/kg (0, 0.00024, 0.00072, and 0.00120 oz/lb) diet was given for 60 days, followed by a 30-day withdrawal period. Fish were necropsied and data recorded. Analysis of the feed obtained from Rangen, Inc, revealed that the MT did not meet the concentrations necessary for this study. The feed contained 1, 2, and 3 times the dosage of 15 mg/kg (0.00024 oz/lb)instead of 1, 3, and 5 times the dosage. Due to the fact that very few laboratories in North America analyze for MT, and there was some question as to the accuracy of the results obtained from the outside testing laboratory, researchers at SIUC developed a method to test MT. The personnel of the Fisheries Research Laboratory at SIUC manufactured the diets utilized in this study.

The diets were color coded and then tested for MT concentration. To verify the accuracy of the testing, Rangen manufactured diets of known concentration and submitted them to the lab for analysis. The results were within 10% of the expected concentrations of MT added to the diets by Rangen.

Since the initial diets did not contain the $5 \times$ dosage, SIUC personnel incorporated 5 and 10 times the initial (15 mg/kg; 0.00024 oz/lb) dosage of MT into two different diets. Walleye were fed these diets for 60 days followed by a 30-day withdrawal. Fish were necropsied and data recorded.

OBJECTIVE 2

At the University of Wisconsin-Madison, groups of walleye fingerlings (initially 35-45 mm [1.4-1.8 in] TL) were fed diets containing MT for 60 consecutive days. Walleyes were then grown to 175 mm (6.9 in) TL and a subsample sacrificed and examined morphologically and histologically for reproductive status. Histological examination of the MT-treated walleye showed that a high percentage had identifiable testicular tissue and only a few fish had identifiable ovarian tissue. These initial observations suggest that the hormone treatments were successful at inducing sex reversal. At this time, however, the gonads of all of the fish are rather poorly developed, making positive phenotypic sex identification difficult. This problem will be resolved by delaying the remaining histological examinations until spring 1998, at which time mature, dark-staining spermatids will be easy to identify.

WORK PLANNED

Treatment groups will be subjected to seasonal temperature and photoperiod changes to promote maturation of the gonads. In the spring of 1998, histological

AQUACULTURE DRUGS: WALLEYE MT EFFICACY

evaluations and data analysis will be completed.

Data is currently being analyzed and the final report is being written.

IMPACTS

MT is not a FDA approved drug. This study will aid in the approval process. Female walleye and other percids grow faster than males, allowing culturists to obtain a market size fish in less time. Sex inversion of the brood stock eliminates the possibility of the public obtaining exogenous hormones, which may be perceived as harmful. The successful production of intersex (masculinized female) walleye brood stock should provide information required for the use of MT for the production of monosex walleye, ultimately resulting in the approval of a NADA for the use of MT in percids. The propagation of all-female populations of percids will result in improved production efficiency for the commercial aquaculture of these species, and a new management tool for resource enhancement programs.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

See the Appendix under Aquaculture Drugs.

SUPPORT

YEARS	NCRAC-	OTHER SUPPORT					TOTAL
	USDA FUNDING	UNIVER- SITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	SUPPORT
1996-97	\$27,000	\$21,375				\$21,375	\$48,375
TOTAL	\$27,000	\$21,375				\$21,375	\$48,375

A LITERATURE REVIEW TO SUPPORT THE INVESTIGATIONAL NEW ANIMAL DRUG EXEMPTION AND NEW ANIMAL DRUG APPLICATION FOR 17α-METHYLTESTOSTERONE¹⁵

Project Termination Report for the Period December 1, 1996 to November 30, 1997

NCRAC Funding Level: \$5,000 (December 1, 1996 to November 30, 1997)

PARTICIPANTS:

Ronald P. Phelps	Auburn University	Alabama
Bartholomew W. Green	Auburn University	Alabama
Industry Advisory Council	Liaison:	
Rosalie A. Schnick		Wisconsin
Extension Liaison:		
Joseph E. Morris	Iowa State University	Iowa

REASON FOR TERMINATION

The objectives of the project have been completed.

PROJECT OBJECTIVES

- (1) Conduct a thorough review of computerized scientific literature data bases.
- (2) Obtain relevant scientific papers and review.
- (3) Prepare the environmental impact statement for submission to the Food and Drug Administration's (FDA) Center for Veterinary Medicine (CVM).

PRINCIPAL ACCOMPLISHMENTS

An environmental impact statement was prepared and submitted to CVM for their review in support of submission of a New Animal Drug Application (NADA) for the use of 17α -methyltestosterone (MT) for the sex inversion of newly hatched tilapia. That document described the safe use of MT for sex inversion (sex reversal) of early life stages of tilapia (*Oreochromis* spp.) based on a thorough review of the scientific literature and interpretation of data presented in the cited papers. Specifically, environmental assessment of the proposed use of MT-treated feed for sex inversion of tilapia was addressed.

The quantity of MT-treated feed ordered by participants in the MT Investigational New Animal Drug (INAD) from March 1996 to August 1997 contained 0.48 kg (1.06 lb) of MT. The domestic tilapia production is estimated annually by the American Tilapia Association with assistance from the USDA Agricultural Statistics Center, and data are reported in the Annual Tilapia Situation and Outlook Report. In 1996, it was reported

¹⁵NCRAC has funded several projects on "Aquaculture Drugs," this being one of them. This project was co-chaired by Ronald P. Phelps and Bartholomew W. Green.

that there was a total of 7,332,396 kg (16,164,894 lb) of live tilapia produced in the United States. Assuming all of the marketable tilapia production was treated with MT, this would require approximately 30,787,000 fry to be sex reversed. The quantity of MT needed to sex reverse this number of fish is 1.3 kg (2.9 lb).

The largest tilapia farms in the United States each sex inverse approximately 4-5 million tilapia per year. Taking a worst case scenario, where all water used to hold fish during treatment is discharged, the following estimate of MT released into the environment was made. For batches of approximately 180,000 fish being treated on a biweekly basis, with an effluent water flow of 63.1 L/min (16.7 gal/min), the quantity of MT released in the effluent would be 4.4 μ g/L (parts per billion; ppb) soon after feeding and 80 ng/L (parts per trillion; ppt) just before the next feeding.

Fate of MT in the environment is not well documented. One study determined that a 17 μ g/L (ppb) testosterone dose added to a recirculating system without fish was below detectable levels after 18 h. Environmental impacts of MT on non-target organisms were not documented in the literature. When tilapia with undifferentiated gonads were held in recirculating water where other fish were being fed MT, sex ratios were altered.

It was found that MT use for sex reversal of tilapia represents only a small fraction of MT use in the United States. The majority is used in human and veterinary medicine.

IMPACTS

The direct review submission to FDA is an essential component in preparing a NADA package for the FDA approval of MT. By being able to prepare a literature review on the environmental aspects of MT use with tilapia, much effort and expense may have been avoided. The review identified areas where the literature was weak in documenting environmental aspects regarding the use of MT.

RECOMMENDED FOLLOW-UP ACTIVITIES

The submission of the environmental impact statement for the use of MT for the sex inversion of newly hatched tilapia is a significant step forward towards approval of a NADA for that compound. The impacts that were identified were considered to be minor but that was based on a minimal amount of documentation. Additional studies may be required to answer questions not well documented in the literature. Specific studies regarding the fate of MT in the environment and its effect on non-target animals may be required.

PUBLICATION

See the Appendix under Aquaculture Drugs.

YEARS	NCRAC-	OTHER SUPPORT					TOTAL
	USDA FUNDING	UNIVER- SITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	SUPPORT
1996-97	\$5,000	\$2,150				\$2,150	\$7,150
TOTAL	\$5,000	\$2,150				\$2,150	\$7,150

SUPPORT

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.

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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., and C.C. Kohler. In review. Pond culture of hybrid striped bass fingerlings in the Midwest. 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.

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- Mittelmark, J. In review. Fish health management. NCRAC Fact Sheet Series #109, NCRAC Publications Office, Iowa State University, Ames.
- Riepe, J.R. 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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- Lichtkoppler, F.P. 1993. Factors to consider in establishing a successful aquaculture business in the North Central Region.
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- Swann, L., and J. R. Riepe. 1994. Niche marketing your aquaculture products. NCRAC Technical Bulletin Series #107, NCRAC Publications Office, Iowa State University, Ames.
- Swann, L., J. Morris, and D. Selock. 1995. Cage culture of fish in the North Central Region. NCRAC Technical Bulletin Series #110, 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.
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 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., editor. 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., editor. 1996. Walleye culture manual. NCRAC Culture Series #101, NCRAC Publications Office, Iowa State University, Ames.

Other Videos

Kayes, T.B., and K. Mathiesen, editors.1994. Investing in freshwateraquaculture: a reprise (part I). VHSformat, 38 min. Cooperative Extension,Institute of Agriculture and Natural

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- 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)
- First North Central Regional 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)
- Second North Central Regional 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)
- North Central Regional Aquaculture Center Hybrid Striped Bass Workshop, Champaign, Illinois, November 2-4,

1995. (Christoper C. Kohler, LaDon Swann, and Joseph E. Morris)

Third North Central Regional Aquaculture Conference, Indianapolis, Indiana, February 6-7, 1997. (LaDon Swann)

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