NORTH CENTRAL
REGIONAL AQUACULTURE CENTER

ANNUAL PROGRESS REPORT 2007-08

JANUARY 2009

ANNUAL PROGRESS REPORT

For the Period
September 1, 2007 to August 31, 2008

January 2009

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A table of commonly used abbreviations and acronyms can be found inside the back cover.

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INTRODUCTION

The U.S. aquaculture industry is an important sector of U.S. agriculture generating a little more than $1.2 billion in 2006 for producers. 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 value of imported fisheries products has substantially increased over the last two decades. In 2007, the U.S. imported $28.7 billion of fisheries products and the trade deficit was $8.7 billion for all fisheries products, most 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 over exploited. 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 Food, Conservation, and Energy Act of 2008 (P.L. 110-246), otherwise known 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:

- Developing and submitting proposals to USDA Cooperative State Research, Education and Extension Service (USDA/CSREES) which, upon approval, becomes a grant to the Center;
- Developing appropriate agreements (sub-contracts) with other parties, including ISU for the Associate Director's office, for purposes of transferring funds for implementation of all projects approved under the grants;
- 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;
- Maintaining liaison with other RACs; and
- Serving on the NCC.

At the present time NCRAC's Office for Publications and Extension Programs at ISU is under the direction of Joseph E. Morris, Associate Director. The Associate Director has the following responsibilities:

- Coordinating, facilitating, and executing regional aquaculture extension program activities;
- Serving as head of Publications for NCRAC, including editor of the fact sheet, technical bulletin, culture manual, and video series as well as of the NCRAC Newsletter;

- Serving as the NCRAC liaison with national aquaculture extension programs, including in particular, extension programs of the other four USDA Regional Aquaculture Centers; 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 four persons from the IAC, a representative from the region's State Agricultural Experiment Stations and Cooperative Extension Services, a member from a non-land grant university, representatives from the two universities responsible for the center: Michigan State and Iowa State, and chairs of the two subcommittees of the Center's Technical Committee. 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 sub-committee 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 a 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 21 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), FY97 (#97-38500-3957), FY98 (#98-38500-5863), FY99 (#99-38500-7376), FY00 (#00-38500-8984), FY2001 (#2001-38500-10369), FY2002 (#2002-38500-11752), FY2003 (#2003-38500-12995), FY2004 (#2004-38500-14269), FY2005 (#2005-38500-15847), FY2006 (#2006-38500-16900), FY2007 (#2007-38500-18569), and FY2008 (#2008-38500-19157) with monies totaling $15,481,706. Currently, five grants are active (FY04-08); the first sixteen grants (FY88-03) have terminated.

- The Center annually coordinates a program planning meeting which typically sets priorities for the next funding cycle and calls for development of project outlines to address priority problem areas.
- Work Groups are formed which submit project outlines to the Center. The projects are peer reviewed by experts from both within and outside the region and a Project Review Committee.
- The BOD, using the Project Review Committee's recommendation and 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, Project Review Committee, 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 agents for both receiving and disbursing funds in accordance with all terms and provisions of the grants.

Through August 31, 2008, the Center has funded or is funding 80 projects through 426 subcontracts from the first 21 grants received. Funding for these Center-supported projects is summarized in Table 1 below (pages 6–8). Information about funded projects is also available at the Center's Web site (http://www.ncrac.org).

During this reporting period, the Publications Office at ISU produced and distributed a number of publications including fact sheets, technical bulletins, and videos. 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; soliciting, in coordination with the other RACs, written testimony for the U.S. House Appropriations Subcommittee on Agriculture, Rural Development, Food and Drug Administration, and Related Agencies and the U.S. Senate Appropriations Subcommittee on Agriculture, Rural Development, and Related Agencies; 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; maintaining the NCRAC Web site.

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 thirteen separately funded projects in regard to Extension and eight on Yellow Perch. 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 POWs or amendments to POWs for the grants as indicated in Table 1. Many times, the projects within a particular area are continuations of previously funded activities while at other times they are addressing new objectives. Presented below are Progress Reports for projects that were underway or completed during the period

September 1, 2007 to August 31, 2008. Projects, or Project components, that terminated prior to September 1, 2007 have been reported on in earlier documents (e.g., 1989-1996 Compendium Report and other Annual Progress Reports).

A cumulative list of all publications, manuscripts, papers presented, or other outputs for all funded NCRAC project areas is contained in the Appendix.

Table 1. North Central Regional Aquaculture Center funded projects.

Project Area	Project Number	Proposed Duration Period	Funding Level	Grant Number
Aquaculture Drugs	1	7/1/96-6/30/97	$27,000	95-38500-1410
	2	12/1/96-11/30/97	$950	95-38500-1410
	3	10/1/99-9/30/00	$8,415	97-38500-3957
	4	6/1/04-11/30/05	$223,677	2003-38500-12995
	5	7/15/04-7/14/05	$60,000	2003-38500-12995
	6	11/1/04-10/31/06	$50,000	2002-38500-11752
	7	1/1/06-12/31/06	$129,936	2005-38500-15847
			$499,978	
Baitfish	1	9/1/92-8/31/94	$61,973	92-38500-6916
	2	9/1/06-8/31/08	$111,997	2006-38500-16900
			$88,003	2005-38500-18547
			$261,973	
Conferences/Workshops/Symposia				
Environmental Strategies Symposium	1	9/1/00-5/31/01	$5,000	96-38500-2631
Nat'l. Aquaculture Exten. Workshop/Conference	1	10/1/91-9/30/92	$3,005	89-38500-4319
	2	12/1/96-11/30/97	$3,700	95-38500-1410
	3	11/1/02-10/31/03	$4,500	00-38500-8984
	4	1/1/06-12/31/06	$5,000	2005-38500-18547
			$16,205	
NCR Aquaculture Conference	1	6/1/90-3/31/91	$7,000	90-38500-5008
	2	12/9/98-6/30/99	$3,000	96-38500-2631
			$10,000	
Percis III	1	11/1/02-10/31/03	$4,000	00-38500-8984
Crayfish	1	9/1/92-8/31/94	$49,677	92-38500-6916
Economics/Marketing	1	5/1/89-12/31/91	$127,338	88-38500-3885
			$34,350	89-38500-4319
	2	9/1/91-8/31/92	$53,300	91-38500-5900
	3	9/1/93-8/31/95	$40,000	93-38500-8392
	4	9/1/99-8/31/01	$47,916	97-38500-3957
	5	9/1/03-8/31/04	$50,000	2002-38500-11752
			$352,904	

Extension	1	5/1/89-4/30/91	$39,221	88-38500-3885
("Base" Extension—Project			$37,089	89-38500-4319
Nos. 1-11; Aquaculture	2	3/17/90-8/31/91	$31,300	89-38500-4319
Regional Extension Facilitator	3	9/1/91-8/31/93	$94,109	91-38500-5900
[AREF]—Project No. 12; and	4	9/1/93-8/31/95	$110,129	91-38500-5900
Regional Aquaculture	5	9/1/95-8/31/97	$10,813	92-38500-6916
Extension Specialist			$20,391	95-38500-1410
[RAES]—Project No. 13)	6	9/1/97-8/31/99	$38,000	97-38500-3957
	7	9/1/99-8/31/01	$94,000	99-38500-7376
	8	9/1/01-8/31/03	$28,500	99-38500-7376
			$18,154	2001-38500-10369
	9	9/1/03-8/31/05	$28,000	2002-38500-11752
	10	9/1/05-8/31/07	$211,545	2003-38500-12995
			$7,735	2005-38500-15847
	11	9/1/07-8/31/09	$21,850	2006-38500-16900
			$92,469	2007-38500-18469
	12	9/1/03-8/31/05	$100,000	2002-38500-11752
	13	9/1/06-8/31/08	$225,000	2004-38500-14269
			$1,208,305	
Feed Training Carnivorous Fish	1	9/1/06-8/31/08	$165,446	2005-38500-15847
			$134,554	2006-38500-16900
			$300,000	
Hybrid Striped Bass	1	5/1/89-8/31/91	$68,296	88-38500-3885
			$68,114	89-38500-4319
	2	6/1/90-8/31/92	$101,000	90-38500-5008
	3	9/1/91-8/31/93	$96,550	91-38500-5900
	4	9/1/93-8/31/95	$168,000	93-38500-8392
	5	9/1/95-8/31/97	$150,000	95-38500-1410
	6	6/1/99-5/31/00	$15,000	96-38500-2631
	7	9/1/01-5/31/04	$98,043	98-38500-5863
			$211,957	2001-38500-10369
			$976,960	
Largemouth Bass	1	9/1/05-8/31/07	$170,000	2004-38500-14269
National Coordinator for	1	9/1/93-8/31/94	$2,000	89-38500-4319
Aquaculture INADs/NADAs		5/15/95-5/14/96	$5,000	94-38500-0048
		5/15/96-5/14/97	$6,669	92-38500-6916
			$3,331	95-38500-1410
		5/15/97-5/14/98	$15,000	96-38500-2631
		5/15/98-5/14/99	$13,241	94-38500-0048
		5/15/99-5/14/00	$10,000	95-38500-1410
	2	7/15/04-7/14/05	$9,000	2003-38500-12995
		9/15/05-8/31/06	$15,000	2004-38500-14269
		9/1/06-8/31/08	$40,000	2006-38500-16900
		5/15/08-5/14/09	$25,000	2007-28500-18469
			$144,241	
Nutrition	1	9/1/04-8/31/06	$200,000	2002-38500-11752
Salmonids	1	6/1/90-8/31/92	$9,000	89-38500-4319
			$120,799	90-38500-5008
	2	9/1/92-8/31/94	$149,997	92-38500-6916
	3	9/1/94-8/31/96	$199,290	94-38500-0048
	4	9/1/97-8/31/99	$158,656	97-38500-3957
			$637,742	

Snail Management/Grub Control	1	9/1/07-8/31/09	$225,000	2007-38500-18469
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	$173,562	94-38500-0048
	4	9/1/96-9/31/98	$199,921	96-38500-2631
	5	9/1/99-8/31/01	$199,748	99-38500-7376
	6	9/1/07-8/31/09	$80,000	2006-38500-16900
			$933,788	
Tilapia	1	9/1/96-8/31/98	$118,791	96-38500-2631
	2	9/1/98-8/31/00	$150,000	98-38500-5863
			$268,791	
Walleye	1	5/1/89-8/31/91	$177,517	89-38500-4319
	2	6/1/90-8/31/92	$111,657	90-38500-5008
	3	9/1/91-8/31/92	$109,223	91-38500-5900
	4	9/1/92-8/31/93	$75,000	89-38500-4319
	5	9/1/93-8/31/95	$150,000	93-38500-8392
	6	9/1/95-8/31/97	$117,395	94-38500-0048
			$59,835	95-38500-1410
	7	9/1/99-6/30/02	$127,000	98-38500-5863
			$927,627	
Wastes/Effluents	1	9/1/92-8/31/94	$153,300	92-38500-6916
	2	9/1/96-8/31/98	$100,000	96-38500-2631
	3	9/1/01-8/31/04	$106,186	00-38500-8984
			$88,814	2001-38500-10369
			$448,300	
White Papers	1	7/1/98-12/31/98	$4,999	96-38500-2631
	2	9/1/99-12/31/99	$17,495	97-38500-3957
			$22,494	
Yellow Perch	1	5/1/89-8/31/91	$76,957	88-38500-3885
			$85,723	89-38500-4319
	2	6/1/90-8/31/92	$92,108	90-38500-5008
	3	9/1/91-8/31/93	$99,997	91-38500-5900
	4	9/1/93-8/31/95	$150,000	93-38500-8392
	5	9/1/95-8/31/97	$199,507	95-38500-1410
	6	9/1/97-8/31/99	$185,458	97-38500-3957
	7	9/1/98-8/31/00	$92,370	98-38500-5863
	8	9/1/01-5/31/04	$326,730	00-38500-8984
			$125,016	2001-38500-10369
			$1,433,866	

PROJECT REPORTS

AQUACULTURE DRUGS: 17α-METHYLTESTOSTERONE TARGET ANIMAL SAFETY STUDY[1]

Project ***Termination Report*** for the Period
December 15, 2004 to August 31, 2008

NCRAC FUNDING: $50,000 (December 15, 2004 to December 31, 2007)

PARTICIPANT:

Anita M. Kelly	Southern Illinois University-Carbondale	Illinois
Industry Advisory Council Liaison:		
Rosalie A. Schnick	National Aquaculture NADA Coordinator	Wisconsin
Extension Liaison:		
Joseph E. Morris	Iowa State University	Iowa

REASON FOR TERMINATION

Anita Kelly is no longer at Southern Illinois University-Carbondale (SIUC) and other University personnel did not want to take over as the Principal Investigator on the grant.

PROJECT OBJECTIVES

(1) Interact with the Center for Veterinary Medicine (CVM) to determine the study design and protocol.

(2) Submit the study protocol to CVM and gain acceptance from CVM for the study protocol.

(3) Conduct a target animal safety study using 17α-methyltestosterone (MT) on tilapia according to CVM guidelines for a target animal safety study in feed under good laboratory practices (GLP).

(4) Write the final study report and submit to CVM through the MT Investigational New Animal Drug (INAD) Coordinator at Auburn University.

(5) Provide progress reports to the North Central Regional Aquaculture Center (NCRAC).

(6) Gain acceptance from CVM for the target animal safety study on MT in tilapia.

PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

The Principal Investigator for this project worked closely with CVM and the U.S. Fish

[1]NCRAC has funded seven Aquaculture Drugs projects. A termination report for the first project is contained in the 1997-98 Annual Progress Report; a termination report for the second project is contained in the 1996-97 Annual Progress Report, a termination report for the third project is contained in the 2001-02 Annual Progress Report, a termination report for the fourth project is contained in the 2006-07 Annual Progress Report, and a termination report for the seventh project is contained elsewhere in this report. A fifth project, which provided $60,000 for a portion of the funds required to purchase sufficient radiolabeled AQUI-S® for use in a total residue depletion study in rainbow trout, is also reported on under the progress report for the National Coordinator for Aquaculture New Animal Drug Applications (NADAs) elsewhere in this report. This termination report is for the sixth Aquaculture Drugs project which was undertaken by Anita M. Kelly. It was a 2-year project that began December 15, 2004.

and Wildlife Service Aquatic Animal Drug Approval Partnership program (USFWS AADAP) which holds the INAD under which this research is being conducted to design and develop an acceptable protocol.

OBJECTIVE 2

The first protocol was submitted on August 8, 2005 to the USFWS AADAP which holds the INAD and must submit all protocols to CVM. They submitted the protocol on August 30, 2005. On December 7, 2005 CVM responded to the protocol submission, to AADAP, and found the protocol unacceptable. The AADAP forwarded the comments to Kelly on January 12, 2006. This correspondence included CVM's detailed explanation with a list of items they wanted corrected. CVM's concerns to the protocol were addressed and the protocol rewritten and sent to AADAP for review and comment. A revised protocol was sent to AADAP on May 2, 2006 and to CVM on May 16, 2006. The revised protocol was reviewed by CVM and the reply sent to AADAP on August 14, 2006. CVM found this protocol unacceptable and the AADAP forwarded the concerns of CVM to Kelly on August 18, 2006. The protocol was revised to address the new concerns of CVM. This revised protocol was accepted by CVM in February 2007.

OBJECTIVES 3-6

The target animal safety study was completed through the necropsy of the fish. Pathology has not been conducted on the fish used in this study. During the study, the laboratory was audited by the Food and Drug Administration (FDA) for GLP compliance. During the audit, the inspectors felt that too many fish were "missing" from the tanks. Cannibalism had been noted when apparent. The fish were netted from the tanks weekly, counted by two individuals, and the lengths of 10 random individuals were measured and recorded. In addition to the missing fish, the FDA audit noted several other noncompliant items including feed discrepancies and lack of an official assignment of a Study Director in the absence of the assigned Study Director.

During the course of the study, the Study Director was in communication with CVM regarding the problems with GLP inspection. It was decided on August 17, 2007 that CVM could not accept the study as conducted due to numerous noncompliant items.

IMPACTS

The ability of culturists to produce fish that exhibit uniform growth while expending little to no energy toward reproduction will increase the profits and production from a facility. Currently, determination of the gender of tilapia by visual inspection is relatively difficult until the fish have attained sexual maturity. Sex reversal of fish prior to sexual differentiation in most cases enables the production of monosex populations. Under an existing INAD, tilapia are being sex reversed to create all male populations using MT. However, in order for this hormone to be approved by the FDA, a target animal safety study must be conducted and approved by CVM.

RECOMMENDED FOLLOW-UP ACTIVITIES

A new MT target animal safety study is to be conducted by personnel at the Harry K. Dupree Stuttgart National Aquaculture Center (SNARC). Kelly will be in contact with the researchers at SNARC to identify areas that were deemed problems in the GLP inspection. This collaborative effort should increase the probability of obtaining approval for the target animal safety study.

SUPPORT

NCRAC provided $50,000 to SIUC which was the entire amount of funding allocated for this project.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

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

AQUACULTURE DRUGS: DETERMINATIVE METHOD FOR THE AQUI-S® MARKER RESIDUE IN FILLET TISSUE[2]

Project ***Termination Report*** for the Period
January 1, 2006 to August 31, 2008

NCRAC FUNDING: $129,936 (January 1, 2006 to December 31, 2006)

PARTICIPANTS:

Jeffrey R. Meinertz	Upper Midwest Environmental Sciences Center	Wisconsin
Industry Advisory Council Liaison:		
Rosalie A. Schnick	National Aquaculture NADA Coordinator	Wisconsin
Extension Liaison:		
Joseph E. Morris	Iowa State University	Iowa

REASON FOR TERMINATION

All work was completed. A final report describing the validation of a proposed determinative method for the AQUI-S® marker residue was submitted to U.S. Food and Drug Administration's Center for Veterinary Medicine (CVM). However because of a ruling identifying isoeugenol (active ingredient of AQUI-S®) as a carcinogen, Objective 4 could not be completed.

PROJECT OBJECTIVES

(1) Interact with the CVM to determine the requirements and procedures to develop and validate a determinative analytical method for the AQUI-S® marker residue in all cool and warm water species of fin fish.

(2) Develop and validate a determinative analytical method for the AQUI-S® marker residue in all cool and warm water species of fin fish according to CVM guidelines for method development under Good Laboratory Practices.

(3) Write the final study report and submit the report to an Investigational New Animal Drug (INAD) number established by CVM for AQUI-S®.

(4) Gain acceptance from CVM for the determinative analytical method for the AQUI-S® marker residue that will help support the approval of AQUI-S® for short-exposure handling for all cool and warm water species of fin fish.

[2]NCRAC has funded seven Aquaculture Drugs projects. A termination report for the first project is contained in the 1997-98 Annual Progress Report; a termination report for the second project is contained in the 1996-97 Annual Progress Report, a termination report for the third project is contained in the 2001-02 Annual Progress Report, a termination report for the fourth project is contained in the 2006-07 Annual Progress Report, and a termination report for the sixth project is contained elsewhere in this report. A fifth project, which provided $60,000 for a portion of the funds required to purchase sufficient radiolabeled AQUI-S® for use in a total residue depletion study in rainbow trout, is also reported on under the progress report for the National Coordinator for Aquaculture New Animal Drug Applications (NADAs) elsewhere in this report. This termination report is for the seventh Aquaculture Drugs project which is being undertaken by Jeffrey R. Meinertz. It was a 1-year project that began January 1, 2006.

PRINCIPAL ACCOMPLISHMENTS

A study protocol was developed and submitted to CVM. The protocol was returned with review comments which were used to revise the protocol.

The chemical purity of the test chemical was verified with high performance liquid chromatography techniques.

The instrument (high performance liquid chromatography system) detection and quantitation limits were determined for isoeugenol analytical standards prepared with 90:10 methanol:water.

The loss of isoeugenol from solutions prepared with 90:10 methanol:water was evaluated periodically through a 21-day storage period.

Fillet tissue from unexposed fish was acquired from the following species: brown trout (*Salmo trutta*), channel catfish (*Ictalurus punctatus*), fall Chinook salmon (*Oncorhynchus tshawytscha*), hybrid striped bass (*Morone saxatilis* × *M. chrysops*), lake trout (*Salvelinus namaycush*), largemouth bass (*Micropterus salmoides*), northern pike (*Esox lucius*), walleye (*Sander vitreus*), and yellow perch (*Perca flavescens*). The fillet tissue from each species was homogenized with dry ice in preparation for impending studies requiring homogenized control fillet tissue.

Homogenized control fillet tissue from lake trout was processed with the proposed determinative method for an evaluation of chromatographic interference that would interfere with the determination of isoeugenol concentrations in lake trout fillet tissue.

Brown trout, channel catfish, hybrid striped bass, lake trout, largemouth bass, northern pike, walleye, and yellow perch were exposed to AQUI-S® (a separate exposure for each species) for the purpose of generating biologically-incurred isoeugenol residues in the fillet tissue. Generation of fillet tissue with biologically-incurred isoeugenol was necessary for the evaluating method precision with fillet tissue containing biologically-incurred isoeugenol residues and for evaluating isoeugenol stability in fillet tissue stored at <-70°C (-94°F).

The precision of the proposed determinative method was evaluated with brown trout, channel catfish, hybrid striped bass, lake trout, largemouth bass, northern pike, walleye, and yellow perch fillet tissue containing biologically-incurred isoeugenol.

The loss of isoeugenol from fillet tissue containing biologically-incurred isoeugenol and stored for about 1 month at <-70°C (-94°F) was evaluated with brown trout, channel catfish, hybrid striped bass, and lake trout fillet tissue.

Samples of homogenized control fillet tissue were processed with the proposed determinative method for an evaluation of fillet constituents that would interfere with the determination of isoeugenol concentrations. Control tissue from the following species was processed: brown trout, channel catfish, fall Chinook salmon, hybrid striped bass, largemouth bass, northern pike, walleye, and yellow perch.

The method detection and quantitation limits were determined with isoeugenol-fortified fillet tissue as were method accuracy and within-day precision from the following species: brown trout, channel catfish, fall Chinook salmon, hybrid striped bass, lake trout, largemouth bass, northern pike, walleye, and yellow perch.

The method accuracy and within-day precision were determined with isoeugenol-fortified fillet tissue from the following species: brown trout, channel catfish, Fall Chinook salmon, hybrid striped bass, lake trout, largemouth bass, northern pike, walleye, and yellow perch.

The method day-to-day precision was determined with isoeugenol fortified fillet tissue from channel catfish.

The loss of isoeugenol from extracts generated with fortified fillet tissue was determined after 1, 7, and 14 days of storage. Control tissue from the following species was fortified with isoeugenol: brown trout, channel catfish, fall Chinook salmon, hybrid striped bass, lake trout, largemouth bass, northern pike, walleye, and yellow perch.

The loss of biologically-incurred isoeugenol from fillet tissue from the following species and stored at <-70°C (-94°F) was determined after 1, 2, 3, 4, 5, and 6 months: largemouth bass, northern pike, walleye, and yellow perch.

The loss of biologically-incurred isoeugenol from fillet tissue stored at <-70°C (-94°F) was determined after 2, 3, 4, 5, and 6 months of storage. Fillet tissue from the following species was assessed: brown trout, channel catfish, hybrid striped bass, and lake trout.

The loss of biologically-incurred isoeugenol from fillet tissue was determined after subjecting fillet tissue to three freeze/thaw cycles. Fillet tissue from the following species was assessed: brown trout, channel catfish, hybrid striped bass, lake trout, largemouth bass, northern pike, walleye, and yellow perch.

In summary, the method was developed to use relatively common procedures and equipment. The procedures include extracting isoeugenol from tissue with acetonitrile, evaporating the acetonitrile from the extract with rotary evaporation techniques, changing the polarity of the extract by adding water, concentrating the isoeugenol with solid phase extraction procedures, and determining concentrations with high pressure liquid chromatography.

The method is robust, i.e., the method will produce accurate and precise results with fillet tissue from the following fish species: brown trout, channel catfish, Chinook salmon, hybrid striped bass, lake trout, largemouth bass, northern pike, walleye, and yellow perch.

The method is accurate, i.e., the percentage of isoeugenol recovered from samples fortified with isoeugenol at nominal concentrations of 1, 50, and 100 µg/g for all species was always >80.3% and <96.5%.

The method is repeatable, i.e., the within-day precision for samples fortified at nominal concentrations of 1, 50, and 100 µg/g for all species was ≤8.5% relative standard deviation (RSD). The day-to-day precision with fillet tissue fortified at a nominal isoeugenol concentration of 1, 50, and 100 µg/g is ≤3.0% RSD. The method precision with tissue from all species containing biologically-incurred isoeugenol was ≤8.1% RSD with the exception of fall Chinook salmon (live fish were not available).

The method is specific, i.e., there are no chromatographic interferences in extracts from control fillet tissue from brown trout, channel catfish, hybrid striped bass, walleye, and yellow perch and only minimal interferences (<0.11 µg/g, isoeugenol

equivalent concentration) in the extracts from control fillet tissue from lake trout, largemouth bass, and northern pike. More notable interference was found in the fillet tissue extracts from fall Chinook salmon (0.20 to 0.52 µg/g, isoeugenol equivalent concentration).

The method is sensitive, i.e., the method detection limits for all species, except for fall Chinook salmon, range from 0.004 to 0.014 µg/g and the quantitation limits range from 0.012 to 0.048 µg/g. The method detection limit for fall Chinook salmon is 0.99 µg/g and the method quantitation limit is 3.3 µg/g.

Isoeugenol in the various matrices was moderately stable. Loss of isoeugenol was insignificant in 90:10 methanol:water solutions with nominal isoeugenol concentrations of 0.1 and 10 µg/mL stored for at least 14 days. Isoeugenol concentration changes are <10% in fillet tissue extracts from 6 of 9 species with nominal isoeugenol concentrations of 1, 50, and 100 µg/mL stored for 14 days. Isoeugenol concentration changes are <10% in fillet tissue from all species stored at <-70°C (-94°F) for 6 months. Isoeugenol concentration changes are <10% in fillet tissue from 6 of 8 species subjected to freeze/thaw cycles.

A comprehensive final report describing the study results was reviewed for accuracy and compliance with FDA regulations for good laboratory practices by the Upper Midwest Environmental Sciences Center (UMESC) Quality Assurance Officer. Because of the following statement issued in late April 2007, submission of the report to CVM for review and submission to INAD number 11-475 for AQUI-S® was postponed:

"Isoeugenol (the active ingredient in AQUI-S®) has been under evaluation by the National Toxicology Program (NTP), an interagency program whose mission is to evaluate chemical agents for potential public health risks. Recently, NTP was forced to delay the review of their nearly completed two-year toxicology studies on isoeugenol until February 2008 because of higher priorities. Although the study data have not been fully analyzed, the preliminary assessments of the data do not eliminate the possibility that isoeugenol residues in treated fish could pose a human health risk.

Because we need to be absolutely certain that there are no human food safety issues that would preclude the approval of AQUI-S®, the U.S. Fish & Wildlife Service (FWS) and the U.S. Geological Survey (USGS) and other participating partner groups have agreed to institute interim measures that will be effective until the NTP meeting in February 2008. Effective April 27, 2007, all ongoing and planned AQUI-S® research funded under the Association of Fish and Wildlife Agencies' Multi-State Conservation Grant, and allied work supported with federal base funds of FWS and USGS will be suspended until the completion of the NTP review. Additionally, FWS will temporarily suspend all field activities under their Investigational New Animal Drug exemption for AQUI-S® until the NTP review is complete.

Although the decision to temporarily suspend all publicly funded AQUI-S® research activities was not an easy decision to make, as responsible stewards of public funds it is the correct course of action. It should be noted that

> significant portions of the data necessary to address many of the original AQUI-S® goals and objectives of the Federal-State Aquaculture Drug Approval Partnership Project have already been generated. It is also important to note that USGS is constrained from further development of residue chemistry data until a tolerance value for the residues has been established by the Center for Veterinary Medicine. This work cannot be initiated until the results of the NTP studies are finalized. We look forward to the opportunity of continuing our collaborative AQUI-S® research efforts in February 2008."

Then in early March 2008, the following statement concerning the status of AQUI-S® was posted:

> "Isoeugenol (the active ingredient in AQUI-S®) has been under evaluation by the National Toxicology Program (NTP), an interagency program whose mission is to evaluate chemical agents for potential public health risks. Initial results reported from the NTP studies resulted in cessation of drug approval efforts for AQUI-S® by the federal partners on the Association of Fish and Wildlife Agencies (AFWA) Drug Approval Working Group (DAWG) on April 27, 2007. On February 28, 2008, the NTP peer review panel confirmed that there is clear evidence of isoeugenol carcinogenicity in male mouse livers; there was no or equivocal evidence of carcinogenicity for the female mouse and male and female rat. Finding clear evidence of carcinogenicity in the male mouse triggered the Delaney Clause, a 1958 amendment to the Food, Drugs, and Cosmetic Act (FDCA). The clause states that "the Secretary of the Food and Drug Administration shall not approve for use in food any chemical additive found to induce cancer in man, or, after tests, found to induce cancer in animals". The Center for Veterinary Medicine (CVM) recently stated that it was "very, very unlikely" that a zero withdrawal period could be gained for isoeugenol based on the NTP interpretation of the results of the male mouse study and the application of the Delaney Clause to the FDCA."

A decision was made to submit to CVM a comprehensive final report that summarized the results of this work. The report was titled "Evaluation of a proposed determinative method for determining concentrations of isoeugenol in fillet tissue from cold, cool, and warm water fish species." The report was submitted for inclusion into INAD number 11-475. We did not request review of the report at this time. We did request that the report be forwarded to the Center for Food Safety and Applied Nutrition for their determination of whether or not the method described in the report could be used in their monitoring program.

IMPACTS

To support FDA approval of a new animal drug for fish, a series of toxicology and residue chemistry studies are conducted to demonstrate the safety of food products derived from treated fish. Mammalian toxicology studies determine if the drug is safe for humans to consume and the amount of drug residues that can be consumed daily for a lifetime without causing adverse effects (acceptable daily intake; ADI). Considering the amount of tissue consumed in a lifetime, the ADI is used to calculate a safe concentration for all of the drug's residues in the edible tissue.

Residue chemistry studies are conducted to assess drug residues in the edible fillet tissue from treated fish. First a total drug residue depletion study is conducted to identify all of the drug's residues in the edible fillet tissue and characterize the depletion of those residues from the fillet. Based on data from this study, a marker residue is selected. The marker residue is one compound or group of compounds that will represent all of the drug's residues in subsequent depletion studies.

After selection of a marker residue, analytical methods for the marker residue are developed and validated. Two methods are required, a determinative method (activities described in this report were conducted to fulfill requirements for a determinative method) and a confirmatory method. The determinative method quantifies concentrations of the marker residue in edible tissue. The confirmatory method confirms the results from the determinative method and provides irrefutable identification of the marker residue in the tissue.

After validating a determinative method, marker residue depletion studies are conducted. Data from these studies are used in conjunction with the safe concentration to determine a tolerance concentration for the marker residue, as well as a withdrawal time. The tolerance concentration is the concentration of the marker residue in the edible tissue that represents the safe concentration (the concentration of all drug residues that is considered to be safe). The withdrawal time is the time it takes for the fish to deplete all drug residues to the safe concentration.

The total residue depletion study for AQUI-S® was completed in 2005. Based on the results from the total residue depletion study, isoeugenol would most likely have been selected as the marker residue. However, because of the information described in the notifications previously presented, all FDA decisions concerning AQUI-S® were postponed, including the selection of a marker residue. Nonetheless, all work validating a determinative method for the probable marker residue was completed. With the completion of that work, we would have been poised to develop and validate a confirmatory method for the probable marker residue as well as conduct the AQUI-S® marker residue depletion studies. Because of the decision to stop all work concerning AQUI-S®, we cannot continue developing data for AQUI-S®.

RECOMMENDED FOLLOW-UP ACTIVITIES

If a decision is made in the future to pursue AQUI-S® as an anesthetic with a longer withdrawal time, the next steps toward an approval will be for FDA to calculate an ADI, calculate a safe concentration, accept data from the total residue depletion study, officially select isoeugenol as the marker residue, and review and accept data from the validation of the determinative method. Additionally, a confirmatory method will need to be developed and validated for the marker residue and at least three marker residue depletion studies will need to be conducted. Data from those studies would be submitted to the FDA for their review and acceptance.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

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SUPPORT

YEAR	NCRAC-USDA FUNDING	OTHER SUPPORT					TOTAL SUPPORT
		UNIVER-SITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
2006	$129,936			$30,044[a]		$30,044	$159,980
TOTAL	$129,936			$30,044		$30,044	$159,980

[a]UMESC salary costs for a GS13 and GS11 (4 pay periods each) that were accrued during the 4th quarter of calendar year 2006.

BAITFISH[3]

Project ***Progress Report*** for the Period
September 1, 2006 to August 31, 2008

NCRAC FUNDING: $200,000 (September 1, 2006 to August 31, 2008)

PARTICIPANTS:

Fred P. Binkowski	University of Wisconsin-Milwaukee	Wisconsin
Gregory J. Fischer	University of Wisconsin-Stevens Point	Wisconsin
Jeffrey L. Gunderson	University of Minnesota-Duluth	Minnesota
Joseph E. Morris	Iowa State University	Iowa
Jeffrey A. Malison	University of Wisconsin-Madison	Wisconsin
Industry Advisory Council Liaison:		
Phil Goeden	Goeden Fisheries, Alexandria	Minnesota
Extension Liaison:		
Jeffrey L. Gunderson	University of Minnesota-Duluth	Minnesota
Non-Funded Collaborators:		
Barkhausen Waterfowl Reserve	Brown County	Wisconsin

PROJECT OBJECTIVES

(1) Determine what techniques and strategies for early season, indoor spawning of golden shiners and subsequent stocking into ponds will result in growth to 76 mm (3 in) by November 1 of that year.

(2) Develop economically viable culture techniques and strategies for growing spotfin shiners to a market size (greater than 51 mm [2 in]).

(3) Provide regular research updates related to this project to the baitfish industry through Web-based technologies, newsletters, fact sheets, workshops, and/or technical bulletins.

ANTICIPATED BENEFITS

This project addresses priority needs identified by the North Central Regional Aquaculture Center (NCRAC) Industry Advisory Council (IAC). In a survey conducted in late 2006, the NCRAC IAC members were asked to rank the value of research topics being undertaken by NCRAC. The members ranked "Baitfish" as being "Very Important," second only to disease and health issues. From the proposed investigation, culture techniques for golden and spotfin shiners will be developed for the North Central Region (NCR) that will aid in overcoming specialized spawning requirements and regional thermal constraints. The development of techniques for producing fry earlier in the growing season so that they can be stocked into ponds concurrent with the onset of natural spawning cycles, will

[3]This is a 2-year project that is chaired by Joseph E. Morris and began September 1, 2006.

allow for grow out to market size within one growing season. This research explores a potentially economically viable solution to advance baitfish culture in the NCR.

PROGESS AND PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

Iowa State University (ISU) staff were successful at developing a strategy for early season spawning of golden shiners. Age-0 and -1 golden shiners brood stock were obtained from the University of Pine Bluff-Arkansas in the fall of 2006. Initially, fish were held indoors under "winter" conditions, i.e., 10°C (50°F) water temperature and a photoperiod of 8 h light/16 h dark. Fish were feed a 32% protein diet at 2% body weight twice weekly. Brood stock were held under these conditions for 10 weeks. Following this "winter" period, temperature and photoperiod were gradually increased over a 2 week transition period to "spring" conditions, i.e., 22°C (72°F) and a photoperiod of 16 h light/8 h dark. Once the tanks were under "spring" conditions, commercial spawning mats were placed into the tanks, just under the water surface. After spawning, the egg-covered mats were then transferred to hatching tanks. Once the eggs had hatched, one of nine commercially available diets were used to feed the newly hatched fry. At this stage, ISU staff determined that too many eggs and fry were not surviving due to the presence of fungus on the mats; cool water temperatures combined with excess feed caused excessive fungal growth. In addition, it was difficult to obtain reliable egg and fry counts using this technique. To overcome this problem, ISU staff began utilizing a technique in which the egg-covered spawning mats were immersed for 2–2½ min in a 1.5% sodium sulfite solution bath. This caused the eggs to drop out of the mat after which they were place in hatching jars. This method allowed for enumeration of the eggs as well as the culture of the fry in tanks without spawning mats, thus eliminating fungal growth.

In 2007 six additional diets were evaluated and in 2008 three more diets were evaluated against the best performing diet from the 2007 trials. Stocking rates ranged from 8–40 fry/L (30–151 fry/gal). In 2007, only one diet, Zeigler AP100™, resulted in any survival of fry. That diet was then used in 2008 as the control for additional pair-wise comparisons of the three additional diets. Results from the 2008 culture season showed the Zeigler AP100™ diet again yielded the best survival; mean survival ranged from 1–28%, while the other three diets had mean survival that ranged from 4–6%. Results from this study show that more effort needs to be put into developing a more nutritionally complete diet for golden shiners. In addition, there is need to refine better culture techniques for growth and survival in indoor tank systems. Both better feeds and improved culture methods are needed to support the growth of the golden shiner industry in indoor systems.

In a related project, the efficacy of hydrogen peroxide (H_2O_2) to control fungal (Saprolegniasis) infections of golden shiner eggs was evaluated in two experiments. Golden shiner eggs were exposed in a 15-min static bath (21°C; 70°F) to 0, 50, 100, and 200 mg/L in the first experiment, and 0, 200, 400, and 800 mg/L in the second experiment. All treatments were based on amount active ingredient (30% active ingredient concentration of H_2O_2) in a single treatment. Three replicates of each concentration were used in both experiments. The objective was to determine the H_2O_2 concentration that would result in optimum hatching successes. The hatching rate significantly increased in

each treatment level until 800 mg/L. The mean percent egg hatchability (± S.E.) at 400 and 800 mg/L was 72.3 ± 8.55 and 68.2 ± 5.03, respectively. Regression analysis revealed the peak treatment level to be between 400 and 800 mg/L.

Because of the low fry survival in both 2007 and 2008, ISU researchers were not able to complete the original project objectives, i.e., use of out-of-season fry in ponds. Instead the objectives of the pond portion of ISU's studies were modified to (1) evaluate the growth of golden shiner fry in ponds using two fertilization regimes, one a combination of organic and inorganic fertilizers and the other organic fertilizer only, and (2) evaluate diet selection of fry in ponds using those two fertilization regimes.

To accomplish these objectives, six 0.08 ha (0.20 acre) ponds were each stocked with 360 golden shiner brood stock, total weight of 4.2 kg (9.3 lb) per pond. The fish were then allowed to spawn naturally on spawning mats that were staked on the edge of the pond slightly below the water surface. After the spawning activity concluded, the brood stock were left in the pond with the resulting fry and cultured for 180 days. All ponds received organic fertilization which consisted of one application of soybean meal at a rate of 9.1 kg (20.1 lb)/pond/week followed by weekly applications at a rate of 4.5 kg (9.9 lb)/pond/week for 5 weeks. Three of the six ponds also received inorganic nitrogen (36-0-0) fertilizer for 4 weeks at a rate which gave a nitrate-nitrogen to total phosphorus ratio (NO_3-N:TP) of 7:1. Water temperature, dissolved oxygen, and pH were all within acceptable ranges for golden shiner pond culture throughout the study period. Nitrite levels were low in both treatments throughout the culture period. Ammonia-nitrogen (TAN) had the largest difference between treatments with the inorganic-organic (mixed) fertilization treatment having elevated TAN levels compared to the organic only fertilizer treatment. Golden shiner fry collected at harvest in the organic only fertilization treatment averaged 71.2 ± 8.8 mm (2.8 ± 0.3 in) in length and 4.6 ± 2.6 g (0.16 ± 0.09 oz) in weight while those harvested in the mixed fertilization treatment averaged 82.2 ± 4.0 mm (3.2 ± 0.2 in) and 4.9 ± 0.8 (0.17 ± 0.03 oz). Both treatments yielded fish in excess of the target size (76 mm; 3 in) for this objective. The average total weight of age-0 golden shiners harvested from the organic only treatment ponds was 43.0 ± 11.9 kg (94.8 ± 26.2 lb) and 43.8 ± 5.1 kg (96.6 ± 11.2 lb) in the mixed fertilization treatment. Production from this experiment in total weight ranged from 239.7–690.2 kg/ha (213.9–615.8 lb/acre) in the organic only treatment and 429.1–646.2 kg/ha (382.8–576.5 lb/acre) in the mixed fertilization treatment. The average length, weight, total weight, and fish numbers were not significantly different ($P<0.1$) between treatments and the preferred food item in both treatments were cyclopoid copepods and the cladoceran, *Chydorus*.

Age-0 and -1 fish from the 2007 culture season were collected from the ponds and placed in the indoor spawning tanks to repeat the earlier tank rearing study using different commercial diets. Results from the 2007 and 2008 feeding trials have been previously noted in this report. As there was again limited fry survival in the spring 2008 feeding trials, the decision was made to stock the ponds with either adults (similar stocking rate used in 2007) or with eggs obtained from out-of season spawning. The objective was to investigate if the use of eggs alone (600,000 eggs/ha; 242,820 eggs/acre) would yield fish that were of a more consistent size distribution compared to the use of brood stock. All ponds in 2008

were fertilized with the combination of organic and inorganic fertilizers that was used in the 2007 study; ponds were then managed for the same time period as 2007.

OBJECTIVE 2

Staff from the University of Wisconsin-Milwaukee facility collected wild brood stock of spotfin shiners from rivers in southeastern Wisconsin which were acclimated to laboratory conditions (temperature, photoperiod, food, and holding tanks). The wild fish accepted standard commercial feeds after 7–10 days of feed training. One group of adults was maintained at seasonal temperature conditions (5–25°C; 41–77°F) and the second group was kept at a constant temperature of 23–25°C (73–77°F). Following seven months of holding adults under laboratory conditions and constant temperature, spawning substrates were placed into each brood stock tank. Within 2–3 days, fish exhibited very active spawning behavior. Gametes were deposited on the spawning substrate apparatus over a period of 10–20 days. Substrate plates with fertilized eggs were placed in incubation tanks and embryos hatched in 7–10 days, resulting in thousands of sac fry.

The young fish were fed Green Tank Water (GTW) and commercial diets from the onset of first-feeding. Survival to the larval stage was estimated to be greater than 50%. However, mortality increased following the post-larval stage resulting in an estimated survival of 5–8% for post larvae to sub adults. These fish were held at a constant temperature 23–25°C (73–77°F) for the first year. Spawning behavior was exhibited by these age-1 fish; however, gamete production was poor.

The group of adult spotfins held at seasonal temperatures exhibited spawning behavior following 10 months of being held under laboratory conditions. Spawning activity was very good and gamete production was excellent. Spawning was observed for the fish from mid-June to mid-August. Embryos hatched in 7-10 days, resulting in thousands of sac fry. The early life stage feeding consisted of GTW and commercial diets. Again survival was estimated to be greater than 50% to the post-larval stage. High mortality following the post-larval stage resulted in an estimated survival of 5–8% for post larvae to sub adults. These fish were held at a constant temperature of 23–25°C (73–77°F) for the first year. Spawning behavior was exhibited by these age-1 fish; however, gamete production was poor.

The young fish from the 2007 season and constant temperature brood stocks were combined and conditioned to seasonal temperatures. In 2008 these age-2 fish exhibited excellent gamete production. From June–August 2008, fertilized egg deposition ranged from excellent to fair. A spawning apparatus was designed to maximize egg deposition and fertilization. This apparatus represents a cabinet/drawer concept. Spawning plates with fertilized eggs could easily be removed without disturbing adjacent plates. This resulted in less fungal growth of egg plates. Fertilized egg plates were removed and placed in an incubation apparatus. This incubation apparatus provided an easy in/out access of the plates resulting in minimal fungal growth and improving hatching success.

The staff at the University of Wisconsin-Stevens Point (UW-Stevens Point) Northern Aquaculture Demonstration Facility (NADF) and the University of Wisconsin-Madison (UW-Madison) could not conduct

their studies as originally planned because of issues regarding the interstate transport of fish that arose subsequent to the outbreak of viral hemorrhagic septicemia (VHS) in the Great Lakes. Because of these issues, the number of adult-sized fish that could be obtained for the 2007 and 2008 studies were limited. The limitation on brood fish, in turn, led to a reduction in number and a delay in time at which fry became available. Additionally, in 2008 the extreme flooding in the region precluded the conduct of any meaningful pond-based studies. Researchers will attempt to conduct pond studies in 2009.

In the spring of 2007, NADF staff set up multiple 227 and 1,514-L (60 and 400-gal) tanks for holding, spawning, and incubation of spotfin shiners and eggs. These tanks were plumbed for both flow through and recirculating aquaculture system capabilities. NADF staff collected adult spotfin shiners in April 2007 from the Wapsipicon River, Iowa with the assistance of Joe Morris, ISU. These fish were examined at the collection site by Dr. Dave Starling, Aquaveterinary Services, Iowa. Additional adult spotfin shiners were obtained from a private Minnesota bait supplier with the assistance of Jeff Gunderson, Minnesota Sea Grant in June 2007. These fish were examined in Minnesota by Dr. Glen Zebarth. All Wisconsin fish import regulations and permits were followed. The Iowa fish were kept separate from the Minnesota fish and both groups were successfully feed trained to a commercial trout diet. Despite the fact that both fish groups were subject to a veterinary fish health inspection, some disease issues have arisen with both groups of fish at NADF.

The fish accepted a commercial trout diet and were kept in temperatures of 18–21°C (64–70°F) during spawning. Water quality parameters were maintained at adequate levels to provide a good rearing environment. Several types of spawning substrates were placed into rearing tanks during the spring of 2008. Adult fish (52–112 mm; 2.0–4.4 in) responded to substrates immediately with active spawning behavior and swarming around the substrates. This behavior was captured with an underwater video camera. Four different types of substrates were utilized for collecting gametes in the tanks, these were:

1) flat style 483 × 101 × 64 mm (19.0 × 4.0 × 2.5 in) with cedar shingles horizontally layered with 2–5 mm (0.08–0.20 in) crevices suspended in the tank on rope and brick
2) square vertical 152 × 152 × 127 mm (6.0 × 6.0 × 5.0 in) cedar shingles layered on threaded rod that was hung on the side of tank with crevices 5–10 mm
3) cinder blocks 25 × 25 × 381 mm (1.0 × 1.0 × 15.0 in) with smaller blocks placed inside with crevices between 3–8 mm
4) aluminum siding layered and suspended on a rope and brick in the tank.

The flat style substrate performed the best for collecting gametes and protecting them from being consumed by fish in the tank. Substrates were removed from tanks within 3–5 days after eggs were deposited on over 50% of available surface to reduce loss to eggs being consumed. Substrates were placed into prepared multiple 227-L (60-gal) tanks connected to the recirculating system for incubation and hatching. NADF staff also utilized some agricultural "horse tanks" set up as a small pond for hatching eggs. Eggs hatched within 5–7 days at 18–21°C (64–71°F), resulting in thousands of <5.0 mm (0.2 in) fry. Newly hatched fry were

initially lethargic and non swimming but became photopositive and strong swimming within a few days. Fry were fed commercial starter diets of several types supplemented with pond water and 24 h lighting. Biomarine Artemac produced the best results with fry at NADF. Fry were observed with feed in stomachs after a few days. Survival of fry to fingerling size was <10%. Average growth rate from fingerlings examined was 0.4 mm/day (0.016 in/day) at 19–21°C (66–70°F) in the recirculating system on a commercial trout diet.

NADF staff also utilized a 1.5% sodium sulfite solution bath and immersed substrates containing eggs for 2–3 min, which caused eggs to drop off the substrates. These eggs were placed into a hatching jar for incubation with no formalin treatment. Within 3 days these eggs were covered in fungus and died.

Multiple age fingerlings from this spring/summer egg hatch are being reared in the NADF recirculating system and growth data is being collected. Brood stock adult spotfins are being held in a commercial recirculating system. These fish will be cold banked throughout the winter in preparation for spawning in the spring of 2009.

The primary problem that has been faced in this project is not being able to produce enough fry at any one time to fully stock production ponds. Despite holding over 2,000 mature brood stock in tanks, staff have not been able to collect more than 5,000 fry in any single week. This has made the conduct of the proposed studies problematic. In our opinion, this is a major problem that will impede the development of this species as a viable commercial baitfish produced in ponds.

Strong swimming, photopositive fry were collected and delivered to the UW-Madison facilities at the Lake Mills State Fish Hatchery at three times during the spawning time frame. These fry were stocked into 2 fertilized outdoor rearing ponds at approximately 25,000 fish/ha (61,774 fish/acre). When the fish in one pond reached 15–25 mm (0.6–1.0 in) staff began regularly feeding them a formulated food, which they readily accepted. In the autumn both ponds were harvested, but only 10% of the stocked fish were recovered. The fish had a mean size of 35 mm (1.4 in). The intent was to continue growing these fish in tanks, but the failure of a water heater resulted in all of the fish being killed.

OBJECTIVE 3

Gunderson, in his role as extension liaison for this project, has presented the results of the baitfish project at the NCRAC Annual Program Planning Meetings in both 2007 and 2008. As stated in the proposal, he was to assist in the procurement of spotfin shiner brood stock. This proved to be difficult in that only one producer was able to provide 7.6-L (2.0-gal) of spotfin shiner brood stock to NADF in June 2007. Gunderson also facilitated one conference call among the researchers to discuss the status of their research efforts and delivered an underwater video camera and recorder to NADF to allow video recording of spotfin shiner spawning activities. Several hours of video have been taken. The primary activities related to this objective will occur once the research has provided results at which point outreach connection with the industry can begin.

WORK PLANNED

OBJECTIVE 2

In November 2008, fish will be harvested from the ponds. Results from the 2008 pond

study will then be analyzed and reported in the termination for this project.

The staff at the University of Wisconsin-Milwaukee have in place a research strategy for 2008–2009 which includes work on the early life stage culture techniques with specific emphasis on nutrition to improve survival. They will also continue to evaluate the new designed spawning and incubation apparatus.

In 2009 UW-Stevens Point NADF and UW-Madison staff will attempt one more time to produce enough fry for pond stocking, and attempt to gather production data on raising this species in ponds and recirculating aquaculture systems.

OBJECTIVE 3
The University of Minnesota-Duluth staff will also examine the video footage of the spawning of spotfin shiners captured at the NADF. This footage will be used to demonstrate the crevice spawning behavior of spotfin shiners so potential culturists can fully understand the unique spawning requirements of this species.

IMPACTS
OBJECTIVE 1
Results from this study show that more effort needs to be put into developing a more nutritionally complete diet for golden shiners. In addition, there is a need to refine better culture techniques for growth and survival in indoor tank systems.

In summary, it is possible to reach a market size in one growing season using a combination of pond fertilizers and a feeding program. This study also showed that even though fish were fed a prepared diet, they still searched for natural prey.

OBJECTIVE 2
Studies demonstrating combined pond and indoor recirculation aquaculture system grow out may provide baitfish producers with an opportunity to produce a new baitfish species, spotfin shiners, for the large and expanding market in the NCR.

However, UW-Stevens Point NADF and UW-Madison studies to date suggest that the limited capacity for producing fry from brood stock may preclude the development of this species as a viable commercial baitfish raised in ponds.

OBJECTIVE 3
The ability to locate and transfer spotfin brood stock to the NADF has helped and will continue to help facilitate this project. This outreach effort will help coordinate the reporting of research results and make this information available to industry representatives who can base business decisions regarding the culture of spotfin shiners and early spawning of golden shiners in the NCR.

SUPPORT
NCRAC has provided $200,000 which is the entire amount allocated for this 2-year project.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED
See the Appendix for a cumulative output for all NCRAC-funded Baitfish activities.

EXTENSION[4]

Project ***Progress Report*** for the Period
May 1, 1989 to August 31, 2008

NCRAC FUNDING LEVEL: $883,305 (May 1, 1989 to August 31, 2008)

PARTICIPANTS:

Dennis E. Bauer	University of Nebraska-Lincoln	Nebraska
Fred P. Binkowski	University of Wisconsin-Milwaukee	Wisconsin
Mark E. Clark	North Dakota State University	North Dakota
Richard D. Clayton	Iowa State University	Iowa
James M. Ebeling	Ohio State University	Ohio
Mark E. Einstein	Purdue University	Indiana
Robert D. Espeseth	University of Illinois	Illinois
Donald L. Garling	Michigan State University	Michigan
Jeffrey L. Gunderson	University of Minnesota-Duluth	Minnesota
F. Robert Henderson	Kansas State University	Kansas
Chester L. Hill	North Dakota State University	North Dakota
John N. Hochheimer	Ohio State University	Ohio
Paul B. Jarvis	North Dakota State University	North Dakota
Anne R. Kapuscinski	University of Minnesota	Minnesota
Terrence B. Kayes	University of Nebraska-Lincoln	Nebraska
David L. Klinkebiel	North Dakota State University	North Dakota
Ronald E. Kinnunen	Michigan State University	Michigan
Christopher C. Kohler	Southern Illinois University-Carbondale	Illinois
David J. Landkamer	University of Minnesota	Minnesota
Charles D. Lee	Kansas State University	Kansas
Frank R. Lichtkoppler	Ohio State University	Ohio
Terry A. Messmer	North Dakota State University	North Dakota
Brian K. Miller	Purdue University	Indiana
Jerry B. Mills	South Dakota State University	South Dakota
Jeff Mittlemark	University of Minnesota	Minnesota
Joseph E. Morris	Iowa State University	Iowa
Kenneth E. Neils	Kansas State University	Kansas

[4]NCRAC has funded a number of Extension activities, both as stand-alone projects or as components of species-or topical-specific projects, including 11 stand-alone projects deemed "Base" Extension. This Progress Report is for components of those 11 "Base" Extension projects. The first three "Base" projects were chaired by Donald L. Garling, the fourth was chaired by Fred P. Binkowski, and projects 5-11 chaired by Joseph E. Morris. A Project Component Termination Report for one of the objectives of the fifth "Base" Extension project is contained in the 1997-98 Annual Progress Report; a Project Component Termination Report for one objective of "Base" Extension projects 1-8 is contained in the 2003-04 Annual Progress Report. The 11th "Base" project is a 2-year project that began September 1, 2007. Fred P. Binkowski chaired the twelfth stand-alone Extension project (the Aquaculture Regional Extension Facilitator); a Termination Report for which was contained in the 2004-05 Annual Progress Report. Laura G. Tiu chairs the thirteenth stand-alone Extension project (the Regional Aquaculture Extension Specialist); a Progress Report for that project is contained elsewhere in this report.

PARTICIPANTS (continued):

Burton F. Pflueger	South Dakota State University	South Dakota
Robert A. Pierce II	University of Missouri	Missouri
Michael D. Plumer	University of Illinois	Illinois
Kwamena K. Quagrainie	Purdue University	Indiana
Shawn H. Sanders	North Dakota State University	North Dakota
Daniel A. Selock	Southern Illinois University-Carbondale	Illinois
John P. Slusher	University of Missouri	Missouri
Fred L. Snyder	Ohio State University	Ohio
Brian R. Stange	North Dakota State University	North Dakota
LaDon Swann	Purdue University	Indiana/Illinois
Laura G.Tiu	Ohio State University	Ohio
Geoffrey Wallat	Ohio State University	Ohio

PROJECT OBJECTIVES

(1) Strengthen linkages between North Central Regional Aquaculture Center (NCRAC) Research and Extension Work Groups.

(2) Enhance the NCRAC extension network for aquaculture information transfer.

(3) Develop and implement aquaculture educational programs for the North Central Region (NCR).

ANTICIPATED BENEFITS

Members of the NCRAC Extension Work Group have promoted and advanced commercial aquaculture in a responsible fashion through an organized education/ training outreach program. The primary benefits are:

- 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 and hybrid striped bass, through hands-on workshops and field demonstration projects;
- Improved lines of communication between interstate aquaculture extension specialists and associated industry contacts;
- Access to aquaculture information by the industry at any time via the Internet, including such things as photographs, publications, and traditional as well as educational streaming videos (which are under development);
- An enhanced legal and socioeconomic atmosphere for aquaculture in the NCR; and
- Continued development of state producer organizations that are engaged in identifying and providing solutions to industry issues.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Examples follow for each of the objectives from the eleven projects funded to date going back to 1989; however, greater emphasis is placed on more recent activities.

OBJECTIVE 1

Aquaculture Extension Work Group members have:

- Served as an extension liaison, if not an active researcher, for every NCRAC-funded project;

- Assisted in developing, writing, and editing several culture manuals as well as fact sheets, book chapters, and videos based on NCRAC-funded research;
- Assisted with the planning, promotion, and implementation of taxa-specific workshops held throughout the region;
- Participated as Steering Committee members for public forums related to revision of the National Aquaculture Development Plan and the four past National Aquaculture Extension Workshops/Conferences; and
- Met with industry representatives and university researchers involved with aquaculture to discuss how the aquaculture industry could grow in the Midwest.

OBJECTIVE 2
The demand for aquaculture extension education programs cannot be met by the few aquaculture-designated specialists in the NCR. A NCRAC white paper on extension presents several strategies to address this concern.

Networking of specialists and Cooperative Extension Service (CES)-designated contacts has maximized the efficiency of education programs and minimized duplication. Individual state extension contacts often respond to 120+ annual calls 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, and detailed responses to both generalized and specialized questions. This extension network is critical to being able to match specific aquaculture questions with the best source of information, e.g., crawfish and leech information with Gunderson; yellow perch information with Garling, Binkowski, and Tiu; and sunfish information with Morris.

The Aquaculture Network Information Center (AquaNIC [http://aquanic.org/]) was established at Purdue University in 1994 through funds from the Cooperative State Research, Education, and Extension Service and the Illinois-Indiana Sea Grant College Program. In subsequent years, NCRAC has provided continued financial support for AquaNIC. The hardware for this Web site is housed in the Department of Animal Sciences at Purdue University and is coordinated by the Mississippi-Alabama Sea Grant Consortium, the Alabama Cooperative Extension System, and the Illinois-Indiana Sea Grant College Program.

AquaNIC was the first U.S. aquaculture Web site and is globally one of the most widely accessed and cited aquaculture Web sites. Approximately 1,200 individual, educational, commercial, and governmental Web sites link to AquaNIC as a source of online aquaculture information. AquaNIC extends beyond the state and regional area, and affects aquaculture stake holders from around the United States; 55.49% of all AquaNIC's usage for the time period September 1, 2007–August 31, 2008 can be verified as belonging to aquaculture stakeholders from the United States and 31.06% of additional usage was from an unknown origin but a fair portion of that usage can be attributed to the United States.

AquaNIC's impacts on the U.S. aquaculture industry for this time, using the known verifiable 55.49% of U.S. usage are:

- at a minimum, 3,450 people from the U.S. visited AquaNIC every day;
- at a minimum, 7,860 pages were viewed every day;
- the average visit length was almost 11 minutes/visitor;

- AquaNIC is currently ranked as the #1 aquaculture electronic resource in the world by ranking.com, a professional Web tracking company that monitors the traffic for the top 1 million Web sites around the world; and
- AquaNIC is also currently ranked in the top 18% of all Web site traffic worldwide by ranking.com.

As with any long-term organization, there have been changes in NCRAC extension personnel since the inception of the project. For instance, Landkamer was the primary aquaculture extension contact for Minnesota. In the intervening years, he has been replaced by Kapuscinski and then by Gunderson. Two other individuals were replaced in 1994. In Kansas, Neils replaced Henderson and in Illinois, Kohler replaced Selock. Lee replaced Neils in Kansas in 1996. Hochheimer, who replaced Ebeling in Ohio, left Ohio State University; Tiu was appointed as the aquaculture extension specialist for Ohio in 1998. Sanders, appointed as the extension contact for North Dakota in 1998, has resigned; Paul Jarvis was appointed in 1999 and he has been replaced by Mark Clark. In 2005 Pflueger replaced Mills as the appointed NCRAC Extension contact for South Dakota. As of 1999, Kayes is no longer with Nebraska Extension; in 2005 Bauer was designated to represent Nebraska. In 2000, Swann resigned from Purdue/Illinois Sea Grant; Felkner served Indiana in the interim. In 2006, Quagrainie was appointed as state extension specialist at Purdue University. Plumer currently serves Illinois. In 2007, two long term extension contacts, Tiu and Morris, were replaced as NCRAC extension contacts by Wallat and Clayton, respectively.

Lee developed and published the 2008-2009 Kansas Aquaculture Association (KAA) Directory as well as maintained the KAA Web site and update material provided by the KAA. He also provided assistance to private pond owners on fish culture, management and aquatic weed control.

Pierce served as the Extension liaison for the Lincoln University Aquaculture Program by co-coordinating aquaculture Extension and outreach educational activities on the culture and production of sunfish for food markets; developing and reviewing Extension publications; and reviewing aquaculture research proposal submissions developed to enhance the capacity of Lincoln University's aquaculture research and outreach program. In addition, in 2008 Pierce undertook a "Pilot Sunfish Production Verification Program – Utilizing Cage Culture Techniques" to begin the process of:

- verifying whether current research-based recommendations can produce profitable yields in cage culture systems;
- estimating cost of production and corresponding feed conversion ratio, yield, and survival;
- identifying future research needs and updating Extension recommendations;
- developing an interdisciplinary management approach to help maximize net profits;
- developing a protocol for future trials; and
- providing practical field experience for researchers and Extension specialists.

In North Dakota, Clark has developed an updated list of state producers for submission to the NCRAC Publications Office.

Continued progress toward enhancing the NCRAC extension network for aquaculture information transfer was accomplished through the North Central Aquaculture Regional Extension Facilitator Web site

(www.ncaref.org) which received 13,045 visits from a wide variety of clients between September 1, 2007–August 31, 2008.

On August 22, 2008, Binkowski and the Great Lakes WATER (Wisconsin Aquatic Technology and Environmental Research) Institute staff hosted the National Aquaculture Association (NAA) Board members and guests for a tour of the WATER Institute's aquaculture facilities followed by a traditional Milwaukee Friday night yellow perch fish fry. The NAA Board members, Wisconsin Sea Grant Director, Anders Andren, and several members of his staff, Colin Scanes, the University of Wisconsin-Milwaukee's Graduate School Dean, USDA/Agricultural Research Service employees, staff from the Urban Aquaculture Center, and WATER Institute researchers were in attendance. This event was chronicled in the Volume 14, Issue 4 Fish Farming News.

OBJECTIVE 3

A number of workshops, conferences, symposia, videos, field-site visits, hands-on training sessions, and other educational programs have been developed and implemented (see the Appendix for a listing of many of these activities). There have been workshops on general aquaculture, fish diseases, early life stage culture, recirculation systems, cage culture, aquaculture business planning, pond management (fish and vegetation), water quality, and taxa-specific topics, e.g., baitfish, channel catfish, crayfish, hybrid striped bass, leach, rainbow trout, sunfish, walleye, and yellow perch culture, as well as in-service training for high school vocational-agricultural teachers. Depending on the workshop, the number in attendance often exceeded 100. Through these workshops, critical issues in the private aquaculture industry have been identified, e.g., market availability, economic returns, and regulatory concerns.

NCRAC Extension contacts have served as editors for regional aquaculture newsletters as well as in-state aquaculture association newsletters; served on state aquaculture advisory councils and state aquaculture task forces; and assisted in the planning and implementation of state aquaculture association meetings.

In addition to the previously mentioned areas, NCRAC Extension contacts have been instrumental in fostering the continued growth of the aquaculture industry in the region through a variety of activities and many have worked with industry and governmental representatives to produce state aquaculture plans and improved governmental regulations.

All fish processors, including those who handle aquaculture products, are now required by law to process their fish following HACCP (hazard analysis and critical control point) guidelines. Kinnunen and Gunderson have conducted numerous HACCP training workshops throughout the NCR. These workshops served to train fish processors on the principles of HACCP and to give them knowledge on how to develop and implement a HACCP plan for their specific facility. Attendees, who come from throughout the NCR, represent both public and private audiences as well as Native American groups.

NCRAC Extension contacts have also been responsive to arising issues for the NCR aquaculture industry. For instance, the aquaculture industry is accused of being an important vector for the further spread of exotic species such as zebra mussels, Eurasian watermilfoil, and round gobies. To better identify the risks of spreading exotic

species and to reduce those risks, an AIS (aquatic invasive species)-HACCP approach has been developed by Kinnunen and Gunderson and taught to private fish farmers, wild bait harvesters, state and federal agency natural resource personnel, and Native Americans. An AIS-HACCP plan has also been developed to address the growing concern of biosecurity, particularly in regard to diseases such as viral hemorrhagic septicemia (VHS). Kinnunen and Gunderson have also taught other members of the NCR aquaculture extension community about their AIS-HACCP program, in essence, they've "trained the trainers" and all AIS-HACCP materials are available at www.seagrant.umn.edu/ais/haccp.

In-service training of secondary teachers has taken place in a number of states. For instance, teachers in Iowa, Ohio, and Wisconsin have received instruction in aquaculture.

Several states have on-site facilities that are used for extension programming, e.g., the Piketon facilities operated by Ohio State University are used to inform the public about aquaculture as well as foster grass root support for this agriculture enterprise. The facilities at Iowa State University and the University of Wisconsin-Milwaukee have also been used in a similar fashion.

The Ohio Center for Aquaculture Research and Development (OCARD) hosts three electronic list serves, the most popular of which is the Aqua-Ohio list serve. Over 150 clients subscribe to this list serve which allows for timely dissemination of aquaculture related news and resources. This information is further disseminated by the list subscribers to additional interested parties.

In early fall 2007 a question was raised by regional producers as to the possibility of bringing aquatic stakeholders together from various backgrounds to discuss the regulatory and administrative discrepancies among states when it comes to aquatic livestock, biosecurity, and commerce. The concept of a meeting/forum evolved into an action plan to try and accomplish this task. A forum was designed to explore federal and state regulations that are impacting the profitable and efficient interstate movement of aquatic livestock for both private and public purposes in hopes of finding consistent uniform methods for the NCR and other states currently under the federal order for VHS. The concept of this Forum was to discuss improvement and revision of state regulations and policies whereby aquatic livestock for both public and private purposes can be enhanced while also maintaining animal health in 2008. The five delegate groups were: private producers, public producers (such as hatchery personal), animal health representative (veterinarians), state natural resources and agriculture state agencies representatives were invited from fourteen states. The states in the NCR (Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin) and those affected by the federal order on VHS (New York and Pennsylvania) were chosen. Issues that the 37 Forum participants were in consensus on in rank order were:

- no uniformity in state regulations;
- limited availability of fish health officials; and
- no uniformity of testing standards among states.

The complete report for this meeting can be found at: www.aquaticlivestock.org/.

This forum impacted the NCR by bringing some of these key players (delegates) to a neutral table to discuss these common issues (never been done before with aquatic livestock producers). Many of the delegate groups had never sat down to discuss their issues with the other stakeholder groups. Some delegates didn't realize that other delegates have the same issues, e.g., private producers and public producers both have to deal with changing transportation regulations.

WORK PLANNED

Efforts will continue in regard to strengthening linkages between research and extension work groups as well as enhancing the network for aquaculture information transfer. Participants will also continue to provide in-service training for CES, Sea Grant, and other land owner assistance personnel.

Educational programs and materials will be developed and implemented including AIS-HACCP workshops that will be planned as needed in the NCR as well as workshops on aquatic plant management for aquaculture facilities, prawn production, and larval fish culture. Any other workshops developed and hosted by state aquaculture extension contacts will be advertised in surrounding states to take advantage of the NCRAC extension network and the individual expertise of the Extension Work Group participants. There are also plans to enhance Web-based communications through the use of streaming videos and electronic fact sheets. Streaming videos will include the following topics:

- yellow perch culture,
- freshwater shrimp culture,
- culture pond construction,
- water quality assessment,
- fry-pond fertilization regimes, and
- aquatic vegetation management.

In addition, a Web site for predator management and fish grub control will be finalized and linked to NCRAC's Web site (http://www.ncrac.org).

IMPACTS

Examples include:

- Development of aquaculture education programs for the NCR has provided "hands-on" opportunities for prospective and experienced producers. More than 10,000 individuals have attended workshops, conferences, or symposia organized and delivered by members of the NCRAC Extension Work Group.
- Fact sheets, technical bulletins, videos, and CDs 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 have been used in numerous regional meetings. The Center's Web site provides immediate availability to many of the products that have been developed by the Extension Work Group (e.g., fact sheets as PDF files) and with the further development of streaming videos, not only will clients have the benefit of being able to read about aquaculture for free on a 24-hour basis, they will also be able to see it in action. This ability to enhance technology transfer should result in a more economically-successful aquaculture industry in the NCR.
- Fish processors who have attended NCRAC-sponsored HACCP Training Workshops have learned the principles of HACCP with regards to its importance in insuring the production of a safe fishery product. HACCP plans have been implemented by workshop

attendees who are now keeping records of their daily processing and Sanitation Standard Operating Procedures. Hundreds of fish processors and/or aquaculturists have attended HACCP Training Workshops.

- AIS-HACCP workshops have been attended by commercial culturists, state and federal natural resource personnel as well as Native Americans, many of whom have implemented the principles of AIS-HACCP into their operations.

PUBLICATIONS, MANUSCRIPTS, WORKSHOPS, AND CONFERENCES

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

SUPPORT

YEARS	NCRAC-USDA FUNDING	OTHER SUPPORT					TOTAL SUPPORT
		UNIVER-SITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
1989-91	$107,610	$237,107				$237,107	$344,717
1991-93	$94,109	$152,952				$152,952	$247,061
1993-95	$110,129	$198,099		$250,000	$55,000	$503,099	$613,228
1995-97	$31,204	$149,325	$5,000	$84,000		$238,325	$269,529
1997-99	$38,000	$110,559				$110,559	$148,559
1999-01	$94,000	$108,124				$108,124	$202,124
2001-03	$46,654	$99,702				$99,702	$146,356
2003-05	$28,000						$28,000
2005-07	$219,280						$219,280
2007-08	$114,139						$114,319
TOTALS	$883,305	$1,055,868	$5,000	$334,000	$55,000	$1,449,868	$2,333.173

REGIONAL AQUACULTURE EXTENSION SPECIALIST (RAES)[5]

Project ***Progress Report*** for the Period
September 1, 2005 to August 31, 2008

NCRAC FUNDING: $151,000 (September 1, 2005 to August 31, 2007)

PARTICIPANTS:

Hanping Wang	Ohio State University	Ohio
Laura G. Tiu	Ohio State University	Ohio
Geoffrey K. Wallat	Ohio State University	Ohio
Industry Advisory Council Liaison:		
Curtis Harrison	Harrison Fish Farm, Hurdland	Missouri
Extension Liaison:		
Laura G. Tiu	Ohio State University	Ohio

PROJECT OBJECTIVES

(1) Provide leadership for the aquaculture industry in the North Central Region (NCR)

(2) Enhance information transfer.

ANTICIPATED BENEFITS

The long term impact of the RAES will be an increase in the value of the aquaculture industry in the NCR. This includes an increased number of successful and sustainable aquaculture operations. Short and medium term impacts include enhanced access by stakeholders to research based information, an increase in the number of regional workshops, a strengthening of state aquaculture associations, and enhanced communication between academia and the industry in the NCR.

PROGESS AND PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

In June 2006, an advisory committee comprised of industry, research and extension individuals in the NCR was convened to create a job description for the RAES and conduct the interview process. A Regional Aquaculture Extension Specialist, located at Purdue University, was hired in August, 2006. The RAES visited many of the states in the NCR and met with several leaders in the aquaculture industry. The RAES attended the annual NCRAC meeting in Columbus, Ohio and the National Aquaculture Extension Conference in Cincinnati, Ohio. Due to circumstances beyond anyone's control, the RAES position was vacated in October 2007. In March 2008, Dr. Chris Weeks accepted the RAES position on a contractual basis though Ohio State University and will continue in that role until August 31, 2009.

[5] NCRAC has funded numerous Extension activities, both as stand-alone projects or as components of species- or topical-specific projects. This progress report is for one of the twelve stand-alone Extension projects which is chaired by Laura G. Tiu. It is a 3-year project that began September 1, 2005.

- The new RAES has attended three state association meetings, represented the NCR industry at an aquatic invasive species (AIS) workshop in Romulus, Michigan, and presented a talk entitled VHS a Regional Industry Perspective, at the Illinois VHS Conference and Workshop, April 2008. He also administers the list serve NCR-Fish-Culture, with 80 plus members from the aquaculture community. His first task as RAES was to provide immediate support to the industry in ways to reduce disruption of commerce due to VHS (viral hemorrhagic septicemia).
- The RAES has made personal contact with principle state regulators responsible for aquaculture and baitfish activities in each NCR state, and solicited members of this group to participate in the VHS Summit at the United States Trout Farmers Association (USTFA) Midwest Aquaculture Conference in September 2008. In this process the RAES has developed a positive working relationship with many state regulators across the region. He also has encouraged members of the industry to contact him for problems or questions related to industry matters. Much of this support has been in regards to fish health issues.
- The RAES has taken an active role in informing the NCR industry on regulatory issues, and soliciting and voicing industry concerns in regards to USDA Animal and Plant Health Inspection Service (APHIS) response to VHS in the Great Lakes.
- The RAES was instrumental in developing a plan with the Michigan Department of Agriculture for the USDA 2008 Cooperative Agreements for VHS. If approved the plan would alleviate VHS testing costs for 30 Michigan producers in 2008 while providing USDA APHIS important VHS surveillance information.

OBJECTIVE 2

- In addition to the transfer of information on issues describe for Objective 1, the RAES has taken responsibility to post and update all aquaculture and baitfish regulations, aquaculture contact information, and fish health certification requirements for the 12 NCR states. This information is complete (for all NCR and several adjacent states) and is now available on the NCRAC Web site, link: North Central Region Aquaculture Contacts, Transport Regulations, and Approved Aquatic Species (http://www.ncrac.org/Info/StateImportRegs/stateregsmain.htm).
- The RAES has actively sought out industry related information from established partnerships, local and regional list severs and Web sites and has distributed this information on the NCR Fish Culture list serve. This is an ongoing project and will continue over the course of the RAES position.
- The RAES is working with other extension outlets in the region to promote aquaculture and disseminate pertinent information to the industry. These include the Aquaculture Regional Extension Facilitator, the Northern Aquaculture Demonstration Facility, Indiana Soybean Alliance, state aquaculture associations and academic extension personnel.
- The RAES has attended a number of state association meetings across the NCR. Often time is provided for discussion on industry needs, NCRAC project funding, and RAES functions and extension programs.
- The RAES is available to answer industry related questions vie e-mail

(weekschr@msu.edu), landline (517-353-2298) or cell phone (517-745-8840).

- The RAES has actively provided support for industry-related conferences and workshops across the region. Examples include the USTFA Midwest Aquaculture Conference VHS Summit held in Milwaukee, Wisconsin (September 2008), and the Largemouth Bass Workshop in West Lafayette, Indiana (October 2008).

WORK PLANNED

Over the course of this project, the RAES has identified four key points to assess and take action on if needed in immediate support of the industry. These include (a) obtaining clear definition of industry related regulatory structure across the region and providing regulatory support to the extent possible, (b) examination of the status of biosecurity development, (c) assessment of availability of fish health support personnel and facilities, and (d) assessment of regional industry development in comparison to national and international aquaculture industry sectors. Items (a) and (b) are considered direct responsibilities of the RAES and will be continually updated and monitored. Biosecurity development (c) appears to be proceeding well across the region in the form of workshops, state association meetings, and national and international organizations (e.g., AIS hazard analysis and critical control point [HAACP], American Fisheries Society and World Aquatic Veterinary Medical Association). The RAES plans to attend the Biosecurity Workshop at the Missouri Aquaculture Association (MOAA) Meeting (January 2009) and will lead a discussion aimed at seeking input from MOAA to carry forward to the 2009 NCRAC annual program planning meeting.

Actions taken by APHIS in response to the finding of VHS in the Great Lakes has caused a considerable amount of concern and disruption in the NCR aquaculture industry. One major concern is whether the region has sufficient qualified fish health inspectors available to conduct mandated test requirements (fish sample collection) imposed on producers in the region. The RAES is currently in discussion with fish health experts to see if a 1-day aquatic animal health clinic/workshop can be given in states across the NCR. The proposed clinic would be geared specifically towards practicing veterinarians not familiar with fish sample collection. It is hopeful that 10 or so veterinarians would attend each workshop from NCR states.

The RAES is also currently looking into forming a strategic planning working group for the purpose of evaluating the progress and status of the NCR industry to date and to strategize as to how to improve the condition of the industry. Potential actions include: assessment of regional, national, and international aquaculture activities for evaluation of successful pathways for increasing production and markets; assessment of failures within the industry and identification of root causes of these failures; identification of potential sources of increased support (funding, political, etc.); and development of strategies to further the development of the NCR aquaculture industry.

IMPACTS

Due to a number of factors (e.g., short time frame, VHS, current economy, etc.), definitive attributes such as increased numbers of aquaculture facilities, production, and/or value is impossible to determine at this time. Moreover, there is a major concern that extrinsic pressures could cause substantial problems in the industry

over the next several years. Indirect positive impacts, however, can be seen. For example, the use by internet users of the NCRAC North Central Region Aquaculture Contacts, Transport Regulations, and Approved Aquatic Species Web site is quite high. This site has been linked to by a number of organizations including APHIS. Also, the VHS Summit at the USTFA conference in Milwaukee was considered a focal point of the conference. The RAES also fields numerous calls and emails from the industry on a daily basis.

SUPPORT

NCRAC funds provided to date total $151,000; a total of $225,000 has been allocated for this 3-year project.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

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

FEED TRAINING CARNIVOROUS FISH[6]

Project ***Progress Report*** for the Period
September 1, 2006 to August 31, 2008

NCRAC FUNDING: $300,000 (September 1, 2006 to August 31, 2008)

PARTICIPANTS:

Fred P. Binkowski	University of Wisconsin-Milwaukee	Wisconsin
Anita M. Kelly	Southern Illinois University-Carbondale	Illinois
Jeffrey A. Malison	University of Wisconsin-Madison	Wisconsin
Robert S. Hayward	University of Missouri-Columbia	Missouri
Gregory W. Whitledge	Southern Illinois University-Carbondale	Illinois
Industry Advisory Council Liaison:		
William W. West	Blue Iris Fish Farm, Black Creek	Wisconsin
Extension Liaison:		
Joseph E. Morris	Iowa State University	Iowa

PROJECT OBJECTIVES

(1) Evaluate strategies including harvest, transport, environmental, and husbandry, to increase survival, growth, to maximize the percent of advanced yellow perch fingerlings trained to accept formulated feeds.

(2) Evaluate strategies including harvest, transport, environmental, and husbandry, to increase survival, growth, to maximize the percent of advanced yellow perch fingerlings and largemouth bass fingerlings retained on formulated feeds after restocking into commercial-scale culture systems.

ANTICIPATED BENEFITS

Studies conducted relating to Objective 1 will document the relative success that can be expected at feed training pond-reared yellow perch fingerlings harvested at different sizes, and using different dietary regimes. These studies will provide valuable information to yellow perch producers for maximizing the productivity and efficiency of their operations.

The proposed studies addressing Objective 2 will document the extent to which repetitive size grading can be used during the feed-training process to improve poststocking survival and growth of age-0 yellow perch and largemouth bass fingerlings. They will also provide key data on the performance of age-0 feed-trained yellow perch and largemouth fingerlings restocked into ponds at different densities. Successful poststocking feeding promotes increased growth and survival. Aggregation of fish through attractants and audible signals could potentially enhance feeding, including the delivery of medication, and facilitate handling and harvest in commercial situations. Increased growth and survival from improved feeding and handling translates into increased profit for producers.

[6]This 2-year project began September 1, 2006 and was originally chaired by Anita M. Kelly who left Southern Illinois University-Carbondale in August 2007, after which Gregory W. Whitledge became chair of the project.

PROGESS AND PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

University of Wisconsin-Madison (UW-Madison)

Two experiments relevant to feed training of pond-raised yellow perch fingerlings were completed. Experiment 1 evaluated the influence of fish size at harvest on habituation success. Yellow perch were harvested at mean total lengths (TLs) of 25.0, 35.0, and 45.0 mm (1.0, 1.4, and 1.8 in). After each harvest, fish were immediately stocked in 750-L (198-gal) tanks (2,500 fish/tank, 4–6 tanks for each size), supplied with tempered water (19°C [66.2°F] 12 L/min flow [3.2 gpm]), and aerated with an airlift pump which created a circular current. Tanks were continually lighted with overhead low intensity lights. All tanks were equipped with an automatic feeder, which continuously delivered the appropriate food type. Additionally, fish were hand-fed 5–8 times daily. During the first four days fish were fed freeze dried krill. The next 10 days, 10% krill was added to the formulated food (#2 Silver Cup Trout Fry diet, Murray Elevators, Murray, Utah). During the balance of the training period, fish were fed only the formulated feed. Length of the training period was defined by mortality due to starvation as well as visual observation of positive feeding activity of all fish in the tanks. To compare the training success of the fry sizes, calculations were made of (1) harvest losses, defined as the percentage of fish which died during the first two days, (2) habituation success, defined as the percentage of fish surviving at the end of the training period (after harvest losses), (3) starvation, defined as the percentage of recovered dead fish, (4) cannibalism, defined as the percentage of fish which were unaccounted for at end of the training period, and (5) overall success, defined as the percentage of fish remaining at the end of the training period (including harvest losses).

Training success was higher for fry harvested at 25.0 and 35.0 mm (1.0 and 1.4 in) TL (93.6% in each case) than for those at 45.0 mm (1.8 in) TL (79.4%). The principal difference in training success is the higher cannibalism rate demonstrated by the larger fish (12.5%) versus those harvested at 35.0 mm (1.4 in) TL (5.5%) or 25.0 mm (1.0 in) TL (2.4%). Higher size variability was recorded in the 45.0 mm (1.8 in) TL group that remained in the production ponds longer than the other groups of fish. This size difference led to a situation where larger fish were able to consume smaller fish. Thus, grading the harvested fingerlings prior to feed training when size differences are apparent is recommended.

Losses due to harvest stress were higher in fingerlings harvested at 25.0 mm (1.0 in) TL (11%), than for those harvested at 35.0 mm (1.4 in) TL (2.4%) or 45.0 mm (1.8 in) TL (1.8%). The fact that no difference in harvest losses was found between fish harvested at 35.0 mm (1.4 in) TL with a seine and fish harvested at 45.0 mm (1.8 in) TL by pond drawdown suggests that losses in the smaller fish were not due to the harvest method, but rather because of the small size and fragile nature of fish harvested at 25.0 mm (1.0 in) TL.

No difference was found in overall success between fish sizes (83.4%, 91.3%, and 78.1%, respectively), for fish harvested at 25.0, 35.0, and 45.0 mm (1.0, 1.4, and 1.8 in) TL. Harvest losses in fish at 25.0 mm (1.0 in) TL were offset by cannibalism losses in fish at 45.0 mm (1.8 in) TL. Fish harvested at 35.0 mm (1.4 in) TL displayed low losses from both harvest stress and cannibalism, and may be recommended as the best size for habituation using the

techniques set forth in this study. From a practical standpoint, it is logistically unfeasible to harvest and train all fingerlings produced at a commercial scale facility at the same size. Techniques should be modified to accommodate the fish on-hand. Low stress harvest methods (e.g., light trapping) for small fish and size grading for larger, more size-diverse populations would likely result in better overall success for both groups of fish.

Experiment 2 compared four different feed regimens using three sizes of fish for each regimen. The feed regimens were: (1) Silver Cup feed, (2) 4 days of INVE feed (Epac 6–8) followed by a 7-day transition to Silver Cup, (3) 4 days of freeze-dried krill followed by a 7-day transition to Silver Cup, and (4) 4 days of krill followed by a 7-day transition to INVE followed by a 7-day transition to Silver Cup. Yellow perch were drain-harvested from the pond at mean TLs of 31.0, 37.0, and 55.0 mm (1.2, 1.5, and 2.2 in). Fingerlings were size-graded prior to being stocked into 113.6-L (30.0-gal) flow-through tanks (200 fish/tank, three tanks/treatment-size). Endpoints examined in experiment 2 were the same as described in experiment 1.

No incidences of loss to harvest stress or cannibalism were noted in any of the treatment groups for this experiment. Overall habituation success was slightly lower in the 31.0 mm (1.2 in) TL group (94.4%) as compared to the two larger sizes (98.7% and 99.4% for 37.0 and 55.0 mm [1.5 and 2.2 in]) TL fingerlings, respectively. No differences in habituation success were found between the four feeding regimens (97.0%, 97.0%, 98.1%, and 98.3% for regimens 1 through 4, respectively), although regimens that included the use of krill (treatments 3 and 4) improved habituation success in the smallest fingerlings by approximately 3.5% (96.0% versus 92.6%). The excellent habituation success demonstrated by all of the treatment groups in this experiment may have been a result of several factors including size-grading prior to training and isolated culture conditions which limited disturbance of the fish.

University of Wisconsin-Milwaukee (UW-Milwaukee)

Extracts of chironomids, zooplankton, redworms (*Eisenia fotida*), artemia, and tubifex worms have been prepared as natural feed attractors. Feeding trials are in progress and the feeding responses will be compared to identify the best positive response for each species (yellow perch and largemouth bass). The top 2–3 extracts will then be selected based on experimental results; these will then be applied to standard diets to be tested for attractant effectiveness.

OBJECTIVE 2

UW-Madison

An experiment on size-grading fingerlings during the habituation period was conducted. Pond-raised fingerlings were habituated according to the conditions described above under Objective 1, experiment 1. Two 750-L (198-gal) tanks containing 3,000 fingerlings each were used for each of three trials during this experiment resulting in three ponds of size-graded and three ponds of non-size-graded fish. For each of the three replicates, the harvest of the fish was staggered in time by 8–12 days. Size grading was conducted on day 7 and day 14 of the training period with the large sized fish removed and stocked into a 0.04 ha (0.1 acre) production pond. The remaining fish were stocked on day 21. Non-size-graded fingerlings were left undisturbed and stocked into a similar size production pond on day 21. All fingerlings

were then raised in ponds for the remainder of the growing season.

Habituation success averaged 81% and was not different between treatment groups. No differences in pond survival (69.7% ± 7.7 versus 67% ± 9.8) or mean fish size (18.3 ± 4.7 g versus 21.0 ± 3.6 g [0.6 ± 0.2 oz versus 0.7 ± 0.1 oz]), for graded and non-graded fish, respectively, were found. A high degree of size variability was noted in all ponds with fish sizes ranging from 3.0–56.0 g (0.1–2.0 oz). After ponds were harvested, the fingerlings were selectively size-graded into three groups: "small" fish (those that passed through a 7.5 mm (19/64 in) size-grader) which averaged 63.5 mm (2.5 in) and 3.0 g (0.1 oz); "medium" fish (those that passed through a 10.7 mm (27/6 4 in) size-grader but were retained by a 7.5 mm (19/64 in) size-grader which averaged 88.9 mm (3.5 in) and 8.0 g (0.2 oz); and "large" fish (those that were retained by a 10.7 mm (27/64 in) size-grader which averaged 132.1 mm (5.2 in) and 24.0 g (0.8 oz).

Size-graded fish were returned to separate tanks to observe their feeding behavior. Nearly 100% of fish in the medium and large groups actively consumed formulated food, while only ~25% of the small fish consumed food. The extent to which these small fish can be "re-trained" to accept formulated food in tanks is being evaluated. Ponds stocked earlier in the season produced larger and more uniformly-sized fingerlings than those stocked later in the season. Pooling data across treatments (size-graded and non-size-graded) fingerlings stocked into the first set of two ponds averaged 24.6 ± 3.5 g (0.9 ± 0.1 oz), fingerlings stocked into the second set of two ponds averaged 21.9 ± 4.5 g (0.8 ± 0.2 oz), and fingerlings stocked into the last set of two ponds averaged 18.4 ± 3.2 g (0.6 ± 0.1 oz). Accordingly, researchers recommend that fingerling producers harvest and feed-train fingerlings as early as possible in the season.

University of Missouri-Columbia (UM-C)
Eight experimental ponds at the Missouri Department of Conservation's Little Dixie Lake (LDL) site were secured for use in 2007 and 2008. Beginning in April 2007, all ponds were longitudinally divided into halves with plastic mesh nets and wire lines spanning pond lengths held the net tops approximately 0.9 m (3.0 ft) above the water surface so that fish could not jump into adjacent pond halves.

The 50,000 pellet-trained, fingerling largemouth bass that were ordered from a commercial producer (for Year 1 activities) to arrive at the LDL facility during the first two weeks of June were not delivered due to a severe weather event at the producer's facility that caused the loss of most of the pellet-trained fingerlings. Substantial efforts were made both by the PI and by the commercial producer to secure fish from another source, but they were not successful in 2007.

From late-April through mid-May 2008, repairs were made to pond dividers that were installed in the LDL ponds during 2007. During mid-May, a graduate student from the University of Missouri traveled to Ostrum Acres Fish Farms in McCook, Nebraska to assist in setting nests, seining brood fish, and stocking brood fish into ponds for reproduction. All ponds at the LDL area were filled and awaiting fish by June 1st.

Due to continued unavailability of fish from the original producer, additional producers were contacted and on August 16, 2008, 30,000 juvenile largemouth bass were purchased and transported from Cambridge, Ohio to the LDL location. Fish were

stocked into eight pond halves (four ponds total) at 37,000 fish/ha (15,000 fish/acre). Each pond is 0.20 ha (0.5 acre), such that each pond-half received 3,750 juvenile largemouth bass. Fish in treatment halves of the ponds were confined to 1/3 of the surface area of these pond halves during the initial two weeks of the study using block nets. This "crowding" in the treatment halves was done on the deep end of the pond to help alleviate any water quality problems that may have ensued from crowding. Four floating creels that were each stocked with 100 largemouth bass were monitored for declines in fish numbers over the initial week to estimate transport mortality.

Twice daily feedings began on August 17 (the morning after stocking). Fish were fed a 50:50 mixture of Silver Cup and Aquamax feeds at 4% of body weight/day. Each week the Silver Cup feed was reduced by 10% and replaced by Aquamax due to its local availability and lower cost. Feeding rates were recalculated for each pond-half each week from mean weight information gathered during weekly samplings. Feedings were shifted from twice daily to once daily on September 15th due to a road wash out that made accessing the ponds difficult. The fish were still fed at 4% of body weight/day. Fish were fed from a stand above each pond that was approximately 38.1 cm (15.0 in) from the bank and located in 20.3 cm (8.0 in) of water. Feed for each pond half was broadcast in approximately a 6.1–6.6 m (20–25 ft) radius from this stand.

Sampling of fish was conducted via corner seining on Wednesdays and Sundays throughout the study period. For each pond half, total catch was recorded, 40 specimens were euthanized, individual lengths and weights were measured and recorded, and specimens were individually tagged and frozen so stomach analysis can be conducted at a later date. This sampling period ran from August 17–October 5. Survival, percentage of fish on feed, and percentage of fish cannibalized will be estimated from catch curve and stomach analysis results. From corresponding length and weight data researchers aim to estimate percent starvation (through use of Wr) and identify largemouth bass size structures that are associated with periods of when cannibalism becomes substantial.

Southern Illinois University-Carbondale (SIUC)

During Year 1 of the project, largemouth bass were produced and feed habituated at Logan Hollow Fish Farm, Murphysboro, Illinois. After the largemouth bass fingerlings were harvested from the nursery ponds, they were placed into a 5,000-L (1,321-gal) grading tank and treated with a 5 ppm potassium permanganate bath for 30 min to prevent introduction of disease or parasites. Fingerlings were then graded through grading boxes to ensure uniform sizes in each tank and to reduce cannibalism. Fish were stocked at a density of 7.9 fish/L (30.0 fish/gal). Freeze-dried krill (Southern Aquaculture Supply, Lake Village, Arkansas) was used as the starter diet and Bio Diet (Bio-Oregon, Inc., Warrenton, Oregon) was the moist pellet feed used in this study. Fish were fed 8% body weight daily. Five different combinations of hand feeding and automatic feeders were examined on three size classes, small, medium, and large (31.0–39.0, 40.0–51.0, 52.0–60.0 mm [1.2–1.5, 1.6–2.0, 2.0–2.4 in] TL, respectively) of largemouth bass fingerlings in an effort to increase the number of fish that were feed-trained and to determine the amount of labor involved in the process. Treatments included: (1) feeding by hand for two weeks, (2) hand feeding for three days and then automatic

feeders only for 11 days, (3) hand feeding for seven days and then automatic feeders only for the remaining seven days, (4) one automatic feeder per tank for 14 days and no hand feeding, and (5) two automatic feeders per tank for 14 days with no hand feeding. This study also examined small fish stocked at 13.2 and 7.9 fish/L (50.0 and 30.0 fish/gal). Treatments did not have a significant effect on survival but did have a highly significant effect on feed training success. Fish size had a highly significant effect on survival as well as feed training success. Small fish had higher feed training success (96.4%) in treatment 3, medium and large fish feed trained better in treatment 2 (97.3% and 86.1%, respectively). Treatments using densities of 13.2 fish/L (50.0 fish/gal) did not differ significantly in terms of survival or feed habituation success compared to tanks stocked at 7.9 fish/L (30.0 fish/gal) with fish of the same size.

The effect of different light intensities on survival and feed habituation success was also examined. Three light intensities were utilized: light = 21 lux, medium = -0.54 lux and dark=-1.08 lux. All treatments were conducted in triplicate.

Light intensity was found to have no impact on feed habituation success and no impact on survival except at the darkest level tested. The number of cannibals differed significantly between the light and dark treatments. Reduced light levels result in decreased ability of culturists to observe fish for health and cannibalism.

The effectiveness of a bird of prey call in deterring fish-eating birds from ponds stocked with largemouth bass fingerlings at a commercial fish farm was evaluated. The Bird Gard Pro was programmed to produce the call of a peregrine falcon (*Falco peregrinus*) at random intervals from 10–30 min apart, 24 h a day. Observations were then made of bird behavior and response to the call. Species, activity before call, response to call, distance from call, and time of day were recorded for each bird observed when the call was activated. Distance from the call was measured using a laser range finder. After testing the peregrine falcon call, the Bird Gard Pro was programmed to produce the call of a sharp-shinned hawk (*Accipiter striatus*) at the same time intervals and durations as the peregrine falcon call. The same observations were made as described for the falcon call. Bird activity at the study ponds was not significantly different among the different observation time periods. Birds of prey calls failed to repel fish-eating birds from the fish farm. Physical barriers are the only demonstrated effective prevention mechanism for bird predation in aquaculture.

Pond studies using different densities of fingerling largemouth bass as outlined in the proposal were begun during summer 2008. Pellet-feed trained largemouth bass fingerlings were obtained from a commercial producer in Arkansas and transported to experimental ponds at SIUC. Two ponds were stocked with fingerlings at a density of 37,000 fish/ha (14,980 fish/acre) and two ponds were stocked at a density of 74,000 fish/ha (29, 960 fish/acre). A sub-sample of 100 fish stocked into each pond was measured for initial length and weight. Fish were fed to satiation several times daily. Pond trials are ongoing during fall 2008.

<u>UW-Milwaukee</u>

Auditory conditioning trials have been conducted on early life stage yellow perch. Auditory signals of low frequency (35–300 Hz) were presented to 12-day post hatched (dph) yellow perch in conjunction with a

commercial fish starter diet. The initial response was recorded as an estimate of the numbers of fish remaining in the feeding area over time. Young fish were exposed to a sound/feeding regime for up to 30 dph. From 30–50 dph, their behavioral response to the auditory signal was measured as a function of time response to the target area involving the food. The auditory signal was presented to the fish when they were randomly distributed in the tank.

Following a brief acclimation period researchers found that more than 90% of the fish responded to the auditory signal associated with food in 2–3 sec. Diets were changed so as not to bias the response to food. Based on these results, it appears that yellow perch can be conditioned to food using an auditory signal. Although these results are estimations, the over-tank video will be used to confirm and measure the behavior pattern. Subsequently, the strength and character of the audible signals will be modified to enhance the response. For example, different volumes and frequencies will be tested to assure that the sound does not cause an unnecessary "startle response."

WORK PLANNED

OBJECTIVES 1 & 2

UW-Madison

Because of the extreme flooding conditions in the Madison, Wisconsin area during 2008, the UW-Madison pond stocking density study will be conducted in 2009 as described in the original proposal.

UM-C

Feeding has continued. The time limit for use of the LDL experimental ponds is approaching. Two, 1.0-ha (0.25-acre) production ponds at a Lincoln University (LU) pond facility in Jefferson City, Missouri have been secured. Ponds at the LDL site (near Columbia) will be drawn down in late-October to more directly determine overall survival. Fish will then be transported to the LU ponds by November 1, and fed until ice-over. Next spring, the fish will be fed for 1–2 weeks and then the LU ponds will be drained and the remaining fish sampled to estimate overwinter survival and the percentage that come back on feed after overwintering.

SIUC

SIUC pond stocking density studies using different densities of fingerling largemouth bass as outlined in the proposal will be completed.

IMPACTS

Studies will provide valuable information to yellow perch fingerling producers for maximizing the productivity and efficiency of their operations. The studies will also provide valuable cost/benefit information on the use of krill and semi-moist feeds as transitional diets.

Studies will also provide valuable information to largemouth bass fingerling producers with respect to stocking densities, size of fish at feed training, light intensity during feed training, and the utility of using bird deterrent devises to reduce labor cost and increase the number of fish that are feed trained.

SUPPORT

NCRAC funds provided to date total $300,000. This is the entire amount of funding allocated for this 2-year project.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

See the Appendix for a cumulative output for all NCRAC-funded Fingerling Feed Training activities.

LARGEMOUTH BASS NUTRITION[7]

Project ***Progress Report*** for the Period
September 1, 2005 to August 31, 2008

NCRAC FUNDING: $170,000 (September 1, 2005 to August 31, 2008)

PARTICIPANTS:

Paul B. Brown	Purdue University	Indiana
Christopher C. Kohler	Southern Illinois University-Carbondale	Illinois
Joseph E. Morris	Iowa State University	Iowa
Industry Advisory Council Liaison:		
William E. Lynch	Mill Creek Perch Farms LLC, Marysville	Ohio
Extension Liaison:		
Joseph E. Morris	Iowa State University	Iowa

PROJECT OBJECTIVES

(1) Assess diet and environmental factors that affect growth and health of largemouth bass raised to 1.5 lb in ponds with formulated feed.

(2) Develop cost-effective finisher diets that enhance health and growth of largemouth bass.

(3) Conduct a region-wide workshop on raising largemouth bass to 1.5 lb in ponds based, at least, on the results of the research activities in Objectives 1 and 2.

ANTICIPATED BENEFITS

Currently, the demand for live largemouth bass in North America is not being met and prices being paid are as high or higher than for virtually any other species raised in or outside the North Central Region (NCR). With producers experiencing difficulties rearing largemouth bass from 0.34–0.68 kg (0.75–1.5 lb) in ponds without using live forage, it is necessary to develop procedures to address this limitation to profitably rear this species. The studies and outreach activities proposed in this project will address this problem by focusing on the two major dietary energy groups, carbohydrates and lipids, as well as feed management and sexual maturation.

Given the previous extension appointment of Morris as the Fisheries and Aquaculture Extension Specialist for Iowa State University (ISU), he is expected to present information garnered from this research in a format acceptable to individuals in the aquaculture industry. Tools used in this activity will allow for the timely dissemination of information.

PROGESS AND PRINCIPAL ACCOMPLISHMENTS

On November 14, 2004 the aquaculture facility at Purdue University (Purdue) was destroyed due to a fire. As a result, all activities proposed by Purdue researchers for Objectives 1 and 2 as well as associated outreach activities by Morris in Objective 3 were postponed until 2008.

[7]This 2-year project is chaired by Christopher C. Kohler and it began September 1, 2005.

OBJECTIVE 1

Current research at Southern Illinois University-Carbondale (SIUC) includes a temperature effect study on largemouth bass feeding and growth during the second growth season. This study involves two treatments with four replicates for each treatment using eight 0.04-ha (0.1-acre) ponds. Each treatment involves feeding fish at a particular time of day with the intent of fish being fed during different water temperatures. In treatment #1, fish are fed within an hour of sunrise each morning while in treatment #2 fish are fed within an hour of sundown each evening. Standard water quality measurements are taken with each treatment feeding, with temperature taken at the surface and at 1.0-m (3.3-ft) depth. Fish in both treatments are fed a commercial 45% crude protein trout diet at 4% of wet body weight. Analysis includes random sampling of 50 fish per replicate per treatment to determine overall growth, densities, and condition. Statistical analysis indicates that there were no significant treatment differences detected.

On-going research also includes a carbohydrate diet study to examine the effects of carbohydrate levels on growth in largemouth bass using an indoor recirculating aquaculture system. This study involves six treatments with four replicates per treatment in 110-L (29-gal) aquaria. The treatments include 0%, 6%, 12%, 18%, 24%, and 30% carbohydrate diets fed to bass averaging 0.25 kg (0.55 lb) at the beginning of a 12-week trial period. Each practical diet is isocaloric and isonitrogenous (40% crude protein) with different levels of dextrin (0%–30%). This current study will be completed in 2008 and results included in the termination report.

An over-winter study was completed including two treatments with four replicates for each treatment using eight 0.04-ha (0.1-acre) ponds to observe whether largemouth bass lose feed training over a winter period of not being fed. One treatment involved feeding largemouth bass 1% of wet body weight of a 45% crude protein commercial trout diet whereas the second treatment received no food. The trial's duration was the entire winter season between the second and third growth seasons. Twenty-five fish from each replicate per treatment were randomly selected and placed in an indoor recirculation system of the same temperature as the research ponds. Tank temperatures were raised 1.0–2.0°C/day (33.8–35.6°F/day) to imitate spring temperature changes. Once spring temperatures were reached, fish were fed at 4% wet body weight and observations of feeding in each treatment were made. No differences in bass recommencing feeding on prepared diets were observed between treatments.

A study was also conducted to determine the effects of pellet size on largemouth bass growth during the third growth season. The pellet-size study included one treatment being fed a 7.5-mm (0.3-in) pellet and a second treatment a 9.5-mm (0.4-in) pellet. Analysis included weight and length gain, as well as feed conversion comparisons between the two treatments from 25 randomly sampled fish/replicate/treatment. There were no significant differences detected in this study as both sizes of pellets yielded similar production data.

Researchers at Purdue stocked nine earthen culture ponds with age 0 fish in the summer of 2007 and acquired an additional group of the same strain for use in laboratory studies. All fish in ponds have been fed a commercial trout diet once per day to satiation. The pond feeding experiment will begin spring 2008.

OBJECTIVE 2
Based on results from Objective 1, one or more "finishing" diets containing carbohydrate levels determined to be suitable for largemouth bass grow out in the second year will be evaluated and compared by Purdue to the industry standard. Research at Purdue was designed to address liver health of bass during the second year of growth. A commercial control diet (Nelson and Sons, Inc., Steelhead Diet) was used as a positive control diet and an experimental diet was developed in collaboration with the feed mill. The experimental diet contained more soybean meal (35%) and more choline than the commercial diet. Both diets were fed to 2+ year old bass raised in earthen culture ponds. Ponds were recently harvested in conjunction with an Extension Field Day (Objective 3). There were no differences in consumption, weight gain, or feed conversion rate of fish fed the two diets. Blood, liver, and muscle samples were collected from fish in each replicate and are being analyzed currently. These data will provide an indication of the effect of increased levels of dietary choline on liver health.

WORK PLANNED
OBJECTIVES 1 & 2
Planned SIUC research includes a carbohydrate diet study to examine the effects of carbohydrate levels on growth in largemouth bass. The carbohydrate diet study involves six treatments with four replicates for each treatment in 110-L (29-gal) aquaria in a recirculation system. The treatments include 0%, 6%, 12%, 18%, 24%, and 30% carbohydrate diets averaging 250.0 g/fish (8.8 oz/fish) at the beginning of a 12-week trial period. Each practical diet will be isonitrogenous (40% crude protein) with different levels of dextrin (0%–30%) used for energy. Analysis will include plasma analysis of insulin and glucose along with liver samples to determine health and condition using hepatosomatic index and overall color and fat deposits. Analysis will also include whole body proximate analyses. The study will end in 2008.

Planned research includes completion of the associated carbohydrate portion of this study by the SIUC investigators. Analyses of biological tissues collected from the pond feeding trial are being analyzed and will provide important insights into the health of the fish.

OBJECTIVE 3
The regional wide workshop on largemouth bass nutrition is scheduled for fall 2008 at Purdue. Supporting materials will be developed using information garnered from this project.

IMPACTS
Developing modified diets for emerging aquaculture species allows feed mills to react to price volatility of commodities and restrain feed costs during times of rapid escalation. Moving toward diets that contain higher concentrations of regionally available ingredients increases the probability of regional manufacturing of diets.

The goal of this project is to evaluate selected carbohydrate levels in diets fed to largemouth bass and the effects of environmental factors such as feed management and onset of sexual maturation on growth in largemouth bass. The long-term benefits of this project will be seen as an improvement in U.S. aquaculture, as the demand for largemouth bass as a market fish increases in North America. The overall outcome of this project will hopefully provide the U.S. aquaculture industry with results that improve and increase growth in largemouth bass production.

SUPPORT

NCRAC funds provided to date total $170,000. This is the entire amount of funding allocated for this 2-year project.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

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

NATIONAL COORDINATOR FOR AQUACULTURE INADs/NADAs[8]

Project ***Progress Report*** for the Period
July 15, 2004 to August 31, 2008

NCRAC FUNDING: $89,000 (July 15, 2004 to August 31, 2008)

PARTICIPANT:
Rosalie A. Schnick Michigan State University Wisconsin

PROJECT OBJECTIVES

(1) Ensure effective communications among groups involved with Investigational New Animal Drug/New Animal Drug Applications (INADs/NADAs), including Canada.

(2) Serve as an information conduit between INAD/NADA applicants and the U.S. Food and Drug Administration's Center for Veterinary Medicine (CVM).

(3) Identify and encourage prospective INAD participants to become involved in specific investigational studies and NADA approval-related research.

(4) Seek the support and participation of pharmaceutical sponsors for INAD studies and NADAs and coordinate with INAD/NADA sponsors to achieve CVM approval more quickly.

(5) Guide prospective and current INAD holders on the format for INAD exemption requests and related submissions to CVM.

(6) Identify existing data and remaining data requirements for NADA approvals.

(7) Review, record, and provide information on the status of INADs and NADAs.

(8) Encourage and seek opportunities for consolidating the INAD/NADA applications.

(9) Coordinate educational efforts on aquaculture drugs as appropriate.

(10) Identify potential funding sources for INAD/NADA activities.

ANTICIPATED BENEFITS

Investigation and approval of safe therapeutic and production drugs for use by the aquaculture industry are some of the highest priorities currently facing the industry. At present, only a few approved compounds are available to the industry and further development of the aquaculture industry is severely constrained by a lack of approved drugs essential for treating more than 50 known aquaculture diseases. CVM has afforded the aquaculture industry

[8]NCRAC has funded two NADA Coordinator projects. The termination report for the first project is contained in the 1999-00 Annual Progress Report. This progress report is for the second NADA Coordinator project. Ted R. Batterson serves as the facilitator for this project interacting with a steering committee in overseeing the Coordinator's activities.

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 Aquaculture NADA Coordinator) serves as a conduit between an INAD/NADA applicant and CVM. The National Aquaculture 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

MAJOR APPROVALS

- **Original NADA approval: 35% PEROX-AID®** for the control of mortality due to (1) saprolegniasis on all finfish eggs, (2) bacterial gill disease on all freshwater-reared salmonids, and (3) external columnaris disease on all coolwater fish and channel catfish (approved January 11, 2007).
- **Supplemental NADA approval: AQUAFLOR®** for the control of mortality in all freshwater-reared salmonids due to coldwater disease (approved March 19, 2007).
- **Conditional approval: AQUAFLOR®** for control of mortality in channel catfish due to columnaris disease associated with *Flavobacterium columnare* (approved April 18, 2007).
- **Abbreviated NADA approval: TETROXY AQUATIC®** for marking in finfish fry and fingerlings (approved April 20, 2007).
- **Abbreviated NADA approval: FORMACIDE-B®** (generic copy of PARASITE-S®, sponsored by Western Chemical, Inc.) for control of certain external parasites on finfish and shrimp and for the control of certain fungi on finfish eggs (approved July 17, 2007).
- **Supplemental NADA approval: Aquaflor®** (florfenicol) for control of furunculosis on freshwater-reared salmonids (Approved October 26, 2007).
- **Supplemental NADA approval: Terramycin® 200 for Fish** (oxytetracycline dihydrate) for control of mortality in (1) all freshwater-reared salmonids due to coldwater disease and (2) *Oncorhynchus mykiss* due to columnaris disease. The limitation on treating salmonids in water temperatures below 9°C was removed. (Approved July 6, 2008).

CHLORAMINE-T (HALAMID®)—EXTERNAL ANTIBACTERIAL

Two initial label claims close to completion: control of mortality due to (1) bacterial gill disease on all freshwater-reared salmonids and (2) external columnaris disease on walleye and possibly largemouth bass.

- On September 15, 2006, CVM granted MUMS designation to Axcentive SARL, the sponsor of HALAMID®, for the following label claim for the control of mortality in freshwater-reared finfish (except freshwater-reared salmonids) due to bacterial gill disease.

- In November 2006, Axcentive SARL submitted the following to CVM: (1) Guidance for Industry Document (GFI) #152 on microbial food safety, (2) GFI document #159 on the safety of residues in human food for all fish, and (3) labeling.
- On April 13, 2007, UMESC submitted the final environmental assessment (EA) on chloramine-T to CVM.
- In May 2007, CVM accepted GFI document #152 on microbial food safety for all finfish from Axcentive SARL.
- On July 23, 2007, Axcentive SARL submitted to CVM a revised GFI document #159 on the safety of residues in human food for all fish for Halamid® (chloramine-T) prepared by the National Aquaculture NADA Coordinator with input from UMESC. The revision was based on the agency's comments.
- On October 11, 2007, UMESC received word that its final EA on Halamid® (chloramine-T) is acceptable to CVM.
- On September 25 and November 21, 2007, the Aquatic Animal Drug Approval Partnership Program (AADAP) submitted pivotal effectiveness studies conducted by Richloam Fish Hatchery (Florida) on chloramine-T to CVM for control of mortality in largemouth bass due to external columnaris disease.
- On October 12, 2007, CVM accepted the EA on HALAMID® AQUA+ developed by the UMESC.
- On December 10, 2007, CVM accepted the GFI #159 on HALAMID® AQUA+ from Axcentive SARL if the sponsor accepts a withdrawal time of 11 days.
- On February 8, 2008, Axcentive SARL submitted the Human Food Safety Technical Section Complete Letter on HALAMID® AQUA+ based on data generated by UMESC.
- On February 13, 2008, Axcentive SARL submitted text for the Labeling on HALAMID® AQUA+ for the following: control of mortality due to (1) bacterial gill disease on all freshwater-reared salmonids and (2) external columnaris disease on walleye.
- On March 3, 2008, CVM accepted from AADAP the Effectiveness Technical Section on chloramine-T as being complete for control of mortality in walleye due to external columnaris disease.
- On May 19, 2008, Axcentive SARL submitted the complete Chemistry, Manufacturing, and Controls Technical Section on HALAMID® AQUA+ to CVM.
- On May 23, 2008, CVM accepted from Axcentive SARL the draft text of Labeling requesting some minor revisions.
- On July 9, 2008, AADAP submitted efficacy data on the control of mortality in bluegill due to external columnaris disease.
- On July 11, 2008, AADAP resubmitted efficacy data on the control of mortality in largemouth bass due to external columnaris disease and requested an Effectiveness Technical Section Complete.
- On August 6, 2008, CVM offered two options to complete Human Food Safety Technical Section: (1) provide human intestinal flora data or (2) improve the determinative method performance so the marker residue can be reliably quantitated to lower levels. If this is accomplished, then CVM would assign an 11-day withdrawal time. In addition, CVM may have other options available to complete this technical section. UMESC is working on improving the method.

CLOVE OIL (MAINLY EUGENOL)—ANESTHETIC

This drug is not currently under development for approval

- On April 24, 2007, CVM revised GFI #150 dealing with concerns related to the use of clove oil (eugenol) as an anesthetic for fish by correcting information on its ingredients and safety.

COPPER SULFATE (TRIANGLE BRAND COPPER SULFATE®)—EXTERNAL MICROBICIDE

One initial label claim close to completion: (1) control of mortality due to ichthyophthiriasis on channel catfish.

- On October 30, 2006, CVM granted MUMS designation to Phelps Dodge Sales Company, the sponsor of Triangle Brand Copper Sulfate®, for the following label claim for the treatment of *Ichthyophthirius multifilis* on channel catfish cultured in earthen ponds.
- In December 2006, the Stuttgart National Aquaculture Research Center (SNARC) submitted the final EA for earthen pond systems to CVM.
- CVM reviewed the copper sulfate final EA for earthen pond systems and required additional changes.
- In December 2007, CVM accepted copper sulfate Human Food Safety Technical Section Complete Letter for channel catfish.
- In January 2008, the sponsor, Phelps Dodge Sales Company, received comments from CVM on the draft Labeling and required revisions for Triangle Brand Copper Sulfate®.
- In April 2008, the SNARC submitted hazard characterization for microbial food safety (GFI #152) to gain a Human Food Safety Technical Section Complete Letter for all finfish
- In May 2008, SNARC submitted a second draft of the Labeling.

ERYTHROMYCIN (AQUAMYCIN 100®) —ORAL ANTIBACTERIAL

One initial label claim close to completion: (1) control of mortality due to bacterial kidney disease in salmonids.

- On October 2, 2006, the University of Idaho submitted to CVM the on the safety of residues in human food for all freshwater-reared salmonids (GFI #159).
- On January 4, 2007, CVM granted MUMS designation to Bimeda, the sponsor of AQUAMYCIN 100®, for the control of mortality in freshwater-reared salmonids due to bacterial kidney disease associated with *Renibacterium salmoninarum*.
- On January 11, 2007, CVM accepted as complete the GFI document #159 on the safety of residues in human food for all freshwater-reared salmonids from the University of Idaho. A right to reference proprietary toxicological data is needed to complete the Human Food Safety Technical Section.
- In May 2007, the University of Idaho submitted the final EA to CVM.
- UMESC is working with the University of Idaho on the revision of the EA.

FLORFENICOL (AQUAFLOR®)—ORAL ANTIBACTERIAL

One supplemental label claim completed, control of mortality due to furunculosis in freshwater-reared salmonids, and two supplemental label claims close to completion: (1) systemic columnaris disease in freshwater-reared salmonids and catfish, and (2) *Streptococcus iniae* in hybrid striped bass and tilapia.

- On March 19, 2007, CVM approved the Florfenicol (Aquaflor®) NADA from Schering-Plough Animal Health for control of mortality in all freshwater-reared salmonids due to coldwater disease.
- On March 19, 2007, CVM accepted the effectiveness data as being complete

from the AADAP for control of mortality due to furunculosis in freshwater-reared salmonids.

- On April 18, 2007, CVM approved Aquaflor® for a conditional approval for control of mortality in channel catfish due to columnaris disease.
- On April 19, 2007, CVM accepted from AADAP as complete the Effectiveness Technical Section on AQUAFLOR® for control of mortality in freshwater-reared salmonids due to furunculosis.
- On October 4, 2007, AADAP requested that CVM consider the Effectiveness Technical Section on AQUAFLOR® to be complete for control of mortality in hybrid striped bass due to *Streptococcus iniae*.
- On October 26, 2007, CVM approved Aquaflor® for a supplemental approval for control of mortality in freshwater-reared salmonids due to furunculosis. Aquaflor® is sponsored by Intervet/Schering-Plough Animal Health.
- On February 22, 2008, CVM granted MUMS designation to Intervet/Schering-Plough Animal Health, the sponsor of emamectin benzoate (Slice®), for the control of sea lice on salmonids.
- On February 23, 2008, the North Central Regional Aquaculture Center (NCRAC) announced that UMESC had received a contract to develop effectiveness data for the control of mortality in coolwater and warmwater finfish due to aeromonad infections with Terramycin 200 for Fish® (oxytetracycline dihydrate) and Aquaflor® (florfenicol).
- On April 4, 2008, AADAP completed the Effectiveness Technical Section for control of mortality in hybrid striped bass due to *Streptococcus iniae* and forwarded the information to Intervet/Schering-Plough Animal Health.

FORMALIN—EXTERNAL MICROBICIDE

One supplemental label claim close to completion: (1) control of mortality due to saprolegniasis on all freshwater-reared fish.

- On July 17, 2007, an Abbreviated NADA (generic copy of Parasite-S®, sponsored by Western Chemical, Inc.) was granted by CVM for Formacide-B® (formalin) for control of certain external parasites on finfish and shrimp and for the control of certain fungi on finfish eggs. Formacide-B® is sponsored by B.L. Mitchell, Inc.
- The CVM Office of Research for the pivotal efficacy studies conducted by for the control of mortality due to saprolegniasis on channel catfish.
- On December 19, 2007, CVM granted MUMS designation to Western Chemical Inc. for their formalin product, PARASITE-S®, for the following label claim: For the control of mortality in freshwater-reared finfish due to saprolegniasis associated with fungi in the family Saprolegniaceae.

HYDROGEN PEROXIDE—EXTERNAL MICROBICIDE

One label claim in progress: (1) control of mortality on all warmwater finfish due to saprolegniasis.

- On September 6, 2006, CVM accepted the all other information technical section for three broad label claims from Eka Chemicals, Inc. in collaboration with the National Aquaculture NADA Coordinator.
- On November 9, 2006, CVM accepted the 35% PEROX-AID® labeling for three broad label claims from Eka Chemicals, Inc.
- On November 22, 2006, CVM accepted the Freedom of Information Summary for 35% PEROX-AID® for three broad label claims from Eka Chemicals, Inc.
- On November 30, 2006, Eka Chemicals, Inc. submitted the original NADA

package for three broad label claims to CVM for approval.

- On January 11, 2007, CVM approved the 35% PEROX-AID® NADA for the control of mortality due to (1) saprolegniasis on all finfish eggs, (2) bacterial gill disease on all freshwater-reared salmonids, and (3) external columnaris disease on all coolwater fish and channel catfish.
- On May 2, 2007, CVM removed hydrogen peroxide from the list of Low Regulatory Priority aquaculture drugs because the drug is now the subject of an approved NADA for 35% PEROX-AID®. This means that 35% PEROX-AID® is the only hydrogen peroxide product that is legal to use.
- On October 15, 2007, CVM accepted the special supplement for minor changes in the 35% PEROX-AID® labeling.
- On October 31, 2007, Eka Chemicals, Inc. submitted a Periodic Drug Experience Report—Six-Month Reporting (21 CFR 514.80) for Original NADA # 142-255 for 35% PEROX-AID®.
- On November 7, 2007, AADAP submitted to CVM effectiveness studies on 35% PEROX-AID® conducted by Richloam Fish Hatchery (Florida) for the control of mortality in largemouth bass due to external columnaris disease.

ISOEUGENOL (AQUI-S®)—ANESTHETIC

One initial label claim was in progress but has been terminated: (1) zero withdrawal anesthetic for sedation to handleable condition of all freshwater fish.

- In February 2004, the National Aquaculture NADA Coordinator obtained $60,000 in funding through NCRAC for the radiolabeled material needed for the total residue depletion study on AQUI-S® to be conducted by the UMESC. The funds were provided to the sponsor, AQUI-S New Zealand LTD., so that the purchase of the material did not have to go through the bidding process as would have been required for a federal agency. The material was purchased in the fall of 2004 and UMESC started the study in early 2005. UMESC completed the laboratory portion of the total residue depletion study on rainbow trout in the spring 2005.
- UMESC submitted the final report to CVM on March 14, 2006. On January 31, 2007, UMESC submitted a response to CVM's August 23, 2006 comments on the total residue depletion studies and a letter requesting the selection of the marker residue. The response was not definitive because of some concern of the radiochemical purity (95%) of the isoeugenol. CVM indicated that the agency cannot determine the significance of using test material with low radiochemical purity until the safe concentration for isoeugenol is calculated. CVM's recommendation is intended to ensure that the reported total radioactivity in tissues is an accurate measurement of total residues. The total residue concentration is then related to the safe concentration determined by the acceptable daily intake (ADI). An ADI daily intake has not been assigned for isoeugenol because the toxicological requirements for isoeugenol have not been completed. This issue will not be resolved until the National Toxicology Program (NTP) has its meeting on isoeugenol toxicology studies in February 2008 and one more toxicology study is completed by the sponsor. If the safe concentration for isoeugenol is much lower than the reported total residues at the time point of concern (in this case 0-h for a zero hour withdrawal anesthetic), the issue of low radiochemical purity may be insignificant. If the safe concentration

for isoeugenol is much higher than the reported total residues at the time point of concern, the low radiochemical purity of the test material may have to be addressed.

- On September 21, 2006, UMESC submitted a final report to Association of Fish and Wildlife Agencies on the development and validation of a determinative method to detect isoeugenol in fish tissues.
- On November 28, 2006, CVM accepted as complete from AADAP the effectiveness studies for all freshwater-reared finfish for sedation to handleable stage. Validation of the dose verification method is required before a technical section complete letter can be granted.
- On December 8, 2006, CVM accepted the target animal safety study protocol on rainbow trout from AADAP.
- On April 27, 2007, AADAP and UMESC announced they were suspending all research until the completion of the NTP review scheduled for February 2008 on studies conducted on mice and rats. The review had originally been scheduled for May 2007 but due to other priorities was delayed.
- The Gibbs method used to detect isoeugenol in efficacy and target animal safety studies was submitted by sponsor to CVM.
- On October 9, 2007, AADAP requested that CVM consider the Target Animal Safety Technical Section on isoeugenol to be complete for freshwater salmonids.
- On January 15, 2008, CVM accepted the Gibbs Method to detect isoeugenol in water with conditions.
- On February 28, 2008, the NTP peer review panel confirmed that there is clear evidence of isoeugenol carcinogenicity in male mouse livers and thus triggered the Delaney Clause, a 1958 amendment to the Food, Drugs, and Cosmetic Act (FDCA). This fact makes it very difficult if not impossible to gain a zero withdrawal period for isoeugenol.
- In a March 19, 2008 conference call between UMESC, National Aquaculture NADA Coordinator, and CVM's Division of Human Food Safety, four potential candidates (benzocaine, eugenol, metomidate, and tricaine methanesulfonate) would require the development of at least some mammalian safety and residue chemistry studies to support a potential approval.
- The Association of Fish and Wildlife Agencies (AFWA) Drug Approval Working Group (DAWG) met on March 26-28, 2008 to discuss the available options for, and limitations to, an isoeugenol approval and to formulate plans to identify an alternative sedative.
- The DAWG and potential sponsors met with CVM to determine the data requirements for prospective candidate sedatives: benzocaine, eugenol, and tricaine on August 20, 2008.

17α-METHYLTESTOSTERONE (MT) —GENDER MANIPULATION AID

One initial label claim in progress: (1) masculinization of female early life-stage tilapia.

- Studies were initiated for effectiveness and target animal safety in late 2006 and early 2007.
- On July 30, 2007, interested parties met in Bozeman, Montana to discuss environmental assessment issues and to determine a course of action.
- On October 1, 2007, UMESC submitted to CVM the environmental safety studies and the water method for MT that were conducted and developed by the University of Wisconsin-Madison (UW-Madison).
- On August 17, 2007, CVM indicated it could not accept the MT target animal

safety study on tilapia conducted at SIUC and that the study needed to be repeated.
- On October 1, 2007, UMESC submitted to CVM the environmental safety studies and the water method for MT that were conducted and developed by UW-Madison.
- On November 5, 2007, AADAP submitted data and requested that CVM consider the Effectiveness Technical Section to be complete for the use of MT to produce predominantly male populations of tilapia.
- In December 2007, AADAP worked with the American Tilapia Association through a survey to develop baseline information relative to hatchery discharge in support of an EA for MT.
- On February 10, 2008, interested parties met in Orlando, Florida to discuss the remaining data requirements for the approval of MT for tilapia, find solutions, and develop schedules for completion.
- On February 23, 2008, the National Aquaculture NADA Coordinator requested $50,000 from NCRAC to fund a repeat of the target animal safety study on tilapia and a feed method transfer study on MT. The NCRAC Board approved up to that amount to fund those two studies, but hoped that another RAC or some other entity would partially or fully fund those studies.
- On March 31, 2008, CVM accepted the MT method validation study in water but not the validation in sediment as developed by UW-Madison.
- On March 31, 2008, CVM requested additional information from UW-Madison concerning the study report on the transformation of MT in aquatic-sediment systems.
- On April 8, 2008, Rangen, Inc. submitted to CVM the data on stability, homogeneity, and segregation of MT feed based on studies by UW-Madison.
- On April 21, 2008, NCRAC requested assistance from the Western Regional Aquaculture Center (WRAC) to help fund the repeat target animal safety study on tilapia and a feed method transfer study on MT. The WRAC Board agreed to split the funding for the two studies.

METOMIDATE (AQUACALM®)—SEDATIVE

One label claim in progress (1) sedative during transport of ornamental finfish.
- On November 13, 2006, CVM granted MUMS designation to Syndel Laboratories, LTD, the sponsor of AQUACALM®, for the label claim for use as a sedative during transport of ornamental (non-food) finfish.

OXYTETRACYCLINE DIHYDRATE (TERRAMYCIN® 200 FOR FISH)—ORAL ANTIBACTERIAL

One supplemental label claim close to completion: skeletal marking for salmonids.
- In December 2007, CVM requested a pivotal efficacy study from AADAP to complete the marking label claim for oxytetracycline dihydrate. This request was in response to an ADDAP request for an effectiveness complete letter for marking data submitted by AADAP.
- On February 6, 2008, CVM accepted the Human Food Safety Technical Section for Terramycin® 200 for Fish from Phibro Animal Health as being complete for all freshwater-reared finfish based on data generated by UMESC.
- On February 14, 2008, Phibro Animal Health submitted an All Other Information Technical Section for Terramycin® 200 for Fish to complete the final technical section submission for an Administrative NADA submission for the following: control of mortality due

to (1) columnaris disease in freshwater-reared *Oncorhynchus mykiss* and (2) coldwater disease in freshwater-reared salmonids**.**

- On February 21, 2008, CVM accepted the Environmental Safety Technical Section for oxytetracycline dihydrate as being complete for all freshwater-reared finfish based on data generated by UMESC.
- On February 23, 2008, NCRAC announced that UMESC had received a contract to develop effectiveness data for the control of mortality in coolwater and warmwater finfish due to aeromonad infections with Terramycin 200 for Fish® (oxytetracycline dihydrate) and Aquaflor® (florfenicol).
- On May 8, 2008, CVM accepted from Phibro Animal Health the Labeling Technical Section for Terramycin® 200 for Fish as being complete control of mortality due to (1) columnaris disease in freshwater-reared *Oncorhynchus mykiss* and (2) coldwater disease in all freshwater-reared salmonids. Phibro Animal Health will add the previously approved label claim (September 23, 1970) for marking skeletal tissue of Pacific salmon to this labeling (250 mg of oxytetracycline per kg of fish per day in fish feed); it was never on any previous labels. Additionally, the temperature restriction on treating salmonids below 9°C. is removed from the label as a result of UMESC data.
- **Supplemental NADA approval: Terramycin® 200 for Fish** (oxytetracycline dihydrate) for control of mortality in (1) all freshwater-reared salmonids due to coldwater disease and (2) *Oncorhynchus mykiss* due to columnaris disease. The limitation on treating salmonids in water temperatures below 9°C was removed (Approved July 6, 2008).

OXYTETRACYCLINE HYDROCHLORIDE (SEVERAL COMMERCIAL PRODUCTS)—MARKING AID

- On April 20, 2007, CVM approved an abbreviated original (generic) NADA approval for TETROXY Aquatic® sponsored by Cross Vetpharm Group LTD. for use as a skeletal marking aid in finfish fry and fingerlings.

OXYTETRACYCLINE HYDROCHLORIDE (TERRAMYCIN-343®)—EXTERNAL ANTIBACTERIAL

One label claim in progress: control of mortality in coolwater and warmwater finfish due to external columnaris disease)

- On March 20, 2007, UMESC submitted to CVM efficacy studies on the control of mortality in coolwater and warmwater finfish due to external columnaris disease.
- On June 7, 2007, CVM granted MUMS designations to Pfizer Animal Health, sponsor of Terramycin-343®, for the following label claims: For the control of mortality in freshwater-reared finfish fry and fingerlings due to (1) external columnaris disease associated with *Flavobacterium columnare*, (2) bacterial gill disease associated with *Flavobacterium branchiophilum*, and (3) systemic columnaris disease associated with *Flavobacterium columnare*.
- On September 14, 2007, CVM accepted from UMESC effectiveness data on oxytetracycline hydrochloride as being supportive for control of mortality in channel catfish due to external columnaris disease.

POTASSIUM PERMANGANATE (CAIROX®)—EXTERNAL MICROBICIDE

One label claim in progress: control of mortality in channel catfish due to external columnaris disease

- On September 12, 2006, CVM granted MUMS designations to Carus Chemical

Company, the sponsor of Cairox®, for the following label claims: For the control of mortality in (1) freshwater-reared finfish due to external columnaris disease associated with *Flavobacterium columnare*, (2) freshwater-reared salmonids due to bacterial gill disease associated with *Flavobacterium branchiophilum*, and (3) freshwater-reared salmonids due to coldwater disease associated with *Flavobacterium psychrophilum*.

SALMON GONADOTROPIN RELEASING HORMONE ANALOG (OVAPLANT®)—SPAWNING AID

One label claim under investigation: For the induction of spawning in ornamental fish

- On July 25, 2007, CVM granted MUMS designation to Syndel Laboratories, LTD, the sponsor of Ovaprim®, for the induction of spawning in ornamental fish.

TRICAINE METHANESULFONATE (TRICAINE-S®)—ANESTHETIC. One label claim under investigation: for the euthanasia of finfish not intended for food.

- On January 4, 2008, CVM granted MUMS designation to Western Chemical Inc., the sponsor of Tricaine-S®, for the euthanasia of finfish not intended for food.

GENERAL

- On December 5, 2007, the National Aquaculture NADA Coordinator gave a two-hour seminar to CVM on the status and background of the aquaculture drug approvals, the roles, responsibilities, and accomplishments of the National Aquaculture NADA Coordinator, and coordination needs after her retirement.
- National Aquaculture NADA Coordinator convened a producer session at Aquaculture America 2008 on February 12, 2008.
- The designation provision of the MUMS Act of 2004 gives sponsors seven years of marketing exclusivity. As of August 31, 2008, the MUMS Office has granted 54 designations, 47 of those are to aquaculture drug sponsors, many of whom have received extensive help from the National Aquaculture NADA Coordinator.
- The 14th Annual Drug Approval Coordination Workshop was held in Bozeman, Montana on July 29-31, 2008. The topics included celebration of approval for Terramycin® 200 for Fish and approval status of all aquaculture drugs. On August 1, 2008, a meeting of the National Aquaculture Drug Research Forum and the organizational meeting of the National Aquaculture Industry Therapeutic Agent Program occurred.
- Because of the potential concern that the Joint Subcommittee on Aquaculture Working Group on Aquaculture Drugs, Biologics, and Pesticides may be acting as a Federal Advisory Committee, an informal meeting was convened on February 9, 2008 to solicit input from non-federal stakeholders on future roles and direction. The new group was tentatively named the National Aquaculture Industry Therapeutic Agent Program (NAI-TAP) and was to be a coalition of aquaculture industry stakeholders and invited non-industry entities who would have addressed and supported the development, approval, availability, and optimal use of drug, biologic, nutritional, and other products that affect the health and production of aquatic animals. However, because of the lack of private aquaculture industry representation on the working group, it became the Working group on Aquaculture Chemicals under the American Fisheries Society Fish Culture Section.

- The National Aquaculture NADA Coordinator began working in February 2008 with CVM, AFWA's DAWG, any potential sponsors, involved researchers, and potential users to determine a course of action for a replacement candidate for a zero withdrawal sedative based on the peer review finding that the NTP study results for the male mouse carcinogenicity study on isoeugenol still stand, i.e., that there is clear evidence of carcinogenicity. The DAWG has also played a major role in these efforts (see details below).
- The DAWG met on September 17, 2007 and March 26-28, 2008 to discuss the approval status of the AFWA Project drugs and on March 26-28, 2008 to discuss available options for, and limitations to, an isoeugenol approval and to formulate plans to identify an alternative sedative.
- The National Aquaculture NADA Coordinator requested help from AquaNic to change her website address to http://aquanic.org/aquadrugs because USDA could no longer be involved because of the Federal Advisory Committee Act.

WORK PLANNED

The Work Plan is to continue meeting Objectives 1–8 and to help aquaculture drug sponsors develop major NADA documents and finalize their NADA submissions for approval.

IMPACTS

Establishment of the National Aquaculture 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 established in 1995 and activities on other new drugs of interest to aquaculture. INAD/NADA sponsors and other entities have initiated new INADs and made progress toward unified efforts on existing and new INADs/NADAs or have renewed their commitment to the INAD/NADA process on their drug products.

This enhanced coordination will help and has helped gain original approvals for new NADAs and extensions and expansions of approved NADAs. These include: (1) Original NADA approvals for human chorionic gonadotropin (Chorulon®), florfenicol (Aquaflor®), and hydrogen peroxide (35% PEROX-AID®); (2) Supplemental NADA approvals for florfenicol (Aquaflor®), formalin (Formalin-F®, Parasite-S®), oxytetracycline dihydrate (Terramycin® 200 for Fish), and oxytetracycline hydrochloride (OxyMarine®, Oxytetracycline HCL Soluble Powder-343®, Terramycin-343®); (3) Abbreviated NADA approvals for tricaine methanesulfonate (Tricaine-S®), formalin (Formacide-B®), and oxytetracycline hydrochloride (TETROXY AQUATIC®); and (4) Conditional approval for florfenicol (Aquaflor®)

The approval of the candidate drugs will aid the aquaculture industry to reduce mortalities associated with infectious and handling diseases and to increase their efficiency by using spawning aids and gender manipulation aids. The domestic aquaculture industry will be better able to compete with foreign producers because there will be more legal drugs for producers to use.

PUBLICATIONS, MANUSCRIPTS, PAPERS PRESENTED, AND REPORTS

See the Appendix for a cumulative output for all NCRAC-funded National Coordinator for Aquaculture INADs/NADAs activities.

SUPPORT

YEAR	NCRAC-USDA FUNDING	OTHER SUPPORT					TOTAL SUPPORT
		UNIVER-SITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
2004-05	$9,000		$22,476	$46,295	$26,000	$94,771	$103,771
2005-06	$15,000		$17,500	$58,527	$21,500	$97,527	$112,527
2006-07	$20,000		$26,980	$52,855	$22,200	$102,035	$122,035
2007-08	$20,000		$32,980	$57,400	$22,200	$112,580	$132,580
2008-09	$25,000		$36,980	$50,000	$22,700	$109,680	$134,680
TOTAL	$89,000		$136,916	$265,077	$92,900	$494,893	$605,593

NUTRITION[9]

Project ***Termination Report*** for the Period
September 1, 2004 to May 31, 2007

NCRAC FUNDING: $200,000 (September 1, 2004 to May 31, 2007)

PARTICIPANTS:

Paul B. Brown	Purdue University	Indiana
Donald L. Garling	Michigan State University	Michigan
Christopher C. Kohler	Southern Illinois University-Carbondale	Illinois
Jeffrey A. Malison	University of Wisconsin-Madison	Wisconsin
Industry Advisory Council Liaison:		
Curtis Harrison	Harrison Fish Farm, Hurdland	Missouri
Extension Liaison:		
Donald L. Garling	Michigan State University	Michigan
Non-Funded Collaborators:		
Mark E. Griffin	Land O'Lakes/Purina Feeds, St. Louis	Missouri

REASON FOR TERMINATION
The project objectives were completed.

PROJECT OBJECTIVES
(1) Develop cost-effective fish meal-free diets for grow out of hybrid striped bass with an initial minimum weight of 100 g (3.5 oz).

(2) Develop cost-effective fish meal-free diets for grow out of yellow perch with an initial weight of 10 g (0.35 oz).

PRINCIPAL ACCOMPLISHMENTS
OBJECTIVE 1
Researchers at Purdue University (Purdue) were delayed in starting the Nutrition project because a fire completely destroyed the Aquaculture Research Laboratory in October 2004 as the project was beginning. Temporary wet lab space was occupied from January 2005 until June 2006, but that space was inadequate for conducting the proposed studies. The Aquaculture Research Laboratory was reconstructed and was occupied in June 2006 at which time the proposed studies with hybrid striped bass and yellow perch commenced. Feedstuffs acquired and analyzed for both studies include distillers dried grains with solubles, sunflower meal, canola meal, soybean meal, corn gluten meal, brewer's yeast, poultry by-product meal/feather meal (1:1 w:w), meat and bone meal, fish meal, and whole ground wheat.

In the study with hybrid striped bass, numerous dietary formulations were attempted. Dietary crude protein concentrations were maintained at 36% of the diet and the essential amino acid concentrations met the established requirements assuming 80% availability from practical ingredients. Dietary lipid concentration was maintained at 10% of the diet using fish oil with ethoxyquin. Use of plant-based ingredients resulted in dietary formulations that would not meet the

[9]This is a 2-year project that was chaired by Paul B. Brown and began September 1, 2004.

requirements in diets containing 36% dietary crude protein. All possible combinations were attempted, including formulations in which all plant-based ingredients were provided. Using the nutrient limitations established above, dietary crude protein must be in the range of 30–34% of the diet. However, combinations of animal-based ingredients and plant-based feedstuffs met the established nutrient restrictions. Two series of diets were established, one series contained meat and bone meal, and the other contained the 1:1 mixture of poultry by-product meal and feather meal in combination with whole wheat and one of the plant-based ingredients. For example, meat and bone meal/soybean meal, meat and bone meal/canola meal, etc. Whole wheat was used as the carbohydrate source, which will be required for extrusion processing of diets. A positive control diet containing only fish meal was also formulated and fed to fish. Initial weight of fish was 110.0 g (3.9 oz), the experimental system was a recirculating system and all fish were fed to satiation twice daily.

Significant differences were detected in mean feed consumption, weight gain, feed conversion ratio (FCR) and specific growth rate (SGR). Statistical analysis revealed significant differences in mean feed consumption across treatments, but the multiple ranking test imposed was unable to differentiate mean values. Mean weight gain of fish fed the positive control diet and the series containing poultry by-product meal was significantly greater than fish fed the series of diets containing meat and bone meal. Similarly, FCR and SGR of fish fed the positive control diet and those fed the poultry by-product series was significantly improved compared to fish fed the meat and bone meal diets. Mean FCR of fish fed the positive control diet was 2.0, mean FCR of fish fed the poultry by-product series of diets was 1.5–1.7, while mean FCR of fish fed the meat and bone meal diets was 2.9–3.0. Mean SGR of fish fed the positive control diet was 0.96, while mean SGR of fish fed the poultry by-product series of diets was 0.98–1.06. Mean SGR of fish fed the meat and bone meal series of diets was 0.67–0.72.

Based on these results, and operating under the formulation assumptions outlined above, fish meal-free diets using blends of animal and plant-based ingredients result in feed acceptance and production parameters that are not different from fish fed fish meal-based diets. Lower dietary crude protein concentrations will allow increased use of plant-based ingredients. The relatively lower results in fish fed the meat and bone meal series of diets was surprising as that ingredient was proven beneficial in a separate series of studies conducted at Purdue. Differences between the previous and current studies may be the result of the uncertainties in source material for meat and bone meal, as well as processing conditions and overall quality.

Research at Southern Illinois University-Carbondale (SIUC) has been conducted to determine the maximum percentage of corn gluten meal that could be used as a substitution for fish meal in hybrid striped bass diets without adversely affecting growth. Two 2-month feeding trials were conducted in a recirculating system with associated mechanical and biological filtration. Isonitrogenous, isocaloric diets containing 40% crude protein and 12% crude lipid were fed twice daily to satiation throughout both trials. During the first trial, ten ~40.0 g (1.41 oz) fish were stocked into each tank and fed five diets ranging from 0–30% fish meal. Based on the results from this study, a second trial was conducted feeding seven diets containing 0–24% fish

meal using ten ~18.0 g (0.63 oz) fish per tank. All practical diets included fish meal, corn gluten meal, soybean meal, wheat middlings, fish and canola oils (50:50), sodium phosphate, dicalcium phosphate, vitamin and mineral mixes, choline, and carboxymethylcellulose.

After the first trial, SIUC researchers observed no significant differences ($P < 0.05$) in growth between the 30 and 22.5% fish meal dietary treatments. At the conclusion of the second feed trial SIUC researchers found that hybrid striped bass fed less than 20% fish meal demonstrated significantly lower ($P < 0.05$) weight gain; however, SGR and FCR were maintained in treatments containing 12 and 16% fish meal, respectively.

SIUC researchers found partially substituting fish meal with corn gluten meal in hybrid striped bass diets is possible without adversely affecting growth. Long-term benefits from this study include an improvement of the efficiency of aquaculture feeds for hybrid striped bass and a reduced reliance on the fish meal industry.

In 2006, SIUC researchers conducted a 10-week feed trial in a 28 tank recirculating system stocked with 10 sunshine bass (9.3 ± 6 g, [0.33 ± 0.21 oz] mean individual weight) per tank. Seven isonitrogenous, isocaloric (40% crude protein and 15% crude lipid) diets containing graded levels (0, 20, 40, 60, 80, or 100%) of menhaden to canola oils with 20% menhaden meal, or 100% canola oil with 20% lipid-extracted menhaden meal (LEMM), were fed twice daily to apparent satiation throughout the trial.

Replacing menhaden oil with canola oil resulted in significant differences ($P < 0.05$) in production parameters. Weight gain, SGR, and FCR were not significantly different in diets containing 60% or less canola oil as a replacement for menhaden oil. The fatty acid (FA) profile of the fillet was highly responsive to dietary FA changes; significant differences were apparent for almost every FA between dietary treatments. Saturated, total n-3, and highly unsaturated FA were highest in fillets from fish fed diets rich in menhaden oil and monounsaturated and total n-6 FA were highest in the fillets of fish fed diets high in canola oil. Liposomatic indices were highest for fish fed 0% menhaden oil with LEMM (5.60 ± 0.27%) compared to fish fed diets containing menhaden oil (range: 3.2–4.4%). Oxidative stability of both liver and fillet tissue decreased in response to dietary menhaden oil inclusion.

SIUC researchers were able to reduce dietary intake of marine oils to 40% without negatively impacting growth of hybrid striped bass fingerlings. Highly unsaturated fatty acid (HUFA) content of the fillet was comparable to wild striped bass when feeding at least 80% menhaden oil with 20% menhaden meal. Data from this study suggest a 40% menhaden oil/20% menhaden meal diet can be used during grow out of sunshine bass fingerlings without altering production. Prior to harvest using an 80% menhaden oil/20% menhaden meal diet may be suitable as a finishing diet to re-establish HUFA levels in the fillet.

In 2007, SIUC conducted a 10-week feed trial in a 28 tank recirculating system stocked with 10 sunshine bass *Morone chrysops* × *M. saxatilis* (5.2 ± 0.22 g [0.18 ± 0.01 oz]; individual mean weight ± standard deviation) per tank. Fish within replicate tanks ($N = 4$) were fed one of seven isonitrogenous, isolipidic (40% crude protein and 15% crude lipid) experimental diets containing graded levels (0, 10, or

20%) of menhaden meal with a feed attractant (1% dry matter) or a control diet containing 30% menhaden meal without a feed attractant. Two feed attractants were evaluated in this study as well, commercially available Finnstim S (Danisco Animal Nutrition, Wiltshire, England) and a plant-based experimental product soluble canola protein concentrate (SCPC; MCN BioProducts, Saskatoon, Saskatchewan, Canada). Corn gluten meal was the alternative dietary protein source used to replace menhaden meal in all experimental feeds containing feed attractants. Fish were fed twice daily to apparent satiation throughout the trial.

Plant-based alternative protein sources replaced up to 67% of menhaden meal fed to sunshine bass without negatively impacting production parameters when Finnstim S or SCPC were added to the diets. FCR of fish fed reduced menhaden meal were not statistically different from control fish ($P <$ 0.05). Additionally, no differences in weight gain, feed intake, or FCR were attributed to feed attractant type. This suggests differences in weight gain observed when fish were fed 0% menhaden meal diets with either attractant are attributable to reductions in feed intake observed in the same treatments.

Previously, Lewis and Kohler found 20% dietary menhaden meal was needed to maintain sunshine bass production performance when corn gluten meal was used as the alternative protein source. However, dietary inclusion of a feeding attractant (Finnstim S or SCPC; 1% dry matter) in this study maintained growth rates and feed conversion ratios when feeding as little as 10% fish meal to sunshine bass. Reducing dietary fish meal by 10% with the addition of soluble canola protein concentrate reduced dietary protein expenditures by 11%.

OBJECTIVE 2

University of Wisconsin-Madison (UW-Madison) investigators conducted a grow-out trial on yellow perch comparing four diets. All diets were formulated to be 41% crude protein and 10.5% crude fat and meet or exceed the nutritional requirements for rainbow trout. The control diet was a commercial trout grower containing a high percentage of fish meal. The experimental diets were similar to the control diet, except that the fish meal was replaced with animal and plant meal mixes in the following ratios: 75% animal meal mix/25% plant meal mix, 55% animal meal mix/45% plant meal mix, and 35% animal meal mix/65% plant meal mix. Each of the experimental diets contained 5% shrimp meal to enhance palatability.

In April 2005, Mark Griffin at Land O'Lakes/Purina Feeds had approximately 31.8 kg (70.0 lb) of each of the experimental diets made into 2.0 mm (0.08 in) sinking pellets. The diets were subsequently shipped to the UW-Madison's facilities at the Lake Mills State Fish Hatchery, Lake Mills, Wisconsin, where they were kept in frozen storage.

In mid-May 2005, UW-Madison investigators set up 12, 220.0-L (58.1-gal) flow through tanks as described in the original proposal. Each tank was stocked with approximately 60 yellow perch having a mean weight of 15.0 g (0.53 oz). The fish in each tank had been fed a sinking commercial trout food (Silver Cup, Murray, Utah). Beginning in early June, the fish were transitioned to the new experimental diets (3 tanks per diet) over a two-week period.

After the transitional period, UW-Madison investigators observed that the feeding behavior of all of the perch in the four treatment groups was extremely poor. After an additional 3-week period all of the fish were weighed and measured. Extremely poor growth rates were noted in all of the groups. Because of the poor feeding response, UW-Madison investigators terminated the experiment, and in July 2005, a new experiment was set up with different fish (mean weight = 28.0 g [1.0 oz]). For this experiment the transition of the fish onto the experimental feeds was altered by mixing equal amounts of Silver Cup™ trout food and the experimental diets and then approximately 5% of freeze-dried krill flakes was added to each mixture. For one month the fish were fed this mixture, and all fish ate well. After one month the Silver Cup diet was eliminated from the mixture, and the fish were fed the experimental feeds for an additional month with a declining amount of krill. After this time, all of the fish were showing a good feeding response to the experimental diets alone. UW-Madison researchers then conducted the grow-out phase of the study as originally proposed. Shortly after the beginning of the grow-out phase, fish that were being fed any of the three new experimental diets began to show a reduced feeding response. The mean weight gains, feed/gain ratios, and survivals (%) of the different groups of fish for the grow-out study were as follows: trout food: 41.0 g (1.45 oz), 1.34, and 91%; 75% animal meal: 22.0 g (0.78 oz), 1.65, and 80%; 55% animal meal: 17.0 g (0.60 oz), 2.8, and 61%; and 35% animal meal: 24.0 g (0.85 oz), 2.5, and 61%.

Clearly, the experimental fish meal-free diets proved sub-optimal for yellow perch growth, survival, and performance. UW-Madison investigators believe that the poor performance of the experimental diets may have been due, at least in part, to low palatability rather than inadequate nutritional properties. This belief is driven by the fact that the fish seemed to readily feed and consume the experimental diets as long as a small amount of krill was mixed into the food. As soon as the krill was eliminated, the feeding responses of the fish declined markedly. This finding was surprising, given that all of the diets contained 5% shrimp meal to enhance palatability. Fillets from all treatment groups were subjected to sensory analysis comparisons by the UW-Madison Department of Food Science, and no differences were found among the four fish groups. The studies on reproduction showed no negative effects of the experimental diets, as fish from each treatment group that were overwintered showed normal egg and sperm development.

Michigan State University (MSU) researchers conducted two experimental studies to examine specific effects of trypsin inhibitors (TIs) on the growth and performance in formulated fish feeds for yellow perch. These studies consisted of a Phase I Growth Study and Phase II Extended Study, and were designed to assess if TIs in soybean meal (SBM) limit its inclusion level in diets for fingerling yellow perch.

A semi-purified control and four experimental diets containing graded levels of TI were used to study effects of TIs associated with SBM on yellow perch fingerlings. Test diets were manufactured by collaborators at Purdue and formulated to be 34% crude protein and 12% crude fiber. TI (Soybean Trypsin Inhibitor CAS #9035-81-8, USB Corporation) inclusion rates were 0, 0.975, 1.95, 2.925, and 3.9 g TI/kg (ppm) feed representing estimated SBM equivalencies of 0, 15, 30, 45, and 60%

SBM (diets TI0, TI15, TI30, TI45, and TI60, respectively). TI inclusion rate SBM equivalencies were based on the average value of 6.5 mg TI/g (ppt) SBM from the range of 5.0–8.0 mg TI/g (ppt) SBM (Dr. Craig Russet, Director of Agri Business with Central Soya).

Young-of-the-year yellow perch were obtained from the Ohio State University Center for Aquaculture Development. The fish were transported to MSU's Aquaculture Research Laboratory and acclimated to water conditions in a 225.0-L (59.4-gal) flow tank system over a 30 day period. Fish were fed a commercial trout diet over the acclimation period. A total of 270 fish were randomly distributed in 15, 225.0-L (59.4-gal) tanks, 18 fish per tank, and acclimated to conditions of a partial recirculating aquaculture system to be used during the feed trial. Fish were fed the experimental control diet over this additional 10-day acclimation period.

The partial recirculating aquaculture system consisted of the fish rearing units, settling basin, rotating biological contactor , and aeration column. Flow rates were maintained between 3.7–5.6 Lpm (1.0–1.5 gpm) based on target exchange rates of 1.0–1.5 water exchanges per hour. Fresh water continual flow to the system varied between 0.5–1.0 Lpm (0.13–0.26 gpm). Water temperature for the Phase I growth study remained between 17.4–19.3°C (63.3–66.7°F), with a mean temperature value of 18.5°C (65.3°F). Water temperature for the Phase II extended study remained between 18.7–22.8°C (65.6–73.0°F), with a mean temperature value of 19.9°C (67.8°F). Dissolved oxygen remained near constant at 95% saturation; total ammonia nitrogen concentrations remained below 1.0 mg/L (ppm) (0.006 ppm unionized ammonia); nitrate concentrations remained below Hach nitrate test kit (colorimetric) detection levels. All other water quality parameters fell within acceptable limits for yellow perch.

For both Phase I and II studies, fish were fed in triplicate, either the control diet, or one of five treatment diets, two-times daily (8:00–9:00 am and 4:30–5:30 pm). Total weight samples were conducted on day-1 of each trial and repeated every 2–4 weeks.
The Phase I Growth Study examined effects of TIs on growth and body composition of yellow perch fingerlings over an initial feed trial period of 85 days. Average initial weight of fish from all tanks was measured to be 4.11 ± 0.36 g (0.14 ± 0.01 oz). Feeding levels were calculated on a constant % body weight (%BW) basis and adjusted every two weeks according to the theoretical optimal feed levels for salmonids at a FCR of 1.0. Feed levels fell both above and below satiation levels of the fish across feeding times based on observations of excess feed in tank bottoms at various times through the feed trial.

At the end of the Phase I study, total weights were taken. Three fish were randomly selected for weight and length measurements and were euthanized in tricaine methanesulfonate (MS-222) at a concentration of 500 mg/L (ppm). The 3 fish were ground, pooled, frozen, and held at -20°C (-4°F) for subsequent whole body composition analysis.

Three mortalities occurred over the 85 day Phase I feed trial: one each for the control, TI15, and TI30 diets. Results show that the TI60 diet resulted in the lowest values for k (condition factor), SGR, and protein efficiency ratio (PER). TI60 body composition samples had the highest composition of ash and lowest composition of lipids. Body compositions showed an

increasing linear trend in ash, y = 0.4029× + 16.563 (R^2 = 0.90), with increased TIs in the diet. Body ash composition was statistically different between TI0 and TI60. No other parameters tested were statistically different (P = 0.10). FCRs ranged from a minimum of 1.43 (TI45) to 1.67 (TI60).

The Phase II study examined long term effects of TIs on yellow perch growth, body composition, and intestinal morphology characteristics. Phase II was conducted over a period of 56 days as an extension to the Phase I trial. Together, these studies combine to examine the affects of SBM TIs for 5 months of continual TI ingestion. Average initial weight of fish from all tanks was measured to be 11.81 ± 1.99 g (0.42 ± 0.07 oz).

Feed levels in Phase II were slightly different than that of Phase I in that %BW was calculated individually for each tank based on a constant k as determined from the Phase I study. This adjustment reduced feed level variations between tanks. Feed levels were adjusted bi-weekly based on total weight samples or FCR rates determined from the last weight sample taken. Feed levels fell both above and below satiation levels of the fish across feeding times based on observations of excess feed in tank bottoms at various times through the feed trial.

At the end of the Phase II study, total weights were taken and all fish were euthanized in tricaine methanesulfonate (MS-222) at a concentration of 500 mg/L (ppm). Ten fish were randomly selected for weight and length measurements, and excision of whole livers which were weighed for hepatosomatic index (HSI) determination. Small intestines were excised from the first three fish samples and fixed in 10% neutral buffered formalin for subsequent intestinal histological examination. Three whole fish subjects, pooled within tank, were frozen at -20°C (-4°F) for proximate body composition analysis.

There were no mortalities observed over the 56-day feed trial. Results of the extended study indicate that there were no significant differences in k, SGR, PER, FCR, or body proximate analysis among diets. The only significant difference observed was for HSI. Yellow perch on the TI15 diet showed significantly lower HSI values than perch fed TI30, TI45, or TI60 diets. No dietary effects were observed on intestinal histopathology samples between 0% SBM and 60% SBM equivalency diets.

Researchers at Purdue attempted to formulate diets for yellow perch with only plant-based ingredients using similar dietary restrictions used in studies with hybrid striped bass (36% dietary crude protein, 8% lipid, quantified methionine, arginine and lysine requirements and predicted requirements for the remaining essential amino acids).

As experienced with hybrid striped bass, dietary formulations with only plant-based proteins could not meet the requirements of yellow perch in diets containing 36% dietary crude protein without substantial supplementation with feed grade amino acids. Thus, a similar series of diets was developed using meat and bone meal or poultry by-product meal blended with plant-based ingredients. Those diets were fed to juvenile all-female yellow perch (mean initial weight 11.2 g; 0.4 oz) held in recirculating systems. All diets were fed to satiation twice daily.

Perch fed the diets containing poultry by-product meal in combination with canola

meal and sunflower meal, and perch fed the diet containing meat and bone meal in combination with canola meal had significantly lower weight gain, SGR and higher feed conversion ratios than in fish fed other diets. There were no clear distinctions in perch fed the experimental diets as seen in similar diets fed to hybrid striped bass. It appears perch may be sensitive to certain combinations of protein-supplying ingredients and selected ones should be evaluated individually.

IMPACTS

The development, testing, and use of fish meal-free diets are critical to the aquaculture industry for two primary reasons. First, some critics of aquaculture have expressed the opinion that wild fish populations are hurt by the growth of aquaculture because of the industry's dependence on fish meal. Second, fish meal is an expensive dietary ingredient that raises the cost of food, and thereby increases overall fish production costs. This project should provide the key information needed by commercial feed producers so that they can begin providing a quality fish meal-free or fish meal-reduced diet to producers.

Work completed by SIUC researchers has demonstrated that plant-based protein and lipid sources can partially replace marine feedstuffs in the diets fed to sunshine bass without negatively impacting production and fillet quality. The results suggest feeding a 40% menhaden oil/20% menhaden meal diet during grow out is sufficient to maintain production. However, it appears menhaden meal can be reduced to 10% with the inclusion of a suitable feed attractant. Regardless, higher concentrations of menhaden oil (80% of the dietary lipid) are needed in the diet to maintain fillet HUFA content. Partial replacement of marine feedstuffs in sunshine bass diets enables producers to utilize fish meal and oil supplies more efficiently, leading to a more cost-effective diet formulation for this industry.

Work completed at UW-Madison clearly indicated that the experimental fish meal-free diets tested proved sub-optimal for yellow perch growth, survival, and performance. The investigators believe that the poor performance of the experimental diets may have been due, at least in part, to low palatability rather than inadequate nutritional properties.

The TI studies conducted at MSU suggest that negative effects of SBM in plant-based feeds may be more of a culmination of anti-nutritional properties, including combined effects of TIs, lectins, phytate, saponins, etc. Based on results with yellow perch, these effects could be more severe than those observed in salmonids. At this time MSU researchers caution the use of SBM for yellow perch diets, and recommend additional research in the area of developing commercial SBM-based feeds and effects of carbohydrates on yellow perch.

Studies conducted at Purdue indicate there is a sufficient body of knowledge for formulating alternative diets for both species. Ingredient choice needs to be carefully considered in new formulations and perch may have sensitivities to certain ingredient combinations.

RECOMMENDED FOLLOW-UP ACTIVITIES

Data for hybrid striped bass indicate alternative dietary formulations can be developed using the available nutrient requirements. Additional nutritional requirements would be beneficial, particularly relating to health status of fish. Data for yellow perch are also promising,

indicating alternative formulations can be developed. However, there appear to be ingredient limitations in perch that should be explored, perhaps in conjunction with ingredient suppliers. Relatively low consumption of feeds by perch remains a significant problem, limiting growth and time to market. Understanding the controlling mechanisms would help alleviate this fundamental problem.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

See the Appendix for a cumulative output for this NCRAC-funded Nutrition project.

SUPPORT

YEAR	NCRAC-USDA FUNDING	OTHER SUPPORT					TOTAL SUPPORT
		UNIVER-SITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
2004-05	$99,250		$1,000			$1,000	$100,250
2005-06	$100,750						$100,750
TOTAL	$200,000		$1,000			$1,000	$201,000

SNAIL MANAGEMENT/GRUB CONTROL[10]

Project ***Progress Report*** for the Period
September 1, 2007 to August 31, 2008

NCRAC FUNDING: $114,138 (September 1, 2007 to August 31, 2008)

PARTICIPANTS:

Gregory W. Whitledge	Southern Illinois University-Carbondale	Illinois
Christopher F. Hartleb	University of Wisconsin-Stevens Point	Wisconsin
Todd Huspeni	University of Wisconsin-Stevens Point	Wisconsin
Joseph E. Morris	Iowa State University	Iowa
Richard D. Clayton	Iowa State University	Iowa
Industry Advisory Council Liaison:		
Rex Ostrum	Ostrum Acres Fish Farm, McCook	Nebraska
Extension Liaison:		
Joseph E. Morris	Iowa State University	Iowa

PROJECT OBJECTIVES

(1) Investigate one or more methods of potentially useful approaches to snail population management and/or grub control. The methods of greatest interest include those that will be effective, economical, and approvable by state and federal regulators at commercial production scale. These methods will include reviewing what has been done elsewhere and designing studies that will address the NCRAC conditions, especially in pond systems for the production of economically important food fish for the region. Attempts will be made to investigate and refine these methods.

(2) Assemble an updatable snail management guide which includes a literature review of known control options, a method of determining snail infestation levels in any water system, and a set of standard operating procedures to reduce snail populations and trematode infestations based on the research cited in Objective 1.

ANTICIPATED BENEFITS

Grub infections in fish culture ponds are extremely relevant to the aquaculture industry in the North Central Region (NCR) as the industry has experienced a loss of income in both commercially important food fish species and baitfish. These economic losses result both directly from fish mortality due to trematode infection, and indirectly because of unappealing visual presentation of food fish fillets containing grubs.

From the proposed investigations, both chemical and biological control methods will be tested for their efficiency and applicability to control grubs and manage snail populations in fish ponds. By utilizing locally available biological control species, e.g., crayfish, and establishing a suitable competitively dominant noninfectious trematode that can both displace the

[10] This is a 2-year project that is chaired by Gregory W. Whitledge and began September 1, 2007.

digenean trematodes and potentially control snail populations through castration of male snails, an economically viable, adaptable, universally applied, and immediate method of snail and grub management can be developed. The proposed work will also permit further experimental testing and demonstration of the dominance hierarchy for intramolluscan competition in larval trematodes and demonstrate another control method which may also have relevance to other trematode infections of veterinarian and human importance.

PROGESS AND PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

University of Wisconsin-Stevens Point

University of Wisconsin-Stevens Point (UW-Stevens Point) investigators collected northern fantail crayfish (*Orconectes virilis*) from lakes in Portage and Vilas Counties, Wisconsin in summer 2007. Baited wire (minnow) traps proved to be the most successful capture method with 455 crayfish (65.2% male, 34.7% female) collected. Additional crayfish were collected in summer 2008 from lakes in Vilas County, Wisconsin, bringing the total number of crayfish collected to 1,255.

The three, original, commercial fish farms, where the field study was to occur in Years 1 & 2, withdrew from the study amid concerns about viral hemorrhagic septicemia (VHS) and because one of the farms implemented a winter draw-down program to control aquatic plants. The study locations were then moved to AquaPoint Fish Farm, Stevens Point, Wisconsin, and Zelinski's Fish Farm, Antigo, Wisconsin. Both are commercial yellow perch (*Perca flavescens*) farms each with four, 0.022-ha (0.05 acre) ponds that are fed with groundwater and are aerated; yellow perch at both facilities have been previously infected with yellow grubs.

Because the total number of crayfish collected in Year 1 was less than the number required for pond stocking, both male and female crayfish were stocked into the treatment ponds in July 2008 as opposed to the original goal of stocking only one sex. Each fish farm also had two control ponds that did not receive crayfish. After introduction into the treatment ponds, crayfish were sampled monthly from each treatment pond by use of baited minnow traps placed out for 24-h periods. Collected data permitted analyses on crayfish growth (measured as carapace length), and catch-per-unit effort as a proxy for crayfish density.

Using an Eckmann grab sampler for benthic sampling, *Planorbella* (=*Helisoma*) and *Physa* snails were recovered from both treatment (crayfish added) and control ponds at both fish farms. *Planorbella* and *Physa* species are potential first intermediate hosts to several digenean trematodes species, and the genera have been described and reported as hosts to grub-causing digenean trematode species (e.g., *Clinostomum*, *Uvulifer*, and *Posthodiplostomum* spp.).

Densities of *Planorbella* at the study ponds were generally low during spring sampling and increased through the summer (see below). Notably, while *Planorbella* densities increased at both control and treatment ponds, the relative increase in densities was significantly greater in the control ponds without crayfish. Densities of *Physa* were always lower than *Planorbella* at all ponds sampled, and unlike *Planorbella*, *Physa* densities were generally static or even witnessed a marginal decline during the summer. In terms of average snail size, both *Planorbella* and *Physa* snails exhibited declines through the summer.

Collections of *Planorbella* and *Physa* snails were assessed for larval digenean infections,

and prevalence values (i.e., % snails infected) were determined for all treatment and control ponds at both fish farms. Surprisingly, none of the snails collected in any sampled pond were infected with *Clinostomum*, the trematode causing "yellow grub" metacercarial infections in yellow perch stocked into these facilities. Similarly, no *Uvulifer* (causing "black spot") or *Posthodiplostomum* were found in any of the snails examined. However, other non-grub causing digenean species were present in *Planorbella*. At AquaPoint, snails from treatment ponds showed an overall trematode prevalence of 3% at the start of the experiment, and a 2% prevalence after 60 days. *Planorbella* snails in control ponds had an initial prevalence of 2%, and a 12% prevalence after 60 days. Trematode prevalence in *Planorbella* snails collected from treatment ponds at Zelinski's had a constant 9% prevalence rate over 60 days, while parasite prevalence rates declined from 10% to 7% in control ponds.

Planorbella snails from both fish farms were also infected with the digenean trematode, *Echinostoma* sp. (likely *Echinostoma trivolvis*). *Planorbella* were infected with *Echinostoma* stages, and these snails served both as first intermediate hosts (possessing redial stages inside the ovotestis), and as second intermediate hosts (with metacercarial stages in the snail pericardial region). In Year 2 of this study *Echinostoma* sp. metacercarial prevalence and intensities in snails serving as second intermediate hosts will be quantified.

All ponds at AquaPoint were stocked with approximately 640 yellow perch, of which 66% were initially infected with yellow grub with an average grub infection of 18.6 grubs/fish. Ponds at Zelinski's were stocked with approximately 1,000 fish, of which 75% were initially infected with yellow grub with an average infection of 2.3 grubs/fish. After two months, average grub infection rates in fish at AquaPoint and Zelinski's were 69% (14.28 grubs/fish) and 68% (4.1 grubs/fish), respectively.

To ensure accurate identification of grub-causing species and evaluate possible cryptic species, metacercarial stages (i.e., "grubs") procured from naturally infected pond fishes were fed to lab-reared ducks. Adult worms were not recovered after 7 and 10 days post exposure. Because of the inability to procure live *Clinostomum* adults in lab exposed animals, and the absence of larval stages in sampled potential first intermediate host *Planorbella* snails, researchers were unable to produce lab controlled exposures of competitor *Echinostoma* sp. miracidia to *Clinostomum* infected snails. However, *Clinostomum* metacercarial stages from infected fishes (i.e., "grubs") will continue to be collected in Year 2 for genetic evaluation and comparison with adults procured from naturally infected herons, and infected first intermediate snails recovered at study ponds in the future.

Southern Illinois University-Carbondale

Laboratory trials were conducted at Southern Illinois University-Carbondale (SIUC) to evaluate the potential of freshwater prawn (*Macrobrachium rosenbergii*) and two hybrid sunfishes (redear sunfish × green sunfish [*Lepomis micolophus* × *L. cyanellus*] and redear sunfish × warmouth [*L. gulosus*]) to serve as biological control agents for *Physa* spp. and *Helisoma* spp. Maximum consumption rates and maximum handling sizes for each of these species or hybrids feeding on *Physa* and *Helisoma* were compared to those of redear sunfish, one of the most common molluscivores native to the NCR. Ten individuals of each species or hybrid were placed individually into 37.8-L (10-gal) aquaria, not fed for 24 h, and then exposed

to known sizes and numbers of *Physa* and *Helisoma* for 48 h; snail sizes represented the full size range commonly found in aquaculture ponds. Species or hybrids that consumed ≥50% of snails in the first set of laboratory trials were used in subsequent trials that assessed maximum snail handling sizes. Ten individuals of each species or hybrid were held individually in 37.8-L (10.0-gal) aquaria, starved for 24 h, and then offered one snail from each 1-mm size increment over the full size range of snails commonly found in ponds (3.0–12.0 mm [0.12–0.47 in] for *Physa* and 3.0–18.0 mm [0.12–0.7 in] for *Helisoma*). Uneaten snails were counted and measured 48 h later to identify sizes of snails that were consumed. Maximum consumption rates for each species or hybrid feeding on *Physa* and *Helisoma* were also determined over a set of 5-day trials in which individually-housed prawns, redear sunfish, or hybrid sunfishes were fed known numbers and sizes of snails *ad libitium* daily. Numbers and sizes of snails consumed were determined daily and mean maximum daily consumption rates were calculated for each species or hybrid.

Redear × warmouth hybrids consumed larger snails than redear sunfish of equivalent body length, but consumed 25% fewer snails on average than redear sunfish. While redear × warmouth hybrids have a larger mouth gape than redear sunfish for a given body size, they do not appear to be sufficiently voracious at consuming snails to represent a significant improvement over redear sunfish as a biological control agent. Freshwater prawn (65.0–85.0 mm [2.6–3.3 in] carapace length) consumed *Physa* up to 12.0 mm (0.5 in) total length and *Helisoma* up to 16.0 mm (0.6 in) total length. However, freshwater prawns showed a strong preference for consuming *Physa* over *Helisoma*; prawns consumed 77% of *Physa* offered in maximum consumption trials but consumed only 20% of *Helisoma* offered. Consumption rates for smaller freshwater prawn feeding on snails were not determined but would likely be considerably lower than those of the harvest-size prawns that were used in laboratory trials. Redear × green sunfish hybrids (120.0–140.0 mm [4.7–5.5 in] total length) consumed *Physa* and *Helisoma* up to 12.0-mm (0.5 in) total length; redear sunfish in this size range only consumed snails <10 mm total length. Maximum consumption rates of redear × green sunfish hybrids were equivalent to those of similar-size redear sunfish.

Laboratory trials were conducted to determine the effectiveness of various concentrations of copper sulfate, hydrated lime, and salt (sodium chloride) for controlling snails given the characteristics (alkalinity, pH, hardness) of ponds at SIUC's pond research facility. All concentrations of hydrated lime (0.83–2.05 kg/m^2 [0.17–0.42 lb/ft^2] of water surface; N = 3 replicate tanks per treatment) yielded 100% snail mortality; mean snail survival rate in control tanks was 71%. Mean survival rate of snails exposed to copper sulfate applied at a rate of 10.23 g/m^2 (0.04 oz/ft^2) was 2% (range 0–6%). Salt concentrations up to 3 ppm were ineffective at controlling snails in laboratory tanks. Based on laboratory trial results and application costs, hydrated lime was chosen as the chemical treatment to be used in subsequent snail control trials in ponds at SIUC.

Pond trials were conducted to evaluate the effectiveness of hydrated lime for controlling snails in research ponds at SIUC. Enclosures were placed in shallow water (0.3 m [1.0 ft] depth) in four ponds and stocked with snails (N = 35 each) obtained from ponds at SIUC. Two ponds contained three enclosures each that served as controls. Two other ponds were treated with hydrated lime (1.07 kg/m^2; 0.22 lb/ft^2) along the pond

edge, including enclosures containing known numbers of snails. Mean snail survival rate in control ponds was 89%, but was only 2% in ponds treated with hydrated lime.

Pond trials were also conducted beginning in July 2008 to assess the effectiveness of redear sunfish and redear × green sunfish hybrids for controlling snail populations in ponds. Three ponds at SIUC's research facility were stocked with redear sunfish at a rate of 247 fish/ha (100 fish/acre), three ponds were stocked with redear sunfish at a rate of 494 fish/ha (200 fish/acre), and three ponds were stocked with redear × green sunfish hybrids at a rate of 200 fish/acre; three ponds were not stocked and served as controls. Grass carp were stocked into each pond to provide control of aquatic macrophytes. Snail population densities and size structure were determined prior to stocking fish and at monthly intervals thereafter. Results to date indicated that snail densities increased or did not significantly change in control ponds or in ponds stocked with redear × green sunfish hybrids; snail densities in ponds stocked with redear sunfish declined significantly over time. Few snails >7.0 mm (0.3 in) total length were present in ponds stocked with redear sunfish following stocking, whereas snails ranging from 3.0–16.0 mm (0.1–0.6 in) total length were relatively abundant in control ponds and ponds stocked with redear × green sunfish hybrids. These pond trials will conclude in October 2008.

OBJECTIVE 2

A search has been initiated by Iowa State University staff to review literature to date concerning the three main control methods for snails: biological, chemical, and mechanical. This information will then be combined with information garnered from this research project to develop an interactive Web page for fish producers to access and obtain information potentially relevant to their snail problems. Among the various options, information regarding effectiveness, application costs, legal implications, and potential for impact on pond general ecology, e.g., zooplankton dynamics in fish fingerling ponds, will be listed. This Web page will be then be hosted on the NCRAC Web site.

WORK PLANNED

UW-Stephens Point

Sample and data collection will be continued in Year 2, with biweekly random sample collection beginning after ice-out (April 2009). Crayfish stocked into treatment ponds during Year 1 will be sampled after ice-out in Year 2, with measurements of size and catch-per-unit effort calculated as an estimate of crayfish density in each pond. Data collection in Year 2 will continue until the ponds freeze.

The field portion of the natural dominant trematode study will begin in Year 2 with three fish-rearing ponds chosen to serve as treatment ponds to which *Echinostoma* eggs will be added, and three additional ponds will serve as controls. Data on snail size-frequency distributions, prevalence of grub infection in snails, and *Echinostoma* prevalence (percent snails with rediae) and *Echinostoma* intensity (number of metacercariae per snail) of in snails will be recorded. Additionally, the prevalence and intensity of grubs in fishes reared in the ponds will be obtained. An analysis of the efficacy of *Echinostoma* trematodes as biocontrol agents will be made by comparing snail populations, prevalence of grub-infected snails for treatment and control ponds, and the prevalence and intensity of grubs in fish at the time of harvest.

SIUC

Pond trials evaluating the effectiveness of biological, chemical, and integrated biological/ chemical controls of snail populations as described in the proposal will be conducted during Year 2. Redear sunfish and redear × green sunfish will be used as biological control agents and hydrated lime will be used as the chemical treatment based on the results of laboratory and pond trials conducted during Year 1. Grass carp will also be stocked into ponds to provide vegetation control. Effectiveness of the snail control treatments will be assessed under production conditions using phase III hybrid striped bass. Prevalence of grub infestation will be assessed at regular intervals for each treatment.

Iowa State University

In 2009 the completed database on snail control will be shared with all project investigators to insure that the information is complete. Following project review of this database, the Web page will then be developed and placed on the NCRAC Web site.

IMPACTS

Results from this project will provide valuable information regarding the effectiveness and efficiency of several potentially useful approaches for controlling snail populations and associated grub infestations in aquaculture ponds in the NCR. Previously untested treatments for snail control in ponds (the crayfish *Orconectes virilis*, freshwater prawn, hybrid sunfishes, biocontrol with natural dominant trematodes, and integrated chemical and biological controls) are being evaluated. Results are anticipated to provide improved and more universally applicable approaches for controlling digenean trematodes and managing snail populations in ponds where food fish are raised.

SUPPORT

NCRAC funds provided to date total $114,138; a total of $225,000 has been allocated for this 2-year project.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

See the Appendix for a cumulative output for all NCRAC-funded Snail Management/Grub Control activities.

SUNFISH[11]

Project ***Progress Report*** for the Period
September 1, 2007 to August 31, 2008

NCRAC FUNDING: $40,000 (September 1, 2007 to August 31, 2008)

PARTICIPANTS:

Robert S. Hayward	University of Missouri-Columbia	Missouri
Jeffre D. Firman	University of Missouri-Columbia	Missouri
Industry Advisory Council Liaison:		
Curtis Harrison	Harrison Fish Farm, Hurdland	Missouri
Extension Liaison:		
Joseph E. Morris	Iowa State University	Iowa

PROJECT OBJECTIVES

(1) Develop a least-cost diet for bluegill *Lepomis macrochirus* by:
   (a) Evaluating amino acid availability of dietary ingredients for bluegills,
   (b) Evaluating amino acid composition of bluegills,
   (c) Evaluating limiting amino acid requirements of bluegills, and
   (d) Making a least-cost diet formulation model available to the industry within a two-year period.

ANTICIPATED BENEFITS

Given the high protein requirement of sunfish, trout diets containing ≥40% crude protein (CP) consisting largely of expensive fish meal are commonly used in intensive sunfish culture. The present study seeks to formulate, within a 2-year period, a complete diet for juvenile-stage sunfish that will yield growth rates that are equivalent to or better than those achieved when best-performing, available trout diets are used. A least-cost analysis will be performed once dietary requirements are determined; minimum-cost diet ingredients will be incorporated to the extent possible while fully maintaining the appropriate diet composition. The resulting diet is expected to yield high sunfish growth rates and to substantially reduce total ingredient costs; improvement of feed conversion is also expected, as is a reduction of the tendency for excessive fat deposition in sunfish livers that has been observed when trout diets have been fed.

The lower cost of dietary ingredients in the developed diet is expected to lead to a lower-cost diet for sunfish production without loss of growth rate relative to trout diets. This will be advantageous to sunfish producers given that feeds represent a substantial portion (≥50%) of variables costs in producer budgets. Most importantly, success in the formulation of this diet for sunfish within a 2-year period would indicate a potential to do likewise for other aquaculture species in the North Central Region (NCR).

[11]This is a 2-year project that is chaired by Robert S. Hayward and began September 1, 2007.

PROGESS AND PRINCIPAL ACCOMPLISHMENTS

Objective 1a

Apparent digestibility of dry matter and energy, and availability of amino acids from blood meal (BM), fish meal (FM), meat and bone meal (MBM), poultry byproduct meal (PBM), soybean meal (SBM), corn, wheat and yellow grease (YG) were determined for bluegill (mean weight 57 g). Feces were collected by a siphoning method. Apparent dry matter digestibility values ranged from 50% (corn) to 87% (BM). Apparent energy digestibility values ranged from 53% (corn) to 92% (BM). Apparent digestibility of most amino acids exceeded 90% for evaluated protein sources, except for MBM which showed slightly lower values (80–90%). Isoleucine digestibility from BM was relatively low (82%) for bluegill. High digestibility values for SBM, PBM, and BM, indicate good potential for replacing FM in diets.

Objective 1b

Whole body amino acid profile was determined for juvenile bluegills (mean weight 31.21 g, N =10) caught from the wild. Amino acid ratio for ten essential amino acids was found to be 2.53 (arginine):0.88 (histidine):2.08 (isoleucine):3.25 (leucine):3.19 (lysine):1.30 (methionine):2.10 (phenylalanine):1.85 (threonine):0.52 (tryptophan):2.51 (valine). Except for leucine, contents of all other amino acids were lower than lysine. This ratio was used for determining essential amino acid requirements (EAAs).

Objective 1c

Requirement for dietary lysine was determined by feeding bluegills seven different diets (45% CP, 4026 kcal gross energy/kg diet) containing graded levels of lysine (0.9%, 1.2%, 1.5%, 1.8%, 2.1%, 2.4%, and 3.0%). Sixteen individually-housed bluegills were used for each dietary treatment, and the experimental design followed a complete randomized block design. The study was run for 60 days. Analysis of data based on segmented regression analysis for specific growth rate showed 2.28% as the lysine requirement. Requirements for other EAAs were determined based on the ratio of whole-body amino acid compositions. Requirements for arginine, histidine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan, and valine were found to be: 1.81%, 0.63%, 1.49%, 2.32%, 0.93%, 1.50%, 1.32%, 0.37%, and 1.79%, respectively.

Objective 1d

Activities in this objective have been postponed until the completion of Objectives 1a-c.

WORK PLANNED

Completion of Objective 1d will involve three experiments to determine protein:energy requirements and to develop a low-cost diet based on the use of alternative protein sources that are highly digestible for bluegill. The overall study will be completed by August 31, 2009.

IMPACTS

The lower cost of dietary ingredients in the diet that will be developed is expected to lead to a lower-cost diet for sunfish production without a reduction in growth rate relative to trout diets. This will be advantageous to sunfish producers given that feeds represent a substantial portion (≥50%) of their variables costs. Most importantly, success in the formulation of this diet for sunfish within a 2-year period would indicate a potential to do likewise for other aquaculture species in the NCR.

SUPPORT

NCRAC funds provided to date total $40,000; a total of $80,000 has been allocated for this 2-year project.

PUBLICATIONS, MANUSCRIPTS, WORKSHOPS, AND CONFERENCES

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

APPENDIX

AQUACULTURE DRUGS

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Workshops/Conferences/Symposia/Papers Presented

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)

1st 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)

1st 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 (World Aquaculture Society special session), San Diego, California, February 7, 1995. (LaDon Swann)

2nd 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, Niagara 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)

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Description of the Aquaculture and Bait Fish Industries: Threat Evaluation and Identification of Critical Control Points, International Joint Commission Workshop on Exotic Policy, Milwaukee, Wisconsin, September 22-26, 1999. (Jeffrey L. Gunderson)

Fisheries Management in the North Central Region, 9th National Extension Wildlife, Fisheries, and Aquaculture Conference, Portland, Maine, September 29-October 2, 1999. (Joseph E. Morris, and S.K. Whitcomb)

Internet Resources for Aquaculture Education and Communications: Present and Future, 9th National Extension Wildlife, Fisheries, and Aquaculture Conference, Portland, Maine, September 29-October 2, 1999. (LaDon Swann)

Yellow Perch Producers' Forum, Hudson, Wisconsin, January 21-22, 2000. (Joseph E. Morris and Jeffrey L. Gunderson)

Organic Aquaculture Standards Workshop, Minneapolis, Minnesota, June 23-24, 2000. (Anne R. Kapuscinski)

"I've got this hog barn..." Videoconference Workshop, Lima, Ohio, November 16, 2002. (Laura G. Tiu)

Applications of HACCP in Aquaculture, Aquaculture America 2003, Louisville, Kentucky, February 18-21, 2003. (Ronald E. Kinnunen)

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Hybrid Walleye Workshop, Jackson, Missouri, March 5, 2003. (Ronald E. Kinnunen and Robert A. Pierce II)

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Great Lakes Native American Involvement in Fisheries Extension Programs, American Fisheries Society Annual Meeting, Madison, Wisconsin, August 25, 2004. (Ronald E. Kinnunen)

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Yellow Perch Aquaculture Workshop, Bad River Tribal Hatchery Program, Milwaukee, Wisconsin, December 2004. (Fred P. Binkowski)

Yellow Perch and Lake Sturgeon Workshop, Lac du Flambeau Tribal Hatchery, Milwaukee, Wisconsin, February 2005. (Fred P. Binkowski)

Yellow Perch Aquaculture Workshop, Kearney, Nebraska, February 26, 2005. (Fred B. Binkowski)

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AIS-HACCP Train-the-Trainer Workshop, Columbus, Ohio, February 9, 2007. (Ronald E. Kinnunen and Jeff Gunderson)

Conversion of Livestock Barns into Fish Production Facilities IP Videoconference, Purdue University, West Lafayette, Indiana, March 8, 2007. (Kwamena K. Quagrainie)

Tri-State Aquaculture Conference/Workshop. Ashland, Nebraska, March 17, 2007. (Fred B. Binkowski and Joseph E. Morris)

Freshwater Prawn Production Workshop, Sellersburg, Indiana, April 14, 2007. (Kwamena K. Quagrainie)

Using Sensory Analysis to Better Position a Fish Product in the Market Place, 4th National Aquaculture Extension Conference, Cincinnati, Ohio, May 1-3, 2007. (Ronald E. Kinnunen)

The HACCP Approach to Prevent the Spread Of Aquatic Invasive Species by Aquaculture and Baitfish Operations, 4th National Aquaculture Extension Conference, Cincinnati, Ohio, May 1-3, 2007. (Ronald E. Kinnunen)

The VHS Virus in the Great Lakes Region, 92nd Annual Meeting and Professional Improvement Conference, National Association of County Agricultural Agents, Grand Rapids, Michigan, July 17, 2007. (Ronald E. Kinnunen)

The HACCP Approach to Prevent the Spread of Aquatic Invasive Species by Aquaculture and Baitfish Operations, 92nd Annual Meeting and Professional Improvement Conference, Association of County Agricultural Agents, Grand Rapids, Michigan, July 17, 2007. (Ronald E. Kinnunen)

AIS-HACCP Training Workshop, Clare, Michigan, July 30, 2007. (Ronald E. Kinnunen)

AIS-HACCP Training Workshop, Rogers, Minnesota, September 6, 2007. (Ronald E. Kinnunen and Jeff Gunderson)

Michigan Aquaculture and Salmonid Aquaculture in the North Central Region, Great Lakes Sea Grant Network Conference, Chicago, Illinois, September 18, 2007. (Ronald E. Kinnunen)

AIS-HACCP Training Workshop, Stevens Point, Wisconsin, October 26, 2007. (Ronald E. Kinnunen and Phil Moy)

AIS-HACCP Training Workshop, Pierre, South Dakota, January 4, 2008. (Ronald E. Kinnunen and Jeff Gunderson)

MarketMaker, Michigan Aquaculture Association Annual Conference, Clare, Michigan, February 12, 2008. (Ronald E. Kinnunen)

North Central Regional Aquaculture Center VHS Project, Michigan Aquaculture Association Annual Conference, Clare, Michigan, February 12, 2008. (Ronald E. Kinnunen)

VHS: a Regional Industry Perspective, Illinois VHS Conference and Workshop, Rend Lake, Indiana, April 26, 2008 (Christopher T. Weeks)

AIS-HACCP Use in the Baitfish and Aquaculture Industries, Organisms in Trade Workshop, Great Lakes Commission, Romulus, Michigan, June 10, 2008. (Ronald E. Kinnunen)

AIS-HACCP Training Workshop, Indianapolis, Indiana, July 23, 2008. (Ronald E. Kinnunen and Kristin TePas)

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Poynter, E.C., R.L. Lane, A.M. Kelly, and C.C. Kohler. 2005. Triglyceride and their constituent fatty acid metabolism of sunshine bass *Morone chrysops* × *M. saxatilis* fed graded levels of long-chain polyunsaturated fatty acids during swimming activity. Aquaculture America 2005, New Orleans, Louisiana, January 18, 2005.

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Ringer, R.K. 1993. Workshop on INADs, NADAs, and the IR-4 Project. California Aquaculture Association, Oakland, October 11, 1993.

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Schnick, R.A. 1995. Idaho Aquaculture Association Annual Meeting, Twin Falls, Idaho, May 19-22, 1995.

Schnick, R.A. 1995. Chemistry in Aquaculture Symposium. Convener and presenter, Cullowhee, North Carolina, May 31-June 2, 1995.

Schnick, R.A. 1995. FWS/INAD Coordination Workshop. Presenter and coordinator, Bozeman, Montana, August 1-4, 1995.

Schnick, R.A. 1995. Funding crisis for drugs/therapeutants and coordination of aquaculture INADs/NADAs. Annual meeting of the U.S. Trout Farmers Association, Twin Falls, Idaho, September 27-30, 1995.

Schnick, R.A. 1995. Activities of the National Coordinator for Aquaculture New Animal Drug Applications. Annual meeting of the National Research Support Program Number 7 (NRSP-7), Rockville, Maryland, October 2, 1995.

Schnick, R.A. 1995. INAD/NADA Coordinators workshop under the sponsorship of CVM. Organizer and presenter, Rockville, Maryland, November 1-2, 1995.

Schnick, R.A. 1996. Status of aquaculture INADs and NADAs. Presenter and coordinator, Midcontinent Warmwater Fish Culture Workshop and INAD/NADA Coordination Meetings, Council Bluffs, Iowa, February 6-8, 1996.

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SOME COMMONLY USED ABBREVIATIONS AND ACRONYMS

×	cross, by, or times
AADAP	Aquatic Animal Drug Approval Partnership Program
ADI	acceptable daily intake
AFWA	Association of fish and Wildlife Agencies
AIS	aquatic invasive species
APHIS	Animal and Plant Health Inspection Service
AquaNIC	Aquaculture Network Information Center
BM	blood meal
BOD	Board of Directors
BW	body weight
°C	degrees Celsius
CES	Cooperative Extension Service
CP	crude protein
CSREES	Cooperative State Research, Education, and Extension Service
CVM	Center for Veterinary Medicine
dph	day(s) post hatch
DAWG	Drug Approval Working Group
EA	environmental assessment
EAA	essential amino acid
°F	degrees Fahrenheit
FA	fatty acid
FCR	feed conversion rate/ratio
FDA	Food and Drug Administration
FDCA	Food, Drugs, and Cosmetic Act
FM	fish meal
ft, ft^2, ft^3	food, square foot, cubic foot
FY	fiscal year
g	gram(s)
gal	gallon(s)
GFI	Guidance for Industry
GLP	Good Laboratory Practices
gpm	gallons per minute
GTW	green tank water
h	hour(s)
ha	hectare(s)
HACCP	Hazard Analysis and Critical Control Point
HIS	hepatosomatic index
HUFA	highly unsaturated fatty acids
IAC	Industry Advisory Council
in	inch(es)
INAD	Investigational New Animal Drug
ISU	Iowa State University
KAA	Kansas State University
kcal	kilocalorie
kg	kilogram(s)
L	liter(s)
lb	pound(s)
LDL	Little Dixie Lake
LEMM	lipid-extracted menhaden meal
Lpm	liters per minute
LU	Lincoln University
m	meter(s)
MBM	meat and blood meal
µg	microgram(s)
mg	milligram(s)
min	minute(s)
ml	milliliter(s)
mm	millimeter(s)
MSU	Michigan State University
MT	methyltestosterone
MUMS	Minor Use and Minor Species
N	number
NAA	National Aquaculture Association
NADA	New Animal Drug Application
NADF	Northern Aquaculture Demonstration Facility
NCC	National Coordinating Council
NCR	North Central Region
NCRAC	North Central Regional Aquaculture Center
NTP	National Toxicology Program
OCARD	Ohio Center for Aquaculture Research and Development
oz	ounce(s)
P	probability
PBM	poultry byproduct meal
PER	protein efficiency ratio
POW	Plan of work
ppm, ppt	parts per million, parts per trillion
Purdue	Purdue University
®	registered
RAC(s)	Regional Aquaculture Center(s)
RAES	Regional Aquaculture Extension Specialist
RSD	relative standard deviation
SBM	soybean meal
SCBC	soluble canola protein concentrate
S.E.	standard error
sec	second(s)
SGR	specific growth rate
SIUC	Southern Illinois University-Carbondale
SNARC	Harry K. Dupree Stuttgart National Aquaculture Research Center
TAN	total ammonia nitrogen
TC	Technical Committee (TC/E = Technical Committee/Extension; TC/R =Technical Committee/Research
TI	trypsin inhibitor
TL	total length
™	trademark
UM-C	University of Missouri-Columbia
UMESC	Upper Midwest Environmental Sciences Center
USDA	U.S. Department of Agriculture
USFWS	U.S. Fish and Wildlife Service
USTFA	U.S. Trout Farmers Association
UW-Stevens Point	University of Wisconsin-Stevens Point
UW-Madison	University of Wisconsin-Madison
UW-Milwaukee	University of Wisconsin-Milwaukee
VHS	viral hemorrhagic septicemia
WATER	Wisconsin Aquatic Technology and Environmental Research
YG	yellow grease