NORTH CENTRAL
REGIONAL AQUACULTURE CENTER

ANNUAL PROGRESS REPORT 2006-07

JANUARY 2008

ANNUAL PROGRESS REPORT

For the Period
September 1, 2006 to August 31, 2007

January 2008

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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 billion in 2005 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 2004, the U.S. imported $22.9 billion of fisheries products and the trade deficit was $9.4 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 Farm Security and Rural Investment Act of 2002 (P.L. 107-171), 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 20 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), and FY2007 (#2007-38500-18569) with monies totaling $14,746,326. Currently, five grants are active (FY03-07); the first fifteen grants (FY88-02) 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, 2007, the Center has funded or is funding 78 projects through 419 subcontracts from the first 20 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; and in conjunction with the Aquaculture Network Information Center (AquaNIC) 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 twelve 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, 2006 to August 31, 2007. Projects, or Project components, that terminated prior to September 1, 2006 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	12/15/04-12/14/06	$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
			$261,173	
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. 1-10; 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. 11; 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. 12)	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	9/1/03-8/31/05	$100,000	2002-38500-11752
	12	9/1/05-8/31/08	$225,000	2004-38500-14269
			$1,093,986	
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
			$119,241	
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	

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
			$853,788	
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 FEED STABILITY AND WATER BIODEGRADATION STUDIES[1]

Project ***Termination Report*** for the Period
June 1, 2004 to August 31, 2007

NCRAC FUNDING: $223,677 (June 1, 2004 to June 30, 2006)

PARTICIPANTS:

Terence P. Barry	University of Wisconsin-Madison	Wisconsin
Ashok Marwah	University of Wisconsin-Madison	Wisconsin
Padma Marwah	University of Wisconsin-Madison	Wisconsin
Industry Advisory Council Liaison:		
Mark Willows	North American Fish Farmers Coop., Binford	North Dakota
Extension Liaison:		
Laura G. Tiu	Ohio State University	Ohio

REASON FOR TERMINATION
The project objectives were completed.

PROJECT OBJECTIVES
(1) Develop a robust and validated high performance liquid chromatography (HPLC) and liquid chromatography-mass spectroscopy (LC-MS) method to measure 17α-methyltestosterone (MT) in fish feed.

(2) Conduct a series of stability studies on MT in fish feed (note: after receiving NCRAC funding to conduct the stability study, it was learned that two additional feed studies must also be completed: (1) a feed homogeneity study and (2) a feed segregation study).

(3) Gain acceptance from the Center for Veterinary Medicine (CVM) for the series of stability studies.

(4) Review and develop an LC-MS method for detecting MT in water.

[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 and a termination report for the third project is contained in the 2001-02 Annual Progress Report. This termination report is for the fourth Aquaculture Drugs project which was chaired by Terence P. Barry. It was an 18-month project that began June 1, 2004. A fifth project, which provided $60,000 for a portion of the funds required to purchase sufficient radiolabelec AQUI-S® for use in a total residue depletion study in rainbow trout, is reported on under the progress report for the National Coordinator for Aquaculture New Animal Drug Applications (NADAs) elsewhere in this report as are progress reports for the sixth and seventh projects.

(5) Conduct a biodegradation study of MT in water.

(6) Gain acceptance from CVM for the biodegradation study on MT.

PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

A method to measure MT in fish feed has been developed. The method has already been published in a peer-reviewed journal. The method was accepted by the CVM for use in the studies proposed under Objective 2. The method has been transferred to CanTest, Ltd (a Canadian analytical company that has worked with MT in the past) for use in efficacy studies.

OBJECTIVE 2

A detailed experimental protocol was written describing the required studies (i.e., the feed stability, homogeneity, segregation experiments) and submitted to and accepted by CVM. All feed studies were completed according to the protocol and samples have been analyzed. The results indicated that (1) MT-treated feed prepared by the manufacturer (Rangen, Inc.) is homogenously mixed during the manufacturing and bagging process, (2) MT remains uniformly distributed throughout the feed during prolonged storage at room temperature, and (3) MT concentrations in fish feed are stable for at least several months when feed is stored at 4.0°C (39.2°F) or lower, but decline linearly with time at higher temperatures. The half-life of MT (i.e., the time required for the MT concentration to fall from 60 mg/kg [ppm] to 30 mg/kg [ppm]) was 1.1 and 4.8 months for feed stored at 40.0°C (104.0°F) and 22.0°C (71.6°F), respectively. A scientific paper on the experimental results has been published.

OBJECTIVE 3

The MT Feed Study Report will be submitted to CVM in November 2007.

OBJECTIVE 4

The LC-MS method to detect MT in water/sediment has been completed and validated. The method will be submitted to the CVM along with the results of the water/sediment experiments.

OBJECTIVE 5

The investigators received final CVM approval for the water/sediment study protocol in November 2005. All of the water/sediment experiments have been conducted. For each experiment, both water and the sediments must be analyzed separately. To date, all of the water samples from both the aerobic and anerobic experiments have been analyzed.

A summary of the biodegradation studies is as follows. Two sediment samples that differed with respect to total organic carbon and texture ("sand" and "clay") were exposed to radioinert MT or [^{14}C]-radiolabeled MT (^{14}C-MT), under both aerobic and anaerobic conditions, for up to 56 days, to characterize the fate of MT in the aquatic environment. Samples were extracted and MT concentrations were quantified by a highly sensitive liquid LC-MS method or by radio-high performance radio chromotography using an on-line flow radiation analyzer. Total radiation was quantified by liquid scintillation counting. The data suggest that MT entering the aquatic environment is converted into metabolites that become tightly associated with the sediment. Half-lives for MT dissipation in the sediment systems ranged from 2–9 days, depending on the sediment type and the presence of oxygen. Sediment type had little effect on MT dissipation. The mineralization of MT under aerobic

conditions was low (<9% conversion of MT to CO_2). A scientific paper on the "MT Water Study" is in preparation.

OBJECTIVE 6
The MT Water Method Development and MT Water Degradation Study Reports were submitted to the CVM in October 2007. All work was conduct according to University of Wisconsin-Madison protocols.

IMPACTS
MT is used to manipulate the gender of a variety of fish species cultured in the U.S., including tilapia, hybrid striped bass, yellow perch, sunfish, and esocids. The U.S. tilapia industry relies on the production of all-male populations, which grow significantly faster than mixed-sex populations. In this regard, this work is a critical contribution to the effort to obtain an original New Animal Drug Application approval for MT in tilapia. Once MT is approved for used in tilapia, approval for other key aquaculture species will follow.

RECOMMENDED FOLLOW-UP ACTIVITIES
None are required or anticipated at this time.

SUPPORT
NCRAC funds provided $223,677 to the University of Wisconsin-Madison 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: 17α-METHYLTESTOSTERONE TARGET ANIMAL SAFETY STUDY[2]

Project ***Progress Report*** for the Period
December 15, 2004 to August 31, 2007

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

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.

[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, and a termination report for the fourth project as well as a progress report for the seventh project are 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 progress report is for the sixth Aquaculture Drugs project which is being undertaken by Anita M. Kelly. It is a 2-year project that began December 15, 2004.

ANTICIPATED BENEFITS

The ability of aquaculturists to produce a fish that is uniform in growth and expends little 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 obtained sexual maturity. It is well known that male tilapia grow faster than female tilapia. Feeding tilapia a diet containing 17α-methyltestosterone (MT) at the onset of exogenous feeding and prior to sexual differentiation has been shown to be a viable method of producing all male populations. Currently, the U.S. Fish and Wildlife Service (USFWS) Aquatic Animal Drug Approval Partnership Program (AADAP) holds the INAD for the use of MT in sex inversion in tilapia. As part of the drug approval process, a target animal safety study must be conducted and approved by CVM. This study will complete the target animal safety study.

PROGESS AND PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

The Principal Investigator for this project was in constant communication with CVM and the 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 Aquatic Animal 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.

WORK PLANNED
Kelly is no longer at Southern Illinois University-Carbondale (SIUC) and the National Coordinator for Aquaculture New Animal Drug Applications will work with CVM, SIUC, and Kelly in regard to finalizing an acceptable target animal safety study on MT in tilapia.

IMPACTS
The ability of aquaculturists to produce a fish that is uniform in growth and expends little 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 would enable the production of monosex populations. Under an existing INAD, tilapia are being sex reversed 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.

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

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTE
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[3]

Project ***Progress Report*** for the Period
January 1, 2006 to August 31, 2007

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

PROJECT OBJECTIVES

(1) Interact with the U.S. Food and Drug Administration's Center for Veterinary Medicine (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®.

[3]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, and a termination report for the fourth project as well as a progress report for the sixth project are 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 progress report is for the seventh Aquaculture Drugs project which is being undertaken by Jeffrey R. Meinertz. It is a 1-year project that began January 1, 2006.

(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.

ANTICIPATED BENEFITS

Currently, Finquel (MS-222) is the only fish anesthetic approved by the U.S. Food and Drug Administration (FDA). Use of this anesthetic is constrained by a 21-day withdrawal period. A critical need for use of an anesthetic with a short withdrawal time in U.S. public aquaculture and fishery management has been expressed. A shorter withdrawal anesthetic would allow anesthetized fish to be handled and released immediately after conducting nearly all aquaculture and fishery management procedures including transport, spawning, marking, harvesting, and grading. AQUI-S® is a fish anesthetic under investigation as a short withdrawal time anesthetic.

PROGESS AND PRINCIPAL ACCOMPLISHMENTS

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

Nearly all supplies needed to conduct the study were procured. Fish-rearing practices were modified to maximize fish growth so fish would be of an adequate size for the study.

A study records system was developed for the storage of data generated during the study. 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, channel catfish, hybrid striped bass, lake trout, largemouth bass, northern pike, walleye, and yellow perch. 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 endogenous isoeugenol residues in the fillet tissue. Generation of fillet tissue with endogenous isoeugenol was necessary for the evaluating method precision with fillet tissue containing endogenous 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 endogenous isoeugenol.

The loss of isoeugenol from fillet tissue containing endogenous 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.

Fillet tissue from unexposed fall Chinook salmon was acquired and homogenized with dry ice in preparation for impending studies requiring homogenized control fillet tissue.

Samples of homogenized control fillet tissue from the following species were processed with the proposed determinative method for an evaluation of fillet constituents that would interfere with the determination of isoeugenol concentrations: 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 fillet tissue extracts from the following species was determined after 1, 7, and 14 days: brown trout, channel catfish, fall Chinook salmon, hybrid striped bass, lake trout, largemouth bass, northern pike, walleye, and yellow perch.

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

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

A comprehensive final report describing the study results has been assembled and reviewed for accuracy and compliance with FDA regulations for good laboratory practices by the UMESC Quality Assurance Officer.

WORK PLANNED

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® has been postponed:

"**STATEMENT ON ISOEUGENOL (AQUI-S®)**

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."

A decision to submit the final report will be made after the National Toxicological Program has made a ruling concerning the status of isoeugenol posing a human health risk.

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 basically 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 experiencing 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 resulting in a identification of all of the drug's residues in the edible fillet tissue and characterization of 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 determines concentrations of the marker residue in edible tissue. The confirmatory method confirms the results from the determinative method and irrefutable identifies 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.

CURRENT STATUS

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

If a decision is made to continue work with AQUI-S®, 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 conduct at least 3 marker residue depletion studies. Data from that work will be submitted to the FDA for their review and acceptance of that data should mark the end of work for an AQUI-S® approval.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

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

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]Estimate of additional UMESC salary costs for a GS13 and GS11 (4 pay periods each) that will be accrued during the 4th quarter of calender year 2006.

BAITFISH[4]

Project ***Progress Report*** for the Period
September 1, 2006 to August 31, 2007

NCRAC FUNDING: $111,997 (September 1, 2006 to August 31, 2007)

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

[4]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 placed under winter conditions, i.e., 10°C (50°F) water temperature and a photoperiod of 8 h light/16 h dark, over a period of two weeks. Fish were feed twice weekly, 2% body weight, 32% protein diet. Brood stock were held under these conditions for 10 weeks. Following this winter period, temperature and photoperiod were gradually increased to spring conditions, i.e., 22°C (72°F) and a photoperiod of 16 h light/8 h dark; a 2-week transition period was used. Once the tanks were under spring conditions, commercial spawning mats were placed into the spawning tanks, just under the water surface.

The egg-covered mats were then transferred to hatching tanks where they then hatched. Once the fry hatched, one of seven 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.

The ISU staff then utilized a 1.5% sodium sulfite solution bath where the spawning mats containing eggs were immersed for 2–2 ½ min that caused the eggs to drop out of the mat. The eggs were then placed in a hatching jar. The method allowed for enumeration of eggs. A total of 11 spawns were collected, however, most of the fry died. The staff tried seven different commercially available fry diets with no success.

The pond portion of the study was not completed as per this proposal because of the limited number of fry available; 60,000 were needed to stock three 0.08-ha (2.0-acre) ponds. The decision was made to stock the brood stock in the tanks into the ponds with the goal of doing a pond fertilization study.

The objectives of the pond fertilization were to (1) evaluate growth of fry in ponds using two fertilization regimes, combination of organic and inorganic versus only organic and (2) evaluate diet selection of fry in ponds using these two fertilization regimes organic.

To accomplish these objectives, 360 brood stock fish, total weight of weighing 4.2 kg (9.3 lb), were stocked into each pond (25.2 kg [55.6 lb] total weight for all fish). 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. All fish in the six ponds were then cultured for 210 days from the beginning of May until the end of November.

Age-0 and -1 fish will be collected from these ponds in November and placed back into indoor spawning tanks with the goal to repeat the earlier tank rearing study using different commercial diets. If successful,

the pond portion of this project will be completed in 2008.

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 these 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 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 were obtained for the 2007 studies was limited. The limitation on brood fish, in turn, led to a reduction in number and a delay in time at which fry became available. Nevertheless, NADF and UW-Madison conducted a set of meaningful studies as described below.

In the spring of 2007, NADF staff set up multiple 227-L (60-gal) and 1,514-L (400-gal) tanks for holding, spawning, and incubating 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 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.

NADF staff constructed spawning substrates out of cedar shingles similar to those described by other researchers, and also experimented with other substrates for spawning. Although the cedar shingle substrates did work, there were issues with food deposition and organic slime loads in the shingle cracks. There was greater success using aluminum siding and concrete blocks as spawning substrates.

In June, spawning substrates were placed into tanks with adult spotfin shiners. Water temperature was kept at 19–21°C (66–70°), natural photoperiod was >12 h daylight, and some current was established in the tanks. Periodically, substrates were removed and examined for eggs. Substrates containing eggs were placed into separate 227-L (60-gal) tanks for incubation and hatch. Hatch occurred within 5–7 days at 19–20°C (66–68°F). Newly hatched fry (<5.0 mm; 0.2 in) were initially lethargic and non swimming, but became photopositive and strong swimming in a few days. Spawning activity was recorded with an underwater camera supplied by Gunderson. Spawning activity continued until September.

Strong swimming, photopositive fry were collected and delivered to the UW-Madison facilities at the Lake Mills State Fish Hatchery three times during the spawning time frame. These fry were stocked into two fertilized outdoor rearing ponds at approximately 25,000 fish/ha. At the present time, the fish in both ponds have reached a length of 15.0–25.0 mm (0.6–1.0 in). The fish in one pond are being regularly fed a formulated food and are showing a significant feeding response.

OBJECTIVE 3

Gunderson, in his role as extension liaison for this project, presented the results of the baitfish project at the NCRAC Annual Program Planning Meeting in Columbus, Ohio. 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

As in the first year of this study, age-0 and -1 pond-run golden shiners will collected from ISU research ponds and placed into the indoor spawning tanks; the same experiment will be done using additional feed sources. If successful, the pond portion of this study will be completed in 2008.

The staff at the University of Wisconsin-Milwaukee have in place a research strategy for the next year which will include photothermal manipulation to initiate spawning and enhance gamete production. Additionally, they will work on the early life stage culture techniques with specific emphasis on nutrition to improve survival.

UW-Stevens Point NADF and UW-Madison staff harvested the ponds at Lake Mills in October. Data will be collected and the fish will be transported to NADF for continued grow out as described in the original proposal. Experience in the first year of this project in spawning spotfin shiners has led to the conclusion that it will be unfeasible to produce large numbers of fry at a single time. Thus, the 2008 studies will not include the evaluation of rearing ponds stocked with fry at only one time during the year. All ponds in 2008 will be stocked at multiple times during the spawning season. A replicated study will be conducted comparing different pond stocking and feeding regimes, similar to that described in the original proposal.

OBJECTIVE 3
The private baitfish producer who supplied 7.6 L (2.0 gal) of spotfin shiner brood stock to the NADF in June, 2007 has captured a large quantity of adult spotfin shiners this fall and the staff from University of Minnesota-Duluth are planning to deliver another 37.9 L (10.0 gal) of adult spotfin shiners to NADF in October or early November. Another conference call with NCRAC baitfish researchers will be conducted this fall to assess progress to date and to compare notes regarding progress on goals. If sufficient information is available regarding results to-date, an effort will be made to get this information out to the industry this winter. More likely, workshops and other outreach activities will be held after results from the research are available.

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
Because all golden shiner fry perished with no success on first-feeding, there is a need to identify a commercial diet that will support fish survival beyond 7–10 day post hatch.

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.

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 funds provided to date total $111,997; a total of $200,000 has been allocated for this 2-year project.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

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

4th NATIONAL AQUACULTURE EXTENSION WORKSHOP/CONFERENCE[5]

Project ***Termination Report*** for the Period
September 1, 2006 to August 31, 2007

NCRAC FUNDING LEVEL: $5,000 (January 1, 2006 to December 31, 2006)

PARTICIPANTS:

Laura G. Tiu	Ohio State University	Ohio
Forest Wynne	Kentucky State University	Kentucky
National Steering Committee:		
Steve Harbell	Washington State University	Washington
Charles Pistis	Michigan State University	Michigan
Michael Rice	University of Rhode Island	Rhode Island
Michael Schwarz	Virginia Tech University	Virginia
George Selden	University of Arkansas-Pine Bluff	Arkansas
Clyde Tamaru	University of Hawaii	Hawaii
Conference Ex-Officio Advisory Committee:		
Kevin Fitzsimmons	University of Arizona	Arizona
Gary L. Jensen	USDA Cooperative State Research, Education, and Extension Service	Washington, D.C.
Andy Lazur	NOAA National Sea Grant College Program	Washington, D.C.
Maxwell H. Mayeaux	USDA Cooperative State Research, Education, and Extension Service	Washington, D.C.
Joseph E. Morris	Iowa State University	Iowa
James Murray	NOAA National Sea Grant College Program	Washington, D.C.
Graham Young	University of Washington	Washington
Local Arrangements Committee:		
Robert Durborow	Kentucky State University	Kentucky
Julie Strawser	Ohio State University	Ohio
Geoffrey Wallat	Ohio State University	Ohio
William Wurts	Kentucky State University	Kentucky

[5]NCRAC has provided funding along with the four other Regional Aquaculture Centers for four national aquaculture extension meetings; the first was called a National Aquaculture Extension Workshop whereas the second through fourth were called National Aquaculture Extension Conferences. This termination report is for the fourth meeting which was held April 30-May 4, 2007 in Cincinnati, Ohio.

REASON FOR TERMINATION
The project objectives were completed.

PROJECT OBJECTIVES
(1) Learn successful approaches to problem-solving through case studies that can be replicated in other states, i.e., lessons learned.

(2) Demonstrate and conduct hands-on experience with state-of-the-art computer applications for improving delivery of extension programs.

(3) Identify national extension priorities and critical issues with development of corresponding action plans for implementation.

(4) Identify potential interregional extension projects, such as curriculum development or national decision-support databases.

(5) Share educational materials and programs in addition to expertise.

(6) Strengthen regional and national communication networks to improve services to clientele.

(7) Examine successful extension components and outcomes to research projects and develop approaches to improve integration nationwide.

(8) Develop a collective strategy to define extension's role in measuring impacts of Regional Aquaculture Center (RAC) projects and collaboration with others in academia and private sector.

(9) Improve business management skills related to aquaculture and enhance knowledge concerning marketing of aquatic products.

PRINCIPAL ACCOMPLISHMENTS
The 4th National Aquaculture Extension Conference was organized by a national Steering Committee which was comprised of representatives from each RAC, the National Association of County Agriculture Agents, U.S. Department of Agriculture's Cooperative Research, Education, and Extension Service, and the National Office of Sea Grant.

The conference was held in Cincinnati, Ohio, April 30- May 4, 2007 and 83 people from 38 states and territories attended.

Multiple presentations made during the conference addressed many of the objectives. Two workshops, dealing with the media and working with www.grants.gov, helped attendees gain new skills. The keynote speaker, Dr. Steve Otwell from the University of Florida, spoke on Seafood Marketing and Consumption issues, identified as a critical issue by the Steering Committee.

A field trip to Jungle Jim's, a large, unique, international market northwest of Cincinnati, Ohio that employs recirculating technology to hold live fish, improved attendees' knowledge concerning marketing of aquatic products. Socials and local tours gave attendees time to share programming ideas and expertise.

A Web site (http://southcenters.osu.edu/aqua/extension%20conference/extension%20conference.htm) with the conference agenda linked to abstracts, attendee list, and photos was created and made accessible to anyone.

IMPACTS
Short term impacts were measured with an on-site evaluation. Highlights include:
- Great networking

- Great program
- Good location
- Well-coordinated sessions
- Diverse variety of topics
- Jungle Jim's field trip amazing!
- Diverse participants
- Great reviews of advances and successful programs
- Time to interact
- Great time span for a conference

RECOMMENDED FOLLOW-UP ACTIVITIES

Attendees will be sent a post-conference evaluation survey to determine how the conference has impacted their actions or activities. Plans for the next conference are being coordinated by the Steering Committee.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

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

SUPPORT

YEARS	NCRAC-USDA FUNDING	OTHER SUPPORT					TOTAL SUPPORT
		UNIVER-SITY	INDUSTRY	OTHER FEDERAL[a]	OTHER	TOTAL	
2006-07	$5,000			$20,000		$20,000	$25,000
TOTAL	$5,000			$20,000		$20,000	$25,000

[a]Each of the four other Regional Aquaculture Centers contributed $5,000 to the conference and some travel grants were supported by NOAA's National Sea Grant College Program.

EXTENSION[6]

Progress Report for the Period
May 1, 1989 to August 31, 2007

NCRAC FUNDING LEVEL: $768,986 (May 1, 1989 to August 31, 2007)

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
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

[6]NCRAC has funded a number of Extension activities, both as stand-alone projects or as components of species-or topical-specific projects, including ten stand-alone projects deemed "Base" Extension. This Progress Report is for components of those ten "Base" Extension projects. The first three "Base" projects were chaired by Donald L. Garling, the fourth was chaired by Fred P. Binkowski, and projects 5-10 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 tenth "Base" project is a 2-year project that began September 1, 2005. Fred P. Binkowski chaired the eleventh 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 twelfth stand-alone Extension project (the Regional Aquaculture Extension Specialist); a Progress Report for that project is contained elsewhere in this report.

PARTICIPANTS (continued):

Burton 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

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 ten 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; and
- 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.

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 (CSREES) 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 site. Approximately 1,200 individual, educational, commercial, and governmental Web sites link to AquaNIC as a source of online aquaculture information. During 2005-2007, AquaNIC was visited by more than 6 million people who viewed almost 16.5 million pages which translates to more than 8,400 visitors/day, each averaging almost 11 minutes/visit. AquaNIC is currently ranked in the top 10% of all Web site traffic worldwide by www.ranking.com – a professional Internet monitoring company that tracks 1,000,000 of the most visited Web sites around the world.

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.

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, 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 has 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, and 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 aquatic invasive species-HACCP (AIS-HACCP) approach has been developed by Kinnunen and Gunderson and taught to private fish farmers, 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."

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 NCR is dotted with unused agriculture buildings harkening to the days when small farms could survive raising small numbers of hogs or chickens. One option that many are exploring is converting the buildings for aquaculture use. To help farmers further explore this option, a videoconference workshop was designed and produced by Tiu to explore the pros and cons of converting existing agricultural buildings into fish culture facilities and a similar sort of videoconference was conducted by Quagrainie on March 8, 2007.

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 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
TOTALS	$768,986	$1,055,868	$5,000	$334,000	$55,000	$1,449,868	$2,218,854

REGIONAL AQUACULTURE EXTENSION SPECIALIST (RAES)[7]

Project ***Progress Report*** for the Period
September 1, 2005 to August 31, 2007

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

(1) 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.

(2) It was determined that JJ Newman Rode was the best candidate and that she would be employed by and housed at Purdue University through a subcontract with Ohio State University.

(3) Newman Rode started working at Purdue University as the RAES on August 15, 2006. Soon thereafter, she began visiting a number of NCR-states where she met with a number of industry leaders and state aquaculture associations. In February 2007 she attended the North Central Regional Aquaculture Center's 2007 Annual

[7] 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.

Program Planning Meeting in Columbus, Ohio where she presented her proposed 12-month work plan as the RAES. Due to unforeseen circumstances she never finalized nor implemented her work plan and on October 15, 2007 ended her role as the RAES.

WORK PLANNED

Tiu will work with the Associate Director of NCRAC, Dr. Joe Morris who is responsible for all Extension activities of the Center, to identify someone who could replace Newman Rode as the RAES. Once there is a new RAES, they will work with Tiu and Morris to develop a plan to address the unfinished objectives of the project.

IMPACTS

None to date.

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[8]

Project ***Progress Report*** for the Period
September 1, 2006 to August 31, 2007

NCRAC FUNDING: $165,446 (September 1, 2006 to August 31, 2007)

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 studies will also provide valuable cost/benefit information on the use of krill and semi-moist feeds as transitional diets. 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. These studies will provide valuable information to producers of these species for maximizing the productivity and efficiency of their operations. The prediction is that this project will explain methods for increasing the efficiency of yellow perch and

[8]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.

largemouth bass fingerling production by 20–40%, and thereby reduce fingerling production costs. This, in turn, will significantly reduce the cost of raising these fish to food size, providing a strong stimulus to the growth of this important industry.

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. The strategies used in this study are likely to be easily transferred from yellow perch and largemouth bass to other fish species produced in the North Central Region. With the success that researchers have had in defining attractants/stimulants and sound cues as they apply to feeding behavior, researchers are confident that the biological and/or physical technology can be developed for yellow perch and largemouth bass.

Understanding the physiological and environmental factors involved in poststocking feed acceptance by yellow perch and largemouth bass fingerlings will aid in the understanding as to whether lack of feed retention is a result of stress, cannibalism, or predation by birds. These areas will further reveal potential changes in husbandry practices that will improve the number of feed-trained fingerlings that are produced annually.

PROGESS AND PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

University of Wisconsin-Madison

University of Wisconsin-Madison (UW-Madison) investigators have completed two experiments relevant to the feed training of pond-raised yellow perch fingerlings. Experiment 1 evaluated the influence of fish size at harvest on habituation success. Yellow perch were harvested from the pond 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, perch 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. Researchers distributed 125.0 g (4.4 oz) of food daily in each tank. 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). The definition of starvation is substantiated by observations that virtually

all dead fish recovered were extremely emaciated and losses attributed to disease or injuries were negligible. The definition of cannibalism is substantiated by observations of cannibalistic behavior and the fact that fish could not escape from the tanks through the standpipe screen or by any other means. Cannibals were not removed during the experiment.

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. This statistical result may have been limited by the low number of replicates (N = 4–6 replicates per fish size) used in the study. 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 114-L (30-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

The University of Wisconsin-Milwaukee (UW-Milwaukee) was unable to conduct research on this project in 2006-2007 due to the state of Wisconsin restricting the movement of fish from ponds to indoor facilities and vice versa. It is anticipated that the research will resume in the coming year.

OBJECTIVE 2

University of Wisconsin-Madison

UW-Madison researchers conducted one experiment on size-grading fingerlings during the habituation period. 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 0.04 ha (0.1 acre) production pond on day 21.

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). Researchers found that ponds stocked earlier in the season produced larger fingerlings than those stocked later in the season. Pooling data across treatments (size-graded and non-size-graded), fingerlings stocked ranged 18.4–24.6 g (0.6–0.9 oz) for the three sets of ponds. It is 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. Substantial effort was put into preparing the ponds for the study. Beginning in April 2007, all ponds were longitudinally divided into halves with plastic mesh nets that were attached to 10.2 × 10.2 cm (4 × 4 in) posts driven approximately 0.9 m (3.0 ft) into pond bottoms. The plastic mesh panels were staked into the pond bottoms to preclude fish passage under the nets. Wire lines spanning pond lengths held the net tops approximately 0.9 m (3.0 ft) above the water surface so that largemouth bass 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, however, attempts to secure this number of fish on relatively short notice were unsuccessful.

Southern Illinois University-Carbondale (SIUC)

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 (1,320-gal) grading tank where they 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. A twenty-tank feed-training system with a randomized block design was utilized. All treatments utilized automatic belt feeders. The treatments were: (1) feeding by hand for the full two weeks, (2) hand feeding for three days and then automatic feeders only for the remaining time, (3) hand feeding for seven days and then automatic feeders only for the remaining seven days, (4) one automatic feeder per tank for the entire time with no hand feeding, and (5) two automatic feeders per tank with no hand feeding for the entire time. 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. More research needs to be done regarding stocking density. Shortly after the initiation of the increased stocking density portion of the study, an outbreak of *Ichthyobodiasis* occurred. As soon as the infestation was detected, an aggressive formalin treatment regimen was instituted. Mortality was reduced to expected levels and the outbreak was contained. For the remainder of the study, formalin treatments were conducted weekly to prevent further disease problems. Because the increased density treatments in this study only utilized the smallest size group of fish, it is not possible to determine whether it was the size, stocking density, an additional factor, or a combination of all three that resulted in the disease outbreak.

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 measurements were taken with a LI-250 LiCor meter (LiCor Biosciences, Lincoln, Nebraska).

Light intensity was found to have no impact on feed habituation success ($P = 0.7249$) and no impact on survival except at the darkest level tested ($P = 0.0261$). The number of cannibals differed significantly between the light and dark treatments ($P = 0.0331$). Reduced light levels result in decreased ability of culturists to observe fish for health and cannibalism.

Additionally, because little research has been done to evaluate bird predation on largemouth bass fingerlings and on non-lethal methods or devices to deter piscivorous birds and prevent their consumption of fingerling fishes, piscivorous bird feeding behavior and the effectiveness of a birds of prey call in deterring fish-eating birds were evaluated. Bird numbers at pond banks were recorded at various times throughout the day during the summer months. Three different bird deterrent devices were evaluated for use on a commercial fish farm. They were an electrical shock device (Scat Mat), a random noise generator, and a birds of prey call.

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 Bushnell Yardage Pro 400 laser range finder (Bushnell Outdoor Products, Overland Park, Kansas). After testing the effectiveness of 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.

Sunrise and sunset were time periods during which bird activity at the study ponds apparently increased, as did bird activity across the farm. Less bird activity was observed during mid-morning and mid-afternoon observations than at other times, although bird activity was not significantly different in the different observation time periods ($P = 0.3493$). It was determined that noise and electrical shock devices placed on the automatic feeders in the ponds would be of little value because a vast majority of the birds feeding at these ponds were wading birds and were not observed utilizing the feeders as perches. Birds of prey calls failed to repel fish-eating birds from the fish farm.

In summary, labor costs may be reduced because hand feeding is not necessary in feed habituation of largemouth bass. Light intensity should remain at intermediate levels at most, as other researchers have shown that extremely bright conditions cause increased stress responses in fish. To date, physical barriers are the only demonstrated effective prevention mechanism for bird predation in aquaculture.

University of Wisconsin-Milwaukee

The UW-Milwaukee was unable to conduct research on this project in 2006-2007 due to the state of Wisconsin restricting the movement of fish from ponds to indoor facilities and vice versa. It is anticipated that the research will resume in the coming year.

WORK PLANNED

OBJECTIVES 1 & 2

The UW-Madison pond stocking density study will be conducted as described in the original proposal.

It is anticipated that the UW-Milwaukee research will begin in the coming year.

Researchers at the UM-C plan to conduct both Year 1 and Year 2 activities in tandem (June-July) at the LDL site in 2008. The fish producer dealt with in Year 1 will again attempt to provide the required numbers of fish, however, efforts are in progress to identify alternative sources of fish as back-up, to ensure that work can move ahead this upcoming spring and summer.

SIUC pond stocking studies using different densities will be conducted as outlined in the proposal.

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.

SUPPORT

NCRAC funds provided to date total $165,446; a total of $300,000 has been allocated for this 2-year project.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

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

LARGEMOUTH BASS NUTRITION[9]

Project ***Progress Report*** for the Period
September 1, 2005 to August 31, 2007

NCRAC FUNDING: $170,000 (September 1, 2005 to August 31, 2007)

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 2007.

[9]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 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. This trial is in the analysis stage as the second growth phase has just ended and data have been collected and are being reviewed.

Current 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%).

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. Data are currently undergoing analysis as the fish were sampled in October 2007.

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. The study will be conducted in triplicated 0.04-ha (0.1-acre) earthen ponds using a density of 1,250 0.275 lb fish (age 1+)/acre. In addition to using experimental diets, the size of feed and timing/number of feedings will be based on results from Objective 1.

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 spring 2008.

In spring 2008, a study will be repeated to assess if the onset of sexual maturation affects growth of largemouth bass. This study had been intended to be part of the current year's work, but the fish all grew to such a size that they were all sexually mature. Fish will be harvested at a smaller size to enhance the odds of some being sexually mature and others not so that an assessment can be made. Determinations of sexual maturation will be done by use of ultrasound and necropsied individuals. Analysis will include determination of sex and gonadosomatic index in each randomly selected individual with comparisons made with overall growth, length and feed conversion.

At Purdue, two separate, but related, studies will be conducted with largemouth bass in the laboratory prior to the pond production experiment. Methionine adequacy and interaction with choline will be addressed. End points will include liver fat concentrations and hepatic metabolism. A second experiment will examine the maximum amount of solvent-extracted soybean meal in diets for largemouth bass. Both studies are in the early stages and should be completed prior to the pond production experiment.

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
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[10]

Project ***Progress Report*** for the Period
July 15, 2004 to August 31, 2007

NCRAC FUNDING: $64,000 (July 15, 2004 to August 31, 2007)

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

[10]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.

than 50 known aquaculture diseases. CVM has afforded the aquaculture industry throughout the United States with a "window of opportunity" to seek approval of legal drugs to be used in their production practices. The need for additional drugs is great, but securing data necessary to satisfy the requirements of CVM for drug approval is time consuming, costly, and procedures are rigorous. The INAD/NADA process is the one method that allows the industry to provide CVM with data on efficacy and also aids producers in their production practices.

Coordination and educational efforts directed toward potential INAD/NADA applicants will save time and effort for both the industry and CVM. The National Coordinator for Aquaculture New Animal Drug Applications (National 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)

CHLORAMINE-T (HALAMID®)—EXTERNAL ANTIBACTERIAL

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

- On September 15, 2006, CVM granted Minor Use and Minor Species (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, the Upper Midwest Environmental Sciences Center (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.

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 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.

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.

FLORFENICOL (AQUAFLOR®)—ORAL ANTIBACTERIAL
Three supplemental label claims close to completion: Control of mortality due to (1) furunculosis in freshwater-reared salmonids, (2) systemic columnaris disease in freshwater-reared salmonids and catfish, and (3) *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 Aquatic Animal Drug Approval Partnership Program (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.

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.

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 August 28-29, 2007, the National Aquaculture NADA Coordinator met with Kona Blue Aquatic Farms in Kona, Hawaii to discuss developing data to support a label claim for controlling certain external parasites with 35% PEROX-AID® (hydrogen peroxide) on the major fish species reared by Kona Blue, Kona Kampachi®.

ISOEUGENOL (AQUI-S®)—ANESTHETIC
One initial label claim in progress: (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 the North Central Regional Aquaculture Center (NCRAC) for the radiolabeled material needed for the total residue depletion study on AQUI-S® to be conducted by the Upper Midwest

Environmental Sciences Center (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.

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 17α-methyltestosterone that were conducted and developed by the University of Wisconsin-Madison.

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 following label claim for use as a sedative during transport of ornamental (non-food) finfish.

OXYTETRACYCLINE DIHYDRATE (TERRAMYCIN® 200 FOR FISH)—ORAL ANTIBACTERIAL
Three supplemental label claims close to completion: control of mortality due to (1) systemic columnaris disease in rainbow trout (*Oncorhynchus mykiss*) and (2) systemic coldwater disease in all freshwater-reared salmonids; (3) skeletal marking for salmonids.

- On August 15, 2006, CVM accepted GFI #159 for penaeid shrimp from the University of Arizona.
- On September 20, 2006, CVM accepted GFI #159 for the safety of residues in all freshwater-reared finfish in human food from Phibro Animal Health.
- On October 13, 2006, CVM requested a revision of the EA from UMESC.
- On March 15, 2007, CVM accepted GFI #152 for the microbial food safety for all freshwater-reared salmonids from AADAP.
- On April 13, 2007, UMESC submitted the amended EA to CVM.
- On July 2, 2007, Phibro Animal Health submitted to CVM a request for a Human Food Safety Complete Letter for that Technical Section.
- On July 23, 2007, Phibro Animal Health submitted to CVM a Labeling Technical Section to add the three new label claims and request the removal of the warning statement concerning use below 9.0°C (48.2°F).

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*.

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.

GENERAL
- On July 18, 2007, the National Aquaculture NADA Coordinator gave an eight-hour presentation to the Veterinary Drugs Directorate (VDD) at its invitation. VDD is the Canadian equivalent of the U.S. CVM. The VDD was interested in (1) the successful aquaculture drug approval processes in the U.S., (2) our experience with various successful partnerships, and (3) insight into expediting the aquaculture drug approval processes in Canada.
- The designation provision of the MUMS Animal Health Act of 2004 gives sponsors seven years of marketing exclusivity. So far, the MUMS Office has granted 50 designations, 44 of those are to aquaculture drug sponsors who received extensive help from the National Coordinator for Aquaculture NADAs. The most recent MUMS designations are three for Pfizer Animal Health's Terramycin 343® (oxytetracycline hydrochloride) on June 7, 2007 and one for Aquatic Life Sciences, Inc.'s Ovaplant® (salmon gonadotropin releasing hormone analog) on May 25, 2007. There have been three NADA approvals of MUMS designations for Eka Chemicals, Inc.'s 35% PEROX-AID® and two NADA approvals and one Conditional Approval of MUMS designations for Schering-Plough Animal Health's Aquaflor®.
- On May 4, 2007, CVM clarified the extra-label use of medicated feeds in minor species under the Compliance Policy Guide (#615.115) to include (1) veterinarian involvement, (2) treatment use only, (3) no production use, and (4) no feed reformulation or relabeling.
- From July to September 2007, the National Aquaculture NADA Coordinator gave presentations on innovations and status of aquaculture drug approvals at the144th American Veterinary Medical Association Convention (Washington, D.C.), 13th Annual Drug Approval Coordination Workshop (Bozeman, Montana), Disease Management Strategies for the Aquatic Environment: Alternative and Innovations Symposium (San Francisco, California), and the Association of Fish and Wildlife Agencies, Drug Approval Working Group meetings (Louisville, Kentucky).

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®) and formalin (Formacide-B®); 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 Aquaculture INAD/NADA Coordinator 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
TOTAL	$64,000		$99,936	$215,077	$92,900	$407,913	$471,913

NUTRITION[11]

Project ***Progress Report*** for the Period
September 1, 2004 to August 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

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).

ANTICIPATED BENEFITS

Concern has been raised whether aquaculture can sustain its rapid growth worldwide if the industry continues to rely on fish meal and oil as the major dietary protein and lipid constituents. Issues have been raised concerning cost, fluctuating availability, and even if aquaculture is growing at the expense of wild fisheries dependent upon the same forage fish being harvested for fish meal. The implications revolving around fish meal and oil are particularly critical in the North Central Region (NCR) because both products must be imported. The completed and on-going studies described here will provide feed manufacturers with the information they will need to produce cost-effective feeds low in or free of fish meal. This line of research is similar to the series of projects funded by the North Central Regional Aquaculture Center (NCRAC) on Salmonids. Those projects were designed to develop fish meal-free diets for rainbow trout. Benefits derived from those studies included a new feed meal specializing in fish meal-free diets for the NCR. That new business is located in Ohio.

PROGESS AND PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

Researchers at Purdue University (Purdue) were delayed in starting the Nutrition project. A fire completely destroyed the Aquaculture Research Laboratory in October 2004 as the project was beginning. Temporary wet lab space was occupied from

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

January 2005 until June 2006, but that space was inadequate for conducting the proposed studies. The Aquaculture Research Laboratory has been reconstructed and was occupied in June 2006. The proposed study with hybrid striped bass has been completed and the proposed study with yellow perch is underway. 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 the plant-based ingredients resulted in dietary formulations that would not meet the 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 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 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 soluble canola protein concentrate; 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 was 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% soybean meal (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 (RBC), 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, $y = 0.4029\times + 16.563$ ($R^2 = 0.90$), 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.

WORK PLANNED

OBJECTIVES 1 & 2

As mentioned earlier, Purdue's aquaculture facility was destroyed by fire in October of 2004 as this project was beginning. Studies with hybrid striped bass have been completed and the proposed research with yellow perch is underway. Researchers 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 and 8% lipid) and the specific or predicted essential amino acid requirements for yellow perch.

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. Plant-based ingredients could not meet the nutrient requirements 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 are currently being fed to all-female yellow perch housed in recirculating systems at the Purdue Aquaculture Research Laboratory.

Work by MSU, SIUC, and UW-Madison has been completed. Results are being prepared for publication or have been published in journals.

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 affects 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.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

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

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

APPENDIX

AQUACULTURE DRUGS

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Lane, R., and C. Kohler. 2006. Hybrid striped bass culture guide. NCRAC Culture Series #104, NCRAC Publications Office, Iowa State University, Ames.

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Boylan, J., and J. Morris. 2003. Invertebrate identification for fish culturists. NCRAC CD-ROM Series #102, NCRAC Publications Office, Iowa State University, Ames.

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Situation and Outlook Report

Hushak, L.J. 1993. North Central Regional aquaculture industry situation and outlook report, volume 1 (revised October 1993). NCRAC Publications Office, Iowa State University, Ames.

Other Publications in Print

Myers, J.J., and R.A. Pierce. 2000. Missouri aquaculture directory. Missouri Department of Agriculture, Jefferson City, Missouri.

Pierce, R.A., and C. Hicks. 2000. Understanding aquaculture businesses and their financial needs. Pages 75-76 *in* R. Plain, editor. Missouri farm financial outlook 2001. University Outreach and Extension, Department of Agricultural Economics, University of Missouri-Columbia.

Swann, D.L., and M.E. Einstein. 2000. User analysis and future directions of the web-based Aquaculture Network Information Center. Journal of Extension 38(5).

Yeo, S.E., F.P. Binkowski, and J.E. Morris. 2004. Aquaculture effluents and waste by-products: characteristics, potential recovery and beneficial reuse. NCRAC Publications Office, Iowa State University, Ames.

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, Niagra Falls, Ontario, May 6, 1995. (LaDon Swann)

AquaNIC. Annual Meeting of the Aquaculture Association of Canada, Nanaimo, British Columbia, June 5, 1995. (LaDon Swann)

Yellow Perch Aquaculture Workshop, Spring Lake, Michigan, June 15-16, 1995. (Donald L. Garling)

Rainbow Trout Production: Indoors/Outdoors, Piketon, Ohio, July 8, 1995. (James E. Ebeling)

North Central Regional Aquaculture Center Hybrid Striped Bass Workshop, Champaign, Illinois, November 2-4, 1995. (Christoper C. Kohler, LaDon Swann, and Joseph E. Morris)

3rd North Central Regional Aquaculture Conference, Indianapolis, Indiana, February 6-7, 1997. (LaDon Swann)

4th North Central Regional Aquaculture Conference, Columbia, Missouri, February 24-26, 1999. (Robert A. Pierce and Joseph E. Morris).

Extension Programming in the North Central Region, SERA-IEG-9, Frankfort, Kentucky, March 14-16, 1999. (Joseph E. Morris)

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)

Food Safety Issues Related to Aquaculture, Aquaculture America 2003, Louisville, Kentucky, February 18-21, 2003. (Ronald E. Kinnunen)

The ANS-HACCP Approach: Reducing the Risk of Spreding Aquatic Nuisance Species, Aquaculture America 2003, Louisville, Kentucky, February 18-21, 2003. (Ronald E. Kinnunen)

Use of Natural Ponds for Fish and Baitfish Production, Aquaculture America 2003, Louisville, Kentucky, February 18-21, 2003. (Ronald E. Kinnunen)

Overviews on Production, Nutrition, Economics, and Fish Health Management for Yellow Perch, *Perca flavescens*, Aquaculture America 2003, Louisville, Kentucky, February 18-21, 2003. (Fred P. Binkowski, Ronald E. Kinnunen, and Geoffrey Wallat)

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

Extension Program Assessment: An Extension Specialist's View, 3rd National Aquaculture Extension Conference, Tucson, Arizona, April 7-11, 2003. (Joseph E. Morris)

Great Lakes Native American Involvement in Fisheries Extension Programs, 3rd National Aquaculture Extension Conference, Tucson, Arizona, April 7-11, 2003. (Ronald E. Kinnunen and Charles Pistis)

On Farm Demonstration of Freshwater Shrimp Culture in Southern Ohio, 3rd National Aquaculture Extension Conference, Tucson, Arizona, April 7-11, 2003. (Laura G. Tiu)

Potential Recovery and Beneficial Use of Aquaculture Effluents and Waste By-Products, Aquaculture 2004, Honolulu, Hawaii, March 1-4, 2004. (Joseph E. Morris and Fred P. Binkowski)

Introduction to Recirculating Aquaculture Workshop, Bellevue, Ohio, March 20, 2004. (Laura G. Tiu)

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

Channel Catfish Culture in Midwestern Plastic-Lined Ponds, American Fisheries Society Annual Meeting, Madison, Wisconsin, August 25, 2004. (Joseph E. Morris)

Aquaculture Field Day, Lincoln University Carver Farm, Missouri, October 2004. (Robert A. Pierce)

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)

Hazard Analysis Critical Control Point (HACCP) Training for Commercial Fish Processors (poster), International Association of Great Lakes Research Conference, Ann Arbor, Michigan, May 24, 2005. (Ronald E. Kinnunen)

Great Lakes Native American Involvement in Fisheries Extension Programs, International Association of Great Lakes Research Conference, Ann Arbor, Michigan, May 24, 2005. (Ronald E. Kinnunen and Charles Pistis)

Why AIS-HACCP? Overview and Rationale, International Association of Great Lakes Research Conference, Ann Arbor, Michigan, May 24, 2005. (Ronald E. Kinnunen and Jeffery L. Gunderson)

Aquaculture Overview, National Farm and Ranch Business Management Education Association Annual Conference, Wooster, Ohio, June 13, 2005. (Laura G. Tiu)

AIS-HACCP Training Workshop, American Fisheries Society Annual Conference, Lake Placid, New York, September 10, 2006. (Ronald E. Kinnunen)

Yellow Perch Spawning Workshop, Milwaukee, Wisconsin, November 2, 2006. (Fred B. Binkowski).

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)

Proceedings

Proceedings of the North Central Regional Aquaculture Conference. 1991. 1st North Central Regional Aquaculture Conference, Kalamazoo, Michigan, March 18-21, 1991.

Gunderson, J., editor. 1995. Proceedings of the Combined North Central and Ninth Annual Minnesota Aquaculture Conference and Tradeshow. 2nd North Central Regional Aquaculture Conference, Minneapolis, Minnesota, February 17-18, 1995.

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FEED TRAINING CARNIVOROUS FISH

Publications in Print

Sims, D.W. 2007. Effects of feed training methods and light intensity on survival and feed training success of largemouth bass*Micropterus salmoides* and effectiveness of new bird repellant devises in a commercial aquaculture setting. Master's thesis. Southern Illinois University-Carbondale.

Papers Presented

Sims, D.W., and A.M. Kelly. 2007. Effects of different feed training methods on survival and feed training success of largemouth bass *Micropterous salmoides*. Aquaculture America 2007, San Antonio, Texas, February 26-March 2, 2007.

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Allyn, M.L., R.J. Sheehan, and C.C. Kohler. 2001. The effects of capture and transportation stress on white bass semen osmolality and their alleviatin via sodium chloride. Transactions of the American Fisheries Society 130:706-711.

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Brown, P.B., R. Twibell, Y. Jonker, and K.A. Wilson. 1997. Evaluation of three soybean products in diets fed to juvenile hybrid striped bass *Morone saxatilis* × *M. chrysops*. Journal of the World Aquaculture Society 28:215-223.

Kasper, C.S., and C.C. Kohler. 2004. Use of finishing diets in indoor hybrid striped bass culture reduces production costs. Pages 507-513 *in* Rakestraw, T., L.S. Douglas, and G.J. Flick, editors. Proceedings of the Fifth International Conference on Recirculating Aquaculture. Virginia Polytechnic Institute and State University, Roanoke, Virginia.

Kelly, A.M., and C.C. Kohler. 1996. Sunshine bass performance in ponds, cages, and indoor tanks. Progressive Fish-Culturist 58:55-58.

Kelly, A.M., and C.C. Kohler. 1999. Cold tolerance and fatty acid composition in striped bass, white bass and their hybrids. North American Journal of Aquaculture 61:278-285.

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Kohler, C.C. 1997. White bass production and broodstock development. Pages 169-185 *in* R.M. Harrell, editor. Striped bass and other *Morone* culture. Elsevier Press, Amsterdam.

Kohler, C.C. 2000. Striped bass and hybrid striped bass culture. Pages 898-907 *in* R.R. Stickney, editor. Encyclopedia of aquaculture. John Wiley & Sons, Inc., New York.

Kohler, C.C., R.J. Sheehan, C. Habicht, J.A. Malison, and T.B. Kayes. 1994. Habituation to captivity and controlled spawning of white bass. Transactions of the American Fisheries Society 123:964-974.

Kohler, C.C., R.J. Sheehan, J.J. Myers, J.B. Rudacille, M.L. Allyn, and A.V. Suresh. 2001. Performance comparison of geographic strains of white bass (*Morone chrysops*) to produce sunshine bass. Aquaculture 202:351-357.

Lane, R.L., and C.C. Kohler. 2006. Effects of dietary lipid and fatty acids on white bass reproductive performance, egg hatchability, and overall quality of progeny. North American Journal of Aquaculture 68:141-150.

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Lane, R.L., J.T. Trushenski, and C.C. Kohler. 2006. Modification of fillet composition and evidence of differential fatty acid turnover in sunshine bass *Morone chrysops* × *M. saxatilis* following change in dietary lipid source. Lipids 41:1029-1038.

Morris, J.E., C.C. Kohler, and C.C. Mischke. 1999. Pond culture of hybrid striped bass in the North Central Region. NCRAC Fact Sheet Series #107, NCRAC Publications Office, Iowa State University, Ames.

Myers, J.J. 1999. Acute responses to salinity for sunshine bass and palmetto bass. Master's thesis. Southern Illinois University-Carbondale.

Myers, J.J., and C.C. Kohler. 2000. Acute responses to salinity for sunshine bass and palmetto bass. North American Journal of Aquaculture 62:195-202.

Rudacille, J.B., and C.C. Kohler. 2000. Aquaculture performance comparison of sunshine bass, palmetto bass, and white bass. North American Journal of Aquaculture 62:114-124.

Settor, K. 1998. Evaluation of different densities for hybrid striped bass (*Morone saxatilis* × *M. chrysops*) in cages and small-scale recirculation system. Master's thesis. Purdue University, West Lafayette, Indiana.

Suresh, A.V., J.B. Rudacille, M.L. Allyn, V. Sheehan, R.J. Sheehan, and C.C. Kohler. 2000. Single injections of human chorionic gonadotropin or mammalian gonadotropin releasing hormone analog at low dosages induce ovulation in white bass. North American Journal of Aquaculture 62:87-94.

Trushenski, J.T., and C.C. Kohler. 2006. Evaluation of natural source vitamin E, d-alpha tocopheryl acetate, as a micronutrient in sunshine bass feed. North American Journal of Aquaculture 68:186-191.

Trushenski, J.T., C.S. Kaspar, and C.C. Kohler. 2006. Challenges and opportunities in finfish nutrition. North American Journal of Aquaculture 68:122-140.

Trushenski, J.T., and C.C. Kohler. 2007. Influence of stress and dietary natural source vitamin E on nonspecific immunocompetence, tissue tocopherol composition, and postslaughter fillet oxidative stability of sunshine bass. North American Journal of Aquaculture 69:330-339.

Volkman, E.T., C.C. Kohler, and S.T. Kohler. 2004. Assessment of floating vertical raceways for the culture of phase-II hybrid striped bass. North American Journal of Aquaculture 66:125-132.

Wetzel, J.E., C.C, Kasper and C.C. Kohler. 2006. Comparison of pond production of phase-III sunshine bass fed 32-, 36-, and 40%-crude-protein diets with fixed energy:protein ratios. North American Journal of Aquaculture 68:264-270.

Woods, L.C., C.C. Kohler, R.J. Sheehan, and C.V. Sullivan. 1995. Volitional tank spawning of female striped bass with male white bass produces hybrid offspring. Transactions of the American Fisheries Society 124:628-632.

Manuscripts

Lewis, H.A., and C.C. Kohler. In press. Corn gluten meal partially replaces fish meal without compromising growth or the fatty acid composition of sunshine bass. North American Journal of Aquaculture.

Lewis, H.A., and C.C. Kohler. Minimizing fish oil and fish meal with plant-based alternatives in sunshine bass diets without negatively impacting growth and fillet fatty acid profile. Journal of the World Aquaculture Society.

Trushenski, J.T., and C.C. Kohler. Influence of stress, exertion, and dietary natural source vitamin E on prostaglandin synthesis, hematology, and tissue fatty acid composition of sunshine bass *Morone chrysops* × *M. saxatilis*. North American Journal of Aqiaculture.

Papers Presented

Brown, B.J., P.B. Brown, S. Hart, J. Curry, and A. Hittle-Hutson. 2005. Comparison of practical diets containing 32, 36, or 40% crude protein fed to hybrid striped bass in earthen culture ponds. Aquaculture America 2005, New Orleans, Louisiana, January 20, 2005.

Brown, P.B., R. Twibell, Y. Hodgin, and K. Wilson. 1995. Soybeans in diets fed to hybrid striped bass. 24th Annual Fish Feed and Nutrition Workshop, Columbus, Ohio, October 19-21, 1995.

Brown, P.B., Y. Hodgin, R. Twibell, and K.A. Wilson. 1996. Use of three soybean products in diets fed to hybrid striped bass. 27th Annual Meeting of the World Aquaculture Society, Bangkok, Thailand, January 29-February 2, 1996.

Brown, G.G., L.D. Brown, K. Dunbar, C. Habicht, R.J. Sheehan, C.C. Kohler, and L. Koutnik. 1991. Evaluation of white bass semen with 31P-NMR for the improvement of transportation, storage, and fertility methods. 53rd Midwest Fish and Wildlife Conference, Des Moines, Iowa, November 30-December 4, 1991.

Brown, G.G., R.J. Sheehan, C.C. Kohler, C. Habicht, L. Koutnik, L. Ellis, and L.D. Brown. 1995. Use of cryopreservatives. North Central Regional Aquaculture Center Hybrid Striped Bass Workshop, Champaign, Illinois, November 2-4, 1995.

Brown, G.G., R.J. Sheehan, C.C. Kohler, C. Habicht, L. Koutnik, L. Ellis, and L.D. Brown. 1998. Short-term storage of striped bass *Morone saxatilis* semen. 29th Annual Meeting of the World Aquaculture Society, Las Vegas, Nevada, February 15-19, 1998.

Habicht, C., R.J. Sheehan, C.C. Kohler, G.G. Brown, and L. Koutnik. 1991. Routine collection, storage, and shipping of white bass sperm. 29th Annual Meeting Illinois Chapter of the American Fisheries Society, Champaign, Illinois, March 5-7, 1991.

Kasper, C.S., and C.C. Kohler. 2004. Use of finishing diets in indoor hybrid striped bass culture reduces production costs. Fifth International Conference on Recirculating Aquaculture, Roanoke, Virginia, July 22-25, 2004.

Kohler, C.C. 1993. The farm fish of the future: hybrid stripers. Aqua '93: 7th Annual Minnesota Aquaculture Conference, Alexandria, Minnesota, March 5-6, 1993. (Invited paper)

Kohler, C.C. 1994. Hybrid striped bass aquaculture. Yellow Perch and Hybrid Striped Bass Production: From Fry to Frying Pan, Piketon, Ohio, July 3, 1994. (Invited speaker)

Kohler, C.C. 1995. Broodstock management of white bass. North Central Regional Aquaculture Center Hybrid Striped Bass Workshop, Champaign, Illinois, November 2-4, 1995.

Kohler, C.C. 1996. Induced out-of-season spawning of fishes. Missouri Aquaculture Industry Association Annual Meeting, Jefferson City, Missouri, February 3-4, 1996.

Kohler, C.C. 1996. Advancing hybrid striped bass culture in the North Central Region and elsewhere. U.S. Chapter of the World Aquaculture Society, Arlington, Texas, February 14-17, 1996.

Kohler, C.C. 1997. Induced spawning of fishes. Third North Central Regional Aquaculture Conference, Indianapolis, Indiana, February 6-7, 1997.

Kohler, C.C. 1998. Hybrid striped bass culture in the Midwest. Joint Missouri-Kansas Aquaculture Conference, Springfield, Missouri, March 4-6, 1998.

Kohler, C.C., and R.J. Sheehan. 1991. Hybrid striped bass culture in the North Central Region. First North Central Regional Aquaculture Conference, Kalamazoo, Michigan, March 18-21, 1991.

Kohler, C.C., R.J. Sheehan, and T.B. Kayes. 1989. Advancing hybrid striped bass culture in the Midwestern United States. 51st Midwest Fish and

Wildlife Conference, Springfield, Illinois, December 5-6, 1989.

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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
ANS	aquatic nuisance species
AquaNIC	Aquaculture Network Information Center
BOD	Board of Directors; biochemical oxygen demand
BW	body weight
°C	degrees Celsius
CES	Cooperative Extension Service
CSREES	Cooperative State Research, Education and Extension Service
CVM	Center for Veterinary Medicine
EA	environmental assessment
°F	degrees Fahrenheit
FA	fatty acid
FCR	feed conversion rate
FDA	Food and Drug Administration
ft, ft^3	foot, cubic foot
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
HPLC	high performance liquid chromatography
HSI	hepatosomatic index
HUFA	highly unsaturated fatty acids
IAC	Industry Advisory Council
in	inch(es)
INAD	Investigational New Animal Drug
ISU	Illinois State University Iowa State University
kg	kilogram(s)
L	liter(s)
lb	pound(s)
LC-MS	liquid chromatography-mass spectroscopy
LDL	Little Dixie Lake
LEMM	lipid-extracted menhaden meal
Lpm	liters per minute
m	meter(s)
min	minute(s)

mm	millimeter(s)
MSU	Michigan State University
MT	methyltestosterone
MUMS	Minor Use and Minor Species
N	number
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
oz	ounce(s)
P	probability
PER	protein efficiency ratio
POW	Plan of Work
ppm, ppt	parts per million, parts per trillion
Purdue	Purdue University
RAC(s)	Regional Aquaculture Center(s)
RAES	Regional Aquaculture Extension Specialist
RBC	rotating biological contactor
SBM	soybean meal
SGR	specific growth rate
SIUC	Southern Illinois University-Carbondale
TC	Technical Committee (TC/E = Technical Committee/Extension; TC/R = Technical Committee/Research)
TI	trypsin inhibitor
TL	total length
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
UW-Stevens Point	University of Wisconsin-Stevens Point
UW-Madison	University of Wisconsin-Madison
UW-Milwaukee	University of Wisconsin-Milwaukee
VDD	Veterinary Drug Directorate
VHS	viral hemorrhagic septicemia