ANNUAL PROGRESS REPORT

For the Period
September 1, 2008 to August 31, 2009

January 2010

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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 almost $1 billion in 2007 for producers. Yet, anticipated growth in the industry, both in magnitude and in species diversity, continues to fall short of expectations.

Much of what is known about aquaculture science is a result of institutional attention given to our traditional capture of wild fisheries with the goal of releasing cultured fishes into public waters for enhancement of declining public stocks. Despite extensive efforts to manage wild populations for a sustained yield, as a nation we consume substantially greater amounts than we produce. Much of the United States' demand for seafood has been met by imports. The value of imported fisheries products has substantially increased over the last two decades. In 2007, the U.S. imported $28.8 billion of fisheries products and the trade deficit was $8.7 billion for all fisheries products, most of which was for edible fish and shellfish.

Landings for most commercial capture fisheries species and recreational fisheries of the United States have been relatively stable during the last decade, with many fish stocks being over exploited. In this situation, aquaculture provides an opportunity to reduce the trade deficit and meet the rising U.S. demand for fish products. A strong domestic aquaculture industry is needed to increase U.S. production of fish and shellfish. This can be achieved by a partnership among the Federal Government, State and local public institutions, and the private sector with expertise in aquaculture development.

Congress recognized the opportunity for making significant progress in aquaculture development in 1980 by passage of the National Aquaculture Act (P.L. 96-362). Congress amended the National Agricultural Research, Extension, and Teaching Policy Act of 1977 (P.L. 95-113) in Title XIV of the Agriculture and Food Act of 1981 (P.L. 97-98) by granting authority to establish aquaculture research, development, and demonstration centers in the United States in association with colleges and universities, State Departments of Agriculture, Federal facilities, and non-profit private research institutions. Five such centers have been established: one in each of the northeastern, north central, southern, western, and tropical/subtropical Pacific regions of the country. The Food, Conservation, and Energy Act of 2008 (P.L. 110-246), otherwise known as the Farm Bill, has reauthorized the Regional Aquaculture Center program at $7.5 million per annum. As used here, a center refers to an administrative center. Centers do not provide monies for brick-and-mortar development. Centers encourage cooperative and collaborative aquaculture research and extension educational programs that have regional or national application. Center programs complement and strengthen other existing research and extension educational programs provided by the U.S. Department of Agriculture (USDA) and other public institutions. As a matter of policy, centers implement their programs by using institutional mechanisms and linkages that are in place in the public and private sector.

The mission of the Regional Aquaculture Centers (RACs) is to support aquaculture research, development, demonstration, and extension education to enhance viable and profitable U.S. aquaculture production which will benefit consumers, producers, service industries, and the American economy.

The North Central Regional Aquaculture Center (NCRAC) was established in February 1988. It serves as a focal point to assess needs, establish priorities, and implement research and extension educational programs in the twelve state agricultural heartland of the United States which includes Illinois, Indiana, Iowa, Kansas, Michigan, Missouri, Minnesota, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin. NCRAC also provides coordination of interregional and national programs through the National Coordinating Council for Aquaculture (NCC). The council is composed of the RAC directors and USDA aquaculture personnel.

ORGANIZATIONAL STRUCTURE

Michigan State University (MSU) and Iowa State University (ISU) work together to develop and administer programs of NCRAC through a memorandum of understanding. MSU is the prime contractor for the Center and has administrative responsibilities for its operation. The Director of NCRAC is located at MSU. ISU shares in leadership of the Center through an office of the Associate Director who is responsible for all aspects of the Center's publications, technology transfer, and outreach activities.

At the present time the staff of NCRAC at MSU includes Ted R. Batterson, Director, and Liz Bartels, Executive Secretary. The Center Director has the following responsibilities:

- Developing and submitting proposals to USDA Cooperative State Research, Education and Extension Service (USDA/CSREES) which, upon approval, becomes a grant to the Center;
- Developing appropriate agreements (sub-contracts) with other parties, including ISU for the Associate Director's office, for purposes of transferring funds for implementation of all projects approved under the grants;
- Serving as executive secretary to the Board of Directors, responsible for preparing agenda and minutes of Board meetings;
- Serving as an ex-officio (non-voting) member of the Technical Committee and Industry Advisory Council;
- Coordinating the development of research and extension plans, budgets, and proposals;
- Coordinating and facilitating interactions among the Administrative Center, Board of Directors, Industry Advisory Council, and Technical Committee;
- Monitoring research and extension activities;
- Arranging for review of proposals for technical and scientific merit, feasibility, and applicability to priority problems and preparing summary budgets and reports as required;
- Recruiting other Administrative Center staff as authorized by the Board of Directors;
- Maintaining liaison with other RACs; and
- Serving on the NCC.

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

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

- Serving as the NCRAC liaison with national aquaculture extension programs, including in particular, extension programs of the other four USDA Regional Aquaculture Centers; and
- Serving as a member of NCRAC's Extension Executive Committee.

The Board of Directors (BOD) is the primary policy-making body of the NCRAC. The BOD has established an Industry Advisory Council (IAC) and Technical Committee (TC). Membership of the BOD consists of four persons from the IAC, a representative from the region's State Agricultural Experiment Stations and Cooperative Extension Services, a member from a non-land grant university, representatives from the two universities responsible for the center: Michigan State and Iowa State, and chairs of the two subcommittees of the Center's Technical Committee. The IAC is composed of representatives from each state's aquaculture association and six at-large members appointed by the BOD who represent various sectors of the aquaculture industry and the region as a whole. The TC is composed of a sub-committee for Extension (TC/E) and a sub-committee for Research (TC/R). Directors of the Cooperative Extension Service within the North Central Region appoint representatives to the TC/E. The TC/R has broad regional make-up and is composed of scientists from universities and state agencies with varied aquacultural expertise who are appointed by the BOD. Each sub-committee of the TC has a chairperson who serves as a member of the BOD.

NCRAC functions in accordance with its *Operations Manual* which is periodically amended and updated with BOD approval. It is an evolving document that has changed as the Center's history lengthens. It is used for the development of the cooperative regional aquaculture and extension projects that NCRAC funds.

ADMINISTRATIVE OPERATIONS

Since inception of NCRAC February 1, 1988, the role of the Administrative Center has been to provide all necessary support services to the BOD, IAC, TC, and project work groups for the North Central Region as well as representing the region on the NCC. As the scope of the NCRAC programs expand, this has entailed a greater work load and continued need for effective communication among all components of the Center and the aquaculture community.

The Center functions in the following manner.

- After BOD approval of Administrative Center costs, the Center submits a grant to USDA/CSREES/Grants Management Branch for approval. To date the Center has received 21 grants from USDA for FY88 (Grant #88-38500-3885), FY89 (Grant #89-38500-4319), FY90 (Grant #90-38500-5008), FY91 (Grant #91-38500-5900), FY92 (Grant #92-38500-6916), FY93 (Grant #93-38500-8392), FY94 (Grant #94-38500-0048), FY95 (Grant #95-38500-1410), FY96 (Grant #96-38500-2631), FY97 (#97-38500-3957), FY98 (#98-38500-5863), FY99 (#99-38500-7376), FY00 (#00-38500-8984), FY2001 (#2001-38500-10369), FY2002 (#2002-38500-11752), FY2003 (#2003-38500-12995), FY2004 (#2004-38500-14269), FY2005 (#2005-38500-15847), FY2006 (#2006-38500-16900), FY2007 (#2007-38500-18569), and FY2008/09 (#2008-38500-19157) with monies totaling $16,217,028. Currently, four grants are active (FY05-08/09); the first 17 grants (FY88-04) 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, 2009, the Center has funded or is funding 89 projects through 472 subcontracts from the first 22 grants received. Funding for these Center-supported projects is summarized in Table 1 below (pages 6–8). Information about funded projects is also available at the Center's Web site (http://www.ncrac.org).

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

Other areas of support by the Administrative Office during this reporting period included: monitoring research and extension activities and developing progress reports; developing liaisons with appropriate institutions, agencies and clientele groups; soliciting, in coordination with the other RACs, written testimony for the U.S. House Appropriations Subcommittee on Agriculture, Rural Development, Food and Drug Administration, and Related Agencies and the U.S. Senate Appropriations Subcommittee on Agriculture, Rural Development, and Related Agencies; participating in the NCC; numerous oral and written presentations to both professional and lay audiences; working with other fisheries and aquaculture programs throughout the North Central Region; maintaining the NCRAC Web site.

PROJECT REPORTING

As indicated in Table 1, NCRAC has funded a number of projects for many of the project areas it has selected for research and extension activities. For example, there have been thirteen separately funded projects in regard to Extension and eight on Yellow Perch. Project outlines have been written for each separate project within an area, or the project area itself if only one project. These project outlines have been submitted in POWs or amendments to POWs for the grants as indicated in Table 1. Many times, the projects within a particular area are continuations of previously funded activities while at other times they are addressing new objectives. Presented below are Progress Reports for projects that were underway or completed during the period

September 1, 2008 to August 31, 2009. Projects, or Project components, that terminated prior to September 1, 2008 have been reported on in earlier documents (e.g., 1989-1996 Compendium Report and other Annual Progress Reports).

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

Table 1. North Central Regional Aquaculture Center funded projects.

Project Area	Project Number	Proposed Duration Period	Funding Level	Grant Number
Aquaculture Drugs	1	7/1/96-6/30/97	$27,000	95-38500-1410
	2	12/1/96-11/30/97	$950	95-38500-1410
	3	10/1/99-9/30/00	$8,415	97-38500-3957
	4	6/1/04-11/30/05	$223,677	2003-38500-12995
	5	7/15/04-7/14/05	$60,000	2003-38500-12995
	6	11/1/04-10/31/06	$50,000	2002-38500-11752
	7	1/1/06-12/31/06	$129,936	2005-38500-15847
	8	9/1/08-8/31/10	$150,000	2008-38500-19157
			$649,978	
Baitfish	1	9/1/92-8/31/94	$61,973	92-38500-6916
	2	9/1/06-8/31/08	$111,997	2006-38500-16900
			$88,003	2005-38500-18547
			$261,973	
Conferences/Workshops/Symposia				
Environmental Strategies Symposium	1	9/1/00-5/31/01	$5,000	96-38500-2631
Nat'l. Aquaculture Exten. Workshop/Conference	1	10/1/91-9/30/92	$3,005	89-38500-4319
	2	12/1/96-11/30/97	$3,700	95-38500-1410
	3	11/1/02-10/31/03	$4,500	00-38500-8984
	4	1/1/06-12/31/06	$5,000	2005-38500-18547
			$16,205	
NCR Aquaculture Conference	1	6/1/90-3/31/91	$7,000	90-38500-5008
	2	12/9/98-6/30/99	$3,000	96-38500-2631
			$10,000	
Percis III	1	11/1/02-10/31/03	$4,000	00-38500-8984
Crayfish	1	9/1/92-8/31/94	$49,677	92-38500-6916
Economics/Marketing	1	5/1/89-12/31/91	$127,338	88-38500-3885
			$34,350	89-38500-4319
	2	9/1/91-8/31/92	$53,300	91-38500-5900
	3	9/1/93-8/31/95	$40,000	93-38500-8392
	4	9/1/99-8/31/01	$47,916	97-38500-3957
	5	9/1/03-8/31/04	$50,000	2002-38500-11752
			$352,904	

Extension	1	5/1/89-4/30/91	$39,221	88-38500-3885
("Base" Extension—Project			$37,089	89-38500-4319
Nos. 1-12; 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. 13; 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. 14)	6	9/1/97-8/31/99	$38,000	97-38500-3957
	7	9/1/99-8/31/01	$94,000	99-38500-7376
	8	9/1/01-8/31/03	$28,500	99-38500-7376
			$18,154	2001-38500-10369
	9	9/1/03-8/31/05	$28,000	2002-38500-11752
	10	9/1/05-8/31/07	$211,545	2003-38500-12995
			$7,735	2005-38500-15847
	11	9/1/07-8/31/09	$21,850	2006-38500-16900
			$92,469	2007-38500-18469
	12	9/1/08-8/31/10	$37,966	2007-38500-18469
			$22,539	2008-38500-19157
	13	9/1/03-8/31/05	$100,000	2002-38500-11752
	14	9/1/06-8/31/09	$199,624	2004-38500-14269
			$1,243,434	
Feed Training Carnivorous Fish	1	9/1/06-8/31/08	$165,446	2005-38500-15847
			$134,554	2006-38500-16900
			$300,000	
Hybrid Striped Bass	1	5/1/89-8/31/91	$68,296	88-38500-3885
			$68,114	89-38500-4319
	2	6/1/90-8/31/92	$101,000	90-38500-5008
	3	9/1/91-8/31/93	$96,550	91-38500-5900
	4	9/1/93-8/31/95	$168,000	93-38500-8392
	5	9/1/95-8/31/97	$150,000	95-38500-1410
	6	6/1/99-5/31/00	$15,000	96-38500-2631
	7	9/1/01-5/31/04	$98,043	98-38500-5863
			$211,957	2001-38500-10369
			$976,960	
Largemouth Bass	1	9/1/05-8/31/07	$170,000	2004-38500-14269
National Coordinator for	1	9/1/93-8/31/94	$2,000	89-38500-4319
Aquaculture INADs/NADAs		5/15/95-5/14/96	$5,000	94-38500-0048
		5/15/96-5/14/97	$6,669	92-38500-6916
			$3,331	95-38500-1410
		5/15/97-5/14/98	$15,000	96-38500-2631
		5/15/98-5/14/99	$13,241	94-38500-0048
		5/15/99-5/14/00	$10,000	95-38500-1410
	2	7/15/04-7/14/05	$9,000	2003-38500-12995
		9/15/05-8/31/06	$15,000	2004-38500-14269
		9/1/06-8/31/08	$40,000	2006-38500-16900
		5/15/08-5/14/09	$25,000	2007-28500-18469
			$144,241	

Nutrition/Diets	1 2	9/1/04-8/31/06 9/1/07-8/31/09	$200,000 $80,000 $280,000	2002-38500-11752 2006-38500-16900
Salmonids	1 2 3 4	6/1/90-8/31/92 9/1/92-8/31/94 9/1/94-8/31/96 9/1/97-8/31/99	$9,000 $120,799 $149,997 $199,290 $158,656 $637,742	89-38500-4319 90-38500-5008 92-38500-6916 94-38500-0048 97-38500-3957
Snail Management/Grub Control	1	9/1/07-8/31/09	$225,000	2007-38500-18469
Sunfish	1 2 3 4 5	6/1/90-8/31/92 9/1/92-8/31/94 9/1/94-8/31/96 9/1/96-9/31/98 9/1/99-8/31/01	$130,758 $149,799 $173,562 $199,921 $199,748 $853,788	90-38500-5008 92-38500-6916 94-38500-0048 96-38500-2631 99-38500-7376
Tilapia	1 2	9/1/96-8/31/98 9/1/98-8/31/00	$118,791 $150,000 $268,791	96-38500-2631 98-38500-5863
Viral Hemorrhagic Septicemia (VHS)	1	9/1/08-8/31/10	$197,960	2008-38500-19157
Walleye	1 2 3 4 5 6 7	5/1/89-8/31/91 6/1/90-8/31/92 9/1/91-8/31/92 9/1/92-8/31/93 9/1/93-8/31/95 9/1/95-8/31/97 9/1/99-6/30/02	$177,517 $111,657 $109,223 $75,000 $150,000 $117,395 $59,835 $127,000 $927,627	89-38500-4319 90-38500-5008 91-38500-5900 89-38500-4319 93-38500-8392 94-38500-0048 95-38500-1410 98-38500-5863
Wastes/Effluents	1 2 3	9/1/92-8/31/94 9/1/96-8/31/98 9/1/01-8/31/04	$153,300 $100,000 $106,186 $88,814 $448,300	92-38500-6916 96-38500-2631 00-38500-8984 2001-38500-10369
White Papers	1 2	7/1/98-12/31/98 9/1/99-12/31/99	$4,999 $17,495 $22,494	96-38500-2631 97-38500-3957
Yellow Perch	1 2 3 4 5 6 7 8	5/1/89-8/31/91 6/1/90-8/31/92 9/1/91-8/31/93 9/1/93-8/31/95 9/1/95-8/31/97 9/1/97-8/31/99 9/1/98-8/31/00 9/1/01-5/31/04	$76,957 $85,723 $92,108 $99,997 $150,000 $199,507 $185,458 $92,370 $326,730 $125,016 $1,433,866	88-38500-3885 89-38500-4319 90-38500-5008 91-38500-5900 93-38500-8392 95-38500-1410 97-38500-3957 98-38500-5863 00-38500-8984 2001-38500-10369

PROJECT REPORTS

AQUACULTURE DRUGS: EFFECTIVENESS RESEARCH LEADING TO APPROVALS FOR CONTROLLING MORTALITY IN COOLWATER AND WARMWATER FINFISH DUE TO AEROMONAD INFECTIONS WITH TERRAMYCIN 200 FOR FISH® (OXYTETRACYCLINE DIHYDRATE) AND AQUIFLOR® (FLORFENICOL)[1]

Project ***Progress Report*** for the Period
September 1, 2008 to August 31, 2009

NCRAC FUNDING: $37,000 (September 1, 2008 to August 31, 2009)

PARTICIPANT:

Mark P. Gaikowski	Upper Midwest Environmental Sciences Center	Wisconsin
Industry Advisory Council Liaison:		
Mark Willows	North American Fish Farmers Cooperative	North Dakota

PROJECT OBJECTIVES

(1) Identify the etiologic agent (*Aeromonas* sp.) from isolates collected from disease outbreaks in the NCR and characterize the disease syndrome before conducting any effectiveness studies

(2) Have active, established INAD exemptions or work with the sponsors of publicly disclosable INADs for Terramycin 200 for Fish® and Aquaflor®.

(3) Develop draft pivotal effectiveness study protocols with the concurrence of the two drug sponsors (Phibro Animal Health=PAH for Terramycin 200 for Fish® and Schering-Plough Animal Health=SPAH for Aquaflor®).

(4) Submit the draft pivotal effectiveness study protocols through established INADs for Terramycin 200 for Fish® and Aquaflor® for protocol concurrence from CVM before beginning the effectiveness studies.

[1]NCRAC has funded eight Aquaculture Drugs projects. A Termination Report for the first project is contained in the 1997-98 Annual Progress Report; a Termination Report for the second project is contained in the 1996-97 Annual Progress Report, a Termination Report for the third project is contained in the 2001-02 Annual Progress Report, a Termination Report for the fourth project is contained in the 2006-07 Annual Progress Report, and Termination Reports for the sixth and seventh projects are contained in the 2007-08 Annual Progress 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 Termination Report for the National Coordinator for Aquaculture New Animal Drug Applications (NADAs) elsewhere in this report. This Progress Report is for the eighth Aquaculture Drugs project which is being undertaken by Mark Gaikowski. It is a 2-year project that began January 1, 2008.

(5) Conduct pivotal effectiveness studies on Terramycin 200 for Fish® and Aquaflor® according to Good Clinical Practice and the CVM concurred protocols.

(6) Analyze the effectiveness data and prepare draft final study reports for Terramycin 200 for Fish® and Aquaflor® no more than four months after the studies are completed.

(7) Submit the respective draft study reports to PAH and SPAH for their review.

(8) Submit the final study reports through established INADs for Terramycin 200 for Fish® and Aquaflor® to CVM for acceptance no more than two months after PAH and SPAH have completed their reviews of the draft study reports.

(9) Ensure that all questions and concerns about the final study reports are answered no more than one month after receiving comments from CVM.

(10) If CVM accepts the data as proving effectiveness for the Aeromonad infections encountered in the NCR (North Central Region), provide the acceptance letter and effectiveness studies to PAH and SPAH so that they can pursue supplemental NADA approvals for their respective drug products.

ANTICIPATED BENEFITS

Disease constitutes the largest single cause of economic losses in aquaculture as represented by some investigators. There are few treatments available for current and emerging aquaculture diseases. The control of mesophilic or motile *Aeromonas* infections (MAI) is extremely relevant to the aquaculture industry in the NCR as it has experienced a loss of income in commercially important food fish species and baitfish. These economic losses result directly from fish mortality due to MAI and from opportunistic secondary infections, and indirectly because of unappealing visual appearance of food fish with gross external lesions.

Both Terramycin 200 For Fish® (oxytetracycline dihydrate) and Aquaflor® (florfenicol) have been shown to be effective against a wide variety of Gram-negative bacterial pathogens of fish including certain *Aeromonas* spp. (e.g. *A. salmonicida*). It is likely that one or both of these antibacterials will effectively reduce mortality associated with motile *Aeromonas* septicemia (MAS) in coolwater and warmwater fish. This research will provide valuable information to commercial and public fish culturists and enable them to effectively reduce production loss in cool- and warmwater fish caused by *Aeromonas* species.

PROGESS AND PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

Etiologic Agent

Observations of clinical signs and gross necropsy were performed on muskellunge and tilapia which exhibited mortality and clinical signs of MAS at two separate NCR fish culture facilities. Clinical signs noted included hemorrhages, ulcerative skin lesions and excess ascetic fluid. At both facilities, the kidney was observed to have discoloration or changes in the overall texture. All observations made were consistent with those previously reported from outbreaks of various motile *Aeromonas* species.

Microbiological samples were obtained from diseased (moribund) fish exhibiting clinical signs of MAS and inoculated onto Tryptic Soy Agar (TSA) plates and incubated at 30°C (86°F) for 24 hours. Creamy to tan, round, shiny colonies were inoculated onto a separate TSA plate to obtain pure cultures. Rimler-Shotts (RS) plates were inoculated with a single colony of a pure culture and incubated at 30°C (86°F) for 24 hours. If yellow colony growth occurred, then cultures were inoculated onto a BBL Crystal™ for identification. Identifications made from isolates obtained from NCR facilities are listed in Table 1.

A third isolate was obtained from walleye exhibiting mortality potentially characteristic of MAS at another NCR fish culture facility. Clinical signs were not obtained as the isolate was submitted directly to the U.S. Fish and Wildlife Service La Crosse Fish Health Center for diagnosis. Identification was accomplished as previously described and is provided in Table 1.

Three additional isolates were obtained from channel catfish exhibiting clinical signs and mortality indicative of MAS. Though outside of the NCR, these isolates were collected from what appears to be a highly virulent strain of *A. hydrophila* which could readily cause mortality in the NCR. Clinical observations provided by the collecting pathologist were consistent with those previously reported for MAS. Identification was accomplished as previously described and is provided in Table 1.

Characterize the Disease Syndrome

Challenge trials will begin in October 2009 to characterize the disease syndrome. Five isolates will be used during each challenge trial, with two species each of cool and warmwater fish. Mortality, morbidity, and clinical signs will be observed for 14 days after challenge initiation. Samples will be collected from mortalities to confirm infection. Isolates will also be tested to determine sensitivity to oxytetracycline dihydrate and forfenicol.

Progress in Year 1 was delayed because there were few public or private aquaculture facilities that had outbreaks of MAI.

OBJECTIVE 2

The Upper Midwest Environmental Sciences Center (UMESC) currently has an established INAD for Terramycin 200 for Fish®. UMESC will request an INAD exemption for Aquaflor® concurrent with submission of pivotal effectiveness protocols.

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Table 1. Species of origin, year and details of Aeromonad isolates obtained for study.

Isolate	Source species	Year	Details	Source
1	Channel catfish	2007	*A. hydrophila*	University of Arkansas, Pine Bluff
2	Tilapia	2009	*A. veronii*	Private fish farm (NCR)
3	Muskellunge	2009	*A. sobria/A. veronii*	Spirit Lake Fish Hatchery (NCR)
4	Walleye	2009	*A. hydrophila*	Rathbun State Fish Hatchery (NCR)
5	Goldfish	2005	*A. hydrophila/A. caviae*	Wisconsin Vet Diagnostic Lab
6	Channel catfish	2009	*A. hydrophila*	Auburn University
7	Channel catfish	2009	*A. hydrophila*	Auburn University
8	Channel catfish	2009	*A. hydrophila*	Auburn University
9	Channel catfish	2007	*Aeromonas* spp.	Mississippi State University

OBJECTIVE 3
Because there were few outbreaks of MAI in Year 1, development of the effectiveness protocol was delayed. A protocol titled "Field effectiveness of Aquaflor® (florfenicol) and Terramycin 200 For Fish® (oxytetracycline dihydrate) to control mortality in coolwater and warmwater finfish due to Motile Aeromonad infections" will be submitted to FDA in October 2009.

WORK PLANNED
UMESC research in Year 2 of the project will focus on the following areas:

OBJECTIVE 4
Submit the draft pivotal effectiveness study protocols through established INADs for Terramycin 200 for Fish® and Aquaflor® for protocol concurrence from CVM before beginning the effectiveness studies.

OBJECTIVE 5
Conduct pivotal effectiveness studies on Terramycin 200 for Fish® and Aquaflor® according to Good Clinical Practice and the CVM concurred protocols.

OBJECTIVE 6
Analyze the effectiveness data and prepare draft final study reports for Terramycin 200 for Fish® and Aquaflor® no more than four months after the studies are completed.

OBJECTIVE 7
Submit the respective draft study reports to PAH and SPAH for their review.

OBJECTIVE 8
Submit the final study reports through established INADs for Terramycin 200 for Fish® and Aquaflor® to CVM for acceptance no more than two months after PAH and SPAH have completed their reviews of the draft study reports.

OBJECTIVE 9
Ensure that all questions and concerns about the final study reports are answered no more than one month after receiving comments from CVM.

OBJECTIVE 10
If CVM accepts the data as proving effectiveness for the aeromonad infections encountered in the NCR, provide the acceptance letter and effectiveness studies to PAH and SPAH so that they can pursue supplemental NADA approvals for their respective drug products.

IMPACTS
The effectiveness studies of this project should lead to supplemental NADA approvals by the U.S. Food and Drug Administration Center for Veterinary Medicine for either, or both, Terramycin 200 for Fish® (oxytetracycline dehydrate) and Aquiflor® (florfenicol), which, if approved, would allow aquaculturists the use of these antibacterials to reduce mortality associated with MAS in coolwater and warmwater fish.

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	
2008-09*	$37,000			$2,600		$2,600	$39,600
TOTAL	$37,000			$2,600		$2,600	$39,600

*Year 1 funding projected to use the $70,000 allocated to complete objectives; however, the remaining $33,000 will be used in Year 2 for completion of efficacy trials.

BAITFISH[2]

Project ***Progress Report*** for the Period
September 1, 2006 to August 31, 2009

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

PARTICIPANTS:

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

PROJECT OBJECTIVES

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

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

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

ANTICIPATED BENEFITS

This project addresses priority needs identified by the North Central Regional Aquaculture Center (NCRAC) Industry Advisory Council (IAC). In a survey conducted in late 2006, the NCRAC IAC members ranked "Baitfish" as being "Very Important," second only to disease and health issues. 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 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.

[2] NCRAC has funded two Baitfish projects. The Termination Report for the first project is contained in the 1989-1996 Compendium Report. This Progress Report is for the second project which is chaired by Joseph E. Morris. It is a 2-year project that began September 1, 2006.

PROGESS AND PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

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

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

In a related project not funded by NCRAC, the efficacy of hydrogen peroxide (H_2O_2) to control fungal (Saprolegniasis) infections of golden shiner eggs was evaluated in two experiments. Golden shiner eggs were exposed in a 15-min static bath (21°C; 70°F) to 0, 50, 100, and 200 mg/L in the first experiment, and 0, 200, 400, and 800 mg/L in the second experiment. All treatments were based on amount of active ingredient (30% active ingredient concentration of H_2O_2) in a single treatment. Three replicates of each concentration were used in both experiments. The objective was to determine the H_2O_2 concentration that would result in optimum hatching successes. The hatching rate significantly increased in each treatment level until 800 mg/L. The mean percent egg hatchability (± S.E.) at 400 and 800 mg/L was 72.3 ± 8.55 and 68.2 ± 5.03, respectively. Regression analysis revealed the peak treatment level to be between 400 and 800 mg/L.

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

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

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

Fish were harvested from all ponds in October 2008. The ponds that were stocked with only eggs yielded larger fish (mean 122 mm [4.8 in]) than ponds stocked with adults (mean 69.2 mm [2.72 in]). However, the ponds stocked with the eggs had a total mean production of 194 kg/ha (1,057 lb/acre) compared to 612 kg/ha (3,333 lb/acre). Both treatments resulted in fish larger than the targeted 76 mm (3 in) size.

OBJECTIVE 2

University of Wisconsin-Milwaukee

Wild adult brood stock were collected during the summer of 2005 from rivers and streams in southeastern Wisconsin. Wild fish were acclimated to 23−25°C (73−77°F) under laboratory conditions. The wild fish accepted standard commercial feeds after several days of feed training. One group of adults was maintained at seasonal (normal) temperatures and a second group was kept at a constant temperature of 23−25°C (73−77°F).

Wild brood stock that were maintained at constant temperatures of 23−25°C (73−77°F) from August 2005 to August 2007 spawned out-of-cycle from March−May and produced progeny in the tens of thousands. The F_1 generation (older fish) produced in 2006 (domesticated brood stock) kept at a constant temperature exhibited spawning behavior but gamete production was poor.

Wild brood stock kept at a seasonal temperature from August 2005 to August 2007 exhibited spawning behavior and produced progeny from May through September resulting in an F_1 generation of 2006. The F_1 generation exhibited spawning behavior and produced numerous 2007 F_1 generations.

Culture techniques for early life stage feeding included: Green Tank Water (GTW), Brine Shrimp Nauplii (BSN), and commercial larval diets. F_1 generations reached an estimated size of 51 mm (2 in) in 12−14 months. Survival was poor throughout the entire post-larvae stage even though tens of thousands were spawned; only several hundred fish survived from each group.

The 2008−2009 work plan focused on improving early life stage survival. However, this research resulted in limited success. At this time, the limiting factor associated with post-larvae survival is providing an appropriate nutritional diet. Also, these experimental results indicate that the spotfin shiner elicited a positive response to temperature manipulation to control reproduction. However, growth and survival remain a question regarding the commercial production of these fish.

University of Wisconsin-Stevens Point (UW-Stevens Point) Northern Aquaculture Demonstration Facility (NADF) and the University of Wisconsin-Madison (UW-Madison)

Researchers could not conduct their studies as originally planned because of issues regarding the interstate transport of fish that arose subsequent to the outbreak of viral hemorrhagic septicemia (VHS) in the Great Lakes. Because of these issues, the number of adult-sized fish that could be obtained for the 2007 and 2008 studies were limited. The limitation on brood fish, in turn, led to a reduction in number and a delay in time at which fry became available. Additionally, in 2008 the extreme flooding in the region precluded the conduct of any meaningful pond-based studies.

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

The fish accepted a commercial trout diet and were kept in temperatures of 18−21°C (64−70°F) during spawning. Water quality parameters were maintained at adequate levels to provide a good rearing environment. Several types of spawning substrates were placed into rearing tanks during the spring of 2008. Adult fish (52.0−112.0 mm; 2.0–4.4 in) responded to substrates immediately with active spawning behavior and swarming around the substrates. This behavior was captured with an underwater video camera. Four different types of substrates were utilized for collecting gametes in the tanks:

(1) flat style 483.0 × 101.0 × 64.0 mm (19.0 × 4.0 × 2.5 in) with cedar shingles horizontally layered with 2.0−5.0 mm (0.08−0.20 in) crevices suspended in the tank on rope and brick;
(2) square vertical 152 × 152 × 127 mm (6 × 6 × 5 in) cedar shingles layered on a threaded rod that was hung on the side of tank with crevices 5.0−10.0 mm (0.20−0.40 in);
(3) cinder blocks 25 × 25 × 381 mm (1 × 1 × 15 in) with smaller blocks placed inside with crevices between 3.0−8.0 mm (0.12−0.31 in); and
(4) aluminum siding layered and suspended on a rope and brick in the tank.

The flat style substrate performed the best for collecting gametes and protecting them from being consumed by fish in the tank. Substrates were removed from tanks within 3−5 days after eggs were deposited on over 50% of available surface to reduce loss to eggs being consumed. Substrates were placed into prepared multiple 227-L (60-gal) tanks connected to the recirculating system for incubation and hatching. NADF staff also utilized some agricultural "horse tanks" set up as a small pond for hatching eggs. Eggs hatched within 5−7 days at 18−21°C (64−70°F), resulting in thousands of <5.0 mm (0.2 in) fry. Newly hatched fry were initially lethargic and non swimming but became photopositive and strong swimming within a few days. Fry were fed commercial starter diets of several types supplemented with pond water and 24 h lighting. Biomarine Artemac produced the best results with fry at NADF. Fry were observed with feed in stomachs after a few days. Survival of fry to fingerling size was <10%. Average growth rate from fingerlings examined was 0.4 mm/day (0.016 in/day) at 19–21°C (66–70°F) in the recirculating system on a commercial trout diet.

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

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

Strong swimming, photopositive fry were collected and delivered to the UW-Madison facilities at the Lake Mills State Fish Hatchery at three times during the spawning time frame. These fry were stocked into two fertilized outdoor rearing ponds at approximately 25,000 fish/ha (61,774 fish/acre). When the fish in one pond reached 15.0–25.0 mm (0.6–1.0 in), staff began regularly feeding them a formulated food, which they readily accepted. In the autumn both ponds were harvested, but only 10% of the stocked fish were recovered. The fish had a mean size of 35.0 mm (1.4 in). The intent was to continue growing these fish in tanks, but the failure of a water heater resulted in all of the fish being killed.

In 2009, a successful attempt was made to conduct the pond-base study onsite at NADF. In May, banked brood stock at NADF and additional brood stock from Minnesota were introduced into the warmwater recirculating aquaculture system at NADF and spawned utilizing equipment and techniques described below from 2007–2008. Using fry garnered from the indoor spawning operations, a nursery pond was stocked in June–August 2009. Prior to being stocked, the pond was fertilized with alfalfa meal and urea. Fry survival in the outdoor pond trial appeared much better than in previous attempts indoors. In two harvest operations in September and October an estimated 20,600 fingerlings (13.0–44.0 mm [0.5-1.7 in]) were harvested and placed into a 20–22°C (68–72°F) recirculating aquaculture system at NADF for further grow out. Fingerling spotfins were fed commercial trout starter diet (Nelson Silvercup Inc., Utah) utilizing 24-h feeders. Monthly growth and condition data was collected on these fish. Spotfins reached >51 mm (2 in) within 60 days in the recirculating aquaculture system. Fish were >51mm (2 in) within 7–8 months by using a combination of indoor spawning in a recirculating aquaculture system, outdoor fry rearing, and final grow out in an indoor recirculating aquaculture system on commercial diets. The combination of recirculating aquaculture systems for brood stock holding and spawning with pond culture for fry and winter grow out back in a recirculating aquaculture system has resulted in the most promising results to date for NADF.

In 2009, newly hatched fry were also utilized for a short term diet study at NADF using three commercial diets (Otohime B1 [Aquasonic PTY, LTD, Wauchope, NSW 2446, Australia]; Inve Proton 2 [INVE Aquaculture, Inc., Salt Lake City, Utah]; and Marisource Artemac [Aquafauna Bio-marine, Inc., Hawthorne, California]). In a 45-day culture period, the first diet resulted in 0% survival but the latter two diets resulted in 19 and 21% survival for Inve Proton 2 and Marisource Artemac, respectively.

OBJECTIVE 3

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

WORK PLANNED

OBJECTIVES 1 & 2

Data collected to date will be further analyzed with the intent to develop protocols for future research into developing golden shiners and spotfin shiners as potential aquaculture species for the NCR.

OBJECTIVE 3

The University of Minnesota-Duluth staff will review the video footage of the spawning of spotfin shiners captured at the NADF for a possible video to demonstrate the crevice spawning behavior of spotfin shiners so potential culturists can fully understand the unique spawning requirements of this species.

IMPACTS

OBJECTIVE 1

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

The potential of using eggs collected from indoor culture operations did result in fish larger than the targeted 76 mm (3 in) size albeit at smaller production levels than ponds stocked with brood stock. It is possible to reach a market size in one growing season using a combination of pond fertilizers, a feeding program, and use of eggs spawned earlier in the season under indoor conditions. This study also showed that even though fish were fed a prepared diet, they still searched for natural prey.

OBJECTIVE 2

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

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

The results from this research do provide some insight to the future direction of research, especially as it relates to nutrition as a function of growth and survival. Additionally, the spawning and egg incubation apparatus developed during this study contributed to improved spawning behavior, egg incubation, and hatching success.

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 on it.

SUPPORT

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

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

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

EXTENSION[3]

Project ***Progress Report*** for the Period
May 1, 1989 to August 31, 2009

NCRAC FUNDING LEVEL: $883,305 (May 1, 1989 to August 31, 2009)

PARTICIPANTS:

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

[3]NCRAC has funded a number of Extension activities, both as stand-alone projects or as components of species-or topical-specific projects, including 12 stand-alone projects deemed “Base” Extension. This Progress Report is for components of the first 11 “Base” Extension projects and a Progress Report for the 12th “Base” Extension project (an “Addendum”) is contained elsewhere in this report. The first three “Base” projects were chaired by Donald L. Garling, the fourth was chaired by Fred P. Binkowski, and projects 5-12 were chaired by Joseph E. Morris. A Project Component Termination Report for one of the objectives of the fifth “Base” Extension project is contained in the 1997-98 Annual Progress Report; a Project Component Termination Report for one objective of “Base” Extension projects 1-8 is contained in the 2003-04 Annual Progress Report. The 11th “Base” project is a 2-year project that began September 1, 2007. Fred P. Binkowski chaired the 13th stand-alone Extension project (the Aquaculture Regional Extension Facilitator [AREF]); a Termination Report for which was contained in the 2004-05 Annual Progress Report. Laura G. Tiu chaired the 14th stand-alone Extension project (the Regional Aquaculture Extension Specialist [RAES]); a Termination Report for that project is contained elsewhere in this report.

PARTICIPANTS (continued):

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

PROJECT OBJECTIVES

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

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

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

ANTICIPATED BENEFITS

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

- Increased public awareness through publications, short courses, and conferences regarding the potential of aquaculture as a viable agricultural enterprise in the NCR;
- Technology transfer to enhance current and future production methodologies for selected species, e.g., walleye and hybrid striped bass, through hands-on workshops and field demonstration projects;
- Improved lines of communication between interstate aquaculture extension specialists and associated industry contacts;
- Access to aquaculture information by the industry at any time via the Internet, including such things as photographs, publications, and traditional as well as educational streaming videos (which are under development);
- An enhanced legal and socioeconomic atmosphere for aquaculture in the NCR; and
- Continued development of state producer organizations that are engaged in identifying and providing solutions to industry issues.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

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

OBJECTIVE 1

Aquaculture Extension Work Group members have:

- Served as an extension liaison, if not an active researcher, for every NCRAC-funded project;
- Assisted in developing, writing, and editing several culture manuals as well as fact sheets, book chapters, and videos based on NCRAC-funded research;
- Assisted with the planning, promotion, and implementation of taxa-specific workshops held throughout the region;
- Participated as Steering Committee members for public forums related to revision of the National Aquaculture Development Plan and the four past National Aquaculture Extension Workshops/Conferences; and
- Met with industry representatives and university researchers involved with aquaculture to discuss how the aquaculture industry could grow in the North Central Region (NCR).

Since the beginning of NCRAC in 1989 numerous publications have been developed that addresses regional aquaculture issues. However, there is now a need to review these past publications. In 2009 the updating process for a series of publications titled "Managing Iowa Fisheries" was completed by Clayton. These publications cover topics from aquatic vegetation and pond management to aquaculture. All are topics that are relevant to fish production in Iowa as well as the NCR. The text in these publications was updated with new pictures included for on-line media delivery. The complete series is now available on-line as well as in print.

On February 26-27, 2009 the members of the NCRAC Regional Aquaculture Extension Team (RAET) and the North Central Region Strategic Planning Group held a joint meeting in Kansas City, Missouri during the NCRAC Annual Program Planning Meeting. The purpose of the meeting was to ascertain whether there are strategies that the team could employ to help the aquaculture industry develop within the region. One conclusion was that there is a need to develop a better communication system to streamline what can be done to help the industry grow. There are many extension and research programs in place, yet a common complaint from people within the aquaculture industry is that the information is either hard to find or not available.

There was a consensus that the following points need to be met in order for the aquaculture industry to grow within the region: demand for a desirable product, a targeted species, opportunity, and an available market. Primary points of discussion included a state-by-state review of the current status of the aquaculture industry in terms of production, availability, and demand of targeted species.

Recommendations to NCRAC were suggested in areas of public education, extension and outreach education, marketing, work with regulatory agencies, and research. An action plan, with the goal of improving growth in the aquaculture industry, was then developed.

A new approach to NCRAC publications was to review past project reports with the aim of identifying possible extension materials. Kinnunen worked with Robert Summerfelt and Richard Clayton (Iowa State University) and Alan Johnson (Iowa DNR) on the final draft of a NCRAC fact sheet on walleye culture for food production. The fact sheet is now out for review.

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 Binkowski, Tiu, and Wallat; and sunfish information with Morris.

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

AquaNIC was the first U.S. aquaculture Web site and is globally one of the most widely accessed and cited aquaculture Web sites. Approximately 1,200 individual, educational, commercial, and governmental Web sites link to AquaNIC as a source of on-line aquaculture information. AquaNIC was visited by more than 3.6 million people who viewed almost 9.8 million pages for the 9/1/07-8/31/09 time period. This translates into more than 4,800 visitors/day, each averaging almost 13 minutes/visit. This is serving to enhance not only NCRAC extension aquaculture information transfer, but to also to meet AquaNIC's stated goal to be the gateway to the world's electronic resources in aquaculture.

AquaNIC is currently ranked in the top 14% 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. There are approximately 7,000 other Web sites that link to AquaNIC.

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, 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. In 2005 Bauer was designated to replace Kayes in Nebraska. In 2000, Swann resigned from Purdue/Illinois Sea Grant; Felkner served

Indiana in the interim. In 2006, Quagrainie was appointed as state extension specialist at Purdue University. Plumer currently serves Illinois. In 2007, two long term extension contacts, Tiu and Morris, were replaced as NCRAC extension contacts by Wallat and Clayton, respectively.

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

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

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

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

Continued progress toward enhancing the NCRAC extension network for aquaculture information transfer has been accomplished through the North Central Aquaculture Regional Extension Facilitator Web site (www.ncaref.org) which continues to receive thousands of visits from a wide variety of clients.

On August 22, 2008, Binkowski and the Great Lakes WATER (Wisconsin Aquatic Technology and Environmental Research) Institute staff hosted the National Aquaculture Association (NAA) Board members and guests for a tour of the WATER Institute's aquaculture facilities followed by a traditional Milwaukee Friday night yellow perch fish fry. In September 2008, the U.S. Trout Farmer's Association held the Midwest Aquaculture Conference in Milwaukee, Wisconsin. The Aquaculture Library was available for participants throughout the course of the conference providing reference books, scientific publications, industry catalogues, and directories pertaining to all aspects of aquaculture. Extension fact sheets were free to all attendees. Due to Binkowski's NCRAC Extension responsibilities, he co-chaired the Planning Committee for this conference developing the program agenda, recruiting speakers, and making local arrangements for the 125+ participants who attended.

OBJECTIVE 3

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

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

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

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

NCRAC Extension contacts have also been responsive to arising issues for the NCR aquaculture industry. For instance, the aquaculture industry is accused of being an important vector for the further spread of exotic species such as zebra mussels, Eurasian watermilfoil, and round gobies. To better identify the risks of spreading exotic species and to reduce those risks, an AIS (aquatic invasive species)-HACCP approach has been developed by Kinnunen and Gunderson and taught to private fish farmers, wild bait harvesters, state and federal agency natural resource personnel, and Native Americans. An AIS-HACCP plan has also been developed to address the growing concern of biosecurity, particularly in regard to diseases such as viral hemorrhagic septicemia (VHS). Kinnunen and Gunderson have also taught other members of the NCR aquaculture extension community about their AIS-HACCP program, in essence, they've "trained the trainers" and all AIS-HACCP materials are available at www.seagrant.umn.edu/ais/haccp.

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

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

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

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

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

The complete report for this meeting can be found at: www.aquaticlivestock.org/.
This forum impacted the NCR by bringing some of these key players (delegates) to a neutral table to discuss these common issues (never been done before with aquatic livestock producers). Many of the delegate groups had never sat down to discuss their issues with the other stakeholder groups. Some delegates didn't realize that other delegates have the same issues, e.g., private producers and public producers both have to deal with changing transportation regulations.

Kinnunen coordinated a 3-day Seafood HACCP Training course that was held at Bay Mills, Michigan, December 9-11, 2008. Formal evaluations from attendees rated the course as excellent. The 33 attendees included state and tribal fishermen/processors, fish farmers, state regulators, along with representatives from major firms from around the U.S. dealing with fishery products.

Kinnunen has been effective in providing outreach/extension materials to many culturists. For instance, he provided preventative information and AIS-HACCP materials to the Colorado Division of Wildlife regarding the control of quagga mussel veligers on Kokanee salmon eggs.

Kinnunen's role in this area is also exemplified by his attendance at the Trade Workshop II that was sponsored by the Great Lakes Commission. Those in attendance learned about the success that we have had with AIS-HACCP and how it has been widely adopted by the baitfish and aquaculture industries and may provide a model for other sectors to follow.

WORK PLANNED

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

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

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

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

IMPACTS

Examples include:

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

Standard Operating Procedures. Hundreds of fish processors and/or aquaculturists have attended HACCP Training Workshops.

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

PUBLICATIONS, MANUSCRIPTS, WORKSHOPS, AND CONFERENCES
See the Appendix for a cumulative output for all NCRAC-funded Extension activities.

SUPPORT

YEARS	NCRAC-USDA FUNDING	OTHER SUPPORT					TOTAL SUPPORT
		UNIVER-SITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
1989-91	$107,610	$237,107				$237,107	$344,717
1991-93	$94,109	$152,952				$152,952	$247,061
1993-95	$110,129	$198,099		$250,000	$55,000	$503,099	$613,228
1995-97	$31,204	$149,325	$5,000	$84,000		$238,325	$269,529
1997-99	$38,000	$110,559				$110,559	$148,559
1999-01	$94,000	$108,124				$108,124	$202,124
2001-03	$46,654	$99,702				$99,702	$146,356
2003-05	$28,000						$28,000
2005-07	$219,280						$219,280
2007-09	$114,139						$114,319
TOTALS	$883,305	$1,055,868	$5,000	$334,000	$55,000	$1,449,868	$2,333,173

EXTENSION ADDENDUM[4]

Project ***Progress Report*** for the Period
September 1, 2008 to August 31, 2009

NCRAC FUNDING: $31,954 (September 1, 2008 to August 31, 2009)

PARTICIPANTS:

Glenda D. Dvorak	Iowa State University	Iowa
Christopher F. Hartleb	University of Wisconsin-Stevens Point	Wisconsin
Myron J. Kebus	Wisconsin Department of Agriculture, Trade, and Consumer Protection	Wisconsin
Ronald E. Kinnunen	Michigan State University	Michigan
Jeannette McDonald	University of Wisconsin-Madison	Wisconsin
Joseph E. Morris	Iowa State University	Iowa
Industry Advisory Council Liaison:		
William West	Blue Iris Fish Farm, Black Creek	Wisconsin

PROJECT OBJECTIVES

(1) To develop an online Fish Health Certificate Program for producers, providing them with relevant risk assessment and management principles and practices to reduce losses due to fish diseases and set up mechanisms to collect data on the impact of the training on the individual fish operations and the industry in general.

(2) Development and presentation on workshops focused on AIS-HACCP training.

ANTICIPATED BENEFITS

Aquatic animal health and fish disease management are extremely relevant to the aquaculture industry in the North Central Region (NCR) because the industry has experienced both long-term and recent disease issues that have resulted in significant changes to the regulation of the industry and economic losses associated with fish mortalities and greater requirements for disease detection and assessment on the farm. As a result, the aquaculture industry has requested more information on fish health and mechanisms by which fish farmers can be better trained to prepare, identify, and manage disease outbreaks on the farm. Though previous attempts at educating and assisting fish farmers with aquatic disease issues have addressed the subject with printed information and workshops, few have had region-wide impact and none have attempted to prepare the aquaculture industry for whole farm disease management.

For this proposed extension project, a series of online fish health learning modules developed for the aquaculture industry will be created and implemented to better educate the fish farmer about aquatic

[4] NCRAC has funded a number of 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 "Base" Extension projects which is chaired by Joseph E. Morris. It is a 2-year project that began September 1, 2008.

diseases and on-farm fish health management. By utilizing an asynchronous delivery system, an Internet-based set of educational modules will present best management practices that will assist the fish farmer in developing biosecurity plans as well as educating about and bringing to the forefront risk factors in farm management and disease control. Fish farmers will not only be shown techniques for evaluating disease introduction, transmission, and basic pathological signs, but they will also be shown how to minimize disease occurrence and prepare for infections and proper disease risk management along with explanations and examples of veterinary inspection, health assessment, and disease treatments. This proposed work will also aid the fish farmer in understanding the veterinary health assessment report and, upon completion of the online learning modules, the fish farmer can obtain a certificate of completion that can help veterinarians recognize the aquaculture facility as one educated on methods of disease prevention and one prepared to cooperate with the veterinarian in implementing proper treatment procedures.

The Aquatic Invasive Species-Hazard Analysis and Critical Control Point (AIS-HACCP) approach has many advantages. It can effectively deal with a diverse industry and diverse risk factors associated with a variety of plant, invertebrate, vertebrate, and pathogen AIS. If it develops as it has in the seafood industry, this approach should prove to be a good partnership between industry and government regulators. It can help avoid overly restrictive regulations, and, if properly applied, can be effective at reducing the risk of spreading AIS via baitfish harvest and aquaculture practices. The HACCP approach concentrates on the points in the process that are critical to the environmental safety of the product, minimizes risks, and stresses communication between regulators and the industry. With proper cooperation among industry representatives, resource management agencies, and other AIS experts, the AIS-HACCP approach will reduce the risk that AIS will be established in new locations while maintaining the economic viability of the baitfish and aquaculture industries. It can provide a mechanism for AIS-free certification, and it can instill confidence in the public that state and federal fish stocking programs are conducting their activities in an environmentally responsible manner.

PROGESS AND PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

Development of an online Fish Health Certificate Program for producers that will provide them with relevant risk assessment and management principles and practices to reduce losses due to fish diseases has begun. Mechanisms to collect data on the impact of the training on the individual fish operations and the industry in general will commence in Year 2 of the project.

Part one of the fish health certificate program included the development of a six module Web-based learning program. A draft version of modules 1-5 of the asynchronous learning program has been completed. This includes modules containing information about:

(1) Introductory principles and practices such as regional fish production, farm types in the NCR, principle culture systems, and the myriad of regulatory agencies involved in U.S. aquaculture.
(2) Risk management and biosecurity methods that can assist producers in reducing the risk of introduction of

diseases at aquaculture facilities. This module reviewed topics such as Best Management Practices, loss events, continuing education, veterinary services, record keeping, and links to state and federal guidelines and policies.

(3) Water quality management, monitoring, and disease prevention which included reviews of water characteristics, physical and chemical water components, and effluent discharge at aquaculture facilities.

(4) Fish health inspections, with particular emphasis on what producers should expect at an inspection, how producers can prepare for inspections, regulatory consequences, supplies and equipment required at an inspection, and how samples are collected, shipped, and what type of voucher specimens may be collected.

(5) Veterinary health assessments and reports are presented showing typical results of a fish health inspection. Information included shows a producer how they can use the information to improve fish health management at their facility. This included a review of treatments and medications and the role of follow-up assessments.

Evaluation and outcome assessment tools are currently in development and will be utilized in Year 2 on the finished products.

OBJECTIVE 2

Kinnunen coordinated a three day AIS HACCP Training course that was held at Bay Mills, Michigan. Formal evaluations from attendees rated the course as excellent. The 33 attendees included state and tribal fishermen/processors, fish farmers, and state regulators along with representatives from major firms from around the U.S. dealing with fishery products. A publication entitled "Biosecurity for Aquaculture Facilities in the North Central Region" was developed and is now available through NCRAC.

Kinnunen has provided preventative information and AIS-HACCP materials to the Colorado Division of Wildlife regarding the control of quagga mussel veligers on Kokanee salmon eggs. Kinnunen's role in this area is also exemplified by his attendance at the Trade Workshop II that was sponsored by the Great Lakes Commission. Those in attendance learned about the success of AIS-HACCP and how it has been widely adopted by the baitfish and aquaculture industries and may provide a model for other sectors to follow.

WORK PLANNED

Module 6, which will present case studies of some well known fish diseases, will be developed showing the emergence, detection, diagnosis, impact, and prevention of these diseases. This module will differ from the previous five in that it can be updated as emerging diseases appear in the aquaculture industry and as additional prevention and preparedness techniques are developed. Rigorous review and editing of all six modules will occur in Year 2.

Evaluation and outcome assessment tools will be finalized and applied to the Web-based program to determine the impact of the Fish Health Certificate Program for producers. Also, significant outside review, beta-testing, and updating of the program will occur in Year 2, with a final launch date for the NCR set for October 2010. As a component of the ongoing extension/outreach aspect of this project, Kinnunen will provide additional AIS-HACCP workshops to regional audiences.

IMPACTS

Part two of the Fish Health Certificate Program directly addresses the impact assessment of this extension project. Part two includes designing and developing tools for evaluating the Web-based program and the level of knowledge gained by the participants through outcome indicators. These indicators include assessments using short evaluation surveys of usefulness, accessibility, and user-friendliness, along with individual module and full-program knowledge indicators based on short-term outcomes surveys. Also, a follow-up survey will be sent to all participants six months after completing the Web-based education program. The follow-up survey will help determine if any actions, changes in practices, policies, or procedures have been implemented at the participant's facility following the completion of the course. The survey will also test the retention of knowledge from the course (i.e., intermediate outcomes).

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.

SUPPORT

NCRAC funds provided to date total $31,954; a total of $60,505 has been allocated for this 2-year project.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

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

REGIONAL AQUACULTURE EXTENSION SPECIALIST (RAES)[5]

Project ***Termination Report*** for the Period
September 1, 2005 to August 31, 2009

NCRAC FUNDING: $199,624 (September 1, 2005 to August 31, 2009)

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

REASON FOR TERMINATION
Project objectives completed.

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

(2) Enhance information transfer.

PRINCIPAL ACCOMPLISHMENTS
OBJECTIVE 1
(1) The RAES, Christopher T. Weeks, has provided direct personal contact to the aquaculture industry by phone, meetings, seminars and workshops across the NCR. In addition to one-on-one communication with industry members, RAES leadership activities included:
- Representation for the NCR industry at a U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) viral hemorrhagic septicemia (VHS) open meeting and an aquatic invasive species workshop (Romulus, Michigan).
- Presentation of talk entitled "VHS a Regional Industry Perspective," at the Illinois VHS Conference and Workshop, April 2008.
- Administration of the NCR-Fish-Culture list serve for providing current and relevant information to subscribing members of the NCR aquaculture industry (current subscription stands at approximately 90 individuals).
- Working with regulators and industry personnel across the region to try to minimize disruption of commerce due to VHS.
- Posting of, and industry solicitation for Federal Register comments in regards to APHIS actions on VHS and the National Aquatic Animal Health Plan.

[5] NCRAC has funded a number of Extension activities, both as stand-alone projects or as components of species- or topical-specific projects. This Termination Report is for one of the 14 stand-alone Extension projects which was chaired by Laura G. Tiu. It was a 3-year project that began September 1, 2005.

(2) In addition to the activities described above, the RAES has made personal contact with principle state regulators responsible for aquaculture and baitfish activities in each NCR state, and solicited members of this group to participate in the VHS Summit at the United States Trout Farmers Association (USTFA) Midwest Aquaculture Conference, September, 2008. In this process the RAES has developed a positive working relationship with many state regulators across the region. He also has encouraged members of the industry to contact him for problems or questions related to industry matters. Much of this support has been in regards to fish production and health related issues.

(3) The RAES was instrumental in developing a plan with the Michigan Department of Agriculture for the USDA 2008 Cooperative Agreements for VHS. This plan alleviated VHS testing costs for 20 Michigan producers in 2008 while providing USDA APHIS with important VHS surveillance information.

OBJECTIVE 2

- In addition to the transfer of information on issues describe for Objective 1, the RAES has taken responsibility to post and update all aquaculture and baitfish regulations, aquaculture contact information, and approved aquatic species and fish health certification requirements for the 12 NCR states and 9 adjacent states. This information is available on the North Central Regional Aquaculture Center (NCRAC) Web site, link: North Central Region Aquaculture Contacts, Transport Regulations, and Approved Aquatic Species (http://www.ncrac.org/Info/StateImportRegs/stateregsmain.htm). This Web site is recognized on the APHIS aquaculture Web site.
- The RAES has actively sought out industry-related information from established partnerships, local and regional list severs and Web sites, and has distributed this information on the NCR Fish Culture list serve. In addition, information obtained from the RAES 2008-2009 project is in the process of being posted in a Web site entitled "NCR Aquaculture Roadmap." This Web site is expected to be live in December 2009 and will be carried forward by the RAES in 2010 and 2011. The NCR Aquaculture Roadmap is intended to provide easy access to aquaculture information including all RAC publications, extension programs and contact information, and other information to internet users.
- The RAES has worked directly with other extension outlets in the region to promote aquaculture and disseminate pertinent information to the industry. These include the Aquaculture Regional Extension Facilitator, the Northern Aquaculture Demonstration Facility, Indiana Soybean Alliance, state aquaculture associations, and academic extension personnel from across the region.
- The RAES has attended a number of state association meetings across the NCR. Often time is provided for discussion on industry needs, NCRAC project funding, and RAES functions and extension programs.
- In January 2009, the project PI, in collaboration with William Lynch, NCRAC Industry Advisory Council Chair, conducted a mail survey to over 200 members of the aquaculture community across the 12 states within the NCR. Potential respondents were

selected randomly from a NCRAC database at a target of 20 individuals per state. The survey was designed specifically to prioritize NCR industry needs of regional significance. Preliminary results were reported at the NCRAC Annual Planning Meeting in February 2009 and passed forward to the Director for distribution to NCRAC members.

- In May 2009, the RAES, in combined effort with the University of Wisconsin, School of Veterinary Medicine (UWSVM) and Wisconsin Department of Agriculture, Trade and Consumer Protection, established a tuition waiver for certified veterinarians to take the online section of the Fish Health Medicine Program offered by UWSVM. This project resulted in 44 certified veterinarians enrolling in the course from across the nation.
- In July 2009, the RAES drafted a report entitled "Improving Information Transfer to the NCR Aquaculture Industry, Extension Priorities and Work Plan Development." This report is an assessment of current information transfer methodologies in the NCR aquaculture industry with focus placed on potential improvement, accessibility, and utilization. The intent of this review is to generate discussion within the NCR aquaculture community towards forming a collaborative effort centered on improving information dissemination into the aquaculture industry. The report is currently in draft form and will be carried forward to the RAES 2010-2011 project.

RECOMMENDED FOLLOW-UP ACTIVITIES

- Identify key members of the NCR aquaculture community who are willing to provide leadership roles and work in improving information transfer methods. Form a work group consisting of researchers, industry members, and extension personnel.
- Identify quantifiable measures of progress in regards to information dissemination efforts.
- Identify hindrances to industry development.
- Identify successful aquaculture ventures inside and outside the region. Identify key characteristics that may have helped lead to success.
- Shore up current Web-based information dissemination methods including assessing what is out there and focusing on how to direct (streamline) specific inquiries to acceptable sources/answers.
- Complete the NCRAC Roadmap Web site and solicit partners, extension material, and research information from across the region to help make the site a valuable information outlet for the NCR aquaculture industry.

Many, if not all, of the recommended follow-up activities will be undertaken in a new 2-year RAES project which began September 1, 2009.

IMPACTS

Due to a number of factors, direct impacts of the RAES activities on the NCR aquaculture industry during the project are difficult to assess. However, the following tasks have either been completed or are being carried forward in the new RAES project:

- The North Central Region Aquaculture Contacts, Transport Regulations, and Approved Aquatic Species Web site has received much attention: with approximately 800 hits per month from August–October 2009. This site is referenced by the APHIS aquaculture Web site.

- RAES activities occurring through this funded project have provided much of the base work for the NCR Aquaculture Roadmap, soon to be launched. It is expected that the Roadmap Web site will effectively complement, and receive as good if not better attendance, than the transport regulation Web site described above.
- Posting of the Roadmap site is expected to increase list serve subscription and help provide collaborative input from across the region in regards to indentifying industry needs and dissemination of research and extension information to the industry.
- Impacts of VHS on the region have been minimized to an extent since the Emergency Order (October 2006). APHIS is expected to come out with a VHS proposed rule in the spring of 2010. While negative impacts of future federal and state regulations are likely, open dialogs do exist between the industry and most agencies holding regulatory authority.

It is hoped that a major portion of the 44 certified veterinarians taking the online Fish Health Medicine Program will remain interested in fish health and actively assist the aquaculture industry.

SUPPORT

NCRAC provided $199,624 to Ohio State University for this 3-year project.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

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

FEED TRAINING CARNIVOROUS FISH[6]

Project ***Progress Report*** for the Period
September 1, 2006 to August 31, 2009

NCRAC FUNDING: $300,000 (September 1, 2006 to August 31, 2009)

PARTICIPANTS:

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

PROJECT OBJECTIVES

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

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

ANTICIPATED BENEFITS

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

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

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

PROGESS AND PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

University of Wisconsin-Madison (UW-Madison)

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

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

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

No difference was found in overall success between fish sizes (83.4%, 91.3%, and 78.1%, respectively) for fish harvested at 25.0, 35.0, and 45.0 mm (1.0, 1.4, and 1.8 in) TL. Harvest losses in fish at 25.0 mm (1.0 in) TL were offset by cannibalism losses in fish at 45.0 mm (1.8 in) TL. Fish harvested at 35.0 mm (1.4 in) TL displayed low losses from both harvest stress and cannibalism, and may be recommended as the best size for habituation using the techniques set forth in this study. From a practical standpoint, it is logistically unfeasible to harvest and train all fingerlings produced at a commercial scale facility at the same size. Techniques should be modified to accommodate the fish on-hand. Low stress harvest methods (e.g., light trapping) for small fish and size grading for larger, more size-diverse populations would likely result in better overall success for both groups of fish.

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

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

<u>University of Wisconsin-Milwaukee (UW-Milwaukee)</u>

Extracts of chironomids, zooplankton, redworms (*Eisenia fotida*), artemia, and tubifex worms have been prepared as natural feed attractors. Feeding trials are in progress and the feeding responses will be compared to identify the best positive response for each species (yellow perch and largemouth bass).

Feeding trials in conjunction with feed attractants such as extracts of chironomids, zooplankton, redworms, artemia and tubifex worms were inconclusive. The feeding behavior appears to be more relative to sight, prey movement, and auditory signals. Consequently, research effort has shifted to investigating these feeding behavior patterns as a function of sight, movement, and sound.

In 2010 investigators will resume their investigation of the olfactory response of yellow perch feeding using a more limited selection of extracts. Also, due to new state fish health policies, investigators are restricted to introducing fish from outside sources into their laboratory. UW-Milwaukee's laboratory has medical separation status which means that introduction of outside fish, e.g., largemouth bass will compromise this status.

OBJECTIVE 2

<u>UW-Madison</u>

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

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

Size-graded fish were returned to separate tanks to observe their feeding behavior. Nearly 100% of fish in the medium and large groups actively consumed formulated food, while only ~25% of the small fish consumed food. The extent to which these small fish can be "re-trained" to accept formulated food in tanks was evaluated in three trials (2007-09). Ponds stocked earlier in the season produced larger and more uniformly-sized fingerlings than those stocked later in the season. Pooling data across treatments (size-graded and non-size-graded) fingerlings stocked into the first set of two ponds averaged 24.6 ± 3.5 g (0.9 ± 0.1 oz), fingerlings stocked into the second set of two ponds averaged 21.9 ± 4.5 g (0.8 ± 0.2 oz), and fingerlings stocked into the last set of two ponds averaged 18.4 ± 3.2 g (0.6 ± 0.1 oz). Accordingly, researchers recommend that fingerling producers harvest and feed-train fingerlings as early as possible in the season.

Also in each of two years investigators stocked two replicate ponds with these small fingerlings for overwintering and second year survival and growth studies. The ponds were stocked in the autumn at 123,579–185,368 fish per ha (50,000–75,000 fish per acre), overwintered, and the ponds were not fed until the following spring. At this time, the ponds were fed daily in a similar fashion to ponds containing larger second-year fingerlings. Ponds were harvested in autumn; the mean survival was 63.2%, and the mean fish size was 182 mm and 75.6 g (7.2 in and 2.7 oz). This finding demonstrates that even the smallest fingerling yellow perch harvested from ponds in autumn have surprisingly good potential for growth if they are exposed to proper environmental and aquacultural conditions.

UW-Madison researchers also conducted a study comparing different pond stocking densities for fingerling yellow perch. After feed-training, groups of yellow perch fingerlings were stocked into six replicate

0.04-ha (0.1-acre) ponds, three ponds stocked ca. 61,789 fish/ha (25,000 fish/acre and three at approximately 185,368 fish/ha (75,000 fish/acre). The fish were reared until the end of the growing season in October, at which time the ponds were harvested. No differences in pond survival (68.7% ± 7.7 versus 70.0% ± 9.8) or mean fish size (22.3 ± 4.7g versus 21.0 ± 3.6 g [0.78 oz ± 0.16 versus 0.74 ± 0.13] for low and high stocking densities, respectively) were found.

Based on the above study, it is recommended that yellow perch fingerling producers can successfully use stocking densities as high as 185,368 fish/ha (75,000 fish/acre) to improve their production. At times, particularly during August and early September when temperatures and feeding rates are at their highest, levels of dissolved oxygen became critical. Accordingly, it is recommend that fingerling producers using high stocking densities regularly monitor levels of dissolved oxygen in the early morning, and be prepared to rapidly employ corrective actions when needed.

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. 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 June 2007 were not delivered. This resulted from 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.

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

Due to continued unavailability of fish from the original producer, additional producers were contacted and on August 16, 2008, 30,000 juvenile largemouth bass were purchased and transported from Cambridge, Ohio to the LDL location. Fish were stocked into eight pond halves (four ponds total) at 37,000 fish/ha (15,000 fish/acre). Each pond is 0.20 ha (0.5 acre), such that each pond-half received 3,750 juvenile largemouth bass. Fish in treatment halves of the ponds were confined to 1/3 of the surface area of these pond halves during the initial two weeks of the study using block nets. This "crowding" in the treatment halves was done on the deep end of the pond to help alleviate any water quality problems that may have ensued from crowding. Four floating creels that were each stocked with 100 largemouth bass were monitored for declines in fish numbers over the initial week to estimate transport mortality.

Twice daily feedings began on August 17 (the morning after stocking). Fish were fed a 50:50 mixture of Silver Cup and Aquamax feeds at 4% of body weight/day. Each week the Silver Cup feed was reduced by 10% and replaced by Aquamax due to its local availability and lower cost. Feeding rates were recalculated for each pond-half each week from mean weight information gathered during weekly samplings. Feedings were shifted from twice daily to once daily on September 15th due to a road wash out that made accessing the ponds difficult; fish were still fed at 4% of body weight/day.

From August-October, for each pond half, total catch from 2× weekly seine samples was recorded, 40 specimens were euthanized, individual lengths and weights were measured and recorded, and specimens were individually tagged and frozen so stomach analysis can be conducted at a later date. Survival, percentage of fish on feed, and percentage of fish cannibalized were estimated from catch curve and stomach contents analysis. Percent starvation was estimated from length and weight data (through use of relative weight [Wr]).

Mortality was estimated to be 3.58% in the first 10 days post-stocking. Within 1 week post-stocking, no fish exhibited Wr <80. Prior to this period, 2.5% of the control population showed Wr <80, while 3.13% of the treatment population had Wrs <80. These fish were assumed to have gone off-feed and to have perished, due to loss of body condition during the 1- to 2-week post-stocking phase. This is supported by the observation that the percentage of fish with commercial feed in their stomachs steadily increased from the third sampling date until the end of the study.

At the end of the 7-week sampling period, the fish exhibited an average length of 102.32 mm (4.03 in) and an average weight of 17.44 g (0.62 oz); initial average fish size was 55.18 mm (2.17 in) and 1.86 g (0.03 oz). Average feed conversion of the ponds during the sampling period was 1.16. There was no statistical difference in total survival between control and treatment pond halves (52–55%).

On average, cannibalized fish were 44.8% as long as the fish that consumed them. In control-pond halves, it required 4 weeks for sufficient size variation to develop to allow cannibalism, while it took 5 weeks for this to occur in treatment-pond halves. Total mortality due to cannibalization during the 7-week sampling period for the treatment was estimated to be 15.65% of the population and 40.38% of the population for the control. It is often believed that cannibals no longer feed on the commercial feed provided in ponds, and that they should be removed from the population. However, every largemouth bass that had cannibalized other fish also had commercial feed in their stomachs. Frequent grading, as often as every 4 weeks, should be conducted to reduce cannibalism.

Individuals found with commercial feed in their stomachs had an average Wr of 136.8, while individuals not found to contain commercial feed had an average Wr of 110.1. A consistent response of statistically higher percentages of fish remaining on feed in the crowded pond halves (79.2%) versus the control halves (54.1%) was indicated. During the crowding phase (the initial 2 weeks of the study), treatment halves also showed statistically higher Wr values (146.3 versus 134.8). Crowding fish to areas where food will be provided is a viable approach for improving feed retention rates and slowing the onset of size disparities.

Two, 0.10-ha (0.25-acre) production ponds at the Lincoln University (LU) pond facility in Jefferson City, Missouri were secured for use during winter 2008-2009. Seventy-five hundred fish from the LDL site were held over-winter in the ponds at LU. Fish were fed at 4% body weight/day until they went off-feed due to declining temperatures. Once the fish resumed feeding activity in the spring, they were fed for two weeks.

Following this period, the fish were sampled and harvested to estimate percentage returning to pelleted feed and overall winter survival. Forty specimens from each pond were collected and individual weight and length data were recorded. Of the fish sampled following the 2-week feeding period in the following spring (at LU), 77.5% contained commercial feed in their stomachs, a percentage slightly less than the 83% of fish "on feed" during the preceding fall at LDL. Of the 7,500 fish stocked in ponds at LU in fall 2008, 87.4% were harvested in the following spring.

Southern Illinois University-Carbondale (SIUC)

During year 1 of the project, largemouth bass were produced and feed habituated at Logan Hollow Fish Farm, Murphysboro, Illinois. After the largemouth bass fingerlings were harvested from the nursery ponds, they were placed into a 5000-L (1,321-gal) grading tank where they were treated with a 5 ppm potassium permanganate bath for 30 minutes to prevent introduction of diseases or parasites.

Fingerlings were then graded through grading boxes to ensure uniform sizes in each tank and to reduce cannibalism. Fish were stocked at a density of 7.9 fish/L (30.0 fish/gal). Freeze-dried krill (Southern Aquaculture Supply, Lake Village, Arkansas) was used as the starter diet and Bio Diet (Bio-Oregon, Inc., Warrenton, Oregon) was the moist pellet feed used in this study. Fish were fed 8% body weight daily. Investigators examined five different combinations of hand feeding and automatic feeders 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] total length, 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 (51.0 and 30.0 fish/gal). Treatments did not have a significant effect on survival but did have a highly significant effect on feed training success. Fish size had a highly significant effect on survival as well as feed training success. Small fish had higher feed training success (96.4%) in treatment 3, medium and large fish feed trained better in treatment 2 (97.3% and 86.1%, respectively). Treatments using densities of 13.2 fish/L (50.0 fish/gal) did not differ significantly in terms of survival or feed habituation success compared to tanks stocked at 7.9 fish/L (30.0 fish/gal) with fish of the same size.

The effect of different light intensities on survival and feed habituation success was also examined. Three light intensities were utilized: light = 21 lux, medium = -0.54 lux and dark = -1.08 lux. All treatments were conducted in triplicate. Light intensity was found to have no impact on feed habituation success and no impact on survival except at the darkest level tested. The number of cannibals differed significantly between the light and dark treatments. Reduced light levels result in decreased ability of culturists to observe fish for health and cannibalism.

The effectiveness of a bird of prey call in deterring fish-eating birds from ponds stocked with largemouth bass fingerlings at a commercial fish farm was evaluated. A Bird Gard Pro™ was programmed to produce the call of a peregrine falcon (*Falco peregrines*) at random intervals from 10–30 minutes apart, 24 hr/day. Observations were then made of bird behavior and response to the call. Species, activity before call, response to call, distance from call, and time of day were recorded for each bird observed when the call was activated. Distance from the call was measured using a laser range finder. After testing the peregrine falcon call, the Bird Gard Pro™ was programmed to produce the call of a sharp-shinned hawk (*Accipiter striatus*) at the same time intervals and durations as the peregrine falcon call. The same observations were made as described for the falcon call. Birds-of-prey calls failed to repel fish-eating birds from the fish farm. Physical barriers are the only demonstrated effective prevention mechanism for bird predation in aquaculture.

Pond studies evaluating the effect of stocking density on growth and survival of feed-trained fingerling largemouth bass were conducted during 2008. Pellet-feed trained largemouth bass fingerlings were obtained from a commercial producer in Arkansas and transported to experimental ponds at SIUC. Two ponds were stocked with fingerlings at a density of 37,000 fish/ha (14,980 fish/acre) and two ponds were stocked at a density of 74,000 fish/ha (29,960 fish/acre). A sub-sample of 100 fish stocked into each pond was measured for initial length and weight. Fish were fed to satiation several times daily. Fish were harvested in fall and counted to determine survival. A random sample of 100 fish from each pond were weighed and measured to estimate growth. Initial size of stocked fish was 59.8 mm (2.35 in) and 2.4 g (0.08 oz). Mean size of fish at harvest was 72.3 mm (2.85 in) and 5.1 g (0.18 oz) and was not significantly different between ponds stocked with different densities of largemouth bass fingerlings. Survival in ponds averaged 73% and was not significantly different between treatments. The percentage of fish that weighed <3.0 g (0.11 oz) (and apparently did not remain "on feed" after stocking into ponds) averaged 28% for the low-density ponds and 47.5% for the high-density ponds.

UW-Milwaukee

Auditory conditioning trials have been conducted on early life stage yellow perch. Auditory signals of low frequency (35–300 Hz) were presented to 12-day post hatched (dph) yellow perch in conjunction with a commercial fish starter diet. The initial response was recorded as an estimate of the numbers of fish remaining in the feeding area over time. Young fish were exposed to a sound/feeding regime for up to 30 dph. From 30–50 dph, their behavioral response to the auditory signal was measured as a function of time response to the target area involving the food. The auditory signal was presented to the fish when they were randomly distributed in the tank.

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

The feeding response of yellow perch as a function of an auditory signal was observed for a range of life stages from sac-fry to adults. A series of five different foods were presented in conjunction with sound, enhanced light, and movement.

The on-set of first feeding was observed for yellow perch sac-fry (6.0 mm; 0.23 in) as a function of live food (green tank water). No auditory signal was used for the first seven days of yellow perch sac-fry and larval feeding of live foods. Between day 8 and 10, a commercial starter diet was presented to the perch larvae, which also included one live food item. Sound, enhanced lighting, and food particle movement was correlated with the observed feeding response. The mean body length of the larvae at this time was 9.3 mm (0.37 in). Between 8 and 24 dph, the young perch elicited a feeding response as a function of sight and food particle movement and to a lesser extent, sound. Between 30 dph (25.0 mm [0.98 in] length) and 40 dph (32.0 mm [1.3 in] length), the auditory signal appears to play a more important role in the young perch feeding response to a commercial diet.

It appears that training to an auditory signal only partially influences their feeding behavior for the first 30 days. However, sight and food particle movement are more important cues for early life stage feeding. The transition of their feeding response to sound was observed at about 50–60 mm (2.0–2.4 in) body length. The fingerling perch were observed to elicit a feeding response in less than 2 seconds. In most cases, when post fingerling perch are introduced into a production system, sight and food particle movement remain the primary cue for 12 to 24 h.

WORK PLANNED

OBJECTIVES 1 & 2

<u>UW-Madison</u>

A video on the feed-training of pond-reared yellow perch fingerlings is being prepared by the extension staff of the University of Wisconsin-Stevens Point Northern Aquaculture Demonstration Facility. All of the footage needed for this video was shot in 2008 and the video should be completed late in 2009. An extension publication on yellow perch fingerling production is planned for 2010.

<u>UM-C</u>

Research has been completed for this project.

<u>SIUC</u>

Research has been completed for this project.

<u>UW-Milwaukee</u>

In 2010 investigators will finish the observation on the feeding response of yellow perch as a function of auditory signals and document them with video.

IMPACTS

Studies will provide valuable information to yellow perch fingerling producers for maximizing the productivity and efficiency of their operations. Efficient and effective yellow perch fingerling production has been identified in numerous regional workshops as being a high priority. The studies will also provide valuable cost/benefit information on the use of krill and semi-moist feeds as transitional diets.

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

SUPPORT

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

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

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

LARGEMOUTH BASS NUTRITION[7]

Project ***Termination Report*** for the Period
September 1, 2005 to August 31, 2009

NCRAC FUNDING: $170,000 (September 1, 2005 to August 31, 2009)

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

REASON FOR TERMINATION

The objectives were completed and funds terminated.

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.

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 anywhere from two to three years.

OBJECTIVE 1

Research at Southern Illinois University-Carbondale (SIUC) included a temperature effect study on largemouth bass feeding and growth during the second growth season. This study involved two treatments with four replicates for each treatment using eight 0.04-ha (0.1-acre) ponds and each treatment involved feeding fish at a particular time of day with the intent of fish being fed during different water temperatures. In treatment #1, fish were fed within an hour of sunrise each morning while in treatment #2 fish were fed within an hour of sundown each evening. Statistical analysis indicates that there were no significant treatment differences in overall growth, densities, or condition.

An over-winter study was completed including two treatments with four replicates for each treatment using eight 0.04-ha (0.1-

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

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. There were no significant differences detected in this study as both sizes of pellets yielded similar production data.

A final study was conducted to examine the effects of carbohydrate levels on growth in largemouth bass reared in an indoor recirculation system. Diets containing 0% (control), 10%, 20%, or 30% dextrin as a carbohydrate source (36% crude protein, 15% crude lipid) were fed to replicate tanks (N = 3) of juvenile largemouth bass (~22.0 g; ~.08 oz) in a recirculation system consisting of 12, 190-L (50-gal) aquaria and associated mechanical and biological filtration units for a period of 10 weeks. In addition to routine measures of production performance, plasma insulin and glucose were monitored periodically throughout the trial. A slight, though significant increase in weight gain and specific growth rate was associated with the 30% dextrin feed, which was primarily associated with an increase in hepatosomatic index (result of excessive glycogen deposition in the liver). Although no diet-related differences in insulin or glucose were noted during the trial, fish fed the dextrin-supplemented feeds exhibited significant pulse in plasma glucose from 6–8 h after feeding. This pulse was not observed in the control group, and was not accompanied by an increase in plasma insulin. This data supports the hypothesis that largemouth bass are poorly suited to carbohydrate-rich diets, and that elevated blood glucose (as opposed to increased plasma amino acid content) is not as strong a trigger for insulin release in carnivorous fishes as it is in other vertebrates.

Complementary pond and laboratory studies were conducted by Purdue in 2007-2008 with largemouth bass to examine new practical dietary formulations and use of alternative protein feedstuffs. Modified practical diets fed to bass in earthen culture ponds resulted in no significant differences in weight gain or other production parameters. The modified diets, manufactured by Nelson and Sons, Inc, Murray, Utah, contained 35% soybean meal. Fish did not display negative influences of fish meal replacement with soybean meal even though diets contained up to 60% soybean meal.

OBJECTIVE 2

Research at Purdue was designed to address liver health of bass during the second year of growth. A commercial control diet (Nelson and Sons, Inc., Steelhead Diet) was used as a positive control diet and an experimental diet was developed in collaboration with a feed mill. The experimental diet contained more soybean meal (35%) and more choline than the commercial diet. Both diets were fed to 2+ year old bass raised in earthen culture ponds. Ponds were harvested in conjunction with an Extension Field Day (Objective 3). There were no differences in consumption, weight gain, or feed conversion rate of fish fed the two diets. None of the modified diets resulted in improved liver health. None of the fish displayed signs of ill health and all gained weight at rates comparable to other studies, but all livers appeared pale and mottled.

OBJECTIVE 3

The region- wide workshop on largemouth bass nutrition was hosted at Purdue on October 17-18, 2008. Speakers included Paul Brown (Purdue), James Tidwell (Kentucky State University), and Kwamena Quagrainie (Purdue). The 20 attendees received supporting materials developed using information garnered from this project and also participated in fish harvests.

IMPACTS

These data indicate that largemouth bass are tolerant of soybean meal in diets and modifications can be made that would save considerable amounts of money on feed costs. However, poor liver health remains an issue.

Results from these studies clearly indicate that the nutritional research conducted over the past 12 years resulted in a sufficient level of knowledge to formulate diets for grow out of largemouth bass using ingredients other than fish meal. However there are some ingredient limitations that need further research.

Information from this project will be used to develop extension materials in conjunction with James Tidwell (Kentucky State University). The associated DVD will contain voice-over PowerPoint presentations as well as footage from the October 2008 workshop.

Developing modified diets for emerging aquaculture species (such as largemouth bass) allows feed mills to react to price volatility of commodities and restrain feed costs during times of rapid escalation. Moving toward diets that contain higher concentrations of regionally available ingredients increases the probability of regional manufacturing of diets. Additionally, formulations containing optimal balance between energy and protein sources improve growth efficiency of aquatic livestock and minimize waste and effluent production.

RECOMMENDED FOLLOW-UP ACTIVITIES

Increased inclusion of carbohydrates, specifically dextin, resulted in physiological dysfunction in largemouth bass. This appears to have been the result of insufficient insulin response to elevated circulating glucose levels. This effect may be attenuated by directly including known piscine insulinotropes (e.g., certain amino acids) in carbohydrate-rich feeds, thereby improving utilization of these feeds. Studies to address this hypothesis are recommended.

Although it is now possible to continue to define critical nutrient requirements for this species, more progress could be made by using alternative methods, i.e., metabolomics and proteomics analytical approaches, to compare wild fish to pellet-fed, cultured fish. These new methods may allow for improved feed formulations in a shorter time period than is often the case in feed development.

SUPPORT

NCRAC funds provided $170,000 which was the entire amount of funding allocated for this 2-year project.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

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

NATIONAL COORDINATOR FOR AQUACULTURE INADs/NADAs[8]

Project ***Termination Report*** for the Period
July 15, 2004 to August 31, 2009

NCRAC FUNDING: $89,000 (July 15, 2004 to August 31, 2009)

PARTICIPANT:
Rosalie A. Schnick Michigan State University Wisconsin

REASON FOR TERMINATION
Funds terminated.

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.

PRINCIPAL ACCOMPLISHMENTS
The major objective of the National Coordinator for Aquaculture New Animal Drug Applications is to help obtain drug approvals for aquatic species through coordination of activities. These activities over the past five years have resulted in a number of NADA approvals and the

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

potential for many more in the near future or that are under development. The major emphasis in the past five years of this position that began in 1995 is to work closely with the drug sponsor to ensure that all activities result in approvals. Following are those drugs and their label claims:[9]

ORIGINAL NADA APPROVALS

1. Florfenicol (Aquaflor®) for control of mortality in channel catfish due to enteric septicemia of catfish associated with *Edwardsiella ictaluri* (NADA #141-246; approved 10/24/05); sponsor: Intervet/Schering-Plough Animal Health (SPAH)✺*

2. Hydrogen peroxide (35% PEROX-AID®) for control of mortality in (a) freshwater-reared finfish eggs due to saprolegniasis, (b) freshwater-reared salmonids due to bacterial gill disease associated with *Flavobacterium branchiophilum*, and (c) freshwater-reared coolwater finfish and channel catfish due to external columnaris disease associated with *Flavobacterium columnare* (*Flexibacter columnaris*) (NADA #141-255; approved 1/11/07); sponsor: Eka Chemicals, Inc.✺*

SUPPLEMENTAL NADA APPROVALS

1. Florfenicol (Aquaflor®) for control of mortality in freshwater-reared salmonids due to coldwater disease associated with *Flavobacterium psychrophilum* (NADA #141-246; approved 3/19/07); sponsor: SPAH*

2. Florfenicol (Aquaflor®) for the control of mortality in all freshwater-reared salmonids due to furunculosis associated with *Aeromonas salmonicida* (NADA #141-246; approved 10/26/07); sponsor: SPAH*

3. Oxytetracycline dihydrate (Terramycin® 200 for Fish) to change formulation, oxytetracycline concentration, product name, and add approved lobster indication to the label for the control of Gaffkemia caused by *Aerococcus viridans* (NADA #038-439; approved 6/30/06); sponsor: Phibro Animal Health (PAH)

4. Oxytetracycline dihydrate (Terramycin® 200 for Fish) for control of mortality in (a) all freshwater-reared salmonids due to coldwater disease associated *Flavobacterium psychrophilum* and (b) *Oncorhynchus mykiss* due to columnaris disease associated with *Flavobacterium columnare*. To remove limitation on treating salmonids in water temperatures below 9°C. To add to the label of the previously approved indication for marking of skeletal tissue in Pacific salmon (NADA #038-439; approved 7/6/08); sponsor: PAH*

5. Oxytetracycline hydrochloride (Oxytetracycline HCL Soluble Powder-343®) for skeletal marking in finfish fry and fingerlings (NADA #200-247; approved 9/15/04); sponsor: IVX Animal Health, Inc., formerly Phoenix Scientific, Inc.

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[9] ✺Denotes a new drug that gained approval for an aquatic species;

*Denotes drug designations under the Minor Use and Minor Species (MUMS) Animal Health Act of 2004 that gives the sponsors of these drugs seven years of marketing exclusivity (as of October 27, 2009, the MUMS Office has granted 83 designations, 71 of those are to aquaculture drug sponsors, many of whom have received extensive help from the National Aquaculture NADA Coordinator); and

❁Denotes a drug which is a candidate sedative (one of the drugs being considered by USDA for development; it is anticipated that there will be eight label claims for the selected sedative).

6. Oxytetracycline hydrochloride (Terramycin 343®) for skeletal marking in finfish fry and fingerlings (NADA #008-622; approved 6/13/05); sponsor: Pfizer Animal Health

ABBREVIATED NADA APPROVALS

1. Formalin (Formacide-B®) for control of (a) external protozoa (*Chilodonella* spp**.,** *Costia* spp., *Epistylis* spp., *Ichthyophthirius* spp. *Scyphidia* spp. and *Trichodina* spp.) on all finfish, (b) monogenetic trematode parasites (*Cleidodiscus* spp., *Dactylogyrus* spp., and *Gyrodactylus* spp.) on all finfish**,** (c) fungi of the family Saprolegniaceae on all finfish eggs**,** and (d) protozoan parasites (*Bodo* spp., *Epistylis* spp., and *Zoothamnium* spp.) on penaeid shrimp (ANADA #200-414; approved July 17, 2007); sponsor: B.L. Mitchell, Inc.

2. Oxytetracycline hydrochloride (TETROXY AQUATIC®) for skeletal marking in finfish fry and fingerlings (ANADA #200-460; approved April 20, 2007); sponsor: Cross Vetpharm Group Ltd., Dublin, Ireland

CONDITIONAL APPROVAL

1. Florfenicol (Aquaflor®) for control of mortality in catfish due to columnaris disease associated with *Flavobacterium columnare* (NADA #141-246 CA1; approved April 18, 2007); sponsor: SPAH*

INDEXED DRUGS

1. Salmon gonadotropin Releasing Hormone analog and Domperidone (Ovaprim®) for use as a spawning aid in ornamental fish (Indexed 3/19/09); sponsor: Western Chemical, Inc.*

2. Metomidate (Aquacalm™) for the sedation and anesthesia of ornamental finfish (Indexed 6/3/09); Sponsor: Western Chemical, Inc.

Totals for NADAs and ANADAs (2004 to October 2009) = 11 NADAs and ANADAs for 5 drugs (2 new) and 14 different label claims

TOTALS for Indexed drugs (2008 to June 2009) = 2 Indexed drugs

NADA APPROVALS ANTICIPATED IN THE NEAR FUTURE

1. Chloramine-T (HALAMID® AQUA) for control of mortality in (a) freshwater-reared salmonids due to bacterial gill disease associated with *Flavobacterium branchiophilum* and (b) walleye and possibly all warmwater finfish due to external columnaris disease associated with *Flavobacterium columnare* (original NADA); sponsor: Axcentive SARL, Bouc Bel Air, France✺*

2. Copper sulfate (Triangle Brand Copper Sulfate®) for control of mortality in channel catfish due to ichthyophthiriasis (original NADA); sponsor: Freeport-McMoRan Copper & Gold✺*

3. Emamectin benzoate (Slice®)for control of sea lice on saltwater-reared salmonids (original NADA); sponsor: SPAH✺*

4. Erythromycin thiocyanate (Aquamycin 100®) for control of mortality in salmonids due to bacterial kidney disease associated with *Renibacterium salmoninarum* (original NADA); sponsor: Bimeda, a Division of Cross Velpharm Group✺*

5. Florfenicol (Aquaflor®) for control of mortality in (a) freshwater-reared salmonids and warmwater finfish due to systemic columnaris disease associated

with *Flavobacterium columnare* and (b) hybrid striped bass and tilapia due to streptococcosis associated with *Streptococcus iniae* (supplemental NADAs); sponsor: SPAH*

6. Formalin (Parasite-S®) for control of mortality in all freshwater-reared fish due to saprolegniasis (supplemental NADA); sponsor: Western Chemical, Inc.*

7. Hydrogen peroxide (35% PEROX-AID®) for control of mortality in (a) all warmwater fish due to saprolegniasis and (2) warmwater finfish due to external columnaris disease associated with *Flavobacterium columnare* (supplemental NADAs); sponsor: Eka Chemicals, Inc.*

8. 17α-methyltestosterone (Masculinizing Feed for Tilapia®) for masculinization of female early life-stage tilapia (original NADA); sponsor: Rangen, Inc.✺*

9. Oxytetracycline dihydrate (Terramycin® 200 for Fish) for skeletal marking in salmonids (supplemental NADA); sponsor: PAH*

Totals anticipated for NADAs (in the near future) = at least 9 NADAs for 9 drugs (5 new) and 12 different label claims

NADAS UNDER DEVELOPMENT FOR APPROVALS

1. Chloramine-T (HALAMID® AQUA) for control of mortality in coolwater finfish, except for walleye, due to external columnaris disease associated with *Flavobacterium columnare* (supplemental NADA); sponsor: Axcentive SARL, Bouc Bel Air, France*

2. Copper sulfate (Triangle Brand Copper Sulfate®) for control of mortality in channel catfish eggs due to saprolegniasis (supplemental NADA); sponsor: Freeport-McMoRan Copper & Gold

3. Benzocaine for anesthesia, sedation during transport, short-term sedative for the purposes of handling for all freshwater-reared finfish (original NADA and supplemental NADAs); sponsor: Frontier Scientific, Inc.✺*❁

4. Benzocaine (BENZOAK®) to sedate to handleable condition all salt-water-reared salmonid finfish and to lightly sedate for transport purposes all salt-water-reared salmonid finfish (original NADA and supplemental NADAs); sponsor: ACD Pharmaceuticals AS, Ålesund, Norway✺*❁

5. Calcein (SE-MARK®) for skeletal marking in freshwater-reared finfish fry and fingerlings (original NADA); sponsor: Western Chemical, Inc.✺*

6. Emamectin benzoate (Slice®)for control of external parasites on freshwater-reared finfish (supplemental NADA); sponsor: SPAH

7. Eugenol (AQUI-S E®) to sedate to handleable condition (a) all freshwater-reared salmonids, (b) all freshwater-reared coolwater finfish, (c) all freshwater-reared warmwater finfish, except freshwater-reared channel catfish, and (d) all salt-water-reared salmonid finfish and to lightly sedate for transport purposes (a) all freshwater-reared salmonids, (b) all freshwater-reared coolwater finfish, (c) all freshwater-reared warmwater finfish, except freshwater-reared channel catfish, and

(d) all salt-water-reared salmonid finfish (original NADA and supplemental NADAs); sponsor: AQUI-S New Zealand Ltd., Lower Hutt, New Zealand❋*❁

8. Fish-eezzz™ for use as a sedative for all freshwater-reared finfish (original NADA); sponsor: Micro Technologies, Inc. ❋❁

9. Florfenicol (Aquaflor®) for control of mortality in (a) freshwater-reared coolwater finfish due to systemic columnaris disease associated with *Flavobacterium columnare*) and (b) freshwater-reared coolwater and warmwater finfish due to motile *Aeromonas* septicemia associated with *Aeromonas* spp. (supplemental NADAs); sponsor: SPAH*

10. Hydrogen peroxide (35% PEROX-AID®) for control of *Ichthyophthirius multifiliis, Ichthyobodo necator,* and *Gyrodactylus spp* in freshwater-reared salmonids, coolwater, and warmwater finfish (supplemental NADA); sponsor: Eka Chemicals, Inc.*

11. 17α-methyltestosterone (Masculinizing Feed for Tilapia®) for masculinization of swordtails and rainbow trout (supplemental NADA); sponsor: Rangen, Inc.*

12. Oxytetracycline dihydrate (Terramycin® 200 for Fish) for control of mortality in (a) freshwater-reared coolwater and warmwater finfish due to motile *Aeromonas* septicemia associated with *Aeromonas* spp., (b) freshwater-reared salmonids due to external columnaris disease associated with *Flavobacterium columnare,* and (c) penaeid shrimp due to necrotizing hepatopancreatitis (supplemental NADAs): sponsor: PAH*

13. Oxytetracycline hydrochloride (Terramycin 343®) for control of mortality in fry and fingerlings of all coolwater and warmwater species of freshwater-reared finfish due to (a) external columnaris disease associated with *Flavobacterium columnare,* (b) systemic columnaris disease associated with *Flavobacterium columnare,* and (c) bacterial gill disease associated with *Flavobacterium branchiophilum* (supplemental NADAs); sponsor: Pfizer Animal Health*

14. Porphyrins (AquaFrin®) for control of mortality in freshwater-reared salmonids due to saprolegniasis (original NADA); sponsor: Frontier Scientific, Inc. ❋

15. Potassium permanganate (Cairox®) for control of mortality in (a) freshwater-reared salmonids due to bacterial gill disease associated with *Flavobacterium branchiophilum,* (b) freshwater-reared finfish due to external columnaris disease associated with *Flavobacterium columnare*, and (c) freshwater-reared salmonids due to coldwater disease associated *Flavobacterium psychrophilum* (original NADA and supplemental NADAs); sponsor: Carus Chemical Company❋*

16. Salmon gonadotropin Releasing Hormone analog (OVAPLANT®) for the induction of spawning in mature salmonids (original NADA); sponsor: Aquatic Life Sciences, c/o Western Chemical, Inc.❋*

17. Tricaine methanesulfonate (TRICAINE-S®) for the euthanasia of finfish not intended for food (supplemental

NADA); sponsor: Western Chemical, Inc.*

Totals anticipated for NADAs under development = at least 12 drugs (+ 1 sedative; 5 new drugs) and 34 (+8 sedative) different label claims

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 high priority 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.

Coordination and educational efforts directed toward potential INAD/NADA applicants saved time and effort for both the industry and CVM. The National Coordinator for Aquaculture New Animal Drug Applications served as a conduit between an INAD/NADA applicant and CVM. The National Aquaculture NADA Coordinator helped 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.
This enhanced coordination will help and has helped gain original, supplemental, and abbreviated NADAs (see the listing above for details).

These approvals aids the aquaculture industries to reduce mortalities associated with infectious and handling diseases and to increase their efficiency by using spawning aids, gender manipulation aids, and sedatives. The domestic aquaculture industry is now better able to compete with foreign producers because there are more legal drugs for producers to use.

RECOMMENDED FOLLOW-UP ACTIVITIES

- Ensure that 17α-methyltestosterone gets approved for use as a gender manipulation aid for tilapia and that effort is made to extend the approval to other finfish species (e.g., rainbow trout) of interest to the North Central Region.
- Ensure that the efficacy studies on florfenicol and oxytetracycline dihydrate are completed so that an approval can be gained for control of mortality in freshwater-reared coolwater and warmwater finfish due to motile *Aeromonas* septicemia associated with *Aeromonas* species.
- Ensure that the private aquaculture sector needs in the North Central Region are met for a sedative or anesthetic that can be used for transport and handling with either a zero or short-term withdrawal period.
- Determine what spawning aids the private aquaculture industries need and provide that information to researchers for action so that those drugs can gain approvals.
- Determine what the remaining drug approval needs are in the North Central Region and ensure that this information is transferred to researchers for action.

PUBLICATIONS, MANUSCRIPTS, PAPERS PRESENTED, AND REPORTS

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

SUPPORT

YEAR	NCRAC-USDA FUNDING	OTHER SUPPORT					TOTAL SUPPORT
		UNIVER-SITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
2004-05	$9,000		$22,476	$46,295	$26,000	$94,771	$103,771
2005-06	$15,000		$17,500	$58,527	$21,500	$97,527	$112,527
2006-07	$20,000		$26,980	$52,855	$22,200	$102,035	$122,035
2007-08	$20,000		$32,980	$57,400	$22,200	$112,580	$132,580
2008-09	$25,000		$36,480	$50,000	$22,700	$109,180	$134,180
TOTAL	$89,000		$136,416	$265,077	$92,900	$494,893	$605,093

NUTRITION/DIETS[10]

Project ***Termination Report*** for the Period
September 1, 2007 to August 31, 2009

NCRAC FUNDING: $80,000 (September 1, 2007 to August 31, 2009)

PARTICIPANTS:

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

REASON FOR TERMINATION

The objectives for this project were completed.

PROJECT OBJECTIVES

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

PRINCIPAL ACCOMPLISHMENTS

Objective 1a

Apparent digestibility of dry matter and energy, and availability of amino acids from blood meal (BM), fish meal (FM), porcine meat and bone meal (MBM), poultry byproduct meal (PBM), soybean meal (SBM), corn gluten meal (CGM), and corn, wheat, and yellow grease (YG) were determined for juvenile bluegill *Lepomis macrochirus* (mean weight, ~57 g [2.01 oz]). Apparent dry matter digestibility values ranged from 50% (corn) to 87% (BM). Apparent energy digestibility values ranged from 53% (corn) to 92% (BM) for bluegill. Apparent digestibility of most amino acids exceeded 90% for evaluated protein sources, except for MBM which showed slightly lower values (80–90%). Isoleucine digestibility from BM was relatively low (82%). High digestibility values for SBM, PBM, and BM indicate good potential for replacing FM in the diets of bluegill.

Objective 1b

Four randomly selected wild-caught juvenile bluegills (31.2 ± 16.4 g [1.10 ± 0.58 oz], mean weight ± SD) were sampled to determine amino acid compositions of whole-body tissue. Amino acid ratio for 10 essential amino acids were found to be 2.53 (arginine), 0.88 (histidine), 2.08 (isoleucine), 3.25 (leucine), 3.19 (lysine), 1.30 (methionine), 2.10 (phenylalanine),

[10]NCRAC has funded two Nutrition/Diets projects. The Termination Report for the first project is contained in the 1997-98 Annual Progress Report. This Termination Report is for the second Nutrition/Diets project which was chaired by Robert S. Hayward. It was a 2-year project that began September 1, 2007.

1.85 (threonine), 0.52 (tryptophan), 2.51 (valine). Except for leucine, contents of all other amino acids were lower than lysine. This ratio was used for determining essential amino acid requirements (EAAs).

Objective 1c

Two, 60-day experiments were conducted to determine: (1) lysine requirement of juvenile bluegill based on the dose-response method, (2) requirements for other EAAs using whole-body amino acid profile, and (3) whether differences in growth rates of group-versus individually-housed bluegills led to different lysine requirement levels due to the presence and absence, respectively, of bluegill social hierarchies. Seven experimental diets (isonitrogenous, isocaloric) were prepared to contain graded levels of digestible lysine (1.0–3.1 %). Experiment-1 involved group-housed bluegills (~27 g [0.95 oz], $N = 10$ fish/chamber, 4 chambers/diet) whereas experiment-2 involved individually-housed bluegills (~30 g [1.05 oz], $N = 1$ fish/chamber, 14 chambers/diet). Fish were fed twice daily to apparent satiation. Bluegill growth responses in both experiments generally improved ($P < 0.05$, ANOVA) with increasing dietary lysine levels from 1.0 to 1.6 %, and then leveled off with further increase in lysine level ($P > 0.05$). Optimal dietary lysine level (digestible basis) was calculated to be 1.5% based on broken-line regression analyses of specific growth rate and feed conversion ratio (FCR) with no differences observed between the two rearing methods. Determined dietary requirement levels for other EAAs ranged from 0.24% (trypophan) to 1.53% (leucine).

Objective 1d

Two, 60-day experiments were conducted to determine optimal levels of protein and energy in the diets of juvenile bluegill. In experiment-1, eight experimental diets were formulated to contain digestible protein levels ranging from 35–49% by 2% increments. Dietary energy level was maintained at 3,800 kcal/kg (digestible basis) across diets. Four bluegill groups (~20 g [0.71 oz], $N = 10$ fish/group) were fed the experimental diets twice daily to apparent satiation. Feed consumption, specific growth rate (SGR), and protein efficiency ratio (PER) were significantly affected by dietary protein levels ($P < 0.05$, ANOVA). Fish fed the diets containing 49% protein exhibited higher feed consumption and SGR than those fed the diets containing 35% and 37% protein. However, fish fed the diets containing 35% and 39% dietary protein levels showed better PER than those fed the diets containing 47% and 49% dietary protein levels ($P < 0.05$, ANOVA). No differences were observed among dietary groups for feed conversion ratio, hepato-somatic index (HSI), viscera-somatic index, or whole-body composition ($P \geq 0.05$, ANOVA). Optimal dietary protein level (digestible basis) was calculated to be 41.3% based on broken-line regression analysis of SGR.

In experiment-2, seven experimental diets were prepared to contain dietary energy levels (digestible basis) ranging from 3,000 kcal/kg–4,200 kcal/kg with digestible protein levels fixed at 41% across diets. Four bluegill groups (~21 g [0.74 oz], $N = 10$ fish/group) were fed experimental diets twice daily to apparent satiation for 60 days. No significant differences were detected among fish groups for feed consumption, SGR, or FCR ($P > 0.05$, ANOVA). However, HSI values and whole body fat content were significantly affected by dietary energy levels with fish fed diets containing <3,600 kcal/kg showing significantly less fat deposition than those fed diets containing ≥4,000 kcal/kg. Diets containing 3,500 kcal/kg produced the maximum SGR. Results of both experiments indicated that bluegills require 41% digestible energy and 3,500 kcal/kg digestible energy in diets for optimal growth performance.

Least-Cost Diet Formulation for Juvenile Bluegill

A 60-day study was conducted to determine the least-cost diet formulation for bluegill based on the ingredients tested for digestibility and nutrient requirement levels from previous experiments. Current market price of the ingredients and the digestible nutrient levels determined for each of the tested ingredients were provided in the software program WUFFDA (Windows-based User Friendly Feed Formulation). A total of seven experimental diets were prepared for evaluation. Diets 1 through 6 were computer-formulated by gradually replacing fish meal with a protein blend; ingredients for the protein blend were determined by the software for the cheapest possible formulation while satisfying the specified minimum nutrient requirement level and the fish meal level. FM inclusion levels in experimental diets 1 through 6 were 55% (diet 1), 40% (diet 2), 30% (diet 3), 20% (diet 4), 10% (diet 5), and 0% (diet 6), respectively. Digestible energy level was maintained at 3,500 kcal/kg and the digestible protein level was maintained at 41% across the six diets. Adequate levels of EAAs were maintained in all the diets. Nutrient levels for vitamins and minerals were maintained at levels generally recommend for fish by the National Research Council in 1993. Ingredient costs of the six diets varied from $865/metric ton ($785/ton) (diet 1) to $527/metric ton ($478/ton) (diet 6). To further reduce the feed cost; diet 7 was prepared by reducing the protein level from 41% to 40% and the energy level from 3,500 kcal /kg to 3,333 kcal/kg, thereby reducing the ingredient cost from $527/metric ton ($478/ton) (0% FM) to $480/metric ton ($435/ton) (0% FM). Protein sources included in the seven experimental diets were FM, PBM, MBM, BM, SBM, and CGM.

The feed formulation program selected MBM, SM, and CGM as the primary protein sources in the protein blend as the FM level was gradually reduced. Three commercial diets (Silver Cup Fish Feed, Nelson & Sons, Inc., Utah) were included in the study (control diets): high-energy trout diet (diet 8: 46% protein, 16% fat, 4,325 kcal digestible energy/kg); low-energy trout diet (diet 9: 42% protein, 12% fat, 3,550 kcal digestible energy/kg); and catfish diet (diet 10: 36% protein, 7% fat, 3,450 kcal digestible energy/kg). Four bluegill groups (~22 g [0.78 oz], N = 10 fish/group) were fed the experimental diets twice daily to apparent satiation. Response variables, SGR, feed consumption, FCR, HSI, viscerosomatic index (VSI), and whole-body composition (moisture, crude lipid, crude protein and crude ash) were determined to evaluate the experimental and commercial diets. Cost of the experimental diets was calculated by multiplying the ingredient cost involved in each of the diets and the amount of diet consumed by the fish group.

Among fish fed computer-formulated diets 1 through 7, no significant differences were observed in SGR, feed consumption, FCR, HSI, and VSI. However, fish fed diets 1 and 2 produced higher body fat and showed lower moisture content than fish fed diets 3 through 7 ($P < 0.05$, ANOVA). No significant differences were observed among the fish in terms of whole-body protein and ash contents. Fish fed diets 1 and 2 consumed slightly higher levels of diet than fish fed the other experimental diets which may have caused higher body fat deposition for those fish.

Comparison of fish fed the experimental diets versus those fed the industry standard diets showed fish fed the catfish feed (diet 10) to have a poorer SGR than those fed diets 1 through 3, and diet 8. Feed consumption was higher for fish fed experimental diets 1 through 7 than for the fish fed diet 10. However, fish fed diets 3 through 7 showed higher FCR than the fish fed diet 8. Similarly, fish diets 3 and 5 showed higher FCR than fish fed diet 10. Fish fed diets 8 and 9 produced significantly higher HSIs than the fish fed diets 4, 5, and 6. Similarly, fish fed diet 10 produced a higher HSI than those fed diets 2 through 6. Fish fed diet 8 produced a higher VSI value than fish fed diets 4 and 5. Similarly, fish group fed diets 8 and 9 showed higher whole-body-lipid deposition than the fish fed diets 3 through 7.

Therefore, fish fed two of the commercial trout feeds (diets 8 and 9) gained body weight more through fat deposition than protein deposition. Higher energy levels in the trout feeds versus the optimal dietary energy level determined in the present study likely caused the increased body-fat deposition. Also, crude lipid level in the experimental diets were maintained at ≤8.3%, whereas estimated lipid levels in trout diets 8 and 9 were 13.7% and 9.7%, respectively.

Fish fed diets 6 and 7 showed significantly higher gain to cost ratios than fish fed diets 1 through 5, while no differences were observed in this ratio between fish fed diets 6 and those fed diet 7. Comparison of ingredient costs of diets 6 and 7 to that of diet 1 showed the cost of diet 6 to be 39% lower, whereas the ingredient cost of diet 7 was 45% lower than the cost of diet 1. However, fish fed diet 7 (6.7%) showed slightly higher whole-body lipid deposition than fish fed diet 6 (7.3%). While the feed cost of diet 6 was ~40% below that of diet 1, diet 6 also reduced whole-body lipid deposition by 25%, 33%, and 27% versus diets 1, 8, and 9, respectively. Cost comparisons against the commercial diets could not be made as the dietary formulations of trout and catfish diets are not reported. Based on these study results diet 6 is recommended as the least-cost diet for bluegill. Formulation of diet 6 is given in Table 1 of the Appendix to this termination report.

IMPACTS

Fish producers have often used trout and catfish diets when rearing bluegill. However, trout diets, as demonstrated in the present study, cause high body-fat deposition in bluegill whereas catfish diets substantially reduce bluegill growth rates. Profit margins, particularly for bluegill producers aiming to rear larger, food-size bluegill, have undoubtedly been negatively impacted in many cases by feeding high-cost trout diets to bluegills to achieve rapid growth. The present study has developed a fish-meal-free diet based on locally available, low-cost ingredients. This diet's ingredient costs are not only ~32% cheaper versus a control diet (55% fish meal diet), but also satisfy optimal dietary nutrient requirements as determined in the present study for juvenile bluegill. The newly developed least-cost diet also produced a ~30% reduction in the whole-body fat deposition compared to the trout diets. Reducing body fat deposition while maintaining desirable fish growth rates (similar to those produced by industry standard diets) should lead to higher fillet yields.

RECOMMENDED FOLLOW-UP ACTIVITIES

The identification of a new least-cost diet for bluegills through laboratory studies still need to be tested under field conditions with production scales used by regional producers. It is anticipated that these fish reared in ponds will not only feed on the provided diets but also on natural feed stuffs including aquatic invertebrates. The combination of a commercial diet with these natural feedstuffs may result in different production parameters. Previous NCRAC-funded projects have revealed the importance of these natural feedstuffs to bluegill reared in ponds.

SUPPORT

NCRAC provided $80,000 to the University of Missouri-Columbia which was the entire amount of funding allocated for this project.

PUBLICATIONS, MANUSCRIPTS, WORKSHOPS, AND CONFERENCES

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

APPENDIX

Table 1. Formulation of a least-cost diet developed for juvenile bluegill.

Ingredients[Source]	Price*		Amount (percent by weight)
	$/metric ton	$/ton	
Porcine meat and bone meal[1]	270.07	245.00	38.01
Soybean meal[2]	336.21	305.00	36.99
Corn gluten meal[3]	518.09	470.00	15.29
Corn[4]	142.45	129.23	3.60
Fish Oil[5]	1,477.10	1,340.00	4.00
Lecithin[6]	4,188.79	3,800.00	0.30
Dicalcium phosphate	11,023.12	10,000.00	0.20
Vitamin premix[7]	11,023.12	10,000.00	1.00
Vitamin C	1,543.24	1,400.00	0.07
Choline chloride	1,543.24	1,400.00	0.14
Mineral mix[7]	1,543.24	1,400.00	0.10
Binder[8]	2,314.86	2,100.00	0.30
			100.00

*Prices in $/ton of porcine meat and bone meal, soybean meal, corn gluten meal, and corn are average values for the price of those ingredients for the first week of every month from the period January 2008 to December 2009 as reported in the weekly newspaper "Feedstuffs." The prices in $/ton of other ingredients are those charged by the respective sources when this diet was prepared.

[1]American Midwest Distributors, LLC, Kansas City, Missouri.
[2]ADM Soybean Meal Plant, Mexico, Missouri.
[3]Grain Processing Corporation, Muscatine, Iowa.
[4]Bourn Feed, Columbia, Missouri.
[5]Refined Menhaden Oil (Virginia Prime Gold), Omega Protein, Inc., Houston, Texas.
[6]Archer Daniels Midland Company, Decatur, Illinois.
[7]Nelson's Silvercup Fish Feed, Nelson & Sons, Inc., Murray, Utah.
[8]Ultra-Bond™, Uniscope, Inc., Johnstown, Colorado.

SNAIL MANAGEMENT/GRUB CONTROL[11]

Project ***Progress Report*** for the Period
September 1, 2007 to August 31, 2009

NCRAC FUNDING: $114,138 (September 1, 2007 to August 31, 2009)

PARTICIPANTS:

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

PROJECT OBJECTIVES

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

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

ANTICIPATED BENEFITS

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

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

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

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

PROGESS AND PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

University of Wisconsin-Stevens Point

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

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

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

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

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

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

Planorbella snails from both fish farms were also infected with the digenean trematode, *Echinostoma* sp. (likely *Echinostoma trivolvis*). *Planorbella* were infected with *Echinostoma* stages, and these snails served both as first intermediate hosts (possessing redial stages inside the ovotestis), and as second intermediate hosts (with metacercarial stages in the snail pericardial region).

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

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

In 2009, monthly monitoring continued from May through September at the treatment (crayfish added) and control ponds (no crayfish) at each of the studied fish farms. No additional crayfish were added to the treatment ponds during the 2009 field season. As in 2008, crayfish were sampled with baited minnow traps left in each pond for 24 h. The number of crayfish caught per trap over a 24-h period during the months sampled in 2009 averaged 0.58 crayfish per trap per 24 h at AquaPoint treatment ponds while averaging 0.71 at Zelinski's treatment ponds. No crayfish were recovered in the control ponds at each of these facilities. Relatively small (<25 mm [1 in] total length) juvenile crayfish were recovered during the summer trapping at treatment ponds at both facilities, implying that successful crayfish reproduction and recruitment had occurred at all treatment ponds since the 2008 crayfish stocking.

In 2009, monthly snail and fish sampling were conducted as described in the 2008 sampling protocol (above). In terms of snail densities, as in 2008, *Planorbella* densities increased during the spring and summer at both treatment and control ponds at both facilities. By September, *Planorbella* densities at AquaPoint averaged 36 snails per sampling event (dredges and sampling frames combined) at treatment ponds while the average across control ponds was 31.5. *Planorbella* were generally denser at both treatment and control ponds at Zelinski's facility. By September, *Planorbella* densities at Zelinski's averaged 67.5 per sampling event while the average across control ponds was 97.5.

Yellow grub (*Clinostomum*) metacercarial infections in yellow perch were measured monthly from May through September of 2009, and prevalence (% of hosts infected with at least one grub) and average intensity (average number of grubs per infected fish) were calculated for ponds at Zelinski's and AquaPoint. Specifically, averaged over May through September, yellow grub prevalence in perch at AquaPoint was 53.7% at treatment ponds while it measured 61.6% at control ponds. Average intensity of yellow grub infections in perch over the same time period was 7.67 grubs per infected fish at AquaPoint treatment ponds and 9.25 grubs per infected fish at the AquaPoint control ponds. At Zelinski's, yellow grub prevalence averaged over May through September was 68.0% at treatment ponds while it measured 74.4% at control ponds. Similarly, average intensity of yellow grub infections in perch over the same time period was 3.71 grubs per infected fish at Zelinski's treatment ponds and 4.03 grubs per infected fish at the Zelinski's control ponds.

For all yellow perch sampled in 2008 and 2009, location of the grub within the fish (operculum, musculature, caudal fin region) was noted for each *Clinostomum* infection encountered. Additionally, data on grub size (length) was also recorded. These data will be used for a comprehensive analysis of distribution of grub infections within a host, and how grub distribution and size relate to site of infection in a fish, size of fish, mass of fish, treatment or control pond, and other measured host variables. The results of this analysis will be provided in the final report.

During 2009 researchers also attempted to test the efficacy of the competitively dominant digenean trematode, *Echinostoma*, at reducing grub infections in other snails and ultimately fish in the treatment ponds. Researchers secured at total of eight 0.019-ha (0.046-acre) ponds at the Hess fish farm facility in New London, Wisconsin. Four ponds were chosen as control ponds and four were chosen as treatment ponds. In May 2009, each of the eight ponds at Hess' facility received 500 yellow perch (provided by Zelinski's Fish Farm). A total of 25 fish from each of the eight ponds (i.e., 200 total fish) were sampled monthly and assessed for grub infections as described above. Snail size frequencies and densities were assessed monthly in the same manner as for AquaPoint and Zelinski's facilities.

To attempt to culture echinostome worms for the production of eggs, researchers dissected *Planorbella* snails naturally infected with echinostome metacercarial cysts. These snails were collected from Blue Iris Fish Farm in Black Creek, Wisconsin. Echinostome metacercarial cysts were removed from these snails and the isolated cysts (~25 cysts per animal) were then orally inserted into to hamsters (15 animals), mice (12 animals), and grasshopper mice (12 animals). Fecal material was then monitored for echinostome eggs using standard ova sedimentation protocols beginning at 2 weeks post-exposure. Unfortunately, while patent infections were easily achieved in hamsters in 2007, no patent infections (i.e., eggs appearing in feces) were achieved in the above described mammals in 2009. Therefore, researchers expanded their infection attempts to mallard ducklings and these were marginally successful using these same techniques. A total of 15 ducklings under 1 week of age were given ~25 echinostome cysts each. As above, the duckling fecal material was then monitored for echinostome eggs, beginning at 2 weeks post-infection. Echinostome eggs first appeared in the fecal material 4 weeks after exposure, and by 5 weeks, egg production of all 15 ducks combined was about 500 eggs/day. By the 6th week, however, these production levels had dropped to less than 300 eggs/day, and by the 7th week, eggs were no longer detected in the fecal material. All eggs produced were distributed equally among the treatment ponds, with each pond receiving roughly 420 echinostome eggs between August 5-17, 2009.

<u>Southern Illinois University-Carbondale (SIUC)</u>

Laboratory trials were conducted at SIUC to evaluate the potential of freshwater prawn (*Macrobrachium rosenbergii*) and two hybrid sunfishes (redear sunfish × green sunfish [*Lepomis micolophus* × *L. cyanellus*] and redear sunfish × warmouth [*L. gulosus*]) to serve as biological control agents for *Physa* spp. and *Helisoma* spp. Maximum consumption rates and maximum handling sizes for each of these species or hybrids feeding on *Physa* and *Helisoma* were compared to those of redear sunfish, one of the most common molluscivores native to the NCR. Ten individuals of each species or hybrid were placed individually into 37.8-L (10-gal) aquaria, not fed for 24 h, and then exposed to known sizes and numbers of *Physa* and *Helisoma* for 48 h; snail sizes represented the full size range commonly found in aquaculture ponds. Species or hybrids that consumed ≥50% of snails in the first set of laboratory trials were used in subsequent trials that assessed maximum snail handling sizes. Ten individuals of each species or hybrid were held individually in 37.8-L (10.0-gal) aquaria, starved for 24 h, and then offered one snail from each 1-mm size increment over the full size range of snails commonly found in ponds (3.0–12.0 mm [0.12–0.47 in] for *Physa* and 3.0–18.0 mm [0.12–0.7 in] for *Helisoma*). Uneaten snails were counted and measured 48 h later to identify sizes of snails that were consumed. Maximum consumption rates for each species or hybrid feeding on *Physa* and *Helisoma* were also determined over a set of 5-day trials in which individually-housed prawns, redear sunfish, or hybrid sunfishes were fed known numbers and sizes of snails *ad libitium* daily. Numbers and sizes of snails consumed were determined daily and mean maximum daily consumption rates were calculated for each species or hybrid.

Redear × warmouth hybrids consumed larger snails than redear sunfish of equivalent body length, but consumed 25% fewer snails on average than redear sunfish. While redear × warmouth hybrids have a larger mouth gape than redear sunfish for a given body size, they do not appear to be sufficiently voracious at consuming snails to represent a significant improvement over redear sunfish as a biological control agent. Freshwater prawn (65.0–85.0 mm [2.6–3.3 in] carapace length) consumed *Physa* up to 12.0 mm (0.5 in) total length and *Helisoma* up to 16.0 mm (0.6 in) total length. However, freshwater prawns showed a strong preference for consuming *Physa* over

Helisoma; prawns consumed 77% of *Physa* offered in maximum consumption trials but consumed only 20% of *Helisoma* offered. Consumption rates for smaller freshwater prawn feeding on snails were not determined but would likely be considerably lower than those of the harvest-size prawns that were used in laboratory trials. Redear × green sunfish hybrids (120.0–140.0 mm [4.7–5.5 in] total length) consumed *Physa* and *Helisoma* up to 12.0-mm (0.5 in) total length; redear sunfish in this size range only consumed snails <10 mm total length. Maximum consumption rates of redear × green sunfish hybrids were equivalent to those of similar-size redear sunfish.

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

Pond trials were conducted to evaluate the effectiveness of hydrated lime for controlling snails in research ponds at SIUC. Enclosures were placed in shallow water (0.3 m [1.0 ft] depth) in four ponds and stocked with snails (N = 35 each) obtained from ponds at SIUC. Two ponds contained three enclosures each that served as controls. Two other ponds were treated with hydrated lime (1.07 kg/m^2; 0.22 lb/ft^2) along the pond edge, including enclosures containing known numbers of snails. Mean snail survival rate in control ponds was 89%, but was only 2% in ponds treated with hydrated lime.

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

Pond trials evaluating the relative effectiveness of biological, chemical, and integrated biological/chemical controls of snail populations were conducted during June through October 2009. Redear sunfish and redear × green sunfish were used as biological control agents and hydrated lime was used as the chemical treatment based on the results of laboratory and pond trials conducted during Year 1. Sixteen ponds at SIUC were used for these pond trials (*N* = 4 ponds each for biological, chemical, biological and chemical combined, and control treatments). Triploid grass carp (*Ctenopharyngodon idella*) were stocked to provide vegetation control. Effectiveness of the snail control treatments (including controls) was assessed under production conditions using hybrid striped bass as a sentinel species. Snail population densities and size structure were determined prior to stocking fish and at monthly intervals thereafter. Prevalence of grub infestation in hybrid striped bass was assessed for each treatment.

OBJECTIVE 2

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

WORK PLANNED

UW-Stevens Point

Researchers are currently analyzing data from 2008 and 2009 on crayfish densities among ponds, effects of crayfish on snail density and size frequency distributions, and grub prevalence and intensity in fish. The results of these analyses will determine the efficacy of using crayfish to control grub infections in aquaculture fishes. The data, albeit limited, on echinostome infectivity in duckling hosts will be used to produce new infections in ducklings and ducks next spring, and researchers will attempt echinostome egg introduction at Hess' ponds in the spring and summer of 2010. Researchers will also continue to collect data on snail density, size frequency, and grub prevalence and intensity in fish at these ponds.

SIUC

Data analysis for pond trials conducted during 2009 is ongoing to assess effectiveness of snail control treatments and to relate snail densities to grub infestation rates in hybrid striped bass. A graduate student working on this project will be completing their thesis and develop a manuscript reporting on the results of the 2009 pond trials.

Iowa State University

In 2010 the completed database on snail control will be shared with all project investigators to insure that the information is complete. Additional information garnered from the ongoing research will be included. Following project review of this database, a Web page will then be developed and placed on the NCRAC Web site.

IMPACTS

Project results will provide valuable information regarding the effectiveness and efficiency of several potentially useful approaches for controlling snail populations and associated grub infestations in aquaculture ponds in the NCR. Previously untested treatments for snail control in ponds (the crayfish *Orconectes virilis*, freshwater prawn, hybrid sunfishes, biocontrol with natural dominant trematodes, and integrated chemical and biological controls) are being evaluated.

Results will provide insight into the degree of snail population control required to limit grub prevalence in cultured fishes and are anticipated to provide improved and more universally applicable approaches for controlling digenean trematodes and managing snail populations in ponds where food fish are raised.

SUPPORT

NCRAC funds provided to date total $225,000. 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 Snail Management/Grub Control activities.

VIRAL HEMORRHAGIC SEPTICEMIA (VHS)[12]

Project ***Progress Report*** for the Period
September 1, 2008 to August 31, 2009

NCRAC FUNDING: $116,870 (September 1, 2008 to August 31, 2009)

PARTICIPANTS:

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Glenda D. Dvorak	Iowa State University	Iowa
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Industry Advisory Council Liaison:		
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PROJECT OBJECTIVES

(1) Determine the safety and efficacy of iodine disinfection on walleye and northern pike eggs infected with VHS.

(2) Prepare and electronically disseminate a VHS "response" packet that specifically targets fish farm producers. The packet will address aspects of the disease (clinical signs, routes of transmission) and prevention practices to minimize introduction and spread. The packet will also contain Web sites and information sources where fish farmers can obtain the most current, up-to-date status of the disease.

(3) Conduct a series of six biosecurity workshops held at different fish farms across the region, targeting different production systems (flow-through, pond, and recirculation systems).

(4) Utilize the existing Aquatic Invasive Species (AIS) Hazard Analysis Critical Control Point (HACCP) Training Curriculum to develop <u>specific</u> fish disease HACCP plans for each of the six facilities involved in the workshops.

(5) Develop and distribute three <u>model</u> fish disease HACCP plans (one each for flow-through, pond, and recirculation systems), relying heavily on the <u>specific</u> plans developed under Objective 4.

(6) Produce a fish farm biosecurity video that incorporates different system types and footage shot at the workshops and distribute this video to end users via DVD and internet streaming videos.

ANTICIPATED BENEFITS

Diseases constitute the largest single cause of economic losses in aquaculture. There are few treatments available for current and emerging aquaculture diseases. This research on egg disinfection will provide valuable information to commercial and public fish culture facilities to make decisions on the safety and efficacy of iodine treatment to eliminate VHS infections from coolwater and warm water fish eggs. If iodophor disinfection can be used to

[12]This 2-year project is chaired by Jeffrey A. Malison and it began September 1, 2008.

safely eliminate VHS virus (VHSv) from eggs, the direct benefits will include: (1) reduction in the risk of movement of VHSv between aquaculture facilities during embryo transfer; (2) potential reduction in restrictions enacted by regulatory agencies on intra- and inter-border egg shipments; (3) maintenance or enhancement of commercial egg production by production of disease free eggs; and (4) ability to maintain genetic diversity of hatchery populations (and thus stocked fish) by supporting the collection (and disinfection) of wild brood fish.

The development of methods for treating fish diseases is greatly needed and disease prevention remains the most important and useful strategy for minimizing disease on fish farms. These projects are proposed to develop an integrated set of educational materials and conduct outreach projects targeted to fish farms and farmers in the North Central Region (NCR) to help protect the region's fish farms by providing farmers with tools and key information needed to help prevent the spread of VHS and other fish diseases onto farms, between farms, and from farms into natural waters.

The proposed use of the AIS-HACCP approach has many advantages. It can effectively deal with a diverse industry and diverse risk factors associated with a variety of plant, invertebrate, vertebrate, and pathogen AIS. If it develops as it has in the seafood industry, it should prove to be a good partnership between industry and government regulators. It can help avoid overly restrictive regulations, and, if properly applied, can be effective at reducing the risk of spreading AIS via baitfish harvest and aquaculture practices. The HACCP approach concentrates on the points in the process that are critical to the environmental safety of the product, minimizes risks, and stresses communication between regulators and the industry. With proper cooperation between industry representatives, resource management agencies, and other AIS experts, the AIS-HACCP approach will reduce the risk that AIS will be established in new locations while maintaining the economic viability of the baitfish and aquaculture industries. It can provide a mechanism for AIS-free certification, and it can instill confidence in the public that state and federal fish stocking programs are conducting their activities in an environmentally responsible manner.

PROGESS AND PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

Adult walleye and northern pike were collected from the Mississippi River (Pool 9) and spawned at the Upper Midwest Environmental Sciences Center (UMESC) by personnel from the U.S. Fish and Wildlife Service (USFWS) Genoa National Fish Hatchery. Immediately after sperm activation, fertilized eggs were taken to a controlled access laboratory with effluent disinfection where egg challenge, disinfection, and incubation activities occurred. Immediately on entry into the laboratory, eggs were challenged at either 10^5 or 10^8 plaque-forming units/mL (PFU/mL) for 30 min. The virus used for this study was isolated by the USFWS La Crosse Fish Health Center from emerald shiners *Notropis atherinoides* collected from Lake Erie in 2006. Eggs challenged at 10^5 PFU/mL were progeny of different male/female pairings than those challenged at 10^8 PFU/mL. Walleye egg adhesion was reduced by immersing the eggs in a bentonite solution for ~2 min during VHSv challenge. Immediately after challenge, eggs were assigned to one of the four treatment groups (Table 1 in the Appendix to this progress report).

Eggs were held in well water for at least 90 min post-fertilization before being distributed to miniature egg jars. Eggs were maintained in egg jars until hatch with no other chemical treatments applied. Egg and fry samples were collected and the presence or absence of VHSv determined using epithelioma papulosum cyprini (EPC) cells. Assays used for determining the presence of VHSv were conducted according to the USFWS Standard Procedures for Aquatic Animal Health Inspections/American Fisheries Society Fish Health Section Blue Book (2007) procedures.

Virus Isolation

VHSv was not isolated from any iodophor-disinfected treatment groups (Appendix Table 1, treatment groups 2-4). However, VHSv was isolated from control eggs (Appendix Table 2) immediately after challenge and for up to four days after challenge in northern pike eggs challenged at 10^8 PFU/mL. The virus was not detected in positive control eggs one day post-challenge for either northern pike or walleye eggs challenged at 10^5 PFU/mL nor was it detected in fry of either control or iodophor-disinfected treatment groups.

Percent Hatch

Though some iodophor treatments reduced hatch, eggs and fry appeared to develop normally. Iodophor disinfection did not substantially reduce northern pike egg hatch but walleye egg hatch was reduced when eggs were held for 30 or 60 min in the iodophor disinfection solution (Appendix Table 3).

Egg iodophor disinfection appears to effectively eliminate VHSv (strain IVb) from the surface of walleye and northern pike eggs. Although iodophor egg disinfection reduced walleye egg hatch in this study, previous UMESC toxicity studies indicated that when applied shortly after fertilization (~5 min), similar iodophor disinfection treatment regimens did not alter egg hatch. Incorporation of iodophor disinfection at 100 ppm during gamete collection from non-salmonid fishes immediately post-fertilization (<5 min) for 30 min or at 90 min after fertlization for 10 min may reduce VHSv (strain IVb) transmission without affecting egg hatch.

OBJECTIVE 2

The VHS “response” packet was developed in April 2009 by Iowa State Univesity. The packet is an 18-page pdf document containing information for aquaculture producers on the signs, susceptible species, and prevention of VHS. A “Biosecurity for Aquaculture Facilities” PowerPoint® presentation (36 slides with speaker notes) was also developed in April 2009. All of the materials have been forwarded to other project participants (Malison and Kinnunen) to be incorporated into the biosecurity workshop objective of this project (Objective 3). Additionally, these materials have been posted for download on the Center for Food Security and Public Health (CFSPH) Web site (http://www.cfsph.iastate.edu/DiseaseInfo/MoreInfo/VHS.htm) and the Focus on Fish Health Web site (www.focusonfishhealth.org). Since April 2009, when the materials were posted, 184 visitors accessed the Web page containing the VHS information. As far as downloads, the “response” packet had a total of 42 downloads, the “prevention” practices document had 59 downloads, and the supplemental checklist had 42 downloads. The Bbiosecurity PowerPoint® has had a total of 284 downloads.

OBJECTIVE 3

In 2009, three of the six planned VHS biosecurity workshops were conducted at aquaculture facilities in Indiana, Missouri, and Michigan. Michigan State University and University of Wisconsin Extension Aquaculture Specialists partnered with local and regional animal health professionals to present information on fish disease transmission, VHS, and HACCP planning specific to developing a biosecurity plan for aquaculture facilities. Details are as follows:

- May 14, Indiana – Bodin State Fish Hatchery (recirculating aquaculture system), 27 total in attendance.
- June 25, Missouri – Crystal Lakes Fisheries (flow through), 29 total in attendance.
- August 20, Michigan – Michigan Bait and Fish Farm (flow through), 24 total in attendance.

Evaluations of the workshops indicated that the participants found the information helpful (average score of 4.56 on a scale of 5), intended to use the information (average score 4.58), and felt the information was presented in an easy to understand format (average score 4.57). HACCP plans were developed for each of the hosting facilities with special emphasis on system type (pond, recirculating aquaculture system, or flow-through) and business activities (wild stocking, egg and fingerling production, or grow out for food). It was interesting to note that the initial skepticism of the participants was overcome by program emphasis on the economic consequences of disease introduction and the critical control point analysis that is the basis of a HACCP plan. This analysis provides the framework to make biosecurity decisions that are effective and economical.

OBJECTIVE 4

- The Bodin State Fish Hatchery already had a HACCP biosecurity plan in place. Comments were made to improve a few critical control points (visitor access and logs).
- Crystal Lakes Fisheries had their own biosecurity plan which was used as a basis for drawing up a HACCP biosecurity plan.
- Michigan Bait and Fish Farms already had a HACCP biosecurity plan in place from previous work with Michigan State University Sea Grant Extension.

OBJECTIVE 6

Video footage was shot at the three workshops held in 2009.

WORK PLANNED

OBJECTIVE 1

- Evaluate the use of iodophor disinfection to eliminate VHSv from yellow perch eggs intentionally challenged with VHSv. Perch eggs will be challenged using methods similar to those used for walleye and northern pike in Year 1 of the project.
- Evaluate the use of iodophor disinfection and VHSv retention on walleye eggs intentionally challenged with VHSv following deadhesion using tannic acid in lieu of bentonite clay.
- Evaluate vertical transmission of VHSv from adult fathead minnows to eggs. Adult fathead minnows will be exposed to VHSv by immersion challenge prior to spawning. Resulting eggs will be tested for the presence/absence of VHSv and selected egg groups will be disinfected with iodophor disinfection prior to hatch to evaluate the effectiveness of iodophor disinfection to eliminate VHSv from eggs of VHS-positive adults.

OBJECTIVE 3

The final three workshops are planned for 2009/2010 in Wisconsin, Ohio, and another site to be determined, possibly Minnesota or South Dakota.

OBJECTIVES 4 and 5
To be completed as described in the original proposal.

OBJECTIVE 6
The video will be completed and distributed as described in the original proposal. Also, following the completion of the biosecurity workshop videos and model HACCP plans, these materials will be posted by Iowa State University for free access on the CFSPH and Focus on Fish Health Web sites.

IMPACTS

- The project demonstrated that coolwater fish eggs retain VHSv for up to 4 days following immersion challenge but that eggs may not retain VHSv through egg hatch (all fry, including controls, were VHSv negative).
- The project demonstrated that iodophor disinfection may safely and effectively reduce the risk associated with VHSv exposure during spawning/egg take operations from wild brood fish.
- A U.S. Geological Survey Fact Sheet is in preparation which summarizes the present research. The Fact Sheet is expected to be available before the 2010 spawning season.
- To date, there have been no reports of VHS having been found in any NCR fish farm or hatchery, nor is there any evidence suggesting that VHS has been spread via fish movements into or out of any fish farms.
- The majority of the attendees at the workshops indicated that they would implement biosecurity/AIS-HACCP plans at their facilities based on the information learned at the workshops.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

See the Appendix.

SUPPORT

YEAR	NCRAC-USDA FUNDING	OTHER SUPPORT					TOTAL SUPPORT
		UNIVER-SITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
2008-09	$116,870			$23,422	$3,900	$27,322	$144,192
TOTAL	$116,870			$23,422	$3,900	$27,322	$144,192

APPENDIX

Table 1. Iodophor disinfection treatment groups. Disinfection was initiated immediately after viral challenge except that disinfection of Treatment group 4 was initiated 60 minutes after challenge.

Treatment group	Iodophor disinfection	Time initiated (min post-fertilization)	Disinfection duration (min)	Iodophor concentration (ppm)
1	No	NA	NA	0
2	Yes	~30	30	100
3	Yes	~30	60	100
4	Yes	~90	10	100

Table 2. Detection of VHSv (# positive samples/# samples tested) in northern pike and walleye positive control eggs and fry.

Day post challenge	Northern pike		Walleye	
	10^5 PFU/mL	10^8 PFU/mL	10^5 PFU/mL	10^8 PFU/mL
Day 0a	3/3	3/3	2/3	3/3
Day 0b	2/3	3/3	2/3	3/3
Day 1	0/6	6/6	0/6	2/6
Day 2	0/6	6/6	NT	NT
Day 3	0/6	6/6	NT	NT
Day 4	0/6	4/6	NT	NT
Day 5	0/6	0/6	NT	NT
Fry	0/6	0/6	0/6	0/6

a-eggs tested during challenge
b-eggs tested after water hardening
NT-Not tested

Table 3. Percent hatch of northern pike and walleye eggs.

Treatment Group	Percent Hatch			
	Northern pike		Walleye	
	10^5 PFU/mL	10^8 PFU/mL	10^5 PFU/mL	10^8 PFU/mL
1	61	65	44	38
2	53	67	56	5
3	49	67	19	0
4	61	69	54	43

APPENDIX

AQUACULTURE DRUGS

Publications in Print

Barry, T.P., A. Marwah, and P. Marwah. 2007. Stability of 17α-methyltestosterone in fish feed. Aquaculture 271:523-529.

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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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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.

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

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

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

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

North Central Regional Aquaculture Center Hybrid Striped Bass Workshop, Champaign, Illinois, November 2-4, 1995. (Christopher 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)

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

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

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

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

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

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

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

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

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

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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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SOME COMMONLY USED ABBREVIATIONS AND ACRONYMS

×	cross, by, or times
AIS	aquatic invasive species
APHIS	Animal and Plant Health Inspection Service
AquaNIC	Aquaculture Network Information Center
BM	blood meal
BOD	Board of Directors
BSN	brine shrimp nauplii
°C	degrees Celsius
CES	Cooperative Extension Service
CGM	corn gluten meal
CSREES	Cooperative State Research, Education, and Extension Service
CVM	Center for Veterinary Medicine
dph	day(s) post hatch
EAA	essential amino acid
EPC	epithelioma papulosum cyprini
°F	degrees Fahrenheit
FCR	feed conversion rate/ratio
FDA	Food and Drug Administration
FM	fish meal
ft, ft^2, ft^3	foot, square foot, cubic foot
FY	fiscal year
g	gram(s)
gal	gallon(s)
gpm	gallons per minute
GTW	green tank water
h	hour(s)
ha	hectare(s)
HACCP	Hazard Analysis and Critical Control Point
HSI	hepatosomatic index
IAC	Industry Advisory Council
in	inch(es)
INAD	Investigational New Animal Drug
ISU	Iowa State University
KAA	Kansas State University
kcal	kilocalorie
kg	kilogram(s)
L	liter(s)
lb	pound(s)
LDL	Little Dixie Lake
LU	Lincoln University
m, m^2, m^3	meter(s), square meter, cubic meter
MAI	motile *Aeromonas* infection
MAS	motile *Aeromonas* septicemia
MBM	meat and bone meal
mg	milligram(s)
min	minute(s)
ml	milliliter(s)
mm	millimeter(s)
MSU	Michigan State University
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
OCARD	Ohio Center for Aquaculture Research and Development
oz	ounce(s)
P	probability
PAH	Phibro Animal Health
PBM	poultry byproduct meal
PFU	plaque-forming units
POW	Plan of Work
ppm, ppt	parts per million, parts per trillion
Purdue	Purdue University
®	registered
RAC(s)	Regional Aquaculture Center(s)
RAES	Regional Aquaculture Extension Specialist
RAET	Regional Aquaculture Extension Team
SBM	soybean meal
sec	second(s)
SGR	specific growth rate
SIUC	Southern Illinois University-Carbondale
SPAH	Schering-Plough Animal Health
TAN	total ammonia nitrogen
TC	Technical Committee (TC/E = Technical Committee/Extension; TC/R =Technical Committee/Research
TL	total length
™	trademark
TSA	Tryptic Soy Agar
UM-C	University of Missouri-Columbia
UMESC	Upper Midwest Environmental Sciences Center
USDA	U.S. Department of Agriculture
USFWS	U.S. Fish and Wildlife Service
USTFA	U.S. Trout Farmers Association
UW-Stevens Point	University of Wisconsin-Stevens Point
UWSVM	University of Wisconsin School of Veterinary Medicine
UW-Madison	University of Wisconsin-Madison
UW-Milwaukee	University of Wisconsin-Milwaukee
VSI	viscerosomatic index
VHS	viral hemorrhagic septicemia
VHSv	viral hemorrhagic septicemia virus
WATER	Wisconsin Aquatic Technology and Environmental Research
Wr	relative weight
YG	yellow grease