

**NORTH CENTRAL
REGIONAL AQUACULTURE CENTER**



ANNUAL PROGRESS REPORT 2009-10

JANUARY 2011

ANNUAL PROGRESS REPORT

For the Period
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NORTH CENTRAL REGIONAL AQUACULTURE CENTER

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 \$937 million in 2008 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 2009, the U.S. imported \$21.8 billion of fisheries products and the trade deficit was \$4.7 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.

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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 the USDA National Institute of Food and Agriculture (NIFA; formerly the Cooperative State Research, Education and Extension Service) 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,

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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/NIFA/Grants Management Branch for approval. To date the Center has received 22 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), FY2008/09 (#2008-38500-19157), and FY2010 (#2010-38500-20929) with monies totaling \$16,952,351. Currently,

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four grants are active (FY06-10); the first 18 grants (FY88-05) 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, 2010, the Center has funded or is funding 89 projects through 487 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

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are Progress Reports for projects that were underway or completed during the period September 1, 2009 to August 31, 2010. Projects, or Project components, that terminated prior to September 1, 2009 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.

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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	
	9	9/1/08-8/31/10	\$150,000	2008-38500-19157	
			9/1/09-8/31/10	<u>\$27,880</u> \$677,858	2008-38500-19157
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	
			<u>\$88,003</u> \$261,973	2005-38500-18547	
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	<u>\$5,000</u> \$16,205	2005-38500-18547
	NCR Aquaculture Conference	1	6/1/90-3/31/91	\$7,000	90-38500-5008
		2	12/9/98-6/30/99	<u>\$3,000</u> \$10,000	96-38500-2631
	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	<u>\$50,000</u> \$352,904	2002-38500-11752	

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Extension ("Base" Extension—Project Nos. 1-13; Aquaculture Regional Extension Facilitator [AREF]—Project No. 14; and Regional Aquaculture Extension Specialist [RAES]—Project Nos. 15 and 16)	1	5/1/89-4/30/91	\$39,221	88-38500-3885
	2	3/17/90-8/31/91	\$37,089	89-38500-4319
	3	9/1/91-8/31/93	\$31,300	89-38500-4319
	4	9/1/93-8/31/95	\$94,109	91-38500-5900
	5	9/1/95-8/31/97	\$110,129	91-38500-5900
	6	9/1/97-8/31/99	\$10,813	92-38500-6916
	7	9/1/99-8/31/01	\$20,391	95-38500-1410
	8	9/1/01-8/31/03	\$38,000	97-38500-3957
	9	9/1/03-8/31/05	\$94,000	99-38500-7376
	10	9/1/05-8/31/07	\$28,500	99-38500-7376
	11	9/1/07-8/31/09	\$18,154	2001-38500-10369
	12	9/1/08-8/31/10	\$28,000	2002-38500-11752
	13	9/1/09-8/31/11	\$211,545	2003-38500-12995
	14	9/1/03-8/31/05	\$7,735	2005-38500-15847
	15	9/1/06-8/31/09	\$21,850	2006-38500-16900
	16	9/1/09-8/31/11	\$92,469	2007-38500-18469
		\$37,966	2007-38500-18469	
		\$22,539	2008-38500-19157	
		\$29,000	2008-38500-19157	
		\$100,000	2002-38500-11752	
		\$199,624	2004-38500-14269	
		\$150,000	2008-38500-19157	
		<u>\$1,422,434</u>		
Hybrid Striped Bass	1	5/1/89-8/31/91	\$68,296	88-38500-3885
	2	6/1/90-8/31/92	\$68,114	89-38500-4319
	3	9/1/91-8/31/93	\$101,000	90-38500-5008
	4	9/1/93-8/31/95	\$96,550	91-38500-5900
	5	9/1/95-8/31/97	\$168,000	93-38500-8392
	6	6/1/99-5/31/00	\$150,000	95-38500-1410
	7	9/1/01-5/31/04	\$15,000	96-38500-2631
		\$98,043	98-38500-5863	
		<u>\$211,957</u>	2001-38500-10369	
		\$976,960		
Largemouth Bass	1	9/1/05-8/31/07	\$170,000	2004-38500-14269
National Coordinator for Aquaculture INADs/NADAs	1	9/1/93-8/31/94	\$2,000	89-38500-4319
		5/15/95-5/14/96	\$5,000	94-38500-0048
		5/15/96-5/14/97	\$6,669	92-38500-6916
		5/15/97-5/14/98	\$3,331	95-38500-1410
		5/15/98-5/14/99	\$15,000	96-38500-2631
	2	5/15/99-5/14/00	\$13,241	94-38500-0048
		7/15/04-7/14/05	\$10,000	95-38500-1410
		9/15/05-8/31/06	\$9,000	2003-38500-12995
		9/1/06-8/31/08	\$15,000	2004-38500-14269
		5/15/08-5/14/09	\$40,000	2006-38500-16900
		<u>\$25,000</u>	2007-28500-18469	
		\$144,241		

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Nutrition/Diets	1	9/1/04-8/31/06	\$200,000	2002-38500-11752
	2	9/1/07-8/31/09	\$80,000	2006-38500-16900
	3	9/1/09-8/31/11	\$80,000	2008-38500-19157
			\$360,000	
Other Feed Training Carnivorous Fish	1	9/1/06-8/31/08	\$165,446	2005-38500-15847
			\$134,554	2006-38500-16900
			\$300,000	
Snail Management/Grub Control	1	9/1/07-8/31/09	\$212,495	2007-38500-18469
RAS Microbial Communities	1	9/1/09-8/31/10	\$65,000	2008-38500-19157
Salmonids	1	6/1/90-8/31/92	\$9,000	89-38500-4319
			\$120,799	90-38500-5008
	2	9/1/92-8/31/94	\$149,997	92-38500-6916
	3	9/1/94-8/31/96	\$199,290	94-38500-0048
	4	9/1/97-8/31/99	\$158,656	97-38500-3957
		\$637,742		
Sunfish	1	6/1/90-8/31/92	\$130,758	90-38500-5008
	2	9/1/92-8/31/94	\$149,799	92-38500-6916
	3	9/1/94-8/31/96	\$173,562	94-38500-0048
	4	9/1/96-9/31/98	\$199,921	96-38500-2631
	5	9/1/99-8/31/01	\$199,748	99-38500-7376
		\$853,788		
Tilapia	1	9/1/96-8/31/98	\$118,791	96-38500-2631
	2	9/1/98-8/31/00	\$150,000	98-38500-5863
		\$268,791		
Viral Hemorrhagic Septicemia (VHS)	1	9/1/08-8/31/10	\$197,960	2008-38500-19157
Walleye	1	5/1/89-8/31/91	\$177,517	89-38500-4319
	2	6/1/90-8/31/92	\$111,657	90-38500-5008
	3	9/1/91-8/31/92	\$109,223	91-38500-5900
	4	9/1/92-8/31/93	\$75,000	89-38500-4319
	5	9/1/93-8/31/95	\$150,000	93-38500-8392
	6	9/1/95-8/31/97	\$117,395	94-38500-0048
			\$59,835	95-38500-1410
	7	9/1/99-6/30/02	\$127,000	98-38500-5863
		\$927,627		
Wastes/Effluents	1	9/1/92-8/31/94	\$153,300	92-38500-6916
	2	9/1/96-8/31/98	\$100,000	96-38500-2631
	3	9/1/01-8/31/04	\$106,186	00-38500-8984
		\$88,814	2001-38500-10369	
		\$448,300		
White Papers	1	7/1/98-12/31/98	\$4,999	96-38500-2631
	2	9/1/99-12/31/99	\$17,495	97-38500-3957
		\$22,494		

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Yellow Perch	1	5/1/89-8/31/91	\$76,957	88-38500-3885
			\$85,723	89-38500-4319
	2	6/1/90-8/31/92	\$92,108	90-38500-5008
	3	9/1/91-8/31/93	\$99,997	91-38500-5900
	4	9/1/93-8/31/95	\$150,000	93-38500-8392
	5	9/1/95-8/31/97	\$199,507	95-38500-1410
	6	9/1/97-8/31/99	\$185,458	97-38500-3957
	7	9/1/98-8/31/00	\$92,370	98-38500-5863
	8	9/1/01-5/31/04	\$326,730	00-38500-8984
		<u>\$125,016</u>	2001-38500-10369	
		\$1,433,866		

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PROJECT REPORTS

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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, 2010

NCRAC FUNDING: \$65,000 (September 1, 2008 to August 31, 2010)

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

- | | |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p>(1) Identify the etiologic agent (<i>Aeromonas</i> spp.) from isolates collected from disease outbreaks in the NCR and characterize the disease syndrome before conducting any effectiveness studies.</p> <p>(2) Have active, established Investigational New Animal Drug (INAD) exemptions or work with the sponsors of publicly disclosable INADs for Terramycin 200 for Fish® and Aquaflor®.</p> | <p>(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®).</p> <p>(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.</p> |
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¹NCRAC has funded nine Aquaculture Drugs projects. This Progress Report is for the eighth Aquaculture Drugs project. It is a 2-year funded project that began January 1, 2008. 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 reported on under the Termination Report for the National Coordinator for Aquaculture New Animal Drug Applications (NADAs) in the 2008-09 Annual Progress Report. A Progress Report for the ninth project is contained elsewhere in this report.

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- (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, 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 North Central

Region (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.

PROGRESS 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

AQUACULTURE DRUGS

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 h. 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 florfenicol.

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.

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®

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

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

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(oxytetracycline dihydrate) to control mortality in coolwater and warmwater finfish due to Motile Aeromonad infections” will be submitted to the U.S. Food and Drug Administration’s Center for Veterinary Medicine (CVM) in October 2009.

OBJECTIVE 4

A protocol was submitted through established INADs for Terramycin 200 for Fish® and Aquaflor® 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” on February 4, 2010 to CVM for concurrence. A stop review request was submitted by UMESC to allow CVM to review an H submission (prepared by UMESC and submitted on July 6, 2010). The H submission summarized literature and data to support the current protocol and requests CVM to consider infection by any motile aeromonad species to be a potential cause of MAI. The H submission is complete and the protocol will be revised in Year 3 and re-submitted for CVM concurrence.

WORK PLANNED

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 new animal drug application (NADA) approvals for their respective drug products.

IMPACTS

The effectiveness studies of this project should lead to supplemental NADA approvals by CVM 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.

AQUACULTURE DRUGS

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
2009-10	\$28,000						\$28,000
TOTAL	\$65,000			\$2,600		\$2,600	\$67,600



AQUACULTURE DRUGS: DRUG APPROVAL RESEARCH ON 17 α -METHYLTESTOSTERONE (OFFICIAL TRANSFER OF 17 α - METHYLTESTOSTERONE [MT] ANALYTICAL METHOD FOR FEED)²

Project *Progress Report* for the Period
September 1, 2009 to August 31, 2010

NCRAC FUNDING: \$33,280 (September 1, 2009 to August 31, 2010)

PARTICIPANTS:

Mark Gaikowski	Upper Midwest Environmental Sciences Center	Wisconsin
Nilmini Wijewickreme	Maxxam Analytics [formerly CANTEST Ltd.]	B.C., Canada
<i>Industry Advisory Council Liaison:</i>		
Mark Willows	Binford Eagle Fisheries	North Dakota
<i>Extension Liaison:</i>		
Kevin Fitzsimmons	University of Arizona	Arizona

PROJECT OBJECTIVES

- | | |
|---------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| (1) Develop study protocols to conduct the MT feed method transfer of the MT analytical feed method. | (5) Assay control and medicated feed samples according to the study protocols concurred with by CVM. |
| (2) Submit method transfer study protocols to the Center for Veterinary Medicine (CVM) for concurrence. | (6) Complete report of analysis and submit along with raw data to the Upper Midwest Environmental Sciences Center (UMESC). |
| (3) Provide final study protocols to participating laboratories. | (7) Compare and discuss the results of both the CANTEST Ltd. (CANTEST) reference (expert) and transferred (naïve) analyses of the MT transfer study samples based on the MT analytical feed method developed by |
| (4) Prepare and ship medicated feed to participating laboratories. | |

² NCRAC has funded nine Aquaculture Drugs projects. This Progress Report is for the ninth Aquaculture Drugs project. It is a 1-year funded project that began January 1, 2009. 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 reported on under the Termination Report for the National Coordinator for Aquaculture New Animal Drug Applications (NADAs) in the 2008-09 Annual Progress Report. A Progress Report for the eighth project is contained elsewhere in this report.

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the University of Wisconsin-Madison (UW-Madison).

- (8) Determine whether any changes are needed to the MT analytical feed method developed by UW-Madison based on the results of the MT feed transfer study.
- (9) Validate that the naïve analyst at CANTEST can analyze the MT feed samples according to the analytical feed method developed by UW-Madison.
- (10) Compile Final Study Report (FSR), archive raw data, and submit FSR to CVM through the UMESC MT investigational new animal drug (INAD) exemption.
- (11) Respond to CVM comments.
- (12) Gain acceptance from CVM for the MT feed method transfer study.

ANTICIPATED BENEFITS

The results from this project will directly affect the potential for approval of MT by the U.S. Food and Drug Administration's Center for Veterinary Medicine (CVM). The data from this study, if accepted by CVM, support the potential approval of MT-medicated feed for use in tilapia. MT-medicated feed is used to produce greater than 80% phenotypic male populations, a significant benefit to U.S. producers because male tilapia generate more biomass with less effort in less time making them more cost efficient to raise.

PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

A study protocol was developed to conduct the work for a method transfer trial for the analytical method to determine MT concentrations in fish feed.

OBJECTIVE 2

The study protocol was submitted CVM for review. The protocol was returned with review comments which were used to revise the protocol to a final draft.

OBJECTIVE 3

The final protocol was provided to Maxxam Analytics (formerly CANTEST; Burnaby, British Columbia, Canada), the company providing the reference and participating laboratories for the work.

OBJECTIVE 4

Control (non-medicated) tilapia feed was prepared at Rangen Inc. The control feed was used to prepare MT-medicated feed at Rangen Inc. Control and MT-medicated feed were shipped from Rangen Inc. to UMESC.

A feed production report was prepared and submitted by UMESC to Rangen Inc. Rangen Inc. submitted the feed production report to Rangen's confidential INAD authorization file.

Control feed samples were shipped from UMESC to Maxxam Analytics to initiate the method familiarization phase of the study.

OBJECTIVE 5

Participating laboratory analysts (analysts with no previous experience performing the method) were involved in a method familiarization analysis session to ensure the participating laboratory analysts could successfully perform the method to determine MT concentrations in feed before analyzing feed samples for the method transfer phase of the study. Control feed samples shipped by UMESC to Maxxam Analytics were stored at $-20 \pm 10^{\circ}\text{C}$. Eight 5 ± 0.1 g of control un-medicated feed samples were weighed. Two samples were not fortified (control samples). The

AQUACULTURE DRUGS

remaining samples were fortified with an appropriate volume of 1,000 µg/g MT stock standard to obtain matrix equivalent MT concentrations of 30, 60, and 90 µg/g. Duplicates were analyzed for each fortification level.

Participating laboratory analysts demonstrated that there was no MT interference from matrix constituents in control feed. Analysts met the acceptance criteria of <15 µg/g of MT equivalent interference in the control feed extract.

Participating laboratory analysts obtained mean percent recoveries of 87.6, 93.8, and 101.6% from samples fortified with MT at 30, 60, and 90 µg/g, respectively. These data were within the method acceptable percent recovery range (>80% and <110%). Based on the results obtained during the method familiarization phase, participating laboratory analysts were successful performing the method for determining MT concentrations in feed.

Thereafter, UMESC shipped to Maxxam Analytics control and MT-medicated feed to be used in the method transfer phase of the study. During the method transfer phase, the participating laboratory analysts were blinded from the identity of the samples they processed and the results from the phase determined if the method could be accurately and precisely used by naïve analysts to determine MT concentrations in fish feed.

Control and medicated feed samples shipped by UMESC to Maxxam Analytics were stored at $-20 \pm 10^{\circ}\text{C}$. Forty control feed samples were weighed. Ten control samples were not fortified (control samples). The remaining control feed samples were fortified with an appropriate volume of 1,000 µg/mL MT stock standard to obtain

matrix equivalent MT concentrations of 30, 60, and 90 µg/g (10 samples/concentration). Ten samples of MT-medicated feed were weighed from each of two MT-medicated feed batches (expected MT concentration of 60 µg/g). The reference laboratory analysts (analysts with previous experience performing the method) processed five control samples, five samples from each fortification level, and five samples from each batch of MT-medicated feed. The participating laboratory analysts processed the same list of samples.

OBJECTIVE 6

Reference and participating laboratory results from the method transfer phase were submitted by Maxxam Analytics to UMESC for review.

OBJECTIVE 7

The method transfer phase results from the reference and participating laboratories were compared. Participating laboratory analysts demonstrated the matrix equivalent MT concentrations in the control feed met the acceptance criteria of <15 µg/g of MT equivalent interference in the control feed extract. In comparison, reference laboratory analysts also demonstrated the matrix equivalent MT concentrations in the control feed met the acceptance criteria of <15 µg/g of MT equivalent interference in the control feed extract.

Participating laboratory analysts obtained mean percent recoveries of 97.6, 99.5, and 109.7% from samples fortified to obtain matrix equivalent MT concentrations of 30, 60, and 90 µg/g, respectively. These data were within the method acceptable percent recovery range (>80% and <110%). In comparison, reference laboratory analysts obtained mean percent recoveries of 81.3, 77.2, and 73.4% from samples fortified to obtain matrix equivalent MT concentrations

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of 30, 60, and 90 µg/g, respectively. Two of the three mean recoveries were not within the method acceptable percent recovery criteria.

Participating laboratory analysts obtained a mean matrix equivalent MT concentration in medicated feed Batch 1 of 58.9 µg/g with a method precision of 9.6% (% relative standard deviation) and a mean matrix equivalent MT concentration in Batch 2 of 60.9 µg/g with a method precision of 6.5%. In comparison, reference laboratory analysts obtained a mean matrix equivalent MT concentration in medicated feed Batch 1 of 43.0 µg/g with a method precision of 8.5% and a mean matrix equivalent MT concentration in Batch 2 of 42.8 µg/g with a method precision of 11%.

OBJECTIVE 8

An investigation was undertaken to determine the cause of failure of the reference laboratory analysts to obtain mean method recoveries from fortified samples in the acceptable range. As a result of the investigation, it was determined that the participating laboratory analysts made slight modifications to the method. Because the method percent recovery data obtained by the participating laboratory analysts were within the method's acceptable range, the modifications were used to revise the existing method.

OBJECTIVE 9

The revised method was used by reference and participating laboratory analysts to process the sample sets described in *OBJECTIVE 5* for the method transfer phase of the study.

Using the revised method, participating and reference laboratory analysts demonstrated the matrix equivalent MT concentrations in the control feed met the acceptance criteria

of <15 µg/g of MT equivalent interference in the control feed extract.

Using the revised method, participating laboratory analysts obtained mean percent recoveries of 96.9, 104, and 105% from samples fortified to obtain matrix equivalent MT concentrations of 30, 60, and 90 µg/g, respectively. These data were within the method acceptable percent recovery range. In comparison, reference laboratory analysts obtained mean percent recoveries of 86.9, 86.5, and 84.9% from samples fortified to obtain matrix equivalent MT concentrations of 30, 60, and 90 µg/g, respectively. These data were also within the method acceptable percent recovery range.

Using the revised method, participating laboratory analysts obtained a mean matrix equivalent MT concentration in medicated feed Batch 1 of 54.3 µg/g with a method precision of 1.7% and a mean matrix equivalent MT concentration in Batch 2 of 58.6 µg/g with a method precision of 8.4%. In comparison, reference laboratory analysts obtained a mean matrix equivalent MT concentration in medicated feed Batch 1 of 47.1 µg/g with a method precision of 2.3% and a mean matrix equivalent MT concentration in Batch 2 of 49.1 µg/g with a method precision of 1.9%.

OBJECTIVE 10

The reference laboratory and the participating laboratory submitted to UMESC reports describing the results from the processing of the sample sets described in *OBJECTIVE 5* for the method transfer phase of the study. UMESC submitted review comments back to the reference and participating laboratory report authors. The raw data and the laboratory reports are undergoing Maxxam Analytics quality assurance review.

AQUACULTURE DRUGS

WORK PLANNED

OBJECTIVE 10

After receiving completed reports from the reference and participating laboratories, UMESC will draft a FSR. Upon completion of all internal reviews, UMESC will submit to CVM through the UMESC MT INAD exemption, the FSR for their review.

OBJECTIVE 11

Upon receipt of CVM's FSR review comments, UMESC will revise the FSR to address CVM review comments.

OBJECTIVE 12

UMESC will submit to CVM the revised FSR and ask that the method transfer study data described in the FSR be accepted.

IMPACTS

Legal use of MT in the U.S. is dependent on CVM approval. Approval is contingent on providing data that will fulfill their data requirements. One of the outstanding data requirements is a method transfer trial where a laboratory naïve to the method for determining MT concentrations in feed must adequately perform the method. The results from this work should fulfill the outstanding data requirement and have a direct affect on the potential for MT approval.

Tilapia is the fifth most consumed seafood in the United States. The approval of MT-medicated feed for use in tilapia to produce greater than 80% phenotypic male populations would be of significant benefit to the U.S. producers. Male tilapia generate more biomass with less effort in less time making them more cost efficient to raise. Approval of MT will provide advantages for those producers who currently do not have the space, time, or money to produce genetically male tilapia populations. The production of male tilapia populations is critical to the U.S. tilapia industry if producers are to remain competitive with foreign tilapia producers.

SUPPORT

NCRAC has provided \$54,615 which is the entire amount allocated for this 1-year project.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

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

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BAITFISH³

Project *Termination Report* for the Period
September 1, 2006 to August 31, 2010

NCRAC FUNDING: \$200,000 (September 1, 2006 to August 31, 2010)

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
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Extension Liaison:

Jeffrey L. Gunderson	University of Minnesota-Duluth	Minnesota
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Non-Funded Collaborators:

Barkhausen Waterfowl Reserve	Brown County	Wisconsin
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REASON FOR TERMINATION

Project objectives completed.

newsletters, fact sheets, workshops,
and/or technical bulletins.

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,

PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

Iowa State University (ISU) staff were successful at developing a strategy for early season spawning of golden shiners. 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 and 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,

³This is a project that had two years of funding and is chaired by Joseph E. Morris. It began September 1, 2006.

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commercial spawning mats were placed into the tanks, just under the water surface. 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. 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 and 2008 nine larval diets were evaluated. Stocking rates ranged from 8–40 fry/L (30–151 fry/gal). Results from the 2007 and 2008 culture seasons showed the Zeigler AP100™ diet yielded the best survival; mean survival ranged from 1–28%.

In a related project, the efficacy of hydrogen peroxide (H₂O₂) to control fungal (*Saprolegniasis*) infections of golden shiner eggs was evaluated in two experiments. Results garnered from this experiment indicate that golden shiner eggs should be exposed in a 15-min static bath at 800 mg/L H₂O₂ (30% active ingredient concentration of H₂O₂) in a single treatment.

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 in 2007 to (1) evaluate the growth of golden shiner fry in ponds using two fertilization regimes, one a mix 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. 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.

In 2008 ponds were stocked 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. The ponds that 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 river 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 (23–25°F).

Wild brood stock that were maintained at 23–25°C (23–25°F) from August 2005 to August 2007 spawned out-of-cycle from March–May and produced progeny in the tens of thousands. The F₁ 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 a F₁ generation of 2006. The F₁ generation exhibited spawning behavior and produced numerous 2007 F₁ generations.

Culture techniques for early life stage feeding included: green tank water (GTW), brine shrimp nauplii (BSN) and commercial larval diets. F₁ generations reached an estimated size of 51 mm (2 in) in 12–14 months. Survival was poor throughout the entire post-larvae stage.

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 southern Wisconsin 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. The fish accepted a commercial trout diet and were kept in temperatures of 18–21°C (64–72°F)

during spawning. 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 a variety substrates immediately with active spawning behavior and swarming around the substrates. Eggs hatched within 5–7 days at 18–21°C (64–71°F), resulting in thousands of <5.0 mm (0.2 in) fry. Newly hatched fry were initially lethargic and non swimming but became photopositive and strong swimming within a few days. Fry were fed commercial starter diets of several types supplemented with pond water and 24-h lighting. Biomarine Artemac produced the best results with fry at NADF in 2008. 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.

Strong swimming, photopositive fry were collected from NADF 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).

In 2009, a successful attempt was made to conduct the pond-based study onsite at NADF. In May, banked brood stock at NADF and additional brood stock from Minnesota were introduced into the warm water recirculating aquaculture system

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operations at NADF and spawned utilizing equipment and techniques described below from 2007-2008. Using fry garnered from the indoor spawning operations, a nursery pond were 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] (95% survival was observed) were harvested and placed into a 20.0–22.0°C (68.0–71.6°F) recirculating aquaculture system in NADF for further grow out. Fingerling spotfins were fed commercial trout starter diet (Nelson Silvercup Inc., Utah) utilizing 24-h feeders. Spotfins reached >51mm (>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.

Methodology that proved successful in 2009 for spawning spotfins included (1) 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 2.0–5.0 mm (0.08–0.2 in) crevices for approximately two days to allow fish to fill with eggs (full substrates removed to separate incubation tanks); (2) recirculating aquaculture system tank temperatures >20°C (>68°F) for spawning; (3) orientation of spawning substrates in direct current in

tanks; (4) separate incubation and hatching tanks without any juvenile or adult fish; (5) 24 h lighting and water temperatures >20°C (>68°F) for incubation/hatching tanks; (6) placing newly hatched fry into prepared outdoor rearing ponds fertilized with alfalfa meal; and (7) draining ponds in fall and bringing fingerlings into indoor recirculating aquaculture systems for final grow out on commercial feed.

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 and Artemac, respectively.

OBJECTIVE 3

Gunderson, in his role as extension liaison for this project, has presented the results of the baitfish project at the 2007 through 2010 North Central Regional Aquaculture Center (NCRAC) Annual Program Planning Meetings. 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.

BAITFISH

A NCRAC Baitfish Workshop was held at the La Crosse Fish Health Center (Onalaska, Wisconsin) on September 21, 2010.

Approximately 30 current and potential fish farmers from around the region attended.

Twelve speakers presented results from the NCRAC Baitfish project and related topics.

A survey of attendees indicated that the workshop achieved its primary objectives which were to present the results of NCRAC baitfish research, provide an overview of the baitfish industry, and to provide related information to help practicing fish farmers. Chris Weeks, the regional Aquaculture Extension Specialist, was instrumental in organizing and facilitating this workshop.

IMPACTS

OBJECTIVE 1

- ▶ The potential of using placing 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 North Central Region (NCR).
- ▶ 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 at the UW-Stevens Point-NADF.

- ▶ 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.
- ▶ The spawning and egg incubation apparatus developed during this study contributed to improved spawning behavior, egg incubation and hatching success.

OBJECTIVE 3

- ▶ This outreach effort helped coordinate the reporting of research results and make the 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. The NCRAC Baitfish Workshop brought together some of the baitfish industry leaders from around the region to learn about research results and related baitfish topics. Several recommendations for future workshops were suggested on the workshop survey. Suggestions included: marketing/business management information, state/federal regulations that negatively impact baitfish production and sales, disease, water quality, and land use impacts to baitfish production.

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RECOMMENDED FOLLOW-UP ACTIVITIES

At this time, the limiting factor associated with post-larvae survival is providing an appropriate nutritional diet for both golden shiners and spot fin shiners. Although there are numerous larval fish diets available to the producer, new research should focus on developing a diet specific for both shiner species.

The spotfin shiner elicited a positive response to temperature manipulation to control reproduction. In spite of this, the yield of over 2,000 mature spotfin shiners in tanks resulted in less than 5,000 fry being

collected in any single week. The researchers' opinion is that this is a major problem that will impede the development of this species as a viable commercial baitfish produced in ponds.

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.

EXTENSION⁴

Project *Progress Report* for the Period
May 1, 1989 to August 31, 2010

NCRAC FUNDING LEVEL: \$897,805 (May 1, 1989 to August 31, 2010)

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

⁴NCRAC has funded a number of Extension activities, both as stand-alone projects or as components of species-or topical-specific projects, including 13 stand-alone projects deemed “Base” Extension. This Progress Report is for components of the first 13 “Base” Extension projects and a Progress Report for the 12th “Base” Extension project (an Addendum to the 11th “Base” Extension project) 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 5-13 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 13th “Base” project is a 2-year funded project that began September 1, 2009. Fred P. Binkowski chaired the 14th 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 15th stand-alone Extension project (Regional Aquaculture Extension Specialist [RAES]); a Termination Report for that project was contained in the 2008-09 Annual Progress Report. Christopher Weeks chairs the 16th stand-alone Extension project (Regional Aquaculture Extension Specialist [RAES]); a Progress Report for that project is contained elsewhere in this report.

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PARTICIPANTS (continued):

Jeff Mittlemark	University of Minnesota	Minnesota
Joseph E. Morris	Iowa State University	Iowa
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. Quagraine	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 thirteen projects funded to date

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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 NCR.

Since the beginning of NCRAC in 1989 numerous publications have been developed that address 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 Department of Natural Resources) on the

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final draft of a NCRAC fact sheet on walleye culture for food production. The fact sheet is now available on the NCRAC Web site

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 and walleye information with Morris.

The Aquaculture Network Information Center (AquaNIC [<http://aquanic.org/>]) was established at Purdue University in 1994 through funds from the U.S. Department of Agriculture's 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 is currently ranked as the #1 aquaculture electronic resource in the world by ranking.com, a professional Web tracking company that monitors the traffic for the top 1 million Web sites around the world. AquaNIC is also currently ranked in the top 18% of all Web site traffic worldwide by ranking.com.

As with any long-term organization, there have been changes in NCRAC extension personnel since the inception of the project. For instance, Landkamer was the primary aquaculture extension contact for Minnesota. In the intervening years, he was replaced by Kapuscinski who was, in turn, replaced 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 since 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

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Indiana in the interim and in 2006 Quagraine was appointed as state extension specialist at Purdue University. Plumer served Illinois until 2010 when, upon his retirement, Dave Shiley was appointed. In 2007, two long term extension contacts, Tiu and Morris, were replaced as NCRAC extension contacts by Wallat and Clayton, respectively. In 2010 Tiu was again appointed as extension contact for Ohio State University.

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 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 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. In 2010 Kinnunen and Morris attended a NCRAC Regional Aquaculture Extension Team Investment Workshop in Milwaukee, Wisconsin, chaired by Fred Binkowski (University of Wisconsin-Milwaukee [UW-Milwaukee]). At this workshop four different types of aquaculture with financial institution representatives were represented. Discussion included possible roadblocks to larger investments in aquaculture in the region.

OBJECTIVE 3

A number of workshops, conferences, symposia, videos, field-site visits, hands-on

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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

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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 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. The five delegate groups represented: 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 two others 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

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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 NCRAC has 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.

A NCR baitfish workshop was hosted by Chris Weeks (Michigan State University [MSU]) and Jeff Gunderson (University of Minnesota-St. Paul) in La Crosse, Wisconsin. Speakers included Kinnunen (MSU), Morris (Iowa State University), Nuese (UW-Milwaukee), Gunderson, Fischer (University of Wisconsin-Stevens Point), Weeks, and Gaikowski (Upper Midwest Environmental Sciences Center) as well as industry representatives such as Barry Thoele. Nathan Stone (University of Arkansas-Pine Bluff) also presented an overview of the baitfish industry. The approximately 30 participants heard presentations regarding new information on baitfish culture as well as associated disease issues.

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 (using information from the recently completed NCRAC snail management/grub control project) 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

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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-h 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
2009-10	\$14,500						\$60,505
TOTALS	\$897,805	\$1,055,868	\$5,000	\$334,000	\$55,000	\$1,449,868	\$2,347,673

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EXTENSION ADDENDUM⁵

Project *Progress Report* for the Period
September 1, 2008 to August 31, 2010

NCRAC FUNDING: \$60,505 (September 1, 2008 to August 31, 2010)

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- and short-term disease issues. These issues have resulted in

both significant changes to the regulation of the industry and economic losses associated with fish mortalities. Thus, greater requirements for disease detection and assessment on the farm are needed. 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

⁵ 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 13 stand-alone "Base" Extension projects and is an Addendum to the 11th "Base" Extension project which is chaired by Joseph E. Morris. This is a project that had two years of funding and began September 1, 2008.

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be created and implemented to better educate the fish farmer about aquatic diseases and on-farm fish health management. 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, 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 fish farmer and his/her 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.

PROGRESS 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 is nearly complete. Mechanisms to collect data on the impact of the training on the individual fish operations and the industry in general are being developed and reviewed.

Part one of the fish health certificate program includes the development of a six-module Web-based learning program. A draft version of modules 1-6 of the asynchronous learning program has been completed and is undergoing peer review. 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.

EXTENSION ADDENDUM

- (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, e.g., Best Management Practices, loss events, continuing education, veterinary services, record keeping, and links to state and federal guidelines and policies.
- (3) Water quality management and monitoring, and disease prevention that includes 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.
- (6) Case studies describing diseases based on water quality problems, environmental diseases, bacterial infections and ectoparasites have been developed. Case studies specific to Koi herpes virus, largemouth bass virus, infectious salmon anemia, spring viraemia of carp, and viral hemorrhagic septicemia have been developed based on actual “real-world” examples.

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

OBJECTIVE 2

A publication entitled “Biosecurity for Aquaculture Facilities in the North Central Region” was developed and is now available through the North Central Regional Aquaculture Center. Kinnunen coordinated a 3-day AIS HACCP Training course that was held at Bay Mills, Michigan in 2008. 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.

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.

In 2010 Kinnunen conducted 1-day AIS-HACCP Training Workshops in Ashland, Nebraska and Spirit Lake, Iowa. Those in attendance included state fish hatchery and fish management personnel, private sector aquaculture personnel, and an aquatic veterinarian from the states of Nebraska and Iowa. Attendees indicated in a written evaluation that they would use the material learned and implement plans at their own facilities within the next several months.

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Kinnunen also coordinated a second 3-day Seafood HACCP Training course at Bay Mills along with Mike Erdman (Menominee County Extension Director) and Jim Thannum (Great Lakes Indian Fish and Wildlife Commission). Formal evaluations from attendees rated the course as excellent. The 40 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.

In addition to the noted workshops, Kinnunen met with the Board of Directors of the Michigan Aquaculture Association to continue the process of developing a strategic plan for aquaculture development in Michigan. Additional contacts made by Kinnunen in support of this objective include a veterinarian who is compiling information for the Michigan Department of Agriculture on the subjects of aquaculture biosecurity and AIS-HACCP and several agency staff and private environmental consultants.

The Michigan Aquaculture Association Conference was held in Mt. Pleasant where Kinnunen gave an update on AIS-HACCP and Aquaculture Biosecurity. In addition, the Michigan Aquaculture Strategic Plan was discussed in conjunction with Ed Mahoney (MSU).

WORK PLANNED

Modules have been sent to outside peer reviewers for content and presentation review. After modifying and editing the modules, the modules will be sent to a fish producer focus group for feedback on applicability and completeness. Final changes and edits will be made to the online program before its official launch in 2011. 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.

IMPACTS

Part two of the Fish Health Certificate Program directly addresses the impact assessment of this extension project. This assessment 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 survey of outcomes. 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 has provided \$60,505 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 Extension activities.

REGIONAL AQUACULTURE EXTENSION SPECIALIST (RAES)⁶

Project *Progress Report* for the Period
September 1, 2009 to August 31, 2010

NCRAC FUNDING: \$74,700 (September 1, 2009 to August 31, 2010)

PARTICIPANTS:

Ronald E. Kinnunen	Michigan State University	Michigan
Joseph E. Morris	Iowa State University	Iowa
Larry G. Olsen	Michigan State University	Michigan
Christopher Weeks	Michigan State University	Michigan
<i>Industry Advisory Council Liaison:</i>		
John Reynolds	Midwest Fish and Crayfish, Merrifield	Wisconsin
<i>Extension Liaison:</i>		
Geoffrey Wallat	Ohio State University	Ohio

PROJECT OBJECTIVES

1. In conjunction with the NCRAC Industry Advisory Council and state aquaculture extension contacts, assess and prioritize North Central Region (NCR) industry needs, focusing on issues with regional significance.
2. Develop and implement strategies to address pertinent needs - interact with pertinent NCRAC and non-NCRAC aquaculture initiatives to accomplish identified strategies.
3. Develop and facilitate “linkages” among agencies, industry, academia, and other relevant entities to foster open, meaningful dialog on critical NCR issues.
4. Coordinate efforts for seeking non-NCRAC support to facilitate information and technology transfer to the industry.

ANTICIPATED BENEFITS

A number of national, state, and regional extension programs exist within the U.S. from which the NCR aquaculture community can draw information. This work plan is intended to help interconnect these efforts and streamline information and technology to the industry.

A number of issues impacting the NCR aquaculture industry (e.g., imports, state and federal budgets, Viral Hemorrhagic Septicemia [VHS]), are driven largely by factors outside the region. In this type of environment, fostering communications and network development in support of the NCR industry is crucial. Project team members and their affiliations have the necessary infrastructure in place to facilitate important dialog and communications for the industry.

⁶ 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 16 stand-alone Extension projects, the second Regional Aquaculture Extension Specialist (RAES) project, which is chaired by Christopher Weeks. It is a 2-year project that began September 1, 2009.

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Information and technology transfer in the form of “deliverables” from research and extension has been identified by the North Central Regional Aquaculture Center’s (NCRAC) Industry Advisory Council (IAC) as a critical component for NCRAC initiatives. In today’s global markets, it is extremely important to stay abreast of national and international developments. Through this work plan the project team will identify critical industry needs, seek avenues of non-NCRAC support, evaluate existing information and technologies relevant to the industry, and help make pertinent information in the form of deliverables more accessible by the industry and the aquaculture community.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

NCR aquaculture industry critical needs were identified through activities such as the 2009 NCRAC Aquaculture Industry Survey, workshops, and direct personal communication. For example, the RAES attended six meetings/workshops in Indiana, Michigan, Ohio, and Wisconsin in 2009-2010. The RAES presented at four of these meetings in topic areas of NCRAC procedures and updates, regulatory fish health information, NCR production information, and NCR producer concerns. These activities also provided important networking opportunities with industry members and extension and research personnel from across the region.

The most frequent response in terms of critical needs as reported by industry members in 2009-2010 is alleviation and clarification of strict regulations imposed on the industry. This response is confirmed also in the 2009 NCRAC Aquaculture Industry Survey, but only by industry members. Academic and agency personnel

surveyed believe top impediments facing NCR aquaculture industry development are other than regulatory. Based on our most current information, impediments as identified by the various aquaculture related groups in the NCR are as follows:

- ▶ All groups combined: lack of funding and loans, lack of industry cooperation, and regulations.
- ▶ Industry: regulations, feed costs, and lack of funding and loans.
- ▶ Academia personnel: lack of industry cooperation, lack of funding and loans, and access to fish health specialists.
- ▶ State and federal agency personnel: effective education and extension programs, technology, and lack of species specific diets.

A noteworthy finding from the assessment is that different sectors of the NCR aquaculture community perceive problem areas substantially different.

OBJECTIVE 2

To date, the RAES has committed substantial effort to help clarify regulations imposed on NCR aquaculture producers.

This effort includes:

- ▶ Monitor and update the NCRAC Web site State Importation and Transportation Requirements for Cultured Aquatic Animals (herein referred to as the NCRAC Regulation Web site). The site was originally developed by the current RAES in 2003 and it was improved in 2009 with help from the NCRAC Associate Director’s office as part of the RAES project. The Regulation Web site receives 500+ views per month and has been given a main link from the Animal and Plant Health Inspection Service (APHIS) aquaculture Web site: http://www.aphis.usda.gov/animal_health/animal_dis_spec/aquaculture/aquastates.shtml.

- ▶ A summary of the import requirements for NCR states has recently been added to the NCR Aquaculture Roadmap Web site in table format. The summary is unique, concise, and has been receiving good feedback:
http://www.ncrac.org/roadmap/webinfo/pdf_downloads/NCRAC%20import%20ref.pdf
- ▶ Seek out information from aquaculture related list serves, news, and personal contact information from across the nation in order to disseminate important up-to-date information to the industry.
- ▶ Work with regulators and industry personnel across the region to try to minimize disruption of commerce due to VHS.
- ▶ Encourage active participation by the NCR aquaculture community in regulatory rule making processes. Examples include posting for, and industry solicitation for Federal Register comments in regards to APHIS actions on VHS and the National Aquatic Animal Health Plan.

Information transfer is another major focus area for the RAES. We recognized that a number of very good aquaculture extension and outreach tools exist across the nation (e.g., the Aquaculture Network Information Center [AquaNIC], University extension Web sites, etc). Yet, through phone calls, e-mails, and conversations, individuals have expressed views that current, pertinent, and useful information for specific aquaculture topics is hard to find. In 2009, the RAES initiated a project to enhance the NCR Aquaculture Roadmap from its original short PDF file, into a user-friendly Web site designed for easy access of information. The Web site provides access to all RAC publications, other non-copyrighted articles, regulations, NCR state associations, extension Web sites, extension, research,

and state agency contacts across the region, upcoming events, and other materials. The NCR Aquaculture Roadmap URL address is <http://www.ncrac.org/roadmap/index.htm>.

Current news items and potentially useful topical information is actively sought from several national and international sources by the RAES for posting to the NCR Fish Culture List Serve. The list serve currently has 125 subscribers and has increased every year since it was facilitated by the RAES in 2008. The NCR Fish Culture List Serve is maintained from Iowa State University and supported by NCRAC's Associate Director's office. Subscription is available through the NCR Aquaculture Roadmap Web site.

Attendance at state association meetings and workshops, and numerous site visits to commercial facilities has proven to be a very useful method for information dissemination and communication regarding industry concerns. In addition, through the course of this project, the RAES has been experiencing a substantial increase in phone calls and e-mail inquiries from across the NCR aquaculture community.

One task initiated under this objective examines effectiveness of past and current research programs and extension utilities in the NCR. Through quarterly conferencing, RAES project team members have concluded that this is an important, but potentially difficult undertaking. The term "impact" was identified as a key parameter for further assessment. Indicators of success include quantifiable components (production, value, number of registered facilities, state association membership, etc.) as well as qualitative items (working relationships with environmental agencies, political environment, etc.). This task will

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be carried forward by the RAES project team (see WORK PLANNED section).

OBJECTIVE 3

In 2009-2010 the RAES gave presentations at the Michigan Aquaculture Association Meeting, Michigan Department of Agriculture Meeting for Aquatic Animal Health, the Wisconsin Workshop for Veterinarians on Fish Regulatory Medicine, and the Indiana Aquaculture Workshop. The RAES also facilitated and presented at the NCRAC Baitfish Workshop held at the La Crosse Fish Health Center, Wisconsin, and participated in the National Aquaculture Association's (NAA) 4P Workshop, as well as a Hazard Analysis and Critical Control Point Workshop on VHS and Biosecurity, both of which were held in Ohio. The primary goal of the RAES throughout the course of these meetings has been to network with industry, academia, and regulatory agency personnel for the purpose of promoting NCR aquaculture industry interests and development.

The RAES project team has identified three organizations where increased membership base among NCRAC members would likely help promote NCR industry interests. These include NAA, Farm Bureau, and the National Association of State Aquaculture Coordinators. In addition, the RAES is currently in the planning stage for an industry development workshop in which we hope to partner with two regional non-NCRAC entities.

OBJECTIVE 4

Tasks identified under Objective 4 include (a) pursuing potential avenues for non-NCRAC support such as funding, information, and technology transfer to the NCR industry, and (b) providing industry access to deliverables from research and extension activities via internet and

extension outlets. Both of these tasks have been initiated as per descriptions of progress for Objectives 1-3 (above). The RAES project team plans to focus on expanding such activities in the coming year.

WORK PLANNED

RAES activities underway will be continued through the course of this project. These include, but are not limited to, the NCR Fish Culture List Serve, the NCRAC Regulation and Roadmap Web sites, and continued liaison services to the industry. Improvements to these outreach methods will be made pending recommendations from the NCR aquaculture community. With the existing information transfer projects functioning and current, the RAES project team plans to shift a good portion of focus over to Objectives 3 and 4, specifically partnership building and seeking additional non-NCRAC support for the industry.

In addition to the 2009 survey, the RAES team plans to facilitate or participate in 1-2 additional region-wide industry development projects. Currently we are assessing the feasibility of holding a walleye industry development workshop within the next 3-4 months. We are also looking to undertake or contribute to an industry economic development project (e.g. Market Maker™).

One of the more difficult tasks in the RAES project plan is to assess how research and extension projects conducted on behalf of industry development can be measured in terms of impacts on the industry. We plan to identify and collect potential deterministic variables (e.g. number of extension personnel, state and federal funding levels, etc.), and indicators of success (trends in production and annual sales, registration numbers, etc.) from individual states across

the NCR. We will then construct a dataset of this information which can be used for assessment purposes. These initial steps should provide important information including the feasibility for obtaining data and maintaining a current dataset. While statistical analysis might be beyond the scope of this RAES project, it is our intent to provide preliminary recommendations as to how measurable outcomes might be better integrated into NCRAC projects.

The RAES project plan calls for a NCR Aquaculture Critical Needs Assessment Report as one of the final deliverable products. This report is expected to be completed by the end of the August 2011.

IMPACTS

Recorded visits to Web pages in the NCRAC regulation Web site totaled 829 for the month of September 2010. Visits to the NCR Aquaculture Roadmap pages totaled 164.

The NCR Baitfish Production Workshop held in September 2010, included 11 speakers and had 22 participants. Participant evaluations showed predominately good to excellent ratings.

The number of USDA-certified veterinarians interested in expanding into aquatic animal health appears to be increasing based on veterinarian attendance at meetings and workshops across the NCR. This trend is due to diligence among many people across the nation, and the RAES team will continue to encourage and support this development to the best of its ability.

RAES industry liaison activities include working with state and federal agency personnel to clarify regulations. On average, this probably occurs about once per month. In addition, updates to the NCRAC regulation Web site are frequent. A side result of these activities is that the RAES has been building working relationships with more regulating agency personnel across the region and nation.

SUPPORT

NCRAC funds provided to date total \$74,700; a total of \$150,000 has been allocated for this 2-year project.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

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

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NUTRITION/DIETS—RAPID DETERMINATION OF AMINO ACID REQUIREMENTS OF YELLOW PERCH AND TILAPIA⁷

Project *Progress Report* for the Period
September 1, 2009 to August 31, 2010

NCRAC FUNDING: \$42,500 (September 1, 2009 to August 31, 2010)

PARTICIPANTS:

Robert S. Hayward	University of Missouri-Columbia	Missouri
<i>Industry Advisory Council Liaison:</i>		
Mark Willows	Binford Eagle Fisheries, Binford	North Dakota
<i>Extension Liaison:</i>		
Joseph E. Morris	Iowa State University	Iowa

PROJECT OBJECTIVES

- (1) Conduct a full literature search on amino acid composition, amino acid requirements, and feed formulations for yellow perch and Nile tilapia.
- (3) Evaluate body amino acid composition of yellow perch and Nile tilapia.
- (4) Evaluate limiting amino acid requirements of yellow perch and Nile tilapia.
- (5) Evaluate amino acid availability of dietary ingredients for yellow perch and Nile tilapia.
- (6) Develop a least-cost formulation model available to the NCR aquaculture industry within a two-year period for yellow perch and Nile tilapia.

- (7) Coordinate findings from this study with the Technical Committee Extension Subcommittee of NCRAC.

ANTICIPATED BENEFITS

Although strong demand exists for both yellow perch and Nile tilapia, current high production costs need to be reduced to enhance profit margins. As with other aquaculture species, feed costs represent a significant percentage of producers' variable costs. Current trout diets contain 4–50% protein, with the majority of the feed costs owing to the use of fish meal based protein. Replacement of fish meal with other animal protein and plant protein sources can result in significant savings in fish feed production costs.

This study, while optimizing nutrient requirements, will remove excess protein from the current diets for both species. This project further seeks to reduce the feed cost by using highly digestible as well as

⁷NCRAC has funded three Nutrition/Diets projects. The Termination Report for the first project is contained in the 1997-98 Annual Progress Report. The Termination Report for the second Nutrition/Diets project is contained in the 2008-09 Annual Progress Report. This Progress Report is for the third project. It is a 2-year project that began September 1, 2009.

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economically available local feedstuffs that benefits both fish producers as well as local grain producers. It is anticipated that this project will reduce current feed costs by at least 40% for yellow perch and by 30% for Nile tilapia while the growth rate of fish will be increased or maintained at current production levels.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

Literature searches have been conducted on feedstuffs' amino acid composition, amino acid requirements, and feed formulations for grow-out-stage yellow perch and Nile tilapia.

OBJECTIVE 2

Activities have been postponed until the second year of the project.

OBJECTIVE 3

The experiment is currently being done to determine lysine requirement of Nile tilapia. The experiment will run for eight weeks, and will ultimately determine the exact lysine requirement of grow-out-stage Nile tilapia.

OBJECTIVE 4

The digestibility study for grow out stage Nile tilapia has been completed. Nine common feedstuffs including fish meal, poultry byproduct meal, meat and bone meal, blood meal, soybean meal, peanut meal, corn gluten meal, as well as wheat and corn, have been tested in this experiment. Digestible energy, protein and amino acids have been evaluated. These data will be important mainly for the ideal protein diet formulation and least-cost diet formulation.

OBJECTIVES 5-6

These objectives will be addressed in the second year of the project.

WORK PLANNED

OBJECTIVE 1

The literature will continued to be searched for additional information on development of least-cost diet formulation for grow out stage Nile tilapia.

OBJECTIVE 2

Whole body amino acid compositions of yellow perch and Nile tilapia will be conducted. The body amino acid profiles will provide a reference for formulating a protein and amino acid balanced diet for the two focal species.

OBJECTIVE 3

Lysine requirement for Nile tilapia will be determined using information from the first year's experiment.

OBJECTIVE 4

The digestibility study for yellow perch will be conducted once an adequate number of yellow perch has been collected.

OBJECTIVES 5-6

Upon the completion of the previous objectives, a low-cost feed formulation for both yellow perch and Nile tilapia will be developed and provided to the extension contacts for distribution.

IMPACTS

Diets for North Central Region species that are effective, economical, and can be developed within reasonable time periods are needed for the advancement of the aquaculture industry in this region. The development of appropriate and effective diets in this project has the potential of greatly increasing the profitability of the aquaculture industry in this region.

NUTRITION/DIETS

SUPPORT

NCRAC funds provide to date total \$42,500; a total of \$80,000 has been allocated for this 2-year project.

PUBLICATIONS, MANUSCRIPTS, WORKSHOPS, AND CONFERENCES

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

NORTH CENTRAL REGIONAL AQUACULTURE CENTER

FEED TRAINING CARNIVOROUS FISH⁸

Project *Termination Report* for the Period
September 1, 2006 to August 31, 2010

NCRAC FUNDING: \$300,000 (September 1, 2006 to August 31, 2010)

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

REASON FOR TERMINATION

The objectives were completed and funds terminated.

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.

PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

University of Wisconsin-Madison (UW-Madison)

UW-Madison investigators completed two experiments relevant to the feed training of pond-raised yellow perch fingerlings. Experiment 1 evaluated the influence of fish size at harvest on habituation success. Yellow perch were harvested 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 (19.0°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

⁸This 2-year funded 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.

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were hand-fed 5–8 times daily. During the first four days fish were fed freeze dried krill. The next 10 days, 10% krill was added to the formulated food (#2 Silver Cup Trout Fry diet, Murray Elevators, Murray, Utah). During the balance of the training period, fish were fed only the formulated feed.

Length of the training period was defined by mortality due to starvation as well as visual observation of positive feeding activity of all fish in the tanks. To compare the training success of the fry sizes, calculations were made of: (1) harvest losses, defined as the percentage of fish which died during the first two days, (2) habituation success, defined as the percentage of fish surviving at the end of the training period (after harvest losses), (3) starvation, defined as the percentage of recovered dead fish, (4) cannibalism, defined as the percentage of fish which were unaccounted for at end of the training period, and (5) overall success, defined as the percentage of fish remaining at the end of the training period (including harvest losses).

Habituation 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 fry (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%). There was higher size variability 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 it is recommended that grading the harvested fingerlings need to be done prior to feed training when size differences are apparent.

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 via 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 among fish sizes (83.4%, 91.3%, and 78.1%, for fish harvested at 25.0, 35.0, and 45.0 mm [1.0, 1.4, and 1.8 in] TL, respectively). This statistical result may have been limited by the low number of replicates ($N = 4-6$ replicates per fish size) used in this study. Harvest losses in fish at 25.0 mm (1.0 in) TL were offset by cannibalism losses in fish at 45.0 mm (1.8 in) TL. Fish harvested at 35.0 mm (1.4 in) TL displayed low losses from both harvest stress and cannibalism, and may be recommended as the best size for habituation using the techniques set forth in this study. From a practical standpoint, however, it may be logistically unfeasible to harvest and train all fingerlings produced at a commercial scale facility at the same size and at the same time. Techniques should be modified to accommodate the fish on-hand. Low stress harvest methods for small fish and size grading for larger more size-diverse populations will likely result in better overall success for both groups of fish. It is suggested that a technique of sequential harvesting of fingerling ponds (i.e., partial seining of fingerlings every 7–10 days, followed by complete final harvest), where possible, will maximize the total fingerling production in most ponds.

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Experiment 2 compared four different feed regimens using three sizes of fish for each regimen. The feed regimens were: (1) Silver Cup feed, (2) four days of INVE feed (Epac 6–8) followed by a seven day transition to Silver Cup, (3) four days of freeze-dried krill followed by a seven day transition to Silver Cup, and (4) four days of krill followed by a seven day transition to INVE followed by a seven 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 [1.4 and 2.2 in] TL fingerlings, respectively). No differences in habituation success were found among 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. UW-Madison researchers recognize the fact that most commercial yellow perch fingerling producers find the use of krill as a

transitional feed to be highly beneficial and cost-effective.

University of Wisconsin-Milwaukee (UW-Milwaukee)

Extracts of midge larvae (chironomids), zooplankton, redworms (*Eisenia fetida*), artemia, and tubifex (*Tubifex tubifex*) worms were prepared as natural feed attractors. Feeding trials were conducted to compare feeding responses among feed attractants and identify the best positive response for each species (yellow perch and largemouth bass). Results of feeding trials were inconclusive although the feeding behavior appears to be more relative to sight, prey movement, and auditory signals. Consequently, research effort was shifted to investigating these feeding behavior patterns as a function of sight, movement, and sound.

OBJECTIVE 2

UW-Madison

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

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differences in pond survival ($69.7\% \pm 7.7$ versus $67\% \pm 9.8$) or mean fish size (18.3 ± 4.7 g [0.7 ± 0.2 oz] versus 21.0 ± 3.6 g [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 harvested in autumn, however, with fish sizes ranging from 3.0–56.0 g (0.1–2.0 oz). The fingerlings were size-graded into three groups: “small” fish passed through a 7.5 mm (19/64 in) size-grader, and averaged 64.0 mm (2.5 in) and 3.0 g (0.1 oz); “medium” fish passed through a 10.7 mm (27/64 in) size-grader but were retained by a 7.5 mm (19/64 in) size-grader, and averaged 89.0 mm (3.5 in) and 8.0 g (0.3 oz); and “large” fish were retained by a 10.7 mm (27/64 in) size-grader, and averaged 132.0 mm (5.2 in) and 24.0 g (0.8 oz).

Size-graded fish were returned to separate tanks to observe their feeding behavior. Almost 100% of fish in the medium and large groups actively consumed formulated food, while only ~25% of the small fish consumed food. Three subsequent trials were then conducted (2007–2009) to evaluate the extent to which the small fish can be “re-trained” in autumn to accept formulated food in tanks. In each trial the fish were stocked into replicate tanks using the procedures described in Objective 1, for the initial feed-training of fingerlings in late-spring/early summer. The fish were then fed for a period of eight weeks. The primary result of these trials was that the survival of these small, autumn-harvested fingerlings in tanks was very low, averaging 37.6%. In each trial the fish showed a steady lingering mortality, despite the fact that the fish had a good condition factor and seemed otherwise to be in good condition. The exposure of these fish to the declining temperatures and photoperiods of autumn prior to pond harvest may have had a negative impact on their subsequent performance in tanks.

Also in each of two years two replicate ponds were stocked with these small fingerlings for overwintering and second year survival and growth studies. The ponds were stocked in the autumn at 124,000–184,000 fish/ha (50,000–75,000 fish/acre). Fish were then overwintered and not fed until the following spring. Fish in these ponds were then fed daily in a similar fashion to ponds containing larger second-year fingerlings. The four ponds were harvested in autumn; the mean survival was 63.2% and the mean fish size was 182 mm (7.2 in) and 75.6 g (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 at approximately 62,000 fish/ha (25,000 fish/acre) and three at approximately 185,000 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 ($68.7\% \pm 7.7$ versus $70.0\% \pm 9.8$) or mean fish size (22.3 ± 4.7 g [0.8 ± 0.2 oz] versus 21.0 ± 3.6 g [0.7 ± 0.1 oz]), 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 185,000 fish/ha (75,000 fish/acre) to improve their production. It is important, however, to include the following caveat regarding this recommendation. In

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parallel with these studies, additional fingerling ponds were stocked at densities of 185,000–250,000 fish/ha (75,000–100,000 fish/acre). At times, particularly during August and early September when temperatures and feeding rates are at their highest, levels of dissolved oxygen became problematic. Accordingly, it is recommended that fingerling producers who use high stocking densities regularly monitor levels of dissolved oxygen in the early morning, and be prepared to rapidly employ corrective actions if needed.

University of Missouri-Columbia

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.

Due to continued unavailability of fish from the original producer, additional producers were contacted; 30,000 juvenile largemouth bass (average 55.18 mm [2.17 in], 1.86 g [0.07 oz]) were purchased and transported from Cambridge, Ohio to the LDL location in August 2008. Fish were stocked into eight pond halves (four ponds total) at 37,000 fish/ha (15,000 fish/acre). 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.

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.

Survival, percentage of fish on feed, and percentage of fish cannibalized were estimated and stomach contents analyses done. Percent starvation was estimated from length and weight data (through use of relative weight (W_r), index of body plumpness with target being 100).

Mortality was estimated to be 3.58% in the first 10 days post-stocking. Fish that were not feeding subsequently perished within 1–2 weeks after stocking. 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.

By the end of the seven week sampling period, the fish exhibited an average length of 102.32 mm (4.03 in) and an average weight of 17.44 g (0.6 oz) and similar survival, ca. 50+% in the two treatments. Average feed conversion of the ponds during the sampling period was 1.16:1.

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On average, cannibalized fish were 44.8% as long as the fish that consumed them. In control-pond halves, it required four weeks for sufficient size variation to develop to allow cannibalism, while it took five weeks for this to occur in treatment-pond halves. Once cannibalism occurred in the control ponds it continued for four weeks, while cannibalism was only present in treatment ponds for two weeks. Total mortality due to cannibalization during the seven week sampling period for the treatment was estimated to be 15.65% of the population and 40.38% of the population for the control. Although 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, every largemouth bass that had cannibalized other fish also had commercial feed in their stomachs in this study. Frequent grading, as often as every four weeks, should be conducted to reduce cannibalism.

Individuals found with commercial feed in their stomachs had an average W_r of 136.8, while individuals not found to contain commercial feed had an average W_r 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 with fish having commercial feeds in their stomachs being relatively plumper. During the crowding phase (the initial two weeks of the study), treatment halves also showed statistically higher W_r 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 to determine their ability to return to feeding on commercial diets in the following spring. 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. The percentage of fish “on feed” (77.5%) was only slightly less than the 83% of fish “on feed” during the preceding fall at LDL; 87.4% of the fish 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 5,000-L (1,321-gal) grading tank where they were treated with a 5 ppm potassium permanganate bath for 30 min 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. 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] TL, respectively), of largemouth bass fingerlings were examined in an effort to increase the number of fish that were feed-trained and to determine the amount of labor involved in

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the process. A 20-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 7.9 and 13.2 fish/L (30.0 and 50.0 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 min apart, 24 h/day. Observations were then made of bird behavior and response to the call. Species, activity before call, response to call, distance from call (using a laser range finder), and time of day were recorded for each bird observed when the call was activated. 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 (<0.1 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 were 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.

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 onset

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 [GTW]). No auditory signal was used for the first seven days of yellow perch sac-fry and larval feeding of live foods. Between days 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 50.0–60.0 mm (2.0–2.4 in) body length. The fingerling perch were observed to elicit a feeding response in less than two 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.

Feeding trials were continued during 2010 to describe behavioral responses to feeding and assess methods for describing and managing transitions from one food type to another during development of intensively cultured larval yellow perch. Observations of feeding movement and whether or not food was visible in the gut for a sample of 100 individuals showed promise for describing

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the timing of transition from GTW prey to feeding on brine shrimp nauplii (BSN). The orange pigmentation of BSN was visible to the naked eye from above the tank. The presence of food in the gut seemed to be more useful than the incidence of feeding movements because it integrates feeding over a wider time period (related to food passage through the gut) rather than quantifying individual feeding movements of fish.

Visual observation from above was ineffective for assessing the transition from live prey to formulated feed (8–24 dph) due to the body walls of the fish becoming more opaque and active avoidance of observers by fish. At this point, fish response to auditory signals of feed provided by automatic feeders becomes more relevant for assessing transition to and acceptance of formulated feed. Beginning at 24 dph, there was a gradual increase in the number of fish that showed an orientation and swimming in the direction of the autofeeders when food was dropped. At about 30 dph, 10–20% of the fish moved toward the feeder when it dropped feed during morning observation. At 35 dph, the fish gradually began to exhibit a feeding behavior relative to the commercial feed as a function of sight and sound. Beyond 40 dph, 100% of the fish responded immediately to the sound and sight of food on the surface of the water.

IMPACTS

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

Studies have also provided 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.

RECOMMENDED FOLLOW-UP ACTIVITIES

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 2009, and the video should be completed sometime in late 2010. An extension publication on yellow perch fingerling production is scheduled for early in 2011.

These studies have developed methods and provided recommendations for yellow perch fingerling producers to improve the efficiency of fingerling production. Despite these improvements, current economic models for yellow perch culture show that fingerling costs remain a high percentage of the overall cost of producing food-size yellow perch. Therefore it is recommended that future studies be aimed at reducing fingerling production costs.

SUPPORT

NCRAC has provided \$300,000 which 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.

NORTH CENTRAL REGIONAL AQUACULTURE CENTER

SNAIL MANAGEMENT/GRUB CONTROL⁹

Project *Progress Report* for the Period
September 1, 2007 to August 31, 2010

NCRAC FUNDING: \$20,500 (September 1, 2007 to August 31, 2010)

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
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Extension Liaison:

Joseph E. Morris	Iowa State University	Iowa
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PROJECT OBJECTIVE

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 (see footnote below).

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. Outcomes of this project should help

culturists in dealing effectively and economically with these infestations.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

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 hosted on the North Central Regional Aquaculture Center (NCRAC) Web site.

⁹ This Progress Report is for the second objective of this project. A project component termination report for the first objective is contained elsewhere in this report. This is a project that had two years of funding and is chaired by Gregory W. Whitledge. It began September 1, 2007.

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WORK PLANNED

Iowa State University

In 2011 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 recently completed 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 also provide insight into the degree of snail population control required to limit grub prevalence in cultured fishes in ponds where food fish are raised.

SUPPORT

To date, NCRAC has provided \$20,500 which is the total amount allocated for this objective.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

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

SNAIL MANAGEMENT/GRUB CONTROL¹⁰

Project Component *Termination Report* for the Period
September 1, 2007 to August 31, 2010

NCRAC FUNDING: \$191,995 (September 1, 2007 to August 31, 2010)

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
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Extension Liaison:

Joseph E. Morris	Iowa State University	Iowa
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REASON FOR TERMINATION

Project objective completed.

PROJECT OBJECTIVE

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.

PRINCIPAL ACCOMPLISHMENTS

University of Wisconsin-Stevens Point (UW-Stevens Point)

Northern fantail crayfish (*Orconectes virilis*) were collected 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 moved to AquaPoint Fish

¹⁰This Project Component Termination Report is for the first of two objectives of this project. A progress report for the second objective is contained elsewhere in this report. This is a project that had two years of funding and is chaired by Gregory W. Whitledge. It began September 1, 2007.

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Farm, Stevens Point, Wisconsin, and Zelinski's Fish Farm, Antigo, Wisconsin. Both are commercial yellow perch (*Perca flavescens*) farms that have four, 0.022 ha (0.05 acre) ponds each that are fed with groundwater and are aerated. Ponds at both facilities had yellow perch that were 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. Two treatment ponds at AquaPoint were stocked with 361 crayfish each (density = 1.66 crayfish/m² [0.15 crayfish/ft²]; 15% female), and two treatment ponds at Zelinski's were stocked with 267 crayfish each (density = 1.20 crayfish/m² [0.11 crayfish/ft²]; 25% female). Each fish farm also had two control ponds that did not receive crayfish. 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 in all treatment ponds after the 2008 crayfish stocking.

Using an Eckmann grab sampler for benthic sampling, *Planorbella* (= *Helisoma*) and *Physa* snails were recovered from both treatment (crayfish added) and control ponds at both fish farms. Densities of *Planorbella* at the study ponds were generally low during spring sampling and increased through the summer. 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 experienced a

marginal decline during the summer. In terms of average snail size, both *Planorbella* and *Physa* snails exhibited declines through the summer.

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*.

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. 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/infected fish at AquaPoint treatment

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ponds and 9.25 grubs/infected fish at the AquaPoint control ponds. At Zelinski's, yellow grub prevalence, averaged over May through September, was 68.0% in treatment ponds while it measured 74.4% in control ponds. Similarly, average intensity of yellow grub infections in perch over the same time period was 3.71 grubs/infected fish at Zelinski's treatment ponds and 4.03 grubs/infected fish at the Zelinski's control ponds. At the completion of the 2-year study, treatment ponds at AquaPoint that received crayfish contained yellow perch that showed 43.9% fewer grub infections than the yellow perch in control ponds. Treatment ponds at Zelinski's contained yellow perch that showed 18.4% fewer grub infections than yellow perch in control ponds after two years.

During 2009 the efficacy of the competitively dominant digenean trematode, *Echinostoma*, at reducing grub infections in other snails and ultimately in fish in the treatment ponds was attempted, i.e., echinostome egg introduction on prevalence of infections in first intermediate host snails (*Planorbella*), and the intensity of echinostome metacercarial infections in snails (*Planorbella*) at both treatment ponds (receiving echinostome eggs) and control ponds (no eggs). Eight 0.02-ha (0.046 acre) study ponds at the Hess fish farm facility in New London, Wisconsin were each stocked with 500 yellow perch. 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.

In an attempt to culture echinostome worms for the production of eggs, *Planorbella* snails naturally infected with echinostome metacercarial cysts were dissected. Echinostome metacercarial cysts from these snails were removed and the isolated cysts were then introduced by oral gavage (~25

cysts/animal) 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 two weeks post-exposure. Unfortunately, while patent infections were achieved in hamsters in 2007, no patent infections (i.e., eggs appearing in feces) were achieved in attempts to infect the above described mammals in 2009. Marginal success was achieved using mallard ducklings. All eggs produced were distributed equally among the treatment ponds, with each pond receiving roughly 420 echinostome eggs between August 5 and August 17, 2009. Comparisons of snail infections between treatment and control ponds showed no significant differences in echinostome-infected snails in treatment versus control ponds.

Southern Illinois University-Carbondale (SIUC)

Laboratory trials were conducted in 2007-2008 to evaluate the potential of freshwater prawn (*Macrobrachium rosenbergii*) and two hybrid sunfishes (redeer sunfish × green sunfish [*Lepomis micolophus* × *L. cyanellus*]) and redear sunfish × warmouth [*L. gulosus*]) to serve as biological control agents for *Physa* spp. and *Planorbella* spp. Maximum consumption rates and maximum handling sizes for each of these taxa feeding on *Physa* and *Planorbella* were compared to those of redear sunfish, one of the most common molluscivores native to the North Central Region (NCR). Laboratory trials followed methods developed by Wang et al. that were published in the Journal of the World Aquaculture Society in 2003.

Redear × warmouth hybrids consumed larger snails than redear sunfish of equivalent body length, but consumed 25% fewer snails on average than redear sunfish.

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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 *Planorbella* up to 16.0 mm (0.6 in) total length. However, freshwater prawns showed a strong preference for consuming *Physa* over *Planorbella*; prawns consumed 77% of *Physa* offered in maximum consumption trials but consumed only 20% of *Planorbella* 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 *Planorbella* up to 12.0 mm (0.5 in) total length; redear sunfish in this size range only consumed snails <10.0 mm (<0.4 in) total length. Maximum consumption rates of redear × green sunfish hybrids were equivalent to those of similar-size redear sunfish.

Laboratory trials were conducted in 2008 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 (0.19–0.47 kg/m² [0.17–0.42 lb/ft²] of water surface; *N* = 3 replicate tanks/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 0.09 g/m² [0.03 oz/ft²] was 2% (range 0–6%). Salt concentrations up to 3 mg/L 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 in 2008 to evaluate the effectiveness of hydrated lime (Ca[OH]₂) for controlling snails in research ponds at SIUC. Enclosures were placed in shallow water (0.3m [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 (0.25 kg/ha² [0.22 lb/ft²]) 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 250 fish/ha (100 fish/acre), three ponds were stocked with redear sunfish at a rate of 500 fish/ha (200 fish/acre), and three ponds were stocked with redear × green sunfish hybrids at a rate of 500 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

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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.

Hydrated lime slurry applied at a rate of 31.7 kg/30.5 m (70.0 lb/100.0 ft) of linear shoreline in a 1.0-m wide (3.3-ft) swath produced a 99% reduction in estimated snail densities during 2009 pond trials. However, estimated snail densities in several ponds rebounded within two months of application. Ponds stocked with redear sunfish redear × green sunfish hybrids at 494 fish/ha (200 fish/acre) experienced a gradual decline in snail densities over four months, resulting in

a 95% overall reduction at the end of the trial period. Ponds treated with both hydrated lime and predator sunfish experienced an abrupt decrease in snail densities, with a less appreciable rebound relative to the hydrated lime treatment group. Lowest abundances of encysted trematodes in hybrid striped bass reared in ponds coincided with low densities of *Planorbella* spp. Estimated *Planorbella* densities during the month in which hybrid striped bass were stocked were most strongly correlated to trematode abundance in crop fish. All three treatment methods reduced snail densities relative to the control. A combination of chemical and biological treatments may produce a rapid reduction of snail densities and maintain low snail numbers over the growing season.

IMPACTS

These results have provided valuable information regarding the effectiveness and efficiency of several 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) were evaluated.

Work completed by UW-Stevens Point researchers has demonstrated that by utilizing locally available biological control species, e.g., crayfish, significant reductions in the snail populations can be achieved. Consequently, with a reduced snail population grub infections in fish contained within the ponds also decreased. Infections in yellow perch reared in ponds along with crayfish showed between 18–43% fewer grub infections after two years. However, it appears that more time is needed to completely eliminate snail populations and

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associated grub infections in fish culture ponds. This is most likely due to the low number of snails that actually harbor *Clinostomum*, the trematode causing “yellow grub” metacercaria. Snail density results showed that between 2–12% of snails actually harbored the yellow grub parasite; however, snails infected with the parasite were capable of releasing tens of thousands of cercaria. It might take time for the crayfish to eliminate all the infected snails especially when snail densities are high.

Crayfish may represent an economically viable, adaptable, and universally applied method of snail and grub management given enough time for the crayfish to be effective. The culturing and introduction of a competitively dominant echinostome trematode proved more difficult and laborious than predicted. Additionally, given the very low average prevalence of *Clinostomum* in snail first intermediate hosts, to possibly achieve measurably effects, the echinostome egg introduction to affected ponds would likely have to be maintained at a level of 10–100× greater than researchers were able to achieve in the field test. In short, this option is likely to be less effective as a biocontrol option compared to the crayfish manipulation.

Work completed at SIUC demonstrated that a combination of chemical (hydrated lime) and biological (native sunfish) treatments was effective for controlling snail abundance in ponds and limiting trematode abundance in cultured fish. Results also indicated that timing of treatment application is important for limiting grub infections. Integrated chemical and biological treatments yielded a rapid reduction in snail density that was maintained over the growing season. Application of a combination of snail control treatments may be a generally

effective strategy for consistently limiting snail abundance in ponds over time, thus reducing the potential for grub infestations in cultured fish.

RECOMMENDED FOLLOW-UP ACTIVITIES

Crayfish as a Biological Control Agent for Snails

Four years after the start of this investigation, producers associated with Zelinski’s fish farm indicated that there are thriving crayfish populations in the treatment ponds, along with crops of yellow perch, and that yellow perch harvested from these ponds have reduced or no grub infections. Control ponds that did not receive crayfish still do not have crayfish and yellow perch harvested from the control ponds continue to have grub infections. Based on the initial results at the completion of the 2-year study and the observations at Zelinski’s fish farm after four years, it appears that using crayfish as a biocontrol mechanism for snails and, ultimately, grubs may require longer than two years to be completely effective. Longer-term funding and further projects are needed to confirm these observations, but data show that crayfish are having an impact on the snail populations in the ponds. Understanding the extent of the crayfish impact and the duration and density of crayfish needed in fish culture ponds to reduce or eliminate grub infections should be further explored.

Integrated Approaches to Snail Management and Grub Control

While a combination of hydrated lime, native sunfish, and triploid grass carp (for vegetation control) was effective in limiting snail abundance and trematode infestation in hybrid striped bass in ponds at SIUC, other combinations of treatment methods may be more effective in ponds with different characteristics or in other geographic

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locations in the NCR. Additional evaluation of integrated pest management approaches (using various combinations of chemical, native biological, and/or physical treatment methods) are recommended to develop snail management and grub control methods tailored to particular settings given the variety of pond systems used for production of food fish across the NCR. Additional studies to assess the degree of snail population control required to limit grub

prevalence in cultured fishes are also recommended.

SUPPORT

NCRAC has provided \$191,995 which is the total amount allocated for this objective.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

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

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COMPARISON, IDENTIFICATION, AND ROLE OF MICROBIAL COMMUNITIES IN RECIRCULATING SYSTEMS IN THE NORTH CENTRAL REGION¹¹

Project *Progress Report* for the Period
September 1, 2009 to August 31, 2010

NCRAC FUNDING: \$65,000 (September 1, 2009 to August 31, 2010)

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PROJECT OBJECTIVES

1. Characterize the microbial communities in established production scale marine and freshwater recirculating aquaculture systems (RAS) units. These systems have been operational and producing aquatic organisms for more than one year.
2. Once these microbial communities have been identified, the role(s) of these microbial communities within the nitrogen cycle will be quantified with the goal of increasing the efficiency of the RAS (increased survival, growth and density, etc. of aquatic organisms).
3. Coordinate the results of this project with the Technical Committee Extension Subcommittee of NCRAC

ANTICIPATED BENEFITS

The goal of this work is to develop a thorough understanding of the microbial ecology and stoichiometry of nitrogen transformations in the biological filters of recirculating aquaculture systems (RAS). This research also seeks to attain a better understanding of the interactions of biofilter microbial communities with fish or shrimp. Knowledge of microbial community composition and dynamics under operational changes will help to determine optimal operational parameters. Process alterations can be made to favor a microbial community composition that will improve stability of nitrification, improve fish or shrimp survival, and allow farmers to increase growth rate or stocking density. A better understanding of the microbial community composition of these systems could also lead to a more rational design methodology. A systematic approach, based on microbial

¹¹This 1-year funded project is chaired by Lutgarde M. Raskin and it began September 1, 2009. In other areas of this report, this project is referred to as RAS Microbial Communities.

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community composition, for the design and optimization of RAS could lead to more widespread implementation and better economic efficiency.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

This research examines the operational conditions and the microbial ecology of two indoor RAS: a freshwater system growing yellow perch and a marine system growing Pacific white shrimp. The freshwater system is composed of a perch production tank, a pH adjustment tank in which sodium bicarbonate is added, a floating bed plastic bead filter, a fluidized bed sand filter, and a whiffle ball aerator. The marine system is composed of several shrimp production tanks, a biofilter containing oyster shells and plastic bioballs used as biofilm attachment media, and a solids settling basin. Much of the work has already been completed for the freshwater system and this progress report summarizes those results.

Three main approaches were taken to attain the objectives of the study for the freshwater system devoted to production of yellow perch. At first, the 26,498-L (7,000-gal) system was fully loaded with 10,000 near market-sized perch and could not be manipulated directly. Water samples for chemical analysis (ammonia and nitrite) were collected daily from five sites, and solid media (sand, plastic beads) were collected at intervals for bench incubation experiments and clone library analyses. More detailed water quality analyses were performed on two occasions. At the end of harvesting in March 2010, the system was idle for four days and we were allowed to make short-term additions during which the capacity of a mature system to transform realistic but large doses of ammonia and nitrite in single pulses through the biofilter system was tested. Another 10,000

fingerlings were added, and weekly water samples were taken along with occasional solid phase bench experiments. After harvesting of this batch in November 2010, the system was idle for more than two months and we were able to make whole-system additions to test capacity decay and recovery from loading starvation.

OBJECTIVE 1

Clone libraries have been completed for each of these filters, targeting the 16S ribosomal RNA genes of the bacterial and archaeal domains, and the *Planctomycetes*. The bacterial clone libraries revealed a diverse group of bacteria was present in both filters. The community in the plastic bead filter fell into 17 different classes, the largest of which were *Alphaproteobacteria*, *Acidobacteria*, and *Planctomycetes*. In the sand bead filter, the community was slightly less diverse. The classified sequences fell into 14 different classes of bacteria. Nitrite oxidizers of the genus *Nitrospira* were found in both the plastic bead and sand filters. Specifically, seven of the 231 sequences obtained from the plastic bead filter and eight of the 239 sequences collected from the sand filter were *Nitrospira*. These data suggest that the potential for nitrite oxidizing activity in the plastic bead filter is similar to that of the sand filter. No ammonia oxidizing bacteria (AOB) were found in either of the filters. Because ammonia oxidation is clearly occurring in this system (see below), one can assume that the fraction of AOB in this system was too low to be detected in a library of ~230 clones. While AOB are typically present at low levels in engineered nitrifying systems, it is still somewhat surprising that no AOB were detected. An upcoming pyrosequencing run (discussed in the WORK PLANNED section) will attempt to address this deficiency by allowing for

deeper sequencing of the microbial community.

The archaeal clone library showed much lower diversity. There were no ammonia-oxidizing archaea (AOA) detected in the system. Classified sequences from both filters represented almost exclusively the class *Thermoprotei*. Archaea will also be represented in the upcoming pyrosequencing run, which will indicate whether AOA are present in the system.

A third set of clone libraries was assembled for the class *Planctomycetes*. The goal of this clone library was to determine if there are any anaerobic ammonium oxidizing bacteria (anammox) present in the system. The plastic bead filter is not aerated and it is possible that there are anaerobic zones within the filter in which anammox bacteria could reside and contribute to nitrification. The clone library indicated the presence of *Planctomycetes* of seven and four different genera in the plastic bead filter and sand filter, respectively. However, none of the populations found in either filter were anammox bacteria.

OBJECTIVE 2

The freshwater RAS was analyzed for various water quality parameters, including ammonia, nitrite, nitrate, total and volatile suspended solids, chemical oxygen demand and alkalinity. Samples were taken at five points throughout the system: the fish production tank, the pH adjustment tank, plastic bead filter effluent, sand filter effluent, and aerator effluent. This analysis indicated that the majority of the nitrification (both ammonia and nitrite oxidation) occurred in the sand filter. The ammonia and nitrite are consistently removed to levels below 0.05 mg/L $\text{NH}_4^+\text{-N}$ and 0.2 mg/L $\text{NO}_2^-\text{-N}$, respectively. Ammonia and nitrite were monitored daily

at all five sites in the system and there was significant daily fluctuation in the production tank, pH adjustment tank, and plastic bead filter effluent, but ammonia and nitrite were both consistently removed to low levels.

In addition to daily monitoring of the system, the system was monitored over the course of a 24-h period to determine whether backwashing had an impact on nitrification performance. Backwashing of the plastic bead filter is performed every morning to remove excess solids. Backwashing results in the washout of some of the biofilm attached to the beads and has the potential to disrupt nitrification if ammonia or nitrite oxidizers are sloughed off in significant numbers. Water was sampled and measured for ammonia, nitrite, and nitrate immediately before and after backwashing. Then, all five previously mentioned sample locations were sampled every ten min for the first hour, every half hour for the next two hours, every two hours for the next eight hours and finally the next morning before backwashing. The ammonia concentrations showed a small increase (0.1 to 0.6 mg/L $\text{NH}_4^+\text{-N}$) for the production tank, pH tank and plastic bead filter effluent within the first three hours after backwashing. However, the concentrations after the sand filter and aerator showed no detectable increase. Nitrite concentrations also showed no significant increase as a result of backwashing. These results indicate that backwashing of the plastic bead filter does not affect nitrification performance of the overall system and suggest that variations may be due to the feeding cycle.

The possible variations due to the feeding cycle were evaluated in more detail and it was found that the ammonia concentration varied according to a daily cycle. Equal

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amounts of food pellets were provided at 30 min intervals from early morning until late afternoon. During this time, ammonia levels in the production tank and in the plastic bead effluent increased continuously, but never reached levels of concern (high values were 0.3–0.4 mg/L $\text{NH}_4^+\text{-N}$). Nitrite was constantly near the limit of detection (0.001 mg/L $\text{NO}_2^-\text{-N}$). As soon as feeding ceased, ammonia decreased to return to morning lows of ca. 0.05 mg/L $\text{NH}_4^+\text{-N}$. Nitrite was elevated in the bead filter effluent but was subsequently removed in the sand filter.

During the protracted three-week harvesting of 10,000 market-size perch, the ammonia concentration in the production tank declined continuously with reduced feeding. The alkalinity also declined, while the nitrite concentration remained at very low levels. Bioassays of nitrification capacity showed a 75% reduction in ammonia oxidation but only 25% reduction in nitrite removal capacity during the two weeks between mid-harvest and new stocking (low loading period). At this time system, alkalinity levels were low and may have contributed to decreased ammonia oxidation potential. Both processes returned to their production-level capacity during grow out.

In the three days between final harvest (February 2010) and new stocking, a series of ammonia and nitrite additions were made as pulses directly into the biofilter stream to test loading capacity. The addition of 1.5 mg/L $\text{NO}_2^-\text{-N}$ was consumed almost completely in one pass through the sand filter, allowing only 0.1 mg/L $\text{NO}_2^-\text{-N}$ to return to the production tank, which was completely removed within 15 min. Increasing the dose to 7 mg/L $\text{NO}_2^-\text{-N}$ allowed slightly more breakthrough of nitrite into the production tank, but the highest value observed (0.3 mg/L $\text{NO}_2^-\text{-N}$) was completely removed in 60 min. Larger

additions of ammonia behaved similarly. With the addition of 14 mg/L $\text{NH}_4^+\text{-N}$, 2.2 mg/L $\text{NH}_4^+\text{-N}$ was detected in the sand filter effluent, but the system reached background levels of 0.2 mg/L $\text{NH}_4^+\text{-N}$ in less than 2 h. Increasing the ammonia dose to 70 mg/L $\text{NH}_4^+\text{-N}$ allowed 11 mg/L $\text{NH}_4^+\text{-N}$ to reach the production tank, and it took 4 h to return to system wide ammonia concentrations less than 1 mg/L $\text{NH}_4^+\text{-N}$. During actual system operation, filter loading occurs primarily through ammonia production in the production tank and does not occur as sudden pulses of high concentrations. However, the above experiments provide valuable information on maximum ammonia and nitrite removal rates that can be achieved in the sand filter.

During grow out of the next 10,000 perch (March–November 2010), occasional assays of sand filter capacity demonstrated return to previous levels of activity. After harvest (November 2010), a series of measurements were undertaken designed to elucidate starvation conditions for the sand filter and to compare bench incubation experiments with whole system progressions in nitrification capacity. After the first week without fish, ammonia oxidation capacity had dropped by 60% even though total alkalinity was adequate. Nitrite removal was much less sensitive to initial starvation. In bench incubation experiments, an additional week of starvation resulted in a small decrease in ammonia removal potential but nitrite potential fell by half. These conditions remained stable for an additional three weeks. Six weeks after fish removal, a week-long series of measurements of the whole system was initiated. Initially, ammonium chloride was added into the production tank to achieve a system wide concentration of 15 mg/L $\text{NH}_4^+\text{-N}$. Samples were collected at 1-min intervals for 1 h, 2-min intervals for another

hour, and then at increased time spans. Analysis of the added chloride (not biologically or chemically active) demonstrated that complete mixing of the system occurred in 30 min. Slow disappearance of ammonia was not accompanied by nitrite buildup, but the initially low alkalinity declined to limiting levels. At 48 h, bicarbonate (5 kg; 11 lb) was added to replenish the alkalinity, and rapid sampling was repeated as before. Shortly after this addition, the rate of ammonia removal began to increase, and a small amount of nitrite began to build up, though the level reached was less than 0.02 mg/L NO_2^- -N. At 96 h, when more than 75% of the ammonia had been removed, an addition of nitrite was made to attain 1.5 mg/L NO_2^- -N. Nitrite was transformed rapidly to completion without lag at rates similar to mid-production measurements.

Bench incubation studies were performed by collecting plastic beads and sand with biofilm from the respective filters and incubating these samples in the presence of ammonia and nitrite. These experiments confirmed that ammonia and nitrite oxidation occurred primarily by the biofilm phases. Ammonia and nitrite removal began immediately in both sand and plastic beads incubations, but overlying water from any site required at least four days to show concentration reduction. Removal rates were directly related to the amount of solid phase material present (as determined by surface area) regardless of the ratio to overlying water. The sand biofilm appeared to contain a balanced assemblage of ammonia and nitrite oxidizers, such that ammonia additions did not result in build-up of the nitrite product and nitrite was also consumed as quickly as it was produced. Plastic beads did not exhibit the expected nitrite removal capacity (which contrasted by the suggested nitrite oxidation capacity

due to the presence of nitrite oxidizers determined by the clone library analysis—Objective 1) and hence nitrite built up to high levels during ammonia addition experiments. This is consistent with the occasional observation of higher nitrite concentrations downstream of the bead filter in the whole system sampling. The maximum ammonia consumption rate was observed for a concentration of ammonia that was 10× lower than the corresponding nitrite concentration. This finding suggests that bacteria could quickly respond to spikes in nitrite loading while ammonia transformation remained nearly constant under the daily conditions of fish production. Both ammonia and nitrite oxidation results in the production of acid: two acid equivalents are produced for each ammonia and one acid equivalent for each nitrite. The bottle experiments confirmed this theoretical production of acid, in that the alkalinity concentration decreased twice as fast as the ammonia concentration. When the alkalinity reached low levels, about 10% of the alkalinity in natural waters, the ammonia oxidation slowed down considerably. The transformation rate could be reestablished through the addition of sodium bicarbonate.

WORK PLANNED

The remaining work for the freshwater yellow perch system is to quantify the diversities and dynamics of bacterial and archaeal nitrifiers through time. Biological media samples have been collected from both the plastic bead and sand biofilters. The samples will be used to quantify the abundance and dynamics of nitrifying populations in each of these filters. This will be accomplished using quantitative polymerase chain reaction (qPCR), which will allow for specific nitrifying populations to be quantified based on the quantity of 16S

rRNA gene sequences in each of the samples.

Similar experiments to those performed for the freshwater system will be performed on the marine shrimp system. Another set of clone libraries for bacteria, archaea, and planctomycetes has been constructed for biomass collected from these biological filters. This will provide information about the microbial populations that inhabit the filters and can indicate which nitrifying populations are most important in the system. To further determine the dominant nitrifying populations, qPCR will be performed on samples from both the oyster shell and the plastic bioball compartments at various points along the depth of the biofilters.

The marine shrimp farm has been shutdown and will be restarted in March. Over the course of the startup, which typically takes six months, the system will be monitored for various water quality parameters to determine how ammonia, nitrite, and nitrate levels change in a system that is not operating at steady state. As the nitrifying biomass establishes itself in the biofilters, the ammonia and nitrite oxidation rates should increase. The biofilters will also be sampled and the various populations quantified to track how the community changes under an increasing ammonia load.

During startup and after the system achieves steady state, water quality will be measured at several points throughout the system to track the change in ammonia and nitrite as the water passes through each shrimp tank and each level in the biofilter. This will show where the majority of ammonia and nitrite oxidation occurs in the system and provide insight into how the system functions.

Batch incubation experiments similar to those discussed for the freshwater system will be performed using the oyster shells and plastic bioball media from these biofilters. These will be used to determine the ammonia and nitrite oxidation potential at various points along the biofilter depth. It could also provide information about whether certain populations tend to grow better on the bioballs or the oyster shells.

In order to sequence the biomass samples to a deeper level than is possible with clone libraries, samples from both the freshwater and marine RAS will be included in a massively parallel sequencing (pyrosequencing) run. This will result in approximately 10,000 sequences for bacterial and archaeal samples for each of the biofilters. This will give a better indication of the AOB, AOA, and nitrite oxidizing bacteria populations present in each of the samples. The results from this sequencing run can also allow for a certain level of quantification of the relative abundance of various microbial populations.

IMPACTS

- ▶ Improve knowledge of nitrogen cycle in RAS and generate means for stable management of toxic nitrogen compounds.
- ▶ Understand how operational changes (e.g., backwashing, change in ammonia load due to feeding) affect biofilter operation.
- ▶ Examine dynamics of nitrification activity and community composition during startup.
- ▶ Highlight similarities and differences in process performance and operation between freshwater and marine RAS.
- ▶ Improve economic viability of RAS by finding ways to improve process efficiency (e.g., underutilized

RAS MICROBIAL COMMUNITIES

nitrification potential suggests that a greater stocking density is possible).

- ▶ In collaboration with a Mathematics-Biology Initiative at the University of Wisconsin-Milwaukee, an undergraduate student has made progress on a model using physical characteristics of the RAS, flow rates, bench-derived process rates, and time series chemical analysis.
- ▶ An Environmental Engineering Master's student has been trained in molecular microbial ecology approaches and studies of RAS.

SUPPORT

NCRAC has provided \$65,000 which is the entire amount allocated for this 1-year project.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

See the Appendix for a cumulative output for all NCRAC-funded RAS Microbial Communities activities.

NORTH CENTRAL REGIONAL AQUACULTURE CENTER

VIRAL HEMORRHAGIC SEPTICEMIA (VHS)¹²

Project *Progress Report* for the Period
September 1, 2008 to August 31, 2010

NCRAC FUNDING: \$197,960 (September 1, 2008 to August 31, 2010)

PARTICIPANTS:

Jeffrey J. Rach	Upper Midwest Environmental Sciences Center	Wisconsin
Glenda D. Dvorak	Iowa State University	Iowa
Ronald E. Kinnunen	Michigan State University	Michigan
Jeffrey A. Malison	University of Wisconsin-Stevens Point	Wisconsin
Industry Advisory Council Liaison:		
Christopher Weeks	Michigan State University	Michigan

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 would 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 specific fish

disease HACCP plans for each of the six facilities involved in the workshops.

- (5) Develop and distribute three model fish disease HACCP plans (one each for flow-through, pond, and recirculation systems), relying heavily on the specific 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 cool and warm water fish eggs. If iodophor disinfection can be used to safely

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

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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 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.

PROGRESS 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.

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.

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 fertilization for 10 min may reduce VHSv (strain IVb) transmission without affecting egg hatch.

In Year 2, adult walleye were collected from North Dakota and spawned at UMESC. Immediately after sperm activation, fertilized eggs were taken to a controlled access laboratory where egg disinfection and incubation activities occurred. The study objective was to determine the safety of iodophor surface disinfection at target doses of 0, 1, or 2× the recommended dose rate (100 mg/L) for multiple exposure durations at various times post fertilization and for 1 or 2 disinfection events (Appendix Table 4). The second disinfection event was administered at the approximate midpoint between fertilization and the first cell division.

The study was conducted in replicate egg jars supplied with well water in a continuous flow system. Egg jars were connected to one of four individually plumbed headbox systems. Each headbox supplied water to 12 egg jars. There were six egg jars per side (block) with one treatment randomly assigned to each block of six egg jars. Eggs (25 ± 5 mL) were assigned to each jar according to a completely randomized distribution scheme.

Very poor fertilization rates were realized. Although adult walleye appeared healthy at spawning and females had free flowing eggs, most male walleye provided very little milt. When notochord development was checked it was apparent that fertilization had not occurred. This trial was terminated

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before embryo hatch due to the low fertilization rate.

Also in Year 2 adult hybrid striped bass were collected from Oklahoma by the Oklahoma Department of Fish and Game and spawned at UMESC. Adult female striped bass were injected with human chorionic gonadotropin (HCG) to stimulate oocyte maturation. After staging, ripe female striped bass eggs were fertilized with male white bass milt. The study objective was to determine the safety of iodophor surface disinfection at target doses of 0, 0.25, 0.50 or 1× the recommended dose rate (100 mg/L) for multiple exposure durations (Appendix Table 5). The study was conducted according to the methods described above for walleye.

The hybrid striped bass eggs were very sensitive to iodophor disinfection. Hatch rate in the 25 mg iodophor/L disinfection group was similar to that of the untreated controls; hatch was very limited in the 50 mg iodophor/L disinfection group and nonexistent in the 100 mg iodophor/L disinfection group.

Iodophor concentrations safe to disinfect hybrid striped bass eggs are substantially less than those used to disinfect the surfaces of eggs of other fish species. Presently it is not clear whether iodophor disinfection is suitable for surface disinfection of hybrid striped bass eggs.

Although walleye safety data were not collected during this spawning year at UMESC, other laboratories did collect data which, when combined with previous UMESC data and data available from the literature, should describe the safe treatment regimens for walleye. UMESC did collaborate with the Missouri Department of Conservation on the effect of iodophor

disinfection on walleye egg hatch and fingerling survival. These data are being summarized and will be submitted to UMESC.

OBJECTIVE 2

The VHS “response” packet was developed by Iowa State University in April 2009. 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 Leaders (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).

OBJECTIVE 3

In 2009/2010, eight planned VHS-biosecurity workshops were conducted at aquaculture facilities in the NCR. 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, 2009, Indiana – Bodin State Fish Hatchery (recirculating aquaculture system), 27 total in attendance.

- ▶ June 25, 2009, Missouri – Crystal Lakes Fisheries (flow through), 29 total in attendance.
- ▶ August 20, 2009, Michigan – Michigan Bait and Fish Farm (flow through), 24 total in attendance.
- ▶ September 17, 2009, Wisconsin – Gollon Bait and Fish Farm (pond), 23 in attendance.
- ▶ April 6, 2010, Wisconsin – U.S. Geological Survey UMESC (research), 50 in attendance.
- ▶ April 21, 2010, Michigan – Keweenaw Bay Indian Fish Hatchery (raceway/pond), 10 in attendance.
- ▶ May 6, 2010, Ohio – Calala’s Water Haven (pond), 12 in attendance.
- ▶ June 17, 2010, South Dakota – Porter’s Bait Farm (pond and flow through), 20 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 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, 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

- ▶ 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.
- ▶ Gollon Bait and Fish Farm had their own biosecurity plan, which was used as a basis for drawing up a HACCP biosecurity plan.
- ▶ U.S. Geological Survey UMESC had a biosecurity plan developed which was reviewed and recommendations for improvement were made.
- ▶ Keweenaw Bay Indian Fish Hatchery is working on developing biosecurity measures and recommendations were made on critical control points.
- ▶ Calala’s Water Haven produces and sells softshell crayfish and an AIS-HACCP plan was developed for this part of their bait operation.
- ▶ Porter’s Bait Farm produces and sells fathead minnows and an AIS-HACCP plan was developed for this part of their bait operation.

WORK PLANNED

OBJECTIVE 1

- ▶ Evaluate vertical transmission of VHSV from adult minnows to eggs. Adult minnows will be exposed to VHSV by injection prior to spawning. Adults and eggs will be tested for the presence/absence of VHSV. A portion of the eggs will be allowed to hatch and the fry retained for approximately one month then tested for the presence or absence of VHSV.
- ▶ Evaluate the use of iodophor disinfection to eliminate VHSV from yellow perch eggs intentionally challenged with

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VHSv. Perch eggs will be challenged using methods similar to those previously reported for walleye and northern pike.

OBJECTIVE 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 ISU 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.
- ▶ The project demonstrated that hybrid striped bass are sensitive to iodophor disinfection.
- ▶ A U.S. Geological Survey Fact Sheet was published in FY 2010 (see <http://pubs.usgs.gov/fs/2009/3107/>).
- ▶ 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. VHS has changed how fish farmers do business in the NCR whether farmers are located in a state directly impacted by the Federal Order or a state that has farmers doing business in the Great Lakes states. Through workshops and educational materials on biosecurity, farmers have become aware of the risks and potential hazards diseases from outside sources bring. Biosecurity was not a word of common vocabulary before 2007 and now is incorporated as part of their business plan. State agencies have responded with their own set of rules requiring additional testing and fish certifications. Farmers have been able to utilize biosecurity strategies to minimize the impacts these rules have or they have been able to continue business by complying with requirements in new rules when biosecurity plans are mandatory.

- ▶ 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.

VHS

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
2009-10	\$8,895			\$50,000			\$50,000
TOTAL	\$125,765			\$73,422	\$3,900	\$27,322	\$194,192

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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 post-fertilization (min)	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

VHS

Table 4. Iodophor disinfection treatment groups for walleye eggs.

Treatment Group	Concentration (mg iodophor/L)	Duration (min)	Time initiated post fertilization (min)	Second iodophor disinfection duration (min)
1	NA	NA	NA	NA
2	100	10	90	NA
3	100	30	immediately	NA
4	200	10	90	NA
5	200	30	immediately	NA
6	NA	NA	NA	NA
7	100	10	90	10
8	100	30	immediately	10

Table 5. Iodophor disinfection treatment groups for striped bass eggs.

Treatment Group	Concentration (mg iodophor/L)	Duration (min)	Time initiated post fertilization (min)
1	NA	NA	NA
2	25	10	90
3	25	30	immediately
4	50	10	90
5	50	30	immediately
6	NA	NA	NA
7	100	10	90
8	100	30	immediately

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APPENDIX

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APPENDIX

AQUACULTURE DRUGS

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- Salmonid Culture, East Lansing, Michigan, March 23-24, 1990. (Donald L. Garling)
- Midwest Regional Cage Fish Culture Workshop, Jasper, Indiana, August 24-25, 1990. (LaDon Swann)
- Aquaculture Leader Training for Great Lakes Sea Grant Extension Agents, Manitowoc, Wisconsin, October 23, 1990. (David J. Landkamer and LaDon Swann)
- Regional Workshop of Commercial Fish Culture Using Water Reuse Systems, Normal, Illinois, November 2-3, 1990. (LaDon Swann)
- 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)

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- 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 Spreading 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)

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- Yellow Perch Aquaculture Workshop, Bad River Tribal Hatchery Program, Milwaukee, Wisconsin, December 2004. (Fred P. Binkowski)
- Yellow Perch and Lake Sturgeon Workshop, Lac du Flambeau Tribal Hatchery, Milwaukee, Wisconsin, February 2005. (Fred P. Binkowski)
- Yellow Perch Aquaculture Workshop, Kearney, Nebraska, February 26, 2005. (Fred B. Binkowski)
- 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. Quagraine)
- 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. Quagraine)
- 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

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- 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)
- Seafood HACCP Training Workshop, Bay Mills, Michigan, December 9-11, 2008. (Ronald E. Kinnunen)
- North Central Regional Aquaculture Center Seeks Input from the Missouri Aquaculture Industry, Missouri Aquaculture Association Meeting and Biosecurity Workshop, Jefferson City, Missouri, January 23, 2009. (Christopher T. Weeks)
- AIS-HACCP Training Workshop, Ashland, Nebraska, October 15, 2009. (Ronald E. Kinnunen and Richard Clayton)
- Aquaculture in Michigan – A Brief Overview of Status, Regulatory Structures and Impacting Factor, Lansing, Michigan, January 22, 2010. (Christopher T. Weeks)
- Michigan Aquaculture Association, Aquaculture Biosecurity/AIS-HACCP Update, Mt. Pleasant, Michigan, February 25, 2010. (Ronald E. Kinnunen)
- Seafood HACCP Training Workshop, Bay Mills, Michigan, March 9-11, 2010. (Ronald E. Kinnunen)
- Certified Pesticide Applicators Workshop, Aquatic Plant Identification and Control, Wetmore, Michigan, March 19, 2010. (Ronald E. Kinnunen and Jim Islieb)
- Interstate Movement of Live Fish Regulations in the North Central U.S., Workshop for Veterinarians, Madison, Wisconsin, April 26-27, 2010. (Christopher T. Weeks)
- AIS-HACCP Training Workshop, Spirit Lake, Iowa, June 16, 2010. (Ronald E. Kinnunen and Joseph Morris)
- Baitfish Regulations and State Industry Contacts, NCRAC Baitfish Workshop, La Crosse, Wisconsin, September 21, 2010. (Christopher T. Weeks)
- Biosecurity and VHS Virus Update, NCRAC Baitfish Workshop, La Crosse, Wisconsin, September 21, 2010. (Ronald E. Kinnunen)
- Early Spawning and Associated Laboratory Practices of Golden Shiners, NCRAC Baitfish Workshop, La Crosse, Wisconsin, September 21, 2010. (Joseph E. Morris)
- Pond Fertilization Strategies for Northern Climates, NCRAC Baitfish Workshop, La Crosse, Wisconsin, September 21, 2010. (Joseph E. Morris)
- Interstate Movement of Live Fish Regulations in the North Central U.S., Workshop for Veterinarians, Frankfort, Indiana, September 30, 2010. (Christopher T. Weeks)
- Online fish health program for fish farmers. The 35th Annual Eastern Fish Health Workshop, Shepherdstown, Shepherdstown, West Virginia, May 24-28, 2010. (Myron J. Kebus)
- NCRAC Baitfish Workshop, La Crosse, Wisconsin, September 21, 2010. (Jeff Gunderson, Joseph E. Morris and Ronald E. Kinnunen)
- Proceedings**
- Proceedings of the North Central Regional Aquaculture Conference. 1st North Central Regional Aquaculture Conference, Kalamazoo, Michigan, March 18-21, 1991.
- Gunderson, J., editor. 1995. Proceedings of the Combined North Central and Ninth Annual Minnesota Aquaculture Conference and Tradeshow. 2nd North Central Regional Aquaculture Conference, Minneapolis, Minnesota, February 17-18, 1995.
- Swann, L., editor. 1997. Proceedings of the 1997 North Central Regional Aquaculture Conference. 3rd North Central Regional Aquaculture Conference, Indianapolis, Indiana, February 6-7, 1997. Illinois-Indiana Sea Grant Program, Publication CES-305. (Also available

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Morris, J.E., editor. 1999. Aquaculture at the crossroads: linking the past to the future. Compilation of abstracts, papers, and supporting articles for the 4th North Central Regional Aquaculture Conference, Columbia, Missouri, February 24-26, 1999.

FEED TRAINING CARNIVOROUS FISH

Publication in Print

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

Paper Presented

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

HYBRID STRIPED BASS

Publications in Print

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 alleviation via sodium chloride. Transactions of the American Fisheries Society 130:706-711.

Anonymous. 1995. Proceedings of the NCRAC Hybrid Striped Bass Workshop. NCRAC Publications Office, Iowa State University, Ames.

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

Brown, P.B., B.J. Brown, S. Hart, J. Curry, and A. Hittle-Hutson. 2008. Comparison of soybean-

based practical diets containing 32, 36, or 40% crude protein fed to hybrid striped bass in earthen culture ponds. North American Journal of Aquaculture 70:128-131.

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

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

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

Kemeh, S., and P.B. Brown. 2001. Evaluation of different stocking densities for hybrid striped bass in small-scale recirculation systems. North American Journal of Aquaculture 63:234-237.

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

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

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

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

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

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- Lane, R.L., and C.C. Kohler. 2007. Influence of organic fertilizer source on fatty acid composition of zooplankton and sunshine bass fingerlings. *North American Journal of Aquaculture* 69:413-418.
- Lane, R.L., J.T. Trushenski, and C.C. Kohler. 2006. Modification of fillet composition and evidence of differential fatty acid turnover in sunshine bass *Morone chrysops* × *M. saxatilis* following change in dietary lipid source. *Lipids* 41:1029-1038.
- Lewis, H.A., and C.C. Kohler. 2008. Corn gluten meal partially replaces fish meal without compromising growth or fatty acid composition of sunshine bass. *North American Journal of Aquaculture* 70:50-60.
- Lewis, H.A., and C.C. Kohler. 2008. Minimizing fish oil and fish meal with plant-based alternatives in sunshine bass diets without negatively impacting growth and muscle fatty acid profile. *Journal of the World Aquaculture Society* 39:573-585.
- Morris, J.E., C.C. Kohler, and C.C. Mischke. 1999. Pond culture of hybrid striped bass in the North Central Region. NCRAC Fact Sheet Series #107, NCRAC Publications Office, Iowa State University, Ames.
- Myers, J.J. 1999. Acute responses to salinity for sunshine bass and palmetto bass. Master's thesis. Southern Illinois University-Carbondale.
- Myers, J.J., and C.C. Kohler. 2000. Acute responses to salinity for sunshine bass and palmetto bass. *North American Journal of Aquaculture* 62:195-202.
- Rudacille, J.B., and C.C. Kohler. 2000. Aquaculture performance comparison of sunshine bass, palmetto bass, and white bass. *North American Journal of Aquaculture* 62:114-124.
- Settor, K. 1998. Evaluation of different densities for hybrid striped bass (*Morone saxatilis* × *M. chrysops*) in cages and small-scale recirculation system. Master's thesis. Purdue University, West Lafayette, Indiana.
- Suresh, A.V., J.B. Rudacille, M.L. Allyn, V. Sheehan, R.J. Sheehan, and C.C. Kohler. 2000. Single injections of human chorionic gonadotropin or mammalian gonadotropin releasing hormone analog at low dosages induce ovulation in white bass. *North American Journal of Aquaculture* 62:87-94.
- Trushenski, J.T., and C.C. Kohler. 2006. Evaluation of natural source vitamin E, d-alpha tocopheryl acetate, as a micronutrient in sunshine bass feed. *North American Journal of Aquaculture* 68:186-191.
- Trushenski, J.T., C.S. Kaspar, and C.C. Kohler. 2006. Challenges and opportunities in finfish nutrition. *North American Journal of Aquaculture* 68:122-140.
- Trushenski, J.T., and C.C. Kohler. 2007. Influence of stress and dietary natural source vitamin E on nonspecific immunocompetence, tissue tocopherol composition, and postslaughter fillet oxidative stability of sunshine bass. *North American Journal of Aquaculture* 69:330-339.
- Trushenski, J.T., and C.C. Kohler. Influence of stress, exertion, and dietary natural source vitamin E on prostaglandin synthesis, hematology, and tissue fatty acid composition of sunshine. *North American Journal of Aquaculture* 70:251-265.
- Volkman, E.T., C.C. Kohler, and S.T. Kohler. 2004. Assessment of floating vertical raceways for the culture of phase-II hybrid striped bass. *North American Journal of Aquaculture* 66:125-132.
- Wetzel, J.E., C.C. Kasper and C.C. Kohler. 2006. Comparison of pond production of phase-III sunshine bass fed 32-, 36-, and 40%-crude-protein diets with fixed energy:protein ratios. *North American Journal of Aquaculture* 68:264-270.
- Woods, L.C., C.C. Kohler, R.J. Sheehan, and C.V. Sullivan. 1995. Volitional tank spawning of female striped bass with male white bass produces hybrid offspring. *Transactions of the American Fisheries Society* 124:628-632.

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- Brown, B.J., P.B. Brown, S. Hart, J. Curry, and A. Hittle-Hutson. 2005. Comparison of practical diets containing 32, 36, or 40% crude protein fed to hybrid striped bass in earthen culture ponds. *Aquaculture America 2005*, New Orleans, Louisiana, January 20, 2005.
- Brown, P.B., R. Twibell, Y. Hodgins, and K. Wilson. 1995. Soybeans in diets fed to hybrid striped bass. 24th Annual Fish Feed and Nutrition

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- Workshop, Columbus, Ohio, October 19-21, 1995.
- Brown, P.B., Y. Hodgin, R. Twibell, and K.A. Wilson. 1996. Use of three soybean products in diets fed to hybrid striped bass. 27th Annual Meeting of the World Aquaculture Society, Bangkok, Thailand, January 29-February 2, 1996.
- Brown, G.G., L.D. Brown, K. Dunbar, C. Habicht, R.J. Sheehan, C.C. Kohler, and L. Koutnik. 1991. Evaluation of white bass semen with 31P-NMR for the improvement of transportation, storage, and fertility methods. 53rd Midwest Fish and Wildlife Conference, Des Moines, Iowa, November 30-December 4, 1991.
- Brown, G.G., R.J. Sheehan, C.C. Kohler, C. Habicht, L. Koutnik, L. Ellis, and L.D. Brown. 1995. Use of cryopreservatives. North Central Regional Aquaculture Center Hybrid Striped Bass Workshop, Champaign, Illinois, November 2-4, 1995.
- Brown, G.G., R.J. Sheehan, C.C. Kohler, C. Habicht, L. Koutnik, L. Ellis, and L.D. Brown. 1998. Short-term storage of striped bass *Morone saxatilis* semen. 29th Annual Meeting of the World Aquaculture Society, Las Vegas, Nevada, February 15-19, 1998.
- Habicht, C., R.J. Sheehan, C.C. Kohler, G.G. Brown, and L. Koutnik. 1991. Routine collection, storage, and shipping of white bass sperm. 29th Annual Meeting Illinois Chapter of the American Fisheries Society, Champaign, Illinois, March 5-7, 1991.
- Kasper, C.S., and C.C. Kohler. 2004. Use of finishing diets in indoor hybrid striped bass culture reduces production costs. Fifth International Conference on Recirculating Aquaculture, Roanoke, Virginia, July 22-25, 2004.
- Kohler, C.C. 1993. The farm fish of the future: hybrid stripers. Aqua '93: 7th Annual Minnesota Aquaculture Conference, Alexandria, Minnesota, March 5-6, 1993. (Invited paper)
- Kohler, C.C. 1994. Hybrid striped bass aquaculture. Yellow Perch and Hybrid Striped Bass Production: From Fry to Frying Pan, Piketon, Ohio, July 3, 1994. (Invited speaker)
- Kohler, C.C. 1995. Broodstock management of white bass. North Central Regional Aquaculture Center Hybrid Striped Bass Workshop, Champaign, Illinois, November 2-4, 1995.
- Kohler, C.C. 1996. Induced out-of-season spawning of fishes. Missouri Aquaculture Industry Association Annual Meeting, Jefferson City, Missouri, February 3-4, 1996.
- Kohler, C.C. 1996. Advancing hybrid striped bass culture in the North Central Region and elsewhere. U.S. Chapter of the World Aquaculture Society, Arlington, Texas, February 14-17, 1996.
- Kohler, C.C. 1997. Induced spawning of fishes. Third North Central Regional Aquaculture Conference, Indianapolis, Indiana, February 6-7, 1997.
- Kohler, C.C. 1998. Hybrid striped bass culture in the Midwest. Joint Missouri-Kansas Aquaculture Conference, Springfield, Missouri, March 4-6, 1998.
- Kohler, C.C., and R.J. Sheehan. 1991. Hybrid striped bass culture in the North Central Region. First North Central Regional Aquaculture Conference, Kalamazoo, Michigan, March 18-21, 1991.
- Kohler, C.C., R.J. Sheehan, and T.B. Kayes. 1989. Advancing hybrid striped bass culture in the Midwestern United States. 51st Midwest Fish and Wildlife Conference, Springfield, Illinois, December 5-6, 1989.
- Kohler, C.C., R.J. Sheehan, V. Sanchez, and A. Suresh. 1994. Evaluation of various dosages of hCG to induce final oocyte maturation and ovulation in white bass. 25th Annual Meeting of the World Aquaculture Society, New Orleans, Louisiana, January 12-18, 1994.
- Kohler, C.C., R.J. Sheehan, M.L. Allyn, J.B. Rudacille, and A. Suresh. 1996. Controlled spawning of white bass. U.S. Chapter of the World Aquaculture Society, Arlington, Texas, February 14-17, 1996.
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SOME COMMONLY USED ABBREVIATIONS AND ACRONYMS

×	cross, by, or times
AIS	aquatic invasive species
anamnox	anaerobic ammonium oxidizing bacteria
AOA	ammonia oxidizing archaea
AOB	ammonia oxidizing bacteria
APHIS	Animal and Plant Health Inspection Service
AREF	Aquaculture Regional Extension Facilitator
AquaNIC	Aquaculture Network Information Center
BOD	Board of Directors
BSN	brine shrimp nauplii
°C	degrees Celsius
CES	Cooperative Extension Service
CSFPH	Center for Food Security and Public Health
CVM	Center for Veterinary Medicine
dph	day(s) post hatch
EPC	epithelioma papulosum cyprini
°F	degrees Fahrenheit
FM	fish meal
FSR	final study report
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
HCG	human chorionic gonadotropin
IAC	Industry Advisory Council
in	inch(es)
INAD	investigational new animal drug
ISU	Iowa State University
KAA	Kansas Aquaculture Association
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 <i>Aeromonas</i> infection
MAS	motile <i>Aeromonas</i> septicemia
μg	microgram(s)
mg	milligram(s)
min	minute(s)
mL	milliliter(s)
mm	millimeter(s)
MSU	Michigan State University
MT	methyltestosterone
N	number
NAA	National Aquaculture Association

NADA	new animal drug application
NADF	Northern Aquaculture Demonstration Facility
NCC	National Coordinating Council
NCR	North Central Region
NCRAC	North Central Regional Aquaculture Center
NIFA	National Institute of Food and Agriculture
oz	ounce(s)
PAH	Phibro Animal Health
PFU	plaque-forming units
POW	Plan of Work
ppm, ppt	parts per million, parts per trillion
qPCR	quantitative polymerase chain reaction
RAC(s)	Regional Aquaculture Center(s)
RAES	Regional Aquaculture Extension Specialist
RAET	Regional Aquaculture Extension Team
RAS	recirculating aquaculture system
RS	Rimler-Stotts
SIUC	Southern Illinois University-Carbondale
SPAH	Schering-Plough Animal Health
TC	Technical Committee (TC/E = Technical Committee/Extension; TC/R = Technical Committee/Research)
TL	total length
™	trademark
TSA	Tryptic Soy Agar
UMESC	Upper Midwest Environmental Sciences Center
USDA	U.S. Department of Agriculture
USFWS	U.S. Fish and Wildlife Service
UW-Stevens Point	University of Wisconsin-Stevens Point
UW-Madison	University of Wisconsin-Madison
UW-Milwaukee	University of Wisconsin-Milwaukee
VHS	viral hemorrhagic septicemia
VHSv	viral hemorrhagic septicemia virus
WATER	Wisconsin Aquatic Technology and Environmental Research
Wr	relative weight