ANNUAL PROGRESS REPORT

For the Period
September 1, 2011 to August 31, 2012

February 2013

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INTRODUCTION

The U.S. aquaculture industry is an important sector of U.S. agriculture generating over $1.16 billion in 2009 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 2010, the U.S. imported $27.4 billion of fisheries products and the trade deficit was $5 billion for all fisheries products, most of which was for edible fish and shellfish.

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

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

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

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

ORGANIZATIONAL STRUCTURE

In the period of 1988 through 2011, Michigan State University (MSU) and Iowa State University (ISU) worked together to develop and administer programs of NCRAC through a memorandum of understanding. MSU was the prime contractor for the Center and had administrative responsibilities for its operation; ISU administered the extension/outreach activities for the Center. In 2012 NCRAC became solely administered by Iowa State University where the Office of the Director is now located.

At the present time the staff of NCRAC at ISU includes Joseph E. Morris, Director; Denise Birney, Administrative Assistant; and D. Allen Pattillo, Program Extension Specialist.

The Center Director has the following responsibilities (0.65 FTE):

- Develop and submit proposals to USDA/NIFA which, upon approval, becomes a grant to the Center;
- Coordination the development of research and extension projects including Work Group formation, review of project outlines for technical and scientific merit, feasibility, and applicability to priority problems and then submission to the Board of Directors for their approval after which, Board-approved project outlines are submitted to USDA/NIFA for approval in a Plan of Work or an Amendment to a Plan of Work;
- Oversee the development of appropriate agreements (sub-contracts) by the Administrative Assistant for purposes of transferring funds for implementation of all projects approved under the grants;
- Serve as executive secretary to the Board of Directors, responsible for preparing the agenda and minutes of Board meetings;
- Coordinate and facilitate interactions among the Administrative Center, Board of Directors, IAC, and TC;
- Monitor research and extension activities;
- Recruit other Administrative Center staff as authorized by the Board of Directors;
- Serve as an additional source of technical information for the regional aquaculture community;
- Maintain liaison with other RACs; and
- Serve on USDA's National Coordinating Council for Aquaculture.

The Center Director also has the following responsibilities (0.25 FTE) for extension/outreach responsibilities for the Center:

- Give regional presentations;
- Develop and distribute (including posting on the Web) news releases for new NCRAC publications;
- Supervise technical editors for NCRAC publications;
- Oversee the development of extension projects;
- Create and publish on-line NCRAC Newsletter – Fin Clips;
- Survey NCR aquaculture industry to guide future NCRAC extension programming; and
- Proofing of "final" draft of new NCRAC publications.

The Administrative Assistant (1.0 FTE) has the following responsibilities:

- Prepare correspondence;
- Maintain the administrative calendar, including scheduling of meetings and making travel arrangements;
- General office management;
- Answer or direct inquiries appropriately relating to aquaculture in general and the Center in particular;
- Maintain and monitor all budgetary matters for both the Center and sponsored projects including developing sub-contracts with other parties for purposes of transferring funds for implementing all approved projects;
- Compile information for periodic reports to the Center's Board of Directors and maintain records of Board business;
- Assist in preparation of Center reports to USDA/NIFA, including annual reports and plans of work;
- Maintain database of persons interested, involved with, or who should be kept informed of the Center's activities; and
- Monitor Web site and keep Director and Program Specialist updated on changes/additions.

The Program Extension Specialist (0.5 FTE) has the following responsibilities:

- Interaction with associated information technology staff NCRAC Web site and NCRAC List Serve (In cooperation with Regional Extension Specialist);
- Coordination with other state extension contacts and the Regional Aquaculture Extension Specialist, Chris Weeks, who cannot address all of the needs in all 12 states of the region equally well because of budgetary and time limitations.
- Regional presentations;
- Representation on NCRAC TC as Iowa's representative on extension;
- Serve as Chair of NCRAC Extension Working Group committee;
- Initial editing of "final" draft of new NCRAC publications;
- Review and prepare responses to e-mail requests sent to NCRAC@iastate.edu;
- Review of all current extension/outreach products for possible deletion or revision; and
- Help with technical and logistical support for the NCRAC Annual Program Planning Meetings.

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 the inception of NCRAC on 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 23 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 (#2008-38500-19157), FY2009 (#2008-38500-19157 extension) FY2010 (#2010-38500-20929), FY2011 (#2010-38500-20929 Amendment) and FY2012 (2012-38500-19550) with monies totaling $17,686,202. Currently, four grants are active (FY07-12); the first 19 grants (FY88-06) 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, 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, 2012, the Center has funded or is funding 100 projects through 514 subcontracts from the first 22 grants received. Funding for these Center-supported projects is summarized in Table 1 below (pages 6-9). 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 fourteen separately funded projects in regard to Extension and nine on Yellow Perch. Project outlines have been written for each separate project within an area, or the project area itself if only one project. These project outlines have been submitted in POWs or amendments to POWs for the grants as indicated in Table 1. Many times, the projects within a particular area are continuations of previously funded activities while at other times they are addressing new objectives. Presented below are Progress Reports for projects that were underway or completed during the period September 1, 2011 to August 31, 2012. Projects, or Project components, that terminated prior to September 1, 2011 have been reported on in earlier documents (e.g., 1989-1996 Compendium Report and other Annual Progress Reports).
A cumulative list of all publications, manuscripts, papers presented, or other outputs for all funded NCRAC project areas is contained in the Appendix.

Table 1. North Central Regional Aquaculture Center funded projects.

Project Area	Project Number	Proposed Duration Period	Funding Level	Grant Number
Aquaculture Drugs	1	7/1/96-6/30/97	$27,000	95-38500-1410
	2	12/1/96-11/30/97	$950	95-38500-1410
	3	10/1/99-9/30/00	$8,415	97-38500-3957
	4	6/1/04-11/30/05	$223,677	2003-38500-12995
	5	7/15/04-7/14/05	$60,000	2003-38500-12995
	6	11/1/04-10/31/06	$50,000	2002-38500-11752
	7	1/1/06-12/31/06	$129,936	2005-38500-15847
	8	9/1/08-8/31/10	$150,000	2008-38500-19157
	9	9/1/09-8/31/10	$27,880	2008-38500-19157
	10	9/1/11-8/31/31	$100,000	2010-38500-20929
	11	9/1/12-8/31/14	$240,000	2012-38500-19550
			$1,017,585	
Baitfish	1	9/1/92-8/31/94	$61,973	92-38500-6916
	2	9/1/06-8/31/08	$111,997	2006-38500-16900
			$88,003	2005-38500-18547
			$261,973	
Conferences/Workshops/Symposia				
Environmental Strategies Symposium	1	9/1/00-5/31/01	$5,000	96-38500-2631
Nat'l. Aquaculture Exten. Workshop/Conference	1	10/1/91-9/30/92	$3,005	89-38500-4319
	2	12/1/96-11/30/97	$3,700	95-38500-1410
	3	11/1/02-10/31/03	$4,500	00-38500-8984
	4	1/1/06-12/31/06	$5,000	2005-38500-18547
	5	9/1/10-8/31/11	$5,000	2008-38500-19157
			$21,205	
NCR Aquaculture Conference	1	6/1/90-3/31/91	$7,000	90-38500-5008
	2	12/9/98-6/30/99	$3,000	96-38500-2631
			$10,000	
Percis III	1	11/1/02-10/31/03	$4,000	00-38500-8984
Crayfish	1	9/1/92-8/31/94	$49,677	92-38500-6916
Economics/Marketing	1	5/1/89-12/31/91	$127,338	88-38500-3885
			$34,350	89-38500-4319
	2	9/1/91-8/31/92	$53,300	91-38500-5900
	3	9/1/93-8/31/95	$40,000	93-38500-8392
	4	9/1/99-8/31/01	$47,916	97-38500-3957
	5	9/1/03-8/31/04	$50,000	2002-38500-11752
	6	9/1/10-8/31/11	$23,565	2010-38500-20929
	7	9/1/12-8/31/14	$115,000	2012-38500-19550
			$376,469	

Project Area	Project Number	Proposed Duration Period	Funding Level	Grant Number
Extension	1	5/1/89-4/30/91	$39,221	88-38500-3885
			$37,089	89-38500-4319
("Base" Extension—Project Nos. 1-14; Aquaculture	2	3/17/90-8/31/91	$31,300	89-38500-4319
Regional Extension Facilitator	3	9/1/91-8/31/93	$94,109	91-38500-5900
[AREF]—Project No. 15; and	4	9/1/93-8/31/95	$110,129	91-38500-5900
Regional Aquaculture	5	9/1/95-8/31/97	$10,813	92-38500-6916
Extension Specialist			$20,391	95-38500-1410
[RAES]—Project Nos. 16, 17	6	9/1/97-8/31/99	$38,000	97-38500-3957
and 18	7	9/1/99-8/31/01	$94,000	99-38500-7376
	8	9/1/01-8/31/03	$28,500	99-38500-7376
			$18,154	2001-38500-10369
	9	9/1/03-8/31/05	$28,000	2002-38500-11752
	10	9/1/05-8/31/07	$211,545	2003-38500-12995
			$7,735	2005-38500-15847
	11	9/1/07-8/31/09	$21,850	2006-38500-16900
			$92,469	2007-38500-18469
	12	9/1/08-8/31/10	$37,966	2007-38500-18469
			$22,539	2008-38500-19157
	13	9/1/09-8/31/11	$29,000	2008-38500-19157
	14	9/1/11-8/31/13	$35,700	2010-35800-20929
	15	9/1/03-8/31/05	$100,000	2002-38500-11752
	16	9/1/05-5/31/09	$199,624	2004-38500-14269
	17	9/1/09-8/31/11	$150,000	2008-38500-19157
	18	9/1/11-8/31/13	$196,612	2010-38500-20929
			$1,619,046	
Hybrid Striped Bass	1	5/1/89-8/31/91	$68,296	88-38500-3885
			$68,114	89-38500-4319
	2	6/1/90-8/31/92	$101,000	90-38500-5008
	3	9/1/91-8/31/93	$96,550	91-38500-5900
	4	9/1/93-8/31/95	$168,000	93-38500-8392
	5	9/1/95-8/31/97	$150,000	95-38500-1410
	6	6/1/99-5/31/00	$15,000	96-38500-2631
	7	9/1/01-5/31/04	$98,043	98-38500-5863
			$211,957	2001-38500-10369
			$976,960	
Largemouth Bass	1	9/1/05-8/31/07	$170,000	2004-38500-14269
National Coordinator for	1	9/1/93-8/31/94	$2,000	89-38500-4319
Aquaculture INADs/NADAs		5/15/95-5/14/96	$5,000	94-38500-0048
		5/15/96-5/14/97	$6,669	92-38500-6916
			$3,331	95-38500-1410
		5/15/97-5/14/98	$15,000	96-38500-2631
		5/15/98-5/14/99	$13,241	94-38500-0048
		5/15/99-5/14/00	$10,000	95-38500-1410
	2	7/15/04-7/14/05	$9,000	2003-38500-12995
		9/15/05-8/31/06	$15,000	2004-38500-14269

Project Area	Project Number	Proposed Duration Period	Funding Level	Grant Number
		9/1/06-8/31/08	$40,000	2006-38500-16900
		5/15/08-5/14/09	$25,000	2007-28500-18469
			$144,241	
Nutrition/Diets	1	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
	4	9/1/10-8/31/12	$43,363	2008-38500-19157
	5	9/1/12-8/31/13	$124,400	2010-28500-20929
			$75,000	2012-38500-20929
			$559,400	
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
Assessment of Winter Kill in Ponds	1	9/1/11-8/31/13	$175,000	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	

Project Area	Project Number	Proposed Duration Period	Funding Level	Grant Number
Wastes/Effluents	1	9/1/92-8/31/94	$153,300	92-38500-6916
	2	9/1/96-8/31/98	$100,000	96-38500-2631
	3	9/1/01-8/31/04	$106,186	00-38500-8984
			$88,814	2001-38500-10369
			$448,300	
White Papers	1	7/1/98-12/31/98	$4,999	96-38500-2631
	2	9/1/99-12/31/99	$17,495	97-38500-3957
			$22,494	
Yellow Perch	1	5/1/89-8/31/91	$76,957	88-38500-3885
			$85,723	89-38500-4319
	2	6/1/90-8/31/92	$92,108	90-38500-5008
	3	9/1/91-8/31/93	$99,997	91-38500-5900
	4	9/1/93-8/31/95	$150,000	93-38500-8392
	5	9/1/95-8/31/97	$199,507	95-38500-1410
	6	9/1/97-8/31/99	$185,458	97-38500-3957
	7	9/1/98-8/31/00	$92,370	98-38500-5863
	8	9/1/01-5/31/04	$326,730	00-38500-8984
			$125,016	2001-38500-10369
	9	9/1/10-8/31/13	$150,000	2010-38500-20929
			$1,583,866	

PROJECT REPORTS

AQUACULTURE DRUGS: DRUG APPROVAL RESEARCH ON 17 α-METHYLTESTOSTERONE (OFFICIAL TRANSFER OF 17 α-METHYLTESTOSTERONE [MT] ANALYTICAL METHOD FOR FEED)[1]

Project ***Progress Report*** for the Period
September 1, 2009 to August 31, 2012

NCRAC FUNDING: $27,880 (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
Industry Advisory Council Liaison:		
Mark Willows	Binford Eagle Fisheries	North Dakota
Extension Liaison:		
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.
(2) Submit method transfer study protocols to the Center for Veterinary Medicine (CVM) for concurrence.
(3) Provide final study protocols to participating laboratories.
(4) Prepare and ship medicated feed to participating laboratories.
(5) Assay control and medicated feed samples according to the study protocols concurred with by CVM.
(6) Complete report of analysis and submit along with raw data to the Upper Midwest Environmental Sciences Center (UMESC).
(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 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.

[1] 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.

(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 to CVM for review. The protocol was returned with review comments which were then 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

The control (non-medicated) 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.

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 (<0.5 oz) of MT equivalent interference in the control feed extract.

Participating laboratory analysts obtained mean percent recoveries that 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. 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 (1.1, 2.1, 3.2 oz) (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).

OBJECTIVE 6
Reference and participating laboratory results from the method transfer phase were submitted by Maxxam Analytics to UMESC for review.

OBJECTIVE 7
Participating laboratory analysts demonstrated the matrix equivalent MT concentrations in the control feed met the acceptance criteria of <15 µg/g (<0.5 oz) 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 (<0.5 oz) of MT equivalent interference in the control feed extract.

Participating laboratory analysts obtained mean percent recoveries that 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 of 30, 60, and 90 µg/g (1.1, 2.1, 3.2 oz), 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. 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.

Participating laboratory analysts obtained mean percent recoveries that 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 final reports describing the results from the processing of sample sets described in *OBJECTIVE* 5 for the method transfer phase of the study. UMESC included those reports in a comprehensive final report. UMESC archived all the raw data, submitted to INAD I-011395 the comprehensive final report, and requested CVM to review the report.

OBJECTIVE 11
The following statement was in a letter UMESC received from CVM on June 21, 2011: "We incomplete your phased investigational new animal drug submission for the proposed MASCULINIZING FEED FOR TILAPIA (17 α-methyltestosterone) Type C Medicated dated December 17, 2010." UMESC efforts to address the review responses are ongoing.

OBJECTIVE 12
UMESC discussed with CVM four comments from the non-concurrence letter. These commentws include: 1) an apparent omission of the description of the pellet size of feed used in the method transfer study; 2) UMESC incorrectly stated that the reference laboratory made modifications to the method, when it actually was the participating laboratory that made changes to the method that resulted in data discrepancies between the two laboratories; 3) an issue of method recovery discrepancies between the reference and participating laboratories; and 4) a reference made to a confidential INAD belonging to Rangen, Inc.

WORK PLANNED
OBJECTIVE 12
UMESC will continue working with Rangen, Inc., Maxxam Analytics, and CVM to develop corrective courses of action. If necessary, the final report will be revised. The revised report will be submitted to CVM and UMESC will request that the method transfer study data described in the final study report be accepted. If substantial work is required to be completed by UMESC then additional resources would be required.

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 effect 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 $27,880 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.

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)[2]

Project ***Progress Report*** for the Period
September 1, 2008 to August 31, 2012

NCRAC FUNDING: $95,000 (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

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

(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®.

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

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

(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.

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2 [2]NCRAC 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.

(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.

PROGESS AND PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

Etiologic Agent. —Observations of clinical signs and gross necropsy were performed on muskellunge and tilapia which exhibited mortality and clinical signs of MAS at two separate NCR fish culture facilities. Microbiological samples were obtained from diseased (moribund) fish exhibiting clinical signs of MAS and inoculated onto Tryptic Soy Agar (TSA) plates and later identified.

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.

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.

Characterize the Disease Syndrome.— Challenge trials began in October 2009 to characterize the disease syndrome. Five

isolates were used during each challenge trial, with two species each of cool and warmwater fish. Mortality, morbidity, and clinical signs were observed for 14 d after challenge initiation. Samples were collected from mortalities to confirm infection. Isolates were also tested to determine sensitivity to oxytetracycline dihydrate and forfenicol.

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

OBJECTIVE 2
The Upper Midwest Environmental Sciences Center (UMESC) currently has an established INAD for both Aquaflor® and Terramycin 200 for Fish®.

OBJECTIVE 3
Because there were few outbreaks of MAI in Year 1, development of the effectiveness protocol was delayed. A protocol titled "Field effectiveness of Aquaflor® (florfenicol) and Terramycin 200 For Fish® (oxytetracycline dihydrate) to control mortality in coolwater and warmwater finfish due to Motile Aeromonad infections" was submitted to the U.S. Food and Drug Administration's Center for Veterinary Medicine (CVM) in October 2009.

OBJECTIVE 4
In Year 2, 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 requested CVM to consider infection by any motile aeromonad species to be a potential cause of MAI. The H submission is complete and the protocol was revised and re-submitted for CVM concurrence in Year 3.

In Year 3, the protocol titled "Field effectiveness of Aquaflor® (florfenicol) and Terramycin 200 For Fish® (oxytetracycline dihydrate) to control mortality in coolwater and warmwater finfish due to Motile Aeromonad infections" was submitted to CVM after review of the H submission described above. Protocol concurrence was received on June 2, 2011. CVM provided protocol concurrence with a protocol titled "Identification of motile aeromonads isolated from Study Number AEH-09-MAS-02" on June 17, 2011. The "Identification of motile aeromonads isolated from Study Number AEH-09-MAS-02" protocol will be used to identify to species the motile aeromonad species isolated in the pivotal effectiveness field trials.

OBJECTIVE 5
In Year 3, a pivotal effectiveness field study was conducted at the Spirit Lake Fish Hatchery, Spirit Lake, IA with juvenile muskellunge which exhibited signs of a motile aeromonad infection. The pivotal field trial is now complete and data generated during the trial are being summarized for inclusion in a final study report.

In Year 4, a pivotal effectiveness field study was conducted at the Spirit Lake Fish Hatchery, Spirit Lake, IA with juvenile walleye which exhibited signs of a motile aeromonad infection. The pivotal field trial is now complete and data generated during

the trial are being summarized for inclusion in a final study report.

WORK PLANNED

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

OBJECTIVE 6
Effectiveness data will be analyzed and draft final study reports will be prepared for Terramycin 200 for Fish® and Aquaflor® no more than four months after the studies are completed.

OBJECTIVE 7
The respective draft study reports will be submitted 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.

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
2010-11	$30,000		$3500	$20,000	$200		$53,700
TOTAL	$95,000			$22,600	$200	$2,600	$121,300

ASSESSMENT OF CARBON DIOXIDE (CO_2) AND INORGANIC NITROGEN COMPOUNDS TO ENHANCE WINTER KILL IN NATURAL REARING PONDS USED FOR FISH PRODUCTION IN THE NORTHCENTRAL REGION

Project ***Progress Report*** for the Period
September 1, 2011 to August 31, 2012

NCRAC FUNDING: $87,500 (September 1, 2011 to August 31, 2012)

PARTICIPANT:

Mark P. Gaikowski	Upper Midwest Environmental Sciences Center	Wisconsin
Konrad Dabrowski	The Ohio State University	Wisconsin
Molly Webb	U.S. Fish and Wildlife Service	Montana
Andy Ray	Montana State University	Montana
Industry Advisory Council Liaison:		
Jeffrey L. Gunderson	Minnesota Sea Grant College Program	Minnesota
Gregory Oswald	Oswald Fisheries	Minnesota
Barnaby Watten, PhD	USGS-Leetown Science Center	West Virginia

PROJECT OBJECTIVES

(1) Conduct a literature review to summarize the toxic effects of carbon dioxide (CO_2) and inorganic nitrogen compounds (e.g. N_2, NO_2^-, NH_3, etc.) during periods of hypoxia on fish with an emphasis on common carp (*Cyprinus carpio*), black bullhead (*Ameiurus melas*) and walleye (*Sander vitreum*).

(2) Estimate the cost per acre of pond treatment using either CO_2 or inorganic nitrogen compounds to enhance winter kill conditions during late winter periods in the North Central Region (NCR).

(3) Consult with EPA to determine the registration eligibility and requirements for the use of CO_2 or inorganic nitrogen compounds to enhance winter kill conditions.

(4) Determine, through laboratory study, application rates required of CO_2 or inorganic nitrogen compounds to enhance winter kill conditions (hypoxia) to remove unwanted fish from natural rearing ponds. Studies required for the registration of CO_2 or inorganic nitrogen compounds to enhance winter kill conditions will be conducted according to GLP regulations (40CFR160).

(5) Evaluate, through laboratory pond experiments, the efficacy of laboratory-derived application rate data for CO_2 or inorganic nitrogen compounds to enhance hypoxic winter kill conditions.

(6) Collect late winter water chemistry condition data in representative NCR natural rearing ponds.

(7) Obtain an experimental use permit (EUP) from the EPA and appropriate state regulatory agencies to conduct experimental applications of CO_2 or inorganic nitrogen compounds,

singularly or in combination, to enhance winter kill conditions in natural rearing ponds to remove populations of unwanted fish.

(8) Compile data into final study reports suitable for submission to the EPA to support potential approval of CO_2 or inorganic nitrogen compounds to enhance winter kill conditions.

(9) Summarize results into appropriate extension materials for dissemination to NCR aquaculturists.

ANTICIPATED BENEFITS

The proposed studies include determining thresholds of the combined effects of CO_2/inorganic nitrogen compounds and oxygen concentrations on survival of three fish species (common carp, black bullhead, and rainbow trout) which will generate, for the first time, results relevant to hypoxic winter conditions. There are no data in the literature directly addressing these interactions (O_2 and N; CO_2 and N). These data will also be extremely useful to predict constraints on fish survival related to winterkill conditions in productive ponds and lakes. They may be used in simulation of thermal/dissolved oxygen/ammonia conditions in pond habitat for fishes under different climate scenarios including severity and duration of winter.

Diffused gases and inorganic nitrogen compounds offer significant piscicidal alternatives to Rotenone for aquaculture. Commonly applied chemical treatments for fish control include antimycin and rotenone, compounds that have traditionally been used but which are receiving greater public scrutiny and which may leave undesired residues in pond sediments, especially when applied in cold water. Most gases are readily available commercially, are inexpensive, have short half-lives, and off-gas from water leaves little residual environmental impact. Carbon dioxide gas, for example, is Generally Regarded as Safe (GRAS) by the Food and Drug Administration (FDA) and is currently used as a humane method of euthanasia in laboratory animals in research, as well as in the aquaculture industry with fish (Pirhonen and Schreck 2003). Many gases have greater binding affinity for hemoglobin than oxygen, providing rapid biological uptake with little bioaccumulation. Determination of appropriate application rates and times to enhance natural winter kill conditions in natural rearing ponds has the potential to substantially reduce fish production costs and reduce dependence on other chemical toxicants like rotenone or antimycin.

PROGESS AND PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

The literature review is being worked on in conjunction with determining toxicity thresholds for designing the experimental research. An initial draft on the toxicity of ammonia nitrogen compounds at different oxygen levels in koi/common carp is currently at the advanced stages. Upon completion of the first year of research the literature review will be combined with the data to make a white paper to be reported to NCRAC and prepared for publication in a peer-reviewed journal.

Similarly, a literature review for the use of CO_2 as a control agent for undesired fish species is currently being completed by U.S. Geological Survey and U.S. Fish and Wildlife Service partners. A draft of this literature review has been completed and incorporates findings from preliminary experimental work with multiple fish species. This effort will complement similar studies by this same research group on

invasive amphibians; findings from that effort (describing the effects of CO_2 on non-native bullfrog larvae; Abbey-Lambertz et al., in review) have been submitted and are currently under review in a peer-reviewed journal. Upon completion of the first year of research, the CO_2 literature review will be summarized as a report to NCRAC and prepared as a *Mini-Review* for *Fisheries* or similar journal.

OBJECTIVE 2

Calculating cost per acre of pond treatment using either CO_2 or inorganic nitrogen compounds to enhance winter kill conditions during late winter periods in the NCR has not been done yet. Such calculations will need the data acquired from objectives 4, 5, and 6. The results from these objectives will provide the concentrations needed to effectively enhance winter kill conditions and accurately calculate the amount of each toxic compound.

OBJECTIVE 4

CO_2 Trials.—Experimental trials to examine the effects of CO_2 were completed with two species of fish; two identical, parallel systems were usedFor both hyperbaric chamber systems, a single water source (same flow rate) and CO_2 source (flow rate dependent on treatment) with separate carbonators were used. CO_2 was introduced from bulk storage and split the gas feed into two independent lines, each regulated by a mass flow meter adjusted until the flow rate reached the desired dCO_2 concentration.

Researchers tested 217 Eastern mosquitofish at multiple concentrations of CO_2 (approx. 50, 60, 75, 100, 125, 150, 300, 450, and 600 mg CO_2/L). CO_2 introductions produced distinct concentrations of dissolved CO_2 (dCO_2) in test waters and pH levels; pH and dCO_2 were inversely correlated. Differences in dissolved oxygen (DO) levels among dCO_2 treatments were also detected and DO levels were inversely correlated with dCO_2. Although dCO_2 and DO levels were correlated, DO in experimental chambers never decreased below 9.36 mg/L (measured in 600 mg CO_2/L treatment) and more than 90% of trials had DO levels greater than 10.00 mg/L. Differences in total gas pressure (TGP) among dCO_2 treatments were detected and dCO_2 was positively correlated with TGP. Temperature did not differ among CO_2 treatments and was not correlated with dCO_2.

Mosquitofish mortality was a function of dCO_2 concentration. Percent mortality differed among dCO_2 treatments. The LC_{50} and LC_{99} for mosquitofish were calculated as 142 and 360 mg CO_2/L, respectively.

One hundred and seven out of 217 test fish (49.3%) survived CO_2 treatments. On average, fish that survived had a condition factor (Fulton's condition factor) that was lower than those that died.

Similar to the work described above, the effects of CO_2 were assessed using 108 walleye fingerlings (3 weeks old) in pressurized hyperbaric chambers. Dissolved CO_2 treatments varied from 40 to 100 mg CO_2/L. Dissolved CO_2 and pH levels were inversely correlated. In these trials, dCO_2 and DO and dCO_2 and temperature were not correlated.

Mortality was a function of dCO_2 concentration: 0.0% and 33.3% of walleye died in control treatments (approximately 40 mg CO_2/L), 55.6% died at 60 and 68 mg CO_2/L, and 100% died at 75 and 100 mg CO_2/L. LC_{50} and LC_{99} were calculated as 58 and 91.2 mg CO_2/L, respectively.

Walleye fingerlings that died (62 out of 108) had a higher Fulton's condition factor and a

higher mass than fish that survived. Dead walleye were commonly found with their mouths locked open, which may have resulted in fish taking on water mass.

Inorganic N Trials.—Six experimental trials were conducted from January to March 2012 at temperatures between 10-15°C (50-59 ^{o}F). A total of three different species, rainbow trout, common carp, and bullhead catfish were used.

Three trials were conducted using common carp using 0.25 mg/L, 0.60 mg/L and 0.80 mg/L un-ionized ammonia (N-NH_3) for 24-h toxicity test, and one trial using both koi carp and bullhead catfish at 0.60 mg N-NH_3/L. Twelve fish were allocated among three experimental groups (normoxia 8.8 ± 1.1 mg O_2/L, moderate-hypoxia 2.6 ± 0.48 mg O_2/L, and severe hypoxia 1.5 ± 0.62 mg O_2/L) with four replicates each. The oxygen levels were slowly lowered over a period of 24h and fish were given 3 d to acclimate to the hypoxic conditions before ammonium exposure.

Two rainbow trout trials were conducted in March 2012. Methods were similar to those used in the carp trial except different ammonia and oxygen levels were used. The trials used 0.3 and 0.28 mg N-NH_3/L. Oxygen levels were 7.2 ± 1.0 mg O_2/L, 3.6 ± 0.7 mg O_2/L, and 2.4 ±0.3 mg O_2/L.

Results indicated that hypoxia increases the toxicity of ammonia. All species of fish experienced higher rates of mortality in severe hypoxia than in the other two groups. Common carp LC-50 values with 95% confidence intervals were 0.74 ± 0.05, 0.59 ± 0.04, and 0.53 ± 0.04 mg N-NH_3/L for normoxia, moderate hypoxia, and severe hypoxia, respectively. LC-50 values for the other species are yet to be determined as more trials need to be conducted at various ammonia levels to calculate LC-50 values.

WORK PLANNED

OBJECTIVE 1

To be completed as described in the original proposal.

OBJECTIVE 2

Upon completions of objectives 4-6, data will be compiled to calculate the amount of CO_2 or inorganic nitrogen compounds needed to effectively enhance winter kill per pond surface area. Once this is calculated, the current pricing of these compounds can then be multiplied by the amount of the compound needed to get a cost estimate for applying these compounds to enhance winter kill.

OBJECTIVE 4

Dose response trials with rainbow trout, common carp, and channel catfish will be conducted at the Bozeman Fish Technology Center (BFTC) during fall 2012 and early winter 2013. In order to determine the LC50 and LC100concentrations, juvenile fish (5-8 cm (2-3 in) long, 5-50 g (0.2-1.8 oz)) will be exposed to each of six treatment levels of CO_2 (background (control), 30, 60, 120, 240, and 480 mg CO_2/L) for a 24-h period. These trials will be conducted at at temperature of 10°C, pH of 7-8, and DO > 8.0 mg/L although researchers recognize that the coldest water temperature that can be maintained at the BFTC (10°C [50.0 F]) is 4-5°C (39.2-41 F) warmer than water temperature under the ice. However, the objectives of this study are to determine the concentration of CO_2 that is lethal to these three species regardless of temperature and to assess the feasibility of using CO_2 at multiple scales. Because colder water will hold more CO_2, the lethal concentration

determined in this study will be effective at colder temperatures.

OBJECTIVE 5
Scale-up trials will be conducted at the BFTC during fall 2012 and early winter 2013. Researchers will test the empirically derived LC100 value at multiple scales (38 - 1,893 L [10 gal to 500 gal]) for rainbow trout. In brief, juvenile rainbow trout ([5-8 cm [2-3 in] long, 5-50 g) will be exposed to the empirically derived LC100 dose of dissolved CO_2 for a period of 24 h using five different volumes of water: 10, 50, 100, 250, and 500 gal. The results from these scale-up trials will be used to examine costs of CO_2 treatment as a function of water volume. These values will be critical for determining whether field applications of CO_2 are an economically feasible means of killing undesirable fish in pond settings.

OBJECTIVE 6
Project participants will identify potential farm participants and sites to collect late winter water chemistry condition data in representative NCR natural rearing ponds.

OBJECTIVE 7
Pending the results of experimental trials in UMESC ponds, work will be completed to obtain an experimental use permit (EUP) from the EPA and appropriate state regulatory agencies to conduct experimental applications of CO_2 or inorganic nitrogen compounds, singularly or in combination, to enhance winter kill conditions in natural rearing ponds to remove populations of unwanted fish.

OBJECTIVE 8
UMESC will coordinate with BFTC and OSU to compile data into final study reports suitable for submission to the EPA to support potential approval of CO_2 or inorganic nitrogen compounds to enhance winter kill conditions.

OBJECTIVE 9
Study participants will coordinate to summarize results into appropriate extension materials for dissemination to NCR aquaculturists.

IMPACTS
The greatest return on investment for this project will be in the removal of undesirable fish from both public and private aquaculture ponds. Removal of remaining fish will enhance production in those ponds. Results of the experiments, where appropriate, will be presented at scientific meetings and extension workshops and may be published in scientific journals, extension bulletins, or NCRAC fact sheets and bulletins. Research results will also be disseminated through the NCRAC Annual Progress Reports. These reports are available on the NCRAC Web site (http://www.ncrac.org)

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

SUPPORT
NCRAC has provided $87,500, which is the total amount allocated for year 1 of this project.

DETERMINATION OF PRODUCTION PARAMETERS OF SELECTED YELLOW PERCH LINES AT COMMERCIAL DENSITIES IN PONDS AT TWO OR MORE FACILITIES IN THE NORTH CENTRAL REGION[3]

Project ***Progress Report*** for the Period
September 1, 2010 to August 31, 2012

NCRAC FUNDING: $133,123 (September 1, 2010 to August 31, 2012)

PARTICIPANTS:

Hanping Wang	Ohio State University	Ohio
Christopher F. Hartleb	University of Wisconsin-Stevens Point	Wisconsin
William E. Lynch, Jr.	Mill Creek Perch Farms, LLC	Ohio
Jeffrey A. Malison	Coolwater Farms, LLC	Wisconsin
Industry Advisory Council Liaison:		
Charles E. Hicks	Lincoln University	Missouri
Extension Liaison:		
Laura G. Tiu	Ohio State University	Ohio

PROJECT OBJECTIVES

(1) Using consistent protocols, assess survival and growth rate of two replications of first-year fingerlings of improved lines of yellow perch as compared to fingerlings from local brood stock (feed-trained fingerlings to be stocked at 60,000/acre (150,000 fish/ha).
(2) Using consistent protocols assess 2nd year survival, growth rate, and market parameters (production, fillet yields, percent market size) of both replications of improved lines of yellow perch as compared to local fish.
(3) Disseminate results to industry and to end-user customers via fact sheets, scientific publications, and an on-farm field day.

ANTICIPATED BENEFITS

The impact of this project will be primarily through the delivery of superior yellow perch strains to farmers for use in a wide range of culture and exposure conditions across the North Central Region (NCR). The greatest return on investment for this project is the ultimate reduction in production costs due to increased growth rate and reduced feed costs by using genetically improved strains. At the completion of this project, multiplication stations will be established to produce enough fry/fingerlings from improved strains for fish farmers in the NCR. Success in this project should be similar to that achieved for striped bass, rainbow trout, and catfish. Improved strains should show increased growth by 20–25% per generation

[3] NCRAC has funded nine Yellow Perch projects. This Progress Report is for the ninth Yellow Perch project. It is a 3-year funded project that is chaired by Hanping Wang and began September 1, 2010. Termination Reports for the first three projects are contained in the 1989-96 Compendium Report; a Termination Report for the fourth and fifth projects is contained in the 1997-98 Annual Progress Report; a Termination Report for the sixth project is contained in the 1999-00 Annual Progress Report; a Termination Report for the seventh project is contained in the 2000-01 Annual Progress Report; and a Termination Report for the eighth project is contained in the 2004-05 Annual Progress Report.

and have a tremendous positive impact on the NCR yellow perch aquaculture industry.

PROGESS AND PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

Ohio.—Using the previously developed 2nd generation of selected brood fish, the 3rd generation of selected lines was created via marker-assisted cohort selection (MACS). When a majority of the 2nd generation improved lines reached harvest size, approximately 500 of the best fish were chosen for selected lines based on their body weight and breeding value, PIT tagged, and genotyped at the Ohio State University (OSU) Center for Aquaculture Research and Development (OCARD) using microsatellite markers. Molecular genetic pedigrees were determined and genetic relatedness charts were constructed for mating. Among the selected fish, about 300 pairs of the least-related fish having the highest breeding value were selected for factorial, mass, and pair mating; 215 survival families and a total of 1.2–1.5 million fry were produced. In addition, a control line of local brood fish from Ohio was also produced.

Factorial and single-pair matings based on the genetic pedigree and relatedness chart were conducted in 50.0 cm-diameter (19.7 in-diameter) flow-through tanks in March 2011 when fish had reached sexual maturity. One or two injections of human chorionic gonadotropin (HCG) at the dosage of 200-600 IU/kg (91-273 IU/lb) body weight based on females' need and maturity were used to synchronize spawning. The fertilized egg ribbon from each mating was collected daily from spawning tanks starting 2 d post-injection.

One hundred egg ribbons were produced from the improved line at OCARD in Piketon. Twenty of them were delivered to Mill Creek (MC) Perch Farms in Ohio on March 16, 2011. No ribbons were shipped to Wisconsin sites because transport permits were not ready by the March spawning time. At both the Piketon Station and MC Perch Farm, egg ribbons were incubated in tanks with flow-through well water. Two days post hatch, fry were siphoned and counted for stocking in the nursery ponds. At the same time, 30 and 18 families from the Ohio control line were produced by mass spawning at MC Perch Farm and OCARD, respectively.

At OCARD, 320,000 improved fry were stocked at 80,000 fry each to four 0.08-ha (0.2 acre) ponds for nursery (1,040,000/ha; 421,000/acre), 50,000 local fry (650,000/ha; 263,000/acre) were stocked into an additional pond. At MC Perch Farm, approximately 200,000 improved fry were stocked to two 0.08-ha (0.2 -acre) ponds, and 200,000 local fry were stocked in two other similar ponds. All ponds were fertilized twice before stocking and once every week during the nursery period. Fry at the two locations were pond-reared until they reached 25.0–35.0 mm (1.0–1.4 in) (~6 weeks), at which time they were harvested and moved into indoor tanks for feed-training.

Feed-training was conducted in 1.8–3.0 m (6.0–10.0 ft) round tanks with a stocking density of 4.0–5.0 kg (8.9–11.0 lb) fingerlings/m^3 at a temperature of 20–24^oC (68–75^oF) for 3–4 weeks. Fish were fed AquaMax® Fry Powder and high protein Starter 100 with high protein using automatic feeders. Feeding rates were about 5% of body weight (BW) for the first 2 d, and then increased to 7–8% BW.

All of the ponds were harvested at the end of October (MC Perch Farm) and in early November 2011 (OCARD). One hundred and fifty fish from each of the separate

ponds at Piketon, and 500 fish from each of the communal ponds at MC Perch Farm were sampled, individually weighed and finclipped. Finclip samples were preserved individually with 95-100% alcohol in small vials. Eight molecular markers were used to assign selected and local-strain yellow perch to their family of origin for communal rearing.

At MC Perch Farm, improved yellow perch grew significantly larger than local yellow perch native to the farm in two communal ponds, where both improved and unimproved fish grew in the same environment. The improved line outweighed the local strain by an average 32.00% at the end of the Year 1 test (October). Fingerling survival in the MC Perch Farm's communal ponds with improved fish was as high as they have ever experienced at that site.

In the OCARD ponds, improved fish exhibited a 27.16% higher survival rate and a 22.01% higher production than the local Ohio strain by the end of October of Year 1. Although the 27.16% higher survival rate of the improved fish resulted in a significantly higher density and lower feed rations (rations were calculated based on the same assumed survival rate for all the ponds), the improved line still had a higher mean body weight (37.82 g; 1.33 oz) than the local Ohio strain (37.62 g; 1.33 oz). A significantly greater reduction of weight variation was observed for the improved line than the unimproved fish, indicating the size variation of the improved fish was smaller, and their percentage of marketable size fish would be higher by the end of Year 2.

At the end of year 1 in October 2011, all the fish in Year 1 ponds were harvested in MC Perch Farms and Piketon Station in Ohio, and re-stocked into Year 2 grow-out ponds.

The two test sites conducted replicated tests of the improved fish vs. the local-strain using two types of rearing tests: 1) at the Piketon Station, the selected line of yellow perch and a local-strain (control) were re-stocked and reared in separate 0.08-ha (0.19-acre) ponds, each having two replicates, with a density of 30,263 fish/ha (12,105/acre); 2) at MC Perch Farms, at the harvesting, commercial grading practice was performed. Each pond of yellow perch was size-graded and the largest fish with mean size of 40.2g (1.42 oz) and 40.9g (1.44 oz) were re-stocked into two 0.30-ha (0.75-acre) ponds, respectively. The selected line and a local-strain were raised communally in the two ponds at a density of 25,000 fish/ha (10,000/acre).

AquaMax Starter 400 to AquaMax Grower 600 feed was used for all experimental ponds with a feeding rate of 2-3% BW (Piketon Station) and satiation feeding (MC Perch Farms) from May to October. Feeding amount and rates were adjusted monthly based on an assumed survival of 80% and calculated biomass using mean weight at the Piketon site. Fish were fed once a day to satiation when water temperature is above 8 ^{o}C (43 ^{o}F) in the afternoon during winter.

All of the ponds were harvested in early (MC Perch Farms) and at the end (Piketon Station) of October 2012. Two hundred fish from each of the separate ponds at Piketon, and 300 fish from each of the communal ponds at MC Perch Farm were sampled, individually weighed, measured and finclip samples removed. Fifty large fish from each of the ponds at both sites were sampled for dress-out percentage analysis. Finclip samples were preserved individually with 95-100% alcohol in small vials.

In MC Perch Farms, communally reared perch from two ponds averaged 217 mm (8.5 in) and 141.1 g (5.0 oz) and 208 mm

(8.2 in) and 122.6 g (4.3 oz), respectively. Eight molecular markers are being used to assign selected and local-strain yellow perch to their family of origin for communal rearing. Family identification for fish collected in October 2012 from MC Perch Farms is still ongoing.

In the Piketon ponds, improved fish exhibited a 12.30% higher survival rate and a 42.07% higher production than the local Ohio strain by the end of October of Year 2. Although the 12.30% higher survival rate of the improved fish resulted in a significantly higher density and lower feed rations (rations were calculated based on the same assumed survival rate for all the ponds), the improved line still had a significant higher mean body weight (126.05 g; 4.4 oz) than the local Ohio strain (100.44 g; 3.5 oz). The improved line grew 25.50% faster than the unimproved fish. There was no significant difference in dress-out percentage between improved line and local Ohio strain (42.0% vs. 42.9%).

Wisconsin.—The co-investigators and collaborators in Wisconsin could not conduct their portion of the studies for Objective 1 as originally planned because of issues regarding the interstate transport of fish and fish eggs that arose from recently changed state and federal viral hemorrhagic septicemia restrictions and testing. Because of these issues, Objectives 1 and 2 at this site were delayed by one year and instead will commence in 2012 and 2013, respectively.

Approximately 200,000 OSU eggs were delivered to the UW-Stevens Point Northern Aquaculture Demonstration Facility (NADF) in Wisconsin on March 28, 2012. Coolwater Frams, LLC (CF) in Wisconsin received 10 OSU egg strands on March 30, 2012 but the eggs suffered significant mortality by April 4, 2012.

Egg quality of shipped eggs was poor as a result of the shipping process. Approximately 14,000 fry at the NADF hatched from these eggs for a hatching success of 7%. On April 11 and April 13, OSU shipped approximately 70,000 fry to the NADF. Many of these fry also arrived in poor condition due to the shipping process. Surviving fry were siphoned by hand and counted using a volumetric method before being stocked in the nursery ponds.

At the same time, 18 strands of eggs from the Wisconsin local strain were produced by mass spawning at CF Perch Farm and distributed to the NADF. The NADF received approximately 160,000 local strain eggs from CF Perch Farm. In contrast to the previous eggs, these tggs arrived in good condition which resulted in >90% hatch rate.

At the NADF, 50,000 and 34,000 OSU improved fry were stocked into two prepared 0.2-ha (0.4- acre) ponds, respectively. Due to pond management issues at the time, approximately 143,000 local fry (212,500-893,750/ha; 85,000-357,500/acre) were stocked into only one prepared 0.2-ha (0.4-acre) pond for early rearing.

All ponds were fertilized twice before stocking and once every week during the nursery period to maintain adequate zooplankton populations for growing fry. Water quality was monitored daily to provide good rearing environment for fry. Fry at the two locations were pond-reared until they reached 35-40 mm (1.4-1.6 in) (~8 weeks), at which time they were harvested from the ponds and moved into indoor tanks for feed-training.

At CF Perch Farm, only 1,000 live fry from OSU were stocked into two 0.08-ha (0.2

acre) ponds, and 200,000 local fry were stocked in two other similar ponds in 2012.

At CF Perch Farm only 5 OSU fry were recovered for feed-training. In 2012, the selected line and a local-strain at CF Farm were not raised communally in two 0.08-ha (0.2-acre) ponds because only five fingerlings were recovered for feed-training.

At the NADF, the two OSU improved fry ponds had 7.5% and 0% (4.5% avg) fry to fingerling survival resulting in 3,750 fingerlings. The one local WI strain pond at the NADF had 20% fry to fingerling survival resulting in 28,000 fingerlings Feed-training was conducted in 1.3- m^3 (4-ft) round fiberglass tanks stocked with fingerlings at 4-5 kg /m^3 (0.03-0.04 lb/gal) at a temperature of 20- 21 C (68 –70 F) for 3-4 weeks. Fish were fed Purina AquaMax Fry Powder and Starter 100 with high protein using automatic feeders supplemented with hand feeding. Feed was top dressed with freeze dried krill for 7 d to improve feed response with the fingerlings. Feeding rates were about 5% body weight per day (BW) for the first 2 d, and then increased to 7-8% BW/d.

Feed training rates for OSU improved and WI local fingerlings at the NADF was 96% and 82%, respectively. Average fingerling size post-feed-training for OSU fish was 44 mm (1.73 in) and 1.3 gm (0.05 oz) ; WI local strain fish was 45 mm (1.77 in) and 1.2 gm (0.04 oz).

Due to the low survival of OSU improved eggs and fry that were shipped earlier, an additional, 15,000 feed trained fingerlings (average 55mm [2.2 in], 2.0 g [0.07 oz]) were hauled by a commercial fish hauler from OSU Piketon Research Facility to the NADF on June 26, 2012. These fish arrived in very good condition with <1% mortality.

For first year (2012) of rearing at the NADF, the selected line of yellow perch and a local-strain (control) were reared in separate ponds, each having two replicates, with a density of 62,500 fish/ha (25,000/acre). Purina AquaMax Starter 100 to AquaMax Grower 400 feed was used for all experimental ponds with a feeding rate of 3% BW/day. Feeding amount and rates were adjusted monthly based on an assumed survival of 75% and calculated biomass using mean weight.

At the NADF, all ponds were drained and fingerlings harvested in early October 2012. Two hundred fish from each pond were sampled, with OSU perch from two ponds averaging 125.1±16.28 mm (4.9 ±0.6 in) and 25.6±13.8 g (0.9±0.5 oz) and 120.3±22.5 mm (4.7±0.9 in) and 22.5±15.6 g (0.8±0.6 oz), respectively. WI strain perch from two ponds averaged 118.6±15.3 mm (4.7±0.6 in) and 20.1±8.9 g (0.7±0.3 oz), and 111.8±21.6 mm (4.4±0.8 in) and 17.9±12.6 g (0.6±0.4 oz), respectively. Finclip samples from 200 fish per pond were preserved individually with 95-100% alcohol in small vials and sent to OSU. Fingerlings were graded and the larger graded fingerlings were stocked back into the ponds for winter. Average fingerling survival was 92.0% for OSU improved strain and 72.0% for WI local strain.

Although the 20.0% higher survival rate of the improved fish resulted in a significantly higher density and lower feed rations (rations were calculated based on the same assumed survival rate for all the ponds), the improved line still grew 26.60% faster than the unimproved fish. Approximately 3,200 graded fingerlings were stocked back into each pond, of appropriate strain, for winter rearing. The percentages of graded fingerlings that were identified as large and could have been restocked into ponds were

73% for OSU improved strain and 56% for WI local strain. This resulted in some medium or small-sized perch being stocked into WI strain ponds to achieve the required density. OSU large fingerlings stocked back into two ponds averaged 142.4±12.5 mm (5.6±0.49 in.) and 41.0±13.8 g (1.4±0.5 oz) and 138.2±14.0 mm (5.44±0.55 in) and 33.8±12.8 g (1.2±0.5 oz), respectively. WI-strainlarge fingerlings stocked back into two ponds averaged 135.2±8.75 mm (5.32±0.35 Inches) and 30.6±7.12 g (1.1±0.3 oz) and 137.2±11.2 mm (5.4±0.44 in) and 32.7±9.8 mm (1.29±0.39 in), respectively.

WORK PLANNED

OBJECTIVE 1

In 2013, researchers will complete family identification to identify improved fish versus local strain fish from the two communal ponds in MC Perch Farm to compare their growth rate, fillet yield, and percent market size with local strain. This will complete the planned work associated with MC Perch Farms. All samples from Piketon Station and Wisconsin site will be genotyped also to evaluate breeding value and family growth. 1-2 journal manuscripts will be prepared.

OBJECTIVE 2

Because of issues regarding the interstate transport of fish and fish eggs, sites in Wisconsin were delayed by one year and instead will commence Objective 2 in 2013. Due to high mortality of OSU improved eggs/fry at the CF Perch Farm site, only the NADF site will be conducting Year 2 project work in Wisconsin. In Year 2, the selected and local lines of large yellow perch fingerlings will be reared in ponds at the NADF. In the autumn of Year 2, all of the ponds will be harvested and the key production parameters (e.g., survival, growth, feed conversion for separate rearing, fillet yield) will be evaluated. Differences between females and males will be measured.

Results from both Ohio and Wisconsin will be disseminated to industry and to end-user customers via fact sheets, scientific publications, and an on-farm field day.

OBJECTIVE 3

Results from both Ohio and Wisconsin will be disseminated to industry and to end-user customers via fact sheets, scientific publications, and an on-farm field day.

IMPACTS

The impact of this project will be primarily through the delivery of superior yellow perch strains to farmers for use in a wide range of culture and exposure conditions across the NCR. The greatest return on investment for this project is the ultimate reduction in production costs due to increased growth rate and reduced feed costs by using genetically improved strains. Success in this project should be similar to that achieved for striped bass, rainbow trout, and catfish. Improved strains should show increased growth by 20–25% per generation and have a tremendous positive impact on the NCR yellow perch aquaculture industry.

SUPPORT

NCRAC funds provided to date total $133,123; a total of $150,000 has been allocated for this 3-year project.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

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

EFFICACY OF EUGENOL TO REDUCE TRANSPORT STRESS AND MORTALITY OF TILAPIA AND YELLOW PERCH

Project ***Progress Report*** for the Period
September 1, 2011 to August 31, 2012

NCRAC FUNDING: $100,000 (September 1, 2011 to August 31, 2013)

PARTICIPANT:

Mark P. Gaikowski	Upper Midwest Environmental Sciences Center	Wisconsin
Christopher F. Hartleb	University of Wisconsin – Stevens Point	Wisconsin
Industry Advisory Council Liaison:		
Mark Willows	North American Fish Farmers Cooperative	North Dakota

PROJECT OBJECTIVES

(1) Interact with CVM to determine the study design and protocol needed to develop the effectiveness data to support a transport sedative claim for eugenol for selected finfish species. The protocol must comply with current CVM Guidance For Industry for the development of pivotal effectiveness data and the study data collection must with CVM Good Clinical Practices regulations.

(2) Obtain fully disclosable Investigational New Animal Drug (INAD) exemptions for the selected sedative to be tested from CVM.

(3) Obtain Categorical Exclusions from the requirement to complete an Environmental Assessment or complete an Environmental Assessment for the selected sedative prior to its use and receive concurrence from CVM Environmental Safety Team.

(4) Submit the pivotal effectiveness protocol to CVM for concurrence.

(5) Conduct pivotal effectiveness studies using the selected sedative on finfish species according to the CVM-concurred protocol and in compliance with CVM Good Clinical Practices regulations.

(6) Summarize the study data into a Final Study Report (FSR) and archive all study data in publicly accessible archives

(7) Submit the FSR to the publicly disclosable INAD file provided by CVM and request CVM review of the FSR and concur that the effectiveness technical section is complete for the selected sedative.

(8) Respond to CVM comments on the FSR to ultimately obtain concurrence that the effectiveness technical section is complete for the use of the selected sedative as a transport sedative for the selected species.

(9) Prepare a Freedom Of Information summary of the submitted data and provide it to CVM.

ANTICIPATED BENEFITS Fish transport costs are a substantial portion of the operational expenses of the aquaculture industry in the North Central Region (NCR), especially as fuel costs continue to increase. Increasing fish loading density during transport could substantially increase the efficiency of NCR aquaculture operations by enabling the transport of more fish per gallon of fuel. Also, gains in operator efficiency may be seen as fewer staff days may be required for transport and hauling with increased loading density. Reducing transport-mediated stress in fish could also improve market sales, especially at live market (either for food fish or baitfish) by improving fish quality and appearance by reducing physical damage of fish during transport and decreasing post-transport disease occurrence. Reducing transport-mediated fish stress may also enhance fillet quality in fish transported to slaughter markets by reducing aerobic metabolism during transport, potentially improving fillet quality by maximizing residual energy stores in the fillet. When hauling juvenile fish for stocking, potential benefits would be realized by increasing loading density during transportation and increasing post-transport survival

PROGESS AND PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

This project assesses the use of eugenol to enhance post-transport survival and increase loading density during long duration ($\leq$10 h) transport events. The Upper Midwest Environmental Science Center (UMESC), starting in September of 2011, initiated conference calls with CVM to discuss necessary study parameters to be included in the study protocols. Through additional revisions and discussions, UMESC and the University of Wisconsin-Stevens Point (UWSP) collaborated with CVM and developed an acceptable protocol and study design for generating eugenol transport data. UMESC has submitted the protocols to CVM through the UMESC publicly-disclosable Investigational New Animal Drug (INAD) permits for eugenol and requested an informal CVM review prior to conducting the study. The staff of CVM were uncertain about how to word the potential label claim, thus official protocol concurrence would not be given until preliminary data could be developed for a eugenol concentration and loading density range finding study.

Yellow perch evaluations were completed in the summer of 2012 and tilapia will be completed in the fall of 2012. Yellow perch were exposed to 0, 10, 20 and 30 mg/L eugenol at 0.12, 0.24, 0.36 kg/L (1, 2 and 3 lb/gal) loading densities. Mortality was monitored as the primary variable of interest. Secondary variables consisted of behavior, water quality (temperature, pH, dissolved oxygen and TAN) and eugenol concentration. Preliminary results suggest that total ammonia – nitrogen (TAN) was generally lower in eugenol treated tanks relative to controls (0 mg/L eugenol). The difference in TAN provides evidence that eugenol is altering stress responses across treatments. Mortality was low in all treatment groups, but was most prevalent at the heaviest loading densities and highest eugenol concentrations. Most treatment combinations had no mortality. The pivotal eugenol transport studies will be conducted in the summer of 2013 based on results from the range finding study. All research has been done in similar accordance to CVM's guidelines for Good Clinical Practices (GCP).

OBJECTIVE 2
UMESC presently has publicly disclosable INADs for eugenol (INAD 011-766) into which UMESC has submitted various data sets relative to the potential approval of eugenol as a fish sedative. All protocols, data, and final study reports submitted to CVM will be submitted by UMESC to INAD 011-766.

WORK PLANNED
OBJECTIVE 1
UMESC will complete the tilapia portion of the initial range finding study in the fall of 2012. Based on those results, UMESC will submit a pivotal protocol for CVM review and concurrence.

OBJECTIVE 3
UMESC will interact with CVM to determine whether a Categorical Exclusions from the requirement to complete an Environmental Assessment will be required. Work within this objective is dependent on progress made by drug sponsor on completion of an original Environmental Assessment for the use of AQUI-S® 20E.

OBJECTIVE 4
UMESC will submit the draft protocols that have been reviewed by the drug sponsors to CVM though UMESC's active INAD files. The CVM concurrence letters will be reviewed and appropriate modifications made to the study protocols before initiation of the effectiveness trials.

OBJECTIVE 5
Following concurrence in Objective 4, UMESC will conduct the pivotal effectiveness studies in the summer of 2013. Under the eugenol INAD 011-766, UMESC will recruit CVM approved fish transporters to promote field supportive data. Results from the initial range finding study will provide guidelines to fish haulers in choosing various loading densities and eugenol concentrations.

OBJECTIVE 6
A final study report will be prepared and its associated data will be audited by the UMESC Quality Assurance Officer before review and acceptance by UMESC management. The final study report will be provided to the appropriate drug sponsor for review prior to submission to CVM. The drug sponsors will have a maximum of 60 days to provide review comments to UMESC before the complete final study report and all trial data are archived according to UMESC Standard Operating Procedures. The final study report will then be submitted to CVM through the UMESC publicly disclosable appropriate INAD file.

OBJECTIVE 7
UMESC will submit the FSRs and associated data to CVM through UMESC's publicly disclosable INAD file. Included with the submission will be appropriate correspondence and CVM-mandated forms to request CVM review to determine whether the submitted data support the potential approval of eugenol as a sedative to improve fish transport loading density and reduce post-transport mortality.

OBJECTIVE 8
UMESC will coordinate with the CVM reviewer to address specific questions during the CVM review of the FSRs as needed. UMESC will address specific study related issues identified in the review letter with an amended final report if needed. If additional data are required that are beyond the scope of this project, UMESC will notify the NCRAC Board of Directors in writing within 30 d of receipt of the CVM response letter.

OBJECTIVE 9
UMESC will provide the CVM response letter to the drug sponsors and will provide draft freedom of information summaries to the drug sponsor for inclusion in a supplemental NADA within 30 days of receipt of the CVM review letter. UMESC will provide access to the study raw data as needed to allow the drug sponsor to prepare the supplemental NADA package.

IMPACTS
The greatest return on investment for this project will be in the reduction of transport related costs to both public and private aquaculture operations. An overall increase in loading densities and decreased stress will benefit the success of fish released into the wild as well as those sold at live markets. Results of the experiments, where appropriate, will be presented at scientific meetings and extension workshops and may be published in scientific journals, extension bulletins, or NCRAC fact sheets and bulletins (see Appendix). Research results will also be disseminated through the NCRAC Annual Progress Reports. These reports are available on the NCRAC Web site (http://www.ncrac.org)

SUPPORT
NCRAC has provided $50,000, which is the total amount allocated for year 1 of this project.

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

EVALUATION OF THE NEWLY-DEVELOPED, LEAST-COST EXPERIMENTAL DIET FOR BLUEGILL AT COMMERCIAL DENSITIES IN PONDS AT TWO OR MORE FACILITIES IN THE NORTH CENTRAL REGION[4]

Project ***Termination Report*** for the Period
September 1, 2010 to August 31, 2012

NCRAC FUNDING LEVEL: $124,400 (September 1, 2010 to August 31, 2012)

PARTICIPANTS:

Paul B. Brown	Purdue University	Indiana
Charles E. Hicks	Lincoln University	Missouri
Joseph E. Morris	Iowa State University	Iowa
Robert A. Rode	Purdue University	Indiana
James E. Wetzel	Lincoln University	Missouri

REASON FOR TERMINATION

Project objectives completed and funds have been terminated.

PROJECT OBJECTIVES

(1) Using as consistent protocols as possible across locations, evaluate/determine performance of recently-developed NCRAC least-cost juvenile (3" minimum total length) bluegill diet versus an "industry standard" diet at two distinct latitude locations at standard pond stocking densities for one growing season. Stocking densities to be determined by the investigator(s) and producer(s).

(2) Coordinate dissemination of project results with the NCRAC Technical Committee/Extension Subcommittee.

PROGRESS ACCOMPLISHMENTS

OBJECTIVE 1

Prior to the initiation of the funded project, Lincoln University (LU) staff conditioned monosex groups of adult northern bluegill and selected brood fish. In late May 2010 half of the brood fish were sent to Iowa State University (ISU) and half were retained by LU for propogation phase of this project. All stocked age-1 fish originated from the same parental stock source.

In October 2010, age-0 fingerlings were harvested from ponds located at both locations with ISU fish being held in the campus facility until the following spring. LU staff over-wintered fish in ponds.

In April-May 2011 ponds located at ISU, LU, and Purdue University (Purdue) were stocked at 19,772 fish/ha (8,000 fish/acre) with ISU ponds stocked with a combination of ISU and LU fish, and Purdue ponds

[4]NCRAC has funded four 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; and the Progress Report for the third project is contained elsewhere in this Annual Report. This Progress Report is for the fourth project. It is a 2-year project that began September 1, 2010.

stocked with LU fish while LU ponds were stocked with LU fish only. Fish were initially graded to minimize the the number of fish smaller than the 76-mm (3-in) length required for this test.
Starting the first week of May 2011fish at all locations were fed either the standard diet (SilverCup Extruded Trout; 40% protein and 12% fat) or the open formula diet to satiation using feeding rings to limit waste. Initial analysis of the fish sampled in late June indicated no significant differences in fish sizes between the two diets at LU and ISU but at Purdue fish, fed the standard diet were significantly larger.

At the end of the approximate 180-d culture period, fish were harvested at all sites. Production data and proximate composition analysis on fillets were completed and summarized and reported at the 2012 Aquaculture American Conference.

Production Data. — Fish fed the standard diet at Purdue were significantly larger (weight and length) than those fed the open formula diet. However, similar results were not obtained from the field trials at ISU and LU. Survival and fillet yield were greatest for fish fed the standard diet at LU; no significant differences were noted at the other two field sites. Food conversion ratio (FCR) was significantly larger for fish fed the open formula diet in the LU ponds; no differences were noted in the ISU or Purdue locations.

In ponds at both Purdue and LU, fish that were fed the open formula diet had significantly larger livers; data for the ISU ponds noted similar relationship although not significant. Proximate analyses indicate fish fed the standard diet had significantly higher percentage of crude fat in fish cultured at Purdue and LU.

At the completion of this study in fall 2011 it became obvious that the final fish size obtained at the three sites were reflective of the different growing conditions among the three sites. Regardless of the diet fed, fish cultured at ISU were smaller than fish cultured at Purdue which were then smaller than fish cultured at LU; relationship related to the number of growing days at each location. In addition, a high percentage of fish did not reach the desired market size 113-150 g (0.25-.033 lb) in the 1-year culture period in this project. The role of genetics should have been minimized in this project since the brood fish were from the same brood source.

In addition to the previously described concerns about fish sizes, the amount of age-0 fish in the ponds upon fish harvests did cause a substantial cost in terms of time and efficiency. Given the submarketable sized fish at harvest, use of the open formula is questionable at best without an additional field season to enable fish reaching market size.

OBJECTIVE 2
Information garnered from this project as well as other regional research projects will be used to develop additional extension materials for bluegill culture. For instance, the ability of these fish to spawn earlier and later in the culture period has been noted as a production concern for this fish in food-fish operations. Additional concerns related to the effect of growing days for the different culture sites are now recognized as important production considerations.

IMPACTS
Results garnered from this research provided the aquaculture industry with relevant field-tested information related to the culture of bluegills using recently developed

experimental diet formulated specifically for bluegill.

Results garnered from this project supported the earlier findings noted by Rob Hayward (University of Missouri-Columbia) in a final NRAC report that described laboratory-reared fish fed the open formula diet as having 25-33% less whole-body lipid deposition compared to the standard diet. However, in this study livers were still larger in fish fed the open formula diet. Implications of these findings include the least diet provides an available nutrient profile that is possibly inferior for bluegill resulting in fish having larger livers and fillets with more moisture.

RECOMMENDED FOLLOW-UP ACTIVITIES

A lack of difference in the growth of the bluegill fed the two diets during their first year could be indicative of similar levels of nutritional completeness provided by both diets or could be an artifact of slow but consistent growth by young-aged bluegill.

A continuation of this project into a second culture period will help to extend the growth cycle and provide clarity as to the effectiveness of the least-cost diet to provide better growth and survival of market-sized bluegill.

SUPPORT

NCRAC- has provided $124,400 which is the entire amount 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.

EXTENSION[5]

Project ***Progress Report*** for the Period
May 1, 1989 to August 31, 2012

NCRAC FUNDING LEVEL: $926,625 (May 1, 1989 to August 31, 2011)

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
James A. Held	University of Wisconsin-Stevens Point	Wisconsin
F. Robert Henderson	Kansas State University	Kansas
Charles E. Hicks	Lincoln University	Missouri
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

[5]NCRAC 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; 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.

PARTICIPANTS (continued):

Brian K. Miller	Purdue University	Indiana
Jerry B. Mills	South Dakota State University	South Dakota
Jeff Mittlemark	University of Minnesota	Minnesota
Joseph E. Morris	Iowa State University	Iowa
Kenneth E. Neils	Kansas State University	Kansas
D. Allen Pattillo	South Dakota State University	South Dakota
Burton F. Pflueger	Iowa State University	Iowa
Robert A. Pierce II	University of Missouri	Missouri
Michael D. Plumer	University of Illinois	Illinois
Kwamena K. Quagrainie	Purdue University	Illinois/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., freshwater shrimp, hybrid striped bass, yellow perch, and walleye, 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 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;
- Served as non-funded collaborator on a variety of projects including the NCRAC Regional Aquaculture Extension Specialist; and
- Met with industry representatives and university researchers involved with aquaculture to discuss how the aquaculture industry could grow in the NCR.

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. 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. A revised NCRAC Web site was developed in 2010 and 2011 to address the need to better present the information to the public. To date, there has been continued refinement to improve information transfer to the aquaculture community.

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.

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.

Enhancing state-wide and regional communication among those in the aquaculture industry is imperative for continued growth of aquaculture in the

NCR. Aquaculture Extension Specialists are important to the distribution of aquaculture extension related materials, providing research-based information to the farmers who will use it. Additionally, promoting networking between public institutions and private aquaculturists helps enhance the transfer of aquaculture information and technology.

The Aquaculture Network Information Center (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 provided continued financial support for AquaNIC. AquaNIC was the first U.S. aquaculture Web site and has globally been one of the most widely accessed and cited aquaculture Web sites. AquaNIC was taken offline April 1, 2011 due to lack of funding.

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. Similar changes iin extension representatives have taken placed since 1988 when NCRAC was first developed. 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. In 2011 there were a number of changes in extension contacts in the NCR with Clayton (Iowa), Pierce (Missouri) and Shiley (Illinois) replaced by Pattillo, Hicks, and Quagrainie, respectively.

To better illustrate individual state extension specialist's role in regional and state extension programs, the following are a partial list. For instance, Lee has continued to assisted the Kansas Aquaculture Association by developing, printing and distributing the Kansas Aquaculture Association Directory. Since the majority of aquaculture in Kansas is from farm ponds and is primarily recreational fisheries, Lee consulted with farm pond owners and provided pond management advice, e.g., location, construction, stocking, management and marketing. Numerous field visits were also conducted to help producers develop strategies to manage aquatic vegetation. Lee also provided information about ponds and fisheries management in eight radio programs as part of my weekly "Outbound Kansas"

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.

Hicks followed up Pierce's activities by developing three aquaculture factsheets: Freshwater Prawn Production in Missouri, Bluegill Sunfish Production in Missouri and Swine Barn Production for Fish Culture. Hicks also conducted a 'Train the Trainer' Workshop on aqualture, a Field Day on prawn and sunfish harvesting and sales, and contributed to the 2012 eXtension Aquaculture Virtual Workshop, bluegill sunfish production.

In North Dakota, Clark developed an updated list of state producers for submission to the NCRAC Publications Office as well as worked with state public agency personnel concerning state/federal regulations for North Dakota producers

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 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]). Discussion included possible roadblocks to larger investments in aquaculture in the region. Starting in n 2010 Kinnunen has continued to be involved in several facets of fish processing and HACCP (hazard analysis and critical control point) training in both aquatic invasive species and food safety.

OBJECTIVE 3

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

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

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

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

NCRAC Extension contacts have also been responsive to arising issues for the NCR aquaculture industry. For instance, the aquaculture industry is accused of being an important vector for the further spread of exotic species such as zebra mussels,

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

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

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

The Aquaculture Technology Transfer (AT2) program at Ohio State University's Ohio Center for Aquaculture Research and Development (OCARD) is dedicated to supporting sustainable development of aquaculture in Ohio through research and extension activities focused on production efficiency, diversification of farm income, emerging species viability, marketing and technical information dissemination for existing and prospective operations. This program strives to provide for the information and training needs of the aquaculture industry. Services include on-line educational materials, workshops, business planning assistance, facility tours and production training. A close working relationship with the Ohio Aquaculture Association, the producer association in Ohio helps drive the growth and success of aquaculture in the state.

OCARD is active nationally and internationally as well. Aquaculture specialists attend and present research at local, national and international conferences. OCARD continues to host three electronic list serves, the most popular of which is the Aqua-Ohio list serve. Regional Aquaculture information (i.e., workshop announcements, fact sheets, and product marketing) is quickly and efficiently distributed to aquaculture clients in Ohio and neighboring states.

OCARD benefits from its close relationship with the industry in Ohio. To support this relationship, OCARD personnel assist in the organization and, promotion of as well as attend and present information at the annual OAA meeting and banquet. NCRAC information is shared at these meetings. Over 150 people attended the February 2012 OAA Tri-State Aquaculture Conference (IN-OH-KY) in Cincinnati, Ohio. A special session on Introduction to Aquaculture was coordinate by Ohio State University staff with Dr. Laura Tiu and Shawn McWhorter presenting. These activities keep fish farmers in Ohio up to date on the latest aquaculture research and extension activities in the Midwest.

OCARD also facilitates the development of the aquaculture industry in Ohio using

traditional and innovative extension methods including fact sheets, workshops, videoconferencing and updated websites and list servesOhio is the one of the largest yellow perch producing states, and many beginning aquaculture farmers look to this species when starting an aquaculture operation.
Twenty participants in a 2012 workshop reported a 30% increase in aquaculture knowledge after completing the event. Eighty-two percent plan to use the knowledge they gained in the future. Attendees estimated the value of the workshop for them personally as between $2000 to $7500.
A conference targeting people interested in learning business strategies to develop and maintain a healthy and sustainable fish farming operation was the product of a regional partnership. The conference, "Planning for Aquaculture Business Success," was held Aug. 6-7, 2012 at Cabela´s Dundee, Michigan store. The 65 attendees hailed from three states (Michigan, Ohio, and Indiana) and two countries (US and Canada), and included producers, educators, agency staff, and government officials. The conference was free and funded through a partnership with the National Aquaculture Association with funding from the United Soybean Board; NCRAC; the Nature Conservancy; Michigan Sea Grant; Michigan Aquaculture Association; Originz; Stoney Creek; Easy Pro; Ohio Aquaculture Association; and the Ohio Aquaculture Research and Development Center at The Ohio State University South Centers. Many of the presentations from the conference are available at http://go.osu.edu/AquaSuccess.

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. 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. 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/.

Kinnunen coordinated a 3-d Seafood HACCP Training course that was held at Bay Mills, Michigan, December 9-11, 2008. 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.

Additional activities by Kinnunen included coordination of a 3-d Seafood HACCP Training course that was held at Keweenaw Bay Indian Community. The 31 attendees included state and tribal fishermen/ processors, fish farmers, Indian Health Service, along with a representative whose

company sells the fish to McDonalds® for their fish sandwiches.

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), Jeff 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.

Kinnunen coordinated a 3-d Seafood HACCP Training course that was held at Little Traverse Bay Indian Community. Instructors that assisted me with teaching this three day course included Mike Erdman (MSU-District 1 Coordinator) and Jim Thannum (Great Lakes Indian Fish and Wildlife Commission). Formal evaluations from attendees rated the course as excellent. The 19 attendees included state and tribal fishermen/processors, MDNR Law Enforcement, along with representatives from Spartan Stores.

Nick Phelps (University of Minnesota Veterinary Diagnostic Lab) and Kinnunen conducted two Aquaculture Biosecurity/AIS-HACCP Workshops (see Extension Addendum for more details).

For the 2011 reporting year the Wisconsin Aquaculture Outreach Specialists (including Held) conducted 56 on-farm visits, 17 high school and university educational presentations, seven workshops including the annual Wisconsin Aquaculture Association Conference, as well as numerous meetings, planning sessions and work group meetings to further the cause of the aquaculture industry. These activities resulted in over 4,000 direct contacts with current and prospective farmers, students, legislators and regulators. As a result of the efforts of the aquaculture specialists the Wisconsin Aquaculture Bill (Act 207) was signed into law. This was the first significant aquaculture legislation to pass since 1997. The law made significant changes to permitting, fees and paperwork requirements that were a burden to expansion of the industry.

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).

Current NCRAC extension materials will continue to be reviewed and updated by regional extension contacts in partnerships with the NCRAC administrative office. The proposed Tribal Aquaculture/Aquaponics Workshop has been rescheduled for Spring of 2013 due to schedule conflicts with the original date. Funding for this objective has been reallocated to the second year of the funding cycle.

IMPACTS

Examples include:

- Development of aquaculture education programs for the NCR has provided "hands-on" opportunities for prospective and experienced producers. More than 10,000 individuals have attended workshops, conferences, or symposia organized and delivered by members of the NCRAC Extension Work Group.
- Fact sheets, technical bulletins, videos, and CDs have served to inform a variety of clients about numerous aquaculture practices for the NCR. For instance, "Making Plans for Commercial Aquaculture in the North Central Region" is often used to provide clients with initial information about aquaculture, while species-specific publications have been used in numerous regional meetings. The Center's Web site provides immediate availability to many of the products that have been developed by the Extension Work Group (e.g., fact sheets as PDF files) and with the further development of streaming videos, not only will clients have the benefit of being able to read about aquaculture for free on a 24-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.
- Quagrainie has developed workshops that were mainly hands-on, which enabled participants to acquire skills in building cages, handling fish, using financial spreadsheets, etc. Some farmers are now building their own cages and using financial spreadsheets for their aquaculture operations.
- Hicks assisted in the establishment of the Gaylord Farms Cage Culture and Prawn business and securing for them a Sustainable Agriculture Research and Education (SARE) grant for expansion of their

operations.

- Regional aquaculture information (i.e., workshop announcements, fact sheets, and product marketing) is quickly and efficiently distributed to aquaculture clients in Ohio and neighboring states. This results in Ohio fish farmers being well informed about activities and information that can enhance the success of their businesses.
    - Nearly 100 fish farmers attended the Ohio Bluegill Workshop in 2011. Farmers were able to network and are now working together, buying feed and fingerlings in bulk and cooperatively marketing together in order to reduce individual costs. The PowerPoint presentations and video/audio from the event are available free and located at http://southcenters.osu.edu/aqua/extension/osu_bluegill_aquaculture_worksho.htm
    - The expansion of the Aqua-Ohio List serve as served to improve the transfer of regional aquaculture information (i.e. workshop announcements, fact sheets, and product marketing) more quickly and efficiently distributed to aquaculture clients in Ohio and neighboring states.

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
2009-11	$29,000						$29,000
TOTALS	$912,125	$1,055,868	$5,000	$334,000	$55,000	$1,449,868	$2,361,993

EXTENSION ADDENDUM[6]

Project ***Termination Report*** for the Period
September 1, 2008 to August 31, 2012

NCRAC FUNDING: $50,505 (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

REASON FOR TERMINATION

The objectives were completed and the funds terminated.

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.

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 now complete. Part one of the fish health certificate program included the development of a six module web-based learning program. Modules 1-6 of the asynchronous learning program have undergone peer review, revisions based on those reviews were made, and the modules have been published, available at http://ce.vetmed.wisc.edu/Fish_Producer_Courses. The modules contain information about:

1. Introductory principles and practices such as regional fish production, farm types in the NCR, principle culture systems, and the myriad of

[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 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.

regulatory agencies involved in U.S. aquaculture.
2. Risk management and biosecurity methods that can assist producers in reducing the risk of introduction of diseases at aquaculture facilities. This module reviewed topics, 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 viremia of carp, and viral hemorrhagic septicemia have been developed based on actual "real-world" examples.
7. Evaluation and outcome assessment tools have been developed. Mechanisms are in place to collect data on the finished products.
8. Free access was provided for the complete online program for those that agreed to complete a pre- and post-program survey.

OBJECTIVE 2

A publication entitled "Biosecurity for Aquaculture Facilities in the North Central Region" was developed and is now available through NCRAC.

Kinnunen has coordinated a number of AIS-HACCP training courses as numerous locations with a varied audience base. For instrance a 3-day AIS HACCP Training course 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 also 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-d 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. 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.

Kinnunen also coordinated a second 3-d Seafood HACCP Training course at Bay Mills, Michigan 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.

Kinnunen also attended a meeting hosted by the Wisconsin Department of Natural Resources in Manitowish Lakes, Wisconsin to discuss the invasion of spiny water fleas into lakes in northern Wisconsin and Michigan's western Upper Peninsula. He shared with the group the AIS-HACCP program and how natural resource management agencies could use this program to prevent the spread of aquatic invasive species by way of their assessment operations. Similar efforts included: (1) Wild Rivers Invasive Species Coalition Annual Meeting; (2) a Central Upper Peninsula all agency meeting that included officials from the U.S. Fish and Wildlife Service, U.S. Forest Service, National Park Service, and Michigan MDNRE where the program efforts were highlighted; and (3) began evaluating the use of AIS-HACCP at Cabala's Master Walleye Circuit fish tournaments and attended events in Escanaba and Sault Ste. Marie, Michigan. Kinnunen developed a display on aquatic invasive species and surveyed tournament anglers on their current practices to prevent the spread of AIS. At these two tournaments he evaluated procedures that could be critical control points to prevent the spread of AIS. Tournaments attract participants from many states and have the potential to spread AIS.

IMPACTSThe complete Fish Health Certificate Program was peer reviewed in the summer of 2011 and was published online (http://www.ncrac.org/node/329) in November of 2011. As of September 15, 2012, 268 participants from over 30 states and eight countries completed the course. As part of the course requirements, participants were asked to complete a short survey prior to taking the course (Pre-Survey), immediately following completion of the course (Post-Survey), and six months after taking the course (Follow-Up Survey). Information gathered was used to assess short-term and intermediate outcome indicators, as well as feedback for improvement of the course.

Pre-Survey.—The majority of course participants indicated they were taking the course to learn how to improve the health of fish on their farm/facility (74%), to learn what biosecurity for fish farms involves (62%), and to learn how to implement biosecurity on their farm/facility (52.5%). Most respondents (90.9%) indicated that implementing biosecurity measures would serve to prevent disease from entering farm/facility. Many (84.2%) also felt it would increase the health of fish. Others (36.4%) indicated it had economic benefit and increases sales of product.

Almost half (47.3%) of the respondents reported never having had a fish health inspection/or fish health assessment conducted for their farm/facility. Of those indicating "yes" for having these procedures for their farm facility, 31.5% had a fish health inspection, 21.2% had a fish health assessment. Of those indicating "no", 13.5% were interested in using them for their farm/facility. The majority (47.2%) of respondents felt current fish health inspection regulatory requirements seemed reasonable. Lastly respondents were asked about the availability and use of fish veterinarians in their area. Approximately one-third of respondents indicated they did not know if there were any aquatic veterinarians in their area. Another third were aware of an aquatic veterinarian in their area; twenty percent replied that no aquatic veterinarian was available for their area. Only 15.7% of respondents indicated ever working with an aquatic veterinarian on their farm/facility.

Post-Survey.—Upon completion of the online course, participants were asked to take a short post-course survey. Only 61 of the 268 (22.7%) participants completed the post-survey. All respondents indicated the information in the course was very useful; almost half (41%) ranked the course as "extremely useful". Prior to this course, the majority (62.7%) of respondents had never attended an aquaculture biosecurity course/workshop. All respondents indicated they would recommend the course to others.

Respondents were asked their opinion about the level of biosecurity used on their farm (after taking the course). The majority (42.4%) felt their level of biosecurity was high, 22% indicated moderate levels of biosecurity, and 8.5% reported low levels of biosecurity.

Respondents were then asked to rate various biosecurity elements. This was a similar question to the pre-survey, and was used to see if the information contained in the modules, changed the participants knowledge or perception of biosecurity measures and importance. In the Post-Survey, all biosecurity elements (water quality, record keeping, visitor control, cleaning and disinfection, diagnostic testing, and fish health assessment) were rated as extremely important. Three parameters (record keeping, visitor control, and diagnostic testing) which were ranked as moderately important in the pre-survey, were increased in rank to extremely important in the post survey, potentially indicating an increased awareness of the importance for these biosecurity measures.

Respondents were asked how likely they were to implement new or enhanced measures of biosecurity on farm/facility after taking course. The majority (75%) indicated highly likely, 18.3% indicated somewhat likely and 3.3% indicated either not likely or it was required. Respondents were asked who should pay for the cost of fish health regulatory requirements. The majority (73.8%) of respondents indicated costs should be a combination of producer financing and State/or Federal funds. Twelve respondents (19.7%) felt producers should be responsible.

Follow-Up Survey.— On August 21, 2012, a follow up survey was emailed out to participants (n=205) that had taken the course at least 6-months prior. Only 43 responses (21%) were received, however not all questions were answered by those responding. The majority of respondents (60.5%) indicated that they had implemented new or enhanced measures of biosecurity on their farm since taking the course. Biosecurity elements that were reported as enhanced from previous measures were cleaning and disinfection (60.7% of respondents), record keeping

(60% of respondents), water quality (42.3% of respondents) and visitor control (40.7% of respondents). Respondents felt that the implementation of biosecurity measures helped in keeping diseases from spreading onto farms (65.5%) and increased the health of fish (58.6%)

The majority of respondents (42.1%) reported they had not worked with an aquatic veterinarian since taking the online course. Additionally, over half (52%) had not had a fish health inspection or assessment since taking the course. 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.

RECOMMENDED FOLLOW-UP ACTIVITIES

Survey results from the Fish Health Certificate Program for Aquaculture Producers online course indicated this can serve as a useful tool to increase education and awareness of fish health and biosecurity issues in aquaculture for producers. For the majority of survey respondents (62.7%), this was the first aquaculture biosecurity course/workshop they had "attended". The course was well received by participants, who also indicated they would recommend the course to others. Many participants reported implementing enhanced fish health and biosecurity measures at their facility or farm after taking the course, and almost half of the respondents on follow-up had had a fish health inspection or assessment conducted on their facility. The survey results suggest there may be a gap of information on where or how to identify aquatic veterinarians in the producers area. There will continue to be a need for additional workshops in AIS-HACCP training, especially given the changing environmental and legal landscape for the aquaculture industry. The utility of these workshops is evident by the wide audience base that has attended the workshops noted in this report.

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.

NCRAC AND MARKETMAKER™[7]

Project ***Progress Report*** for the Period
September 1, 2010 to August 31, 2012

NCRAC FUNDING: $23,565 (September 1, 2010 to August 31, 2011)

PARTICIPANTS:

Joseph E. Morris	Iowa State University	Iowa
Extension Liaison:		
Richard Clayton	Iowa State University	Iowa

PROJECT OBJECTIVES

(1) Conduct a survey of all North Central Region (NCR) aquaculture producers for data that will be assimilated into the MarketMaker™ system.
(2) Undertake outreach activities to educate and register NCR producers into the MarketMaker™ system.
(3) Develop a "how to" tutorial case study tool that will instruct NCR producers on how to conduct market research using the MarketMaker™ system.

ANTICIPATED BENEFITS

This project will result in producer to consumer value-chain visibility that will immediately begin to address all of the five stated goals of the NCRAC program:

- Develop transferable (marketing/outreach)* technology to enable producers to be profitable;
- Disseminate relevant educational materials to achieve profitable margins of operation (through increased market exposure, visibility, outreach and delivery efficiency);
- Engage in research (cooperative market-research and outreach initiative that incorporates detailed industry value-chain data) partnerships between industry, universities, and public agencies;
- Deliver demonstrations and regular aquaculture extension programs (with respect to Market-Maker training and communication skills); and
- Foster open dialogue and networking throughout the North Central aquaculture community.

* Information in parenthesis qualifies the goal with respect to this proposed project.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

Activities in this objective have been delayed until Spring 2012 due to limited number of staff with Clayton's departure.

OBJECTIVE 2

An effort to host a ½-d meeting on the use of MarketMaker™ with a walleye workshop was developed for summer 2011 in conjunction with Chris Weeks in January 2011. However, due to limited number of

[7] NCRAC has funded six Economics/Marketing projects. Termination Reports for the first two projects are contained in the 1989-1996 Compendium Report; a Termination Report for the third project is contained in the 1996-97 Annual Progress Report; a Termination Report for the fourth project is contained in the 2002-03 Annual Progress Report; and a Termination Report for the fifth project is contained in the 2003-04 Annual Progress Report. This Progress Report is for the sixth project. It is a 1-year project that began September 1, 2010.

projected anticipants, the decision was made to pursue an on-line portal for MartketMaker training.

OBJECTIVE 3
The MarketMaker™ tutorial has been developed by Iowa State University Value Added Agriculture Program. This information will also be developed as a web-based document for subsequent use by the aquaculture community.

WORK PLANNED
OBJECTIVE 1
In 2013, Allen Pattillo will continue to work with individual aquaculturists to assist them in placing their information into the Market-Maker system.

OBJECTIVE 2
A final online educational module that will assist in the registration of individual operations in the region will be placed onto the NCRAC web site.

OBJECTIVE 3
The final draft of the MarketMaker™ tutorial will be provided to regional and national audiences.

SUPPORT
NCRAC has provided $23,565 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 Economics/ Marketing activities.

RAPID DETERMINATION OF AMINO ACID REQUIREMENTS OF YELLOW PERCH AND TILAPIA[8]

Project ***Termination Report*** for the Period
September 1, 2009 to August 31, 2011

NCRAC FUNDING: $80,000 (September 1, 2009 to August 31, 2011)

PARTICIPANTS:

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

REASON FOR TERMINATION

The objectives have been completed and the funds terminated.

PROJECT OBJECTIVES

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

[8]NCRAC 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.

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. Although trout diets are practical diets that are often used by yellow perch producers, these diets may not be appropriate for that species. For instance, the lipid requirement for yellow perch is 6-8%, compared to diets that typically contain, 16-17% lipid. Feeding trout diets to yellow perch may promote substantial fat deposition. Excess fat deposition not only reduces fillet yield, but also decreases feed consumption and ultimately, fish production. Moreover, the costs of trout diets threaten the economic sustainability of yellow perch farming.

OBJECTIVE 2

A study was conducted to model dietary essential amino acid requirement for yellow perch, based on digestible dietary lysine requirement (1.25%) and whole-body amino acids profile. Eighteen wild-cultured juvenile yellow perch (22±1.3 g) were fasted for 24 h and then euthanatized, ground and stored for analysis. Six fish were grouped as one sample, with three samples being used to determine the EAA profile of whole-body tissue. The modeled essential amino acids requirement was similar to that determined by the dose-response approach which, when expressed relative to the lysine A/E ratio, was estimated to be: Arginine 1.07%, methionine 0.44%, leucine 1.11%, isoleucine 0.61%, valine 0.7%, Phenylalanine 0.6%, trptophan 0.48%, histidine 0.36%, and threonine 0.65%.

The tilapia lysine experiment for grow-out-size male tilapia was not completed even though researchers attempted this project twice in 2010 and 2012, respectively. Grow-out-size male tilapia exhibited strong social hierarchies, wherein the larger fish continued to chase and bite the relatively small individuals, until they perished. Mortality reached 80% in first experiment, which ran until 2010. In the second effort, researchers used various barriers and stones to reduce the internal conflict and increase fish density; however, mortality remained up to 70% after a 6-week experimental period. It was this difficulty that also prevented the completion of objectives 3-5 for tilapia, and hence, only information related to yellow perch will be presented.

OBJECTIVE 3

A study was also conducted to determine the dietary lysine requirement of yellow perch in an indoor, water flow-through system. Six isonitrogenous and isoenergetic experimental diets were formulated to contain graded levels of lysine (1.1, 1.3, 1.5, 1.7, 1.9, 2.2) from commercial ingredients and crystalline lysine. Crystalline, methionine, and arginine were supplemented in all experimental diets, to satisfy requirements according to previous work on yellow perch. Results showed that relative growth rate (RGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and protein retention (PR), significantly increased with increasing dietary lysine level, from 1.1 to 1.5% of the diet, and then leveled off. Whole-body crude protein (14.48–15.9%), as well as lysine (1.15-1.22%), increased significantly with increasing dietary lysine level ($P<0.05$), whereas moisture, fat and ash, showed no significant differences among the dietary treatments. The lysine requirement of yellow perch was estimated as 1.33% of dry diet and 1.25% at the digestible level based on growth performance.

A study was conducted to model dietary essential amino acid requirement for yellow perch, based on digestible dietary lysine requirement (1.25%) and whole-body amino acids profile. Eighteen, wild-cultured juvenile yellow perch (22±1.3 g [size]) were fasted for 24 h and then euthanatized, ground and stored for analysis. The modeled essential amino acid requirement was similar to that determined by the dose response approach which, when expressed relative to the lysine A/E ratio, was estimated to be: Arginine 1.07%, methionine 0.44%, leucine 1.11%, isoleucine 0.61%, valine 0.7%, Phenylalanine 0.6%, tryptophan 0.48%, histidine 0.36%, and threonine 0.65%.

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.

Apparent digestibility of dry matter, energy, and amino acids in fish meal, meat and bone meal, poultry byproduct meal, soybean meal, corn gluten meal and wheat, were determined for yellow perch (body weight, 19.7±1.5 g) by using single test ingredients. Apparent dry matter digestibility ranged from 58% (meat and bone meal) to 79% (fish meal) for animal products and from 47% (wheat) to 72% (corn gluten meal) for plant products. Apparent energy digestibility values ranged from 52% (wheat) to 88% (fish meal) for yellow perch. Apparent digestibility of crude protein for animal products and plant products ranged from 85%-88% and 82%-90%, respectively. Apparent digestibilities of most essential amino acids exceeded 90% except for meat and bone meal, and corn gluten meal. These data provide more precise information on nutrient and energy availability for yellow perch, and hence, should enable aquaculture nutritionists to design yellow perch formulae based upon digestible nutrients.

OBJECTIVE 5

A study was conducted to determine the feasibility of replacing fish meal with poultry byproduct meal in yellow perch, in an indoor re-circulating system. Five experimental diets were formulated with poultry byproduct meal replacing 0, 25, 50, 75, and 100% of the fish meal protein (PBM0 control), PBM25, PBM50, PBM75, and PBM100, respectively; all diets were compared against a commercial diet (Aquamax grower 400 [minimum 45% of crude protein]; Purina Mills). Experimental diets were formulated to contain required digestible requirement values of 33-34% crude protein, and 3,420 kcal/kg of digestible energy for yellow perch. Crystalline methionine and lysine were supplemented in all experimental diets to satisfy the dietary requirements according to previous research on yellow perch.

Results indicated that fish fed with PBM75 and PBM100 showed a significantly lower specific growth rate (SGR) and weight gain (WG) than fish fed PBM0 ($P<0.05$) or the commercial diet. Fish fed PBM100 showed a significantly lower feed intake (FI), feed efficiency (FE) and protein efficiency ratio (PER), than fish fed with PMB0 ($P<0.05$). Whole body protein and lipid contents declined linearly with increasing inclusion of PBM, and there was no significant difference in whole body moisture content shown in this study.

There were no significant differences in survival rates among the experimental treatments.

In conclusion, yellow perch appear capable of using high quality poultry byproduct meal at high inclusion levels. PBM is able to replace 50% of fish meal protein without impeding growth performance. Moreover, replacement could be as high as 75% without reducing FE and PER.

OBJECTIVE 6
The least-cost formulation will be presented to the NCRAC community for later field testing.

IMPACTS

- A cost-effective diet for yellow perch has been developed for future analyses as a diet for fish cultured under commercial conditions. Yellow perch appear capable of using high-quality poultry byproduct meal (PBM) at high inclusion levels. PBM is able to replace as much as 50% of fish meal protein without impairing growth performance. In addition, replacement could be up to 75% without reducing feed efficiency and protein efficiency ratio.

RECOMMENDED FOLLOW-UP ACTIVITIES
The identification of a new, cost-effective diet for yellow perch through laboratory studies, still warrants testing under field conditions with production scales used by regional producers. It is anticipated that these fish reared in ponds will not only feed on the provided diets, but also on natural feed stuffs including aquatic invertebrates. The combination of a commercial diet with these natural feedstuffs may result in different production parameters.

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

REGIONAL AQUACULTURE EXTENSION SPECIALIST (RAES)

Project ***Progress Report*** for the Period
September 1, 2011 to August 31, 2012

NCRAC FUNDING: $196,612 (September 1, 2011 to August 31, 2013)

PARTICIPANTS:

Joseph Morris	Iowa State University	Iowa
Ronald Kinnunen	Michigan Sea Grant	
Industry Advisory Council Liaison:		
William E. Lynch		Ohio
Extension Liaison:		
Kwamena Quagrainie	Purdue University	Indiana

PROJECT OBJECTIVES

(1) Continue RAES support to the NCR aquaculture community through ongoing activities in areas of services, leadership, assessing and addressing industry needs, and information transfer.
(2) Develop and implement strategies to address and promote aquaculture sustainability in the NCR.
(3) Develop and strengthen partnerships from within the NCR and outside the region among regulatory agencies, industry, academia, and other relevant entities to foster open, meaningful dialog on critical issues and build support for the NCR aquaculture industry.
(4) Coordinate efforts for seeking non-NCRAC support for NCR aquaculture development.
(5) Examine regional aquaculture development and assess NCRAC research and extension activities in terms of impacts on the NCR aquaculture industry. Make recommendations for improving NCRAC projects in terms of incorporating measures of program success.

ANTICIPATED BENEFITS

The RAES project team has identified internal and external factors influencing industry development in previous assessments. Information access, management practices, partnerships, organization, support from within the industry, willingness to participate, ingenuity, and environmental sustainability are examples of industry internal factors. External factors include regulations, funding opportunities, support from outside the industry, epizootics, exotic species, etc. This work plan takes a slightly different approach than previous NCRAC projects by focusing on aquaculture sustainability, which is a common denominator of many, if not all, of the current topical issues facing the industry.

Sustainability is a key term used to describe long term viability of industrial development. Opponents often use environmental degradation as a means to discredit industrial activities across the general population. Within the NCR, aquaculture producers have identified environmental regulations as a serious concern. Also, aquaculture and baitfish industry sectors are being singled out as vectors needing stricter regulatory control due to aquatic invasive species (AIS) issues.

Through discussion opportunities, presentations, workshops, list serves, and other outreach activities the RAES will demonstrate that further industry development necessitates environmental, social, and economic sustainability. Furthermore, the NCR aquaculture community must be more proactive in demonstrating sustainable methods and practices, and communicating such standards to regulatory agencies and environmental groups. An anticipated benefit of this project is for increased awareness of sustainable aquaculture across the industry, regulatory agencies, and the general public.

Partnerships, alliances, and endorsements are extremely important at this phase of NCR aquaculture development. Through direct interactions, the RAES project will help to strengthen alliances with groups such as the National Aquaculture Association, Farm Bureau, and other agricultural commodity groups. The PI will also work with various regulatory advisory committees to the extent possible to provide voice and viewpoint of the NCR aquaculture industry. The RAES will encourage, through discussion and outreach, other members of the NCR aquaculture community to take a more proactive approach in networking and partnership building. In addition, the RAES will actively seek outside funding through collaborative efforts with the goal of securing 1-2 funded projects through pursuit of non NCRAC grant opportunities. Increased support for NCR aquaculture is anticipated on local, state, and national levels with the potential for 1-2 funded research projects from outside the NCR through these activities.

One conclusion by the current RAES team is that measuring outreach program success is difficult, specifically measuring success in terms of impact on the NCR aquaculture industry. One objective of this work plan is designed to help gain a better understanding of the effectiveness of NCRAC extension, outreach, and research activities in terms industry impact. The structure and objectives of recent and current NCRAC projects (RAES project included) will be reviewed and assessed as to whether anticipated benefits or measured outcomes are identified and/or being realized. A database will be constructed of the number of state registered aquaculture facilities in each state over time. Other potential indicators (e.g. economics, federal funding, extension full time equivalents) will also be identified and examined for their feasibility for use as measurements of program development. In year-2 of this project the RAES will report assessment results and make recommendations for future NCRAC activities. Anticipated benefits from this activity will be a written review describing the status of NCR aquaculture based on acquired data and how aquaculture research and NCRAC extension activities might be improved in the region through a better understanding of industry impacts.

PROGESS AND PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1 To date, the RAES has committed substantial effort in continued support to the NCR aquaculture community. This effort includes:

- Monitor and update the NCRAC website *State Importation and Transportation Requirements for Cultured Aquatic Animals* (herein referred to as the NCRAC Regulation Website). The site was originally developed by the current RAES back in 2003, and improved in 2009 with help from the NCRAC Associate Director's office as part of the RAES project. The Regulation Website receives 500+ views per month and has been given a main link from APHIS aquaculture: http://www.aphis.usda.gov/animal_health/animal_dis_spec/aquaculture/aquastates.shtml.
- 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 interstate transport laws and developing AIS issues.
- 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 Lacey Act and the National Aquaculture Research and Development Strategic Plan.
- PI continues to attend and support state association meetings and workshops in the region. The RAES also visits commercial facilities across the region to meet with seasoned and new producers. In addition the RAES is building more and more network opportunities, and receiving a substantial increase in phone calls and email inquiries from across the NCR aquaculture community.

OBJECTIVE 2 From February - March 2012, the RAES presented a workshop-style discussion on sustainable aquaculture during state association meetings in Michigan, Missouri, and Kentucky (tri-state conference with OH, IN and KY). This workshop exercise was designed to provide an example of how to establish sustainable goals and objectives on a farm by farm basis. Perceptions and recommendations regarding status of sustainable aquaculture in the NCR were obtained from individuals attending the workshops have been summarized in a review entitled *Sustainable Aquaculture in the NCR - a Review of Perceptions and Recommendations from the Aquaculture Community*. The final draft is complete and will be submitted for publication or NCRAC technical paper. Judging by the responses from attendees, it appears that collectively the NCR aquaculture community has a good grasp on sustainable aquaculture issues. The RAES will continue to strive for increasing awareness for sustainable aquaculture development in year-2 activities. Specifically, the aquaculture community as a whole, must strive to address three principle accomplishment areas of sustainability over the long term: environmental conservation + social benefits + economic viability.

OBJECTIVE 3
PI continues to expand personal relation/partnerships aiming to increase NCR aquaculture industry support. In 2011-2012, the RAES received appointments to the Great Lakes Panel of the National Aquatic Nuisance Species Task Force, the Michigan Farm Bureau Aquaculture Advisory Committee, and recently, to the NSF International Food Division Advisory Council as industry representative to the program's Regulatory/Seafood sector. The PI is also a full member of the National Aquaculture Association and has participated in projects with Michigan Soybean and Indiana Soybean Associations and Soy Aquaculture Alliance. The RAES is also a member of the USDA sponsored eXtension Freshwater Aquaculture Group and serves as an "ask the expert" role on regional issues.

Currently the RAES is focusing to large extent on AIS, working closely with Ron Johnson, member of the National Aquatic Nuisance Species Task Force, and Nathan Stone, member of National Invasive Species Advisory Committee. The RAES recently gave a presentation entitled "AIS Impacts on Regional Aquaculture – the Need for Effective but Fair Regulations and a Proactive Industry" at the Upper Midwest Invasive Species Conference, La Crosse, Wisconsin. PI is also working with NCRAC TC Members, Nicholas Phelps and Ronald Kinnunen in strategic planning towards development of AIS HACCP into a potentially certifiable program, recognized by state and federal environmental protection agencies.

OBJECTIVE 4
The PI was awarded $268,000 for a 2-year Michigan Sea Grant project entitled "Integrated Assessment - Expansion of Michigan's Existing Commercial Aquaculture Activities into a Major Sustainable Seafood Production Industry". The project is underway and the RAES acts in supervisory role only. In 2012-2013 PI will search for additional means of support for one or more other states in the region. The RAES has also served on various grant review panels for US aquaculture program development.

OBJECTIVES 5
A greater focus has been placed on AIS regulatory issues facing the industry than anticipated in 2011-2012. Objective 5 will be further addressed in 2012-2013.

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 and continued liaison services to the industry. Starting November 2012 the RAES will review all state regulations and links in the NCRAC regulation website for updates. Outreach methods will be improved pending recommendations from the NCR aquaculture community. With the regulation website current, the RAES project team will place focus on partnership building, seeking additional non-NCRAC support for the industry, and impact assessment. Notwithstanding, since members of the project team and NCRAC IAC and TC members have identified AIS issues as a critical need at this time, the RAES will continue to address AIS as a top priority in 2013.

IMPACTS

- Increased knowledge, awareness, and accessibility of information regarding aquaculture, aquaculture sustainability, interstate transport regulations, and health certification requirements to the industry and aquaculture community in the NCR.
- New partnerships for aquaculture support in the NCR.
- Increase level of awareness regarding AIS impacts on the NCR aquaculture industry.
- Increased non NCRAC funding support for NCR aquaculture industry development.

SUPPORT

To date, NCRAC has provided $96,770 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 Extension activities.

SNAIL MANAGEMENT/GRUB CONTROL[9]

Project ***Progress Report*** for the Period
September 1, 2007 to August 31, 2012

NCRAC FUNDING: $20,500 (September 1, 2007 to August 31, 2011)

PARTICIPANTS:

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

PROJECT OBJECTIVE

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

PROGESS 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

[9] This Progress Report is for the second objective of this project. A Project Component Termination Report for the first objective is contained in the 2009-10 Annual Progress Report. This is a project that had two years of funding and is chaired by Gregory W. Whitledge. It began September 1, 2007.

revised North Central Regional Aquaculture Center (NCRAC) Web site.

WORK PLANNED

In 2013 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.

VIRAL HEMORRHAGIC SEPTICEMIA (VHS)[10]

Project ***Progress Report*** for the Period
September 1, 2008 to August 31, 2012

NCRAC FUNDING: $197,960 (September 1, 2008 to August 31, 2011)

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 <u>specific</u> fish disease HACCP plans for each of the six facilities involved in the workshops.
(5) Develop and distribute three <u>model</u> fish disease HACCP plans (one each for flow-through, pond, and recirculation systems), relying heavily on the <u>specific</u> plans developed under Objective 4.
(6) Produce a fish farm biosecurity video that incorporates different system types and footage shot at the workshops and distribute this video to end users via DVD and internet streaming videos.

ANTICIPATED BENEFITS

Diseases constitute the largest single cause of economic losses in aquaculture. There are few treatments available for current and emerging aquaculture diseases. This research on egg disinfection will provide valuable information to commercial and public fish culture facilities to make decisions on the safety and efficacy of iodine treatment to eliminate VHS infections from cool and warm water fish eggs. If iodophor disinfection can be used to safely eliminate VHS virus (VHSv) from eggs, the direct benefits will include: (1) reduction in the risk of movement of VHSv between aquaculture facilities during embryo

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

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.

PROGESS AND PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

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

Eggs were held in well water for at least 90 min post-fertilization before being distributed to miniature egg jars and later 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 . However, VHSv was isolated from control eggs 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

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

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 .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. 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 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 . 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.

In Year 3, UMESC will evaluate vertical transmission of VHSv from adult spotfin shiners (*Notropis spilopterus*) to eggs. Adult spotfin shiners will be obtained from Woodside Farms (Bellevue, Ohio) and 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 tested for the presence or absence of VHSv.

In late winter 2012, UMESC was approached by the University of Wisconsin Stevens Point – Northern Aquaculture Demonstration Facility (UWSP-NADF) to request participation in a study to develop rearing methods of lake herring *Coregonus artedi*, specifically to evaluate the use of iodophor disinfection of eggs to eliminate the virus that causes VHS. UMESC received permission from NCRAC in March 2012 to stop work on spotfin shiners and focus on the lake herring project since there was a high probability of obtaining adult herring that were positive carriers; all remaining project funds at UMESC were expended in preparation for the work with lake herring. In December 2012, UWSP-NADF, UMESC, and USFWS La Crosse Fish Health Center (LFHC) personnel plan to evaluate the effectiveness of iodophor treatments on lake herring eggs obtained from wild VHSv positive adults collected from Lake Superior.

Egg groups will be subjected to one of six treatment options, with 3 replicates:

1) prewater hardening iodophor treatment of 100ppm/15min, dead eggs hand picked

2) postwater hardening iodophor treatment of 100ppm/15min, dead eggs hand picked

3) postwater hardening iodophor treatment of 50ppm/30 min, dead eggs hand picked

4) Control group –no iodophor treatment, dead eggs hand picked

5) postwater hardening iodophor treatment of 100ppm/15min, formalin treatments for fungus control

6) Control group –no iodophor treatment, formalin treatments for fungus control

The four treatment groups will be incubated in a mini-egg jar system set-up at UWSP-NADF through hatching and monitored for eye-up, hatch rate, and survival. The efficiency of iodophor disinfection procedures will be evaluated on egg/fry survival and fish health protocols with the assistance of UMESC. UMESC will assist UWSP-NADF with iodophor disinfection, egg placement and verification of iodophor concentrations . Personnel at LFHC will be responsible for determining the presence or absence of VHSv. Egg and fry samples will be pooled and tested using standard protocols to isolate and identify fish viruses (USFWS/AFS-FHS Standard Procedures for Aquatic Animal Health Inspections, Section 2, Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogen, 2007; and OIE Manual of Diagnostic Tests for Aquatic Animals, 2006). The pooled samples will be tested using standard tissue cell culture methods for isolation of virus and confirmation by polymerase chain reaction (PCR).

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

- 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 5

To be completed as described in the original proposal.

OBJECTIVE 6

Production of the HACCP biosecurity video is nearing completion. The DVD script has undergone review and the appropriate edits have been incorporated, the audio portion of the DVD has been recorded and the video components have been selected. The final editing and production has been completed. Final distribution will be done once associated HACCP plans have been completed. 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.
- The VHS-HACCP instructional DVD will further increase the ability of aquaculture producers to develop effective and economical HACCP-based biosecurity plans to control the spread of VHSV as well as address potential AIS and disease concerns in the future.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

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

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
2010-11	$29,600						$29,600
TOTAL	$155,365			$73,422	$3,900	$27,322	$223,792

APPENDIX

AQUACULTURE DRUGS

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Barry, T.P., A. Marwah, and P. Marwah. 2007. Measurement and stability of 17α-methyltestosterone in fish feed. Aquaculture 2007, San Antonio, Texas, February 26-March 2, 2007.

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BAITFISH

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CONFERENCES/WORKSHOPS/ SYMPOSIA

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CD-ROM

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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)

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

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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)

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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)

Missouri Aquaculture – Status, Progress, Priorities, Information Access and Concerns. Missouri Aquaculture Association Annual Meeting, Lincoln University Carver Farm, January 10, 2011. (Christopher T. Weeks)

Ohio State University Bluegill Aquaculture Workshop, Columbus, Ohio, February 11, 2011. (Charles Hicks, Laura Tiu)

Seafood HACCP Training Workshop, Baraga, Michigan, February 15-18, 2011. (Ronald E. Kinnunen)

Growing Power Conference, Fish Processing, Milwaukee, Wisconsin, September 11, 2010 . (Ronald E. Kinnunen)

Seafood HACCP Training Workshop, Petoskey, Michigan, December 6-8, 2011. (Ronald E. Kinnunen)

Michigan Aquaculture Association Annual Conference, Aquaculture Biosecurity/AIS-HACCP/Seafood HACCP/Fish Processing and Marketing, Tustin, Michigan, February 7, 2012 (Ronald E. Kinnunen)

Aquaculture Biosecurity/AIS-HACCP Workshop, Ellendale, Minnesota, July 12, 2012. (Ronald E. Kinnunen and Nick Phelps)

Aquaculture Biosecurity/AIS-HACCP Workshop, La Crosse, Wisconsin, July 30, 2012. (Ronald E. Kinnunen and Nick Phelps)

American Fisheries Society Fish Health Section Annual Conference, North Central Regional Aquaculture Center VHS Project, La Crosse, Wisconsin, August 1, 2012. (Ronald E. Kinnunen)

Aquaculture Biosecurity/AIS-HACCP Workshop, Dundee, Michigan, August 6, 2012. (Ronald E. Kinnunen and Chris Weeks)

American Fisheries Society Annual Conference, North Central Regional Aquaculture Center VHS Project, St. Paul, Minnesota, August 20, 2012. (Ronald E. Kinnunen)

Proceedings

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

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

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 electronically at: http://ag.ansc.purdue.edu/aquanic/publicat/state/il-in/ces-305.htm)

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.

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Brown, P.B., R. Twibell, Y. Jonker, and K.A. Wilson. 1997. Evaluation of three soybean products in diets fed to juvenile hybrid striped bass *Morone saxatilis* H *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.

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

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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.

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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.

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

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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.

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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.

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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.

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

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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.

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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.

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

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

Kohler, C.C., R.J. Sheehan, and T.B. Kayes. 1989. Advancing hybrid striped bass culture in the Midwestern United States. 51st Midwest Fish and Wildlife Conference, Springfield, Illinois, December 5-6, 1989.

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the World Aquaculture Society, New Orleans, Louisiana, January 12-18, 1994.

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Kohler, C.C., R.J. Sheehan, A. Suresh, L. Allyn, and J. Rudacliffe. 1996. Effect of hCG dosage on hatching success in white bass. International Congress on the Biology of Fishes, San Francisco, California, July 15-18, 1996.

Kohler, C.C., R.J. Sheehan, J.J. Myers, J.B. Rudacille, M.L. Allyn, and A.V. Suresh. 1998. Performance comparison of geographically distinct strains of white bass to produce sunshine bass. Striper 2000, College Park, Maryland, June 6-7, 1998.

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Rudacille, J.B., and C.C. Kohler. 1997. Relative performance of Phase III sunshine bass, palmetto bass, and white bass in an indoor recirculating system. 35th Annual Meeting of the Illinois Chapter of the American Fisheries Society, Collinsville, Illinois, March 4-6, 1997. (Awarded Lewis L. Osborne Best Student Paper)

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LARGEMOUTH BASS

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Group Meeting, Portland, Oregon, March 22, 2007.

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APPENDIX

SOME COMMONLY USED ABBREVIATIONS AND ACRONYMS

×	cross, by, or times
AIS	aquatic invasive species
anammox	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
BW	body weight
EC	degrees Celsius
CES	Cooperative Extension Service
COD	chemical oxygen demand
CSFPH	Center for Food Security and Public Health
CVM	Center for Veterinary Medicine
EPC	epithelioma papulosum cyprini
EF	degrees Fahrenheit
FSR	final study report
ft, ft^2, ft^3	foot, square foot, cubic foot
FY	fiscal year
g	gram(s)
gal	gallon(s)
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)
LU	Lincoln University
m, m^2, m^3	meter(s), square meter, cubic meter
MAI	motile *Aeromonas* infection
MAS	motile *Aeromonas* septicemia
MDNRE	Michigan Department of Natural Resources and Environment
µg	microgram(s)
mg	milligram(s)
MC	Mill Creek
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
NASAC	National Association of State Aquaculture Coordinators
NCC	National Coordinating Council
NCR	North Central Region
NCRAC	North Central Regional Aquaculture Center
NIFA	National Institute of Food and Agriculture
NOB	nitrite oxidizing bacterial
OCARD	Ohio Center for Aquaculture Research and Development
OSU	Ohio State University
oz	ounce(s)
PAH	Phibro Animal Health
PCR	polymerase chain reaction
PFU	plaque-forming units
POW	Plan of Work
ppm, ppt	parts per million, parts per trillion
Purdue	Purdue University
RAC(s)	Regional Aquaculture Center(s)
RAES	Regional Aquaculture Extension Specialist
RAET	Regional Aquaculture Extension Team
RAS	recirculating aquaculture system
RS	Rimler-Stotts
SPAH	Schering-Plough Animal Health
TC	Technical Committee (TC/E = Technical Committee/Extension; TC/R =Technical Committee/Research
™	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-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