ANNUAL PROGRESS REPORT

For the Period
September 1, 2012 to August 31, 2013

June 2014

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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 104 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 fifteen separately funded projects in regard to Extension and ten 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, 2012 to August 31, 2013. Projects, or Project components, that terminated prior to September 1, 2012 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 located at http://ncrac.org .

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
			$777,858	
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 Ext. 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
			$432,935	
Extension ("Base" Extension—Project Nos. 1-15; Aquaculture Regional Extension Facilitator [AREF]—Project No. 17; and	1	5/1/89-4/30/91	$39,221	88-38500-3885
			$37,089	89-38500-4319
	2	3/17/90-8/31/91	$31,300	89-38500-4319
	3	9/1/91-8/31/93	$94,109	91-38500-5900
	4	9/1/93-8/31/95	$110,129	91-38500-5900

Project Area	Project Number	Proposed Duration Period	Funding Level	Grant Number
Regional Aquaculture Extension Specialist [RAES]—Project Nos. 18-22	5	9/1/95-8/31/97	$10,813	92-38500-6916
			$20,391	95-38500-1410
	6	9/1/97-8/31/99	$38,000	97-38500-3957
	7	9/1/99-8/31/01	$94,000	99-38500-7376
	8	9/1/01-8/31/03	$28,500	99-38500-7376
			$18,154	2001-38500-10369
	9	9/1/03-8/31/05	$28,000	2002-38500-11752
	10	9/1/05-8/31/07	$211,545	2003-38500-12995
			$7,735	2005-38500-15847
	11	9/1/07-8/31/09	$21,850	2006-38500-16900
			$92,469	2007-38500-18469
	12	9/1/08-8/31/10	$37,966	2007-38500-18469
			$22,539	2008-38500-19157
	13	9/1/09-8/31/11	$29,000	2008-38500-19157
	14	9/1/11-8/31/13	$35,700	2010-35800-20929
	17	9/1/03-8/31/05	$100,000	2002-38500-11752
	18	9/1/05-5/31/09	$199,624	2004-38500-14269
	19	9/1/09-8/31/11	$150,000	2008-38500-19157
	20	9/1/11-8/31/13	$196,612	2010-38500-20929
			$1,654,746	
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
			$170,000	
National Coordinator for Aquaculture INADs/NADAs	1	9/1/93-8/31/94	$2,000	89-38500-4319
		5/15/95-5/14/96	$5,000	94-38500-0048
		5/15/96-5/14/97	$6,669	92-38500-6916
			$3,331	95-38500-1410
		5/15/97-5/14/98	$15,000	96-38500-2631
		5/15/98-5/14/99	$13,241	94-38500-0048
		5/15/99-5/14/00	$10,000	95-38500-1410
	2	7/15/04-7/14/05	$9,000	2003-38500-12995
		9/15/05-8/31/06	$15,000	2004-38500-14269
		9/1/06-8/31/08	$40,000	2006-38500-16900
		5/15/08-5/14/09	$25,000	2007-28500-18469
			$144,241	

Project Area	Project Number	Proposed Duration Period	Funding Level	Grant Number
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	$124,400	2008-38500-19157
	5	9/1/12-8/31/13	$75,000	2010-28500-20929
			$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	$225,000	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
			$240,000	
Salmonids	1	6/1/90-8/31/92	$9,000	89-38500-4319
			$120,799	90-38500-5008
	2	9/1/92-8/31/94	$149,997	92-38500-6916
	3	9/1/94-8/31/96	$199,290	94-38500-0048
	4	9/1/97-8/31/99	$158,656	97-38500-3957
			$637,742	
Sunfish	1	6/1/90-8/31/92	$130,758	90-38500-5008
	2	9/1/92-8/31/94	$149,799	92-38500-6916
	3	9/1/94-8/31/96	$173,562	94-38500-0048
	4	9/1/96-9/31/98	$199,921	96-38500-2631
	5	9/1/99-8/31/01	$199,748	99-38500-7376
			$853.,788	
Tilapia	1	9/1/96-8/31/98	$118,791	96-38500-2631
	2	9/1/98-8/31/00	$150,000	98-38500-5863
			$268,791	
Viral Hemorrhagic Septicemia (VHS)	1	9/1/08-8/31/10	$197,960	2008-38500-19157
Walleye	1	5/1/89-8/31/91	$177,517	89-38500-4319
	2	6/1/90-8/31/92	$111,657	90-38500-5008
	3	9/1/91-8/31/92	$109,223	91-38500-5900
	4	9/1/92-8/31/93	$75,000	89-38500-4319
	5	9/1/93-8/31/95	$150,000	93-38500-8392
	6	9/1/95-8/31/97	$117,395	94-38500-0048
			$59,835	95-38500-1410
	7	9/1/99-6/30/02	$127,000	98-38500-5863
			$927,627	
Wastes/Effluents	1	9/1/92-8/31/94	$153,300	92-38500-6916
	2	9/1/96-8/31/98	$100,000	96-38500-2631
	3	9/1/01-8/31/04	$106,186	00-38500-8984
			$88,814	2001-38500-10369
			$448,300	

Project Area	Project Number	Proposed Duration Period	Funding Level	Grant Number
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
			$85,7231	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	
TOTAL			$11,810,175	

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, 2013

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.
(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.

[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.

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

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.

UMESC is continuing to work with Rangen, Inc., Maxxam Analytics, and CVM to develop a course of action to respond to the CVM information needs. UMESC, in coordination with Rangen, Inc. and Maxxam Analytics, completed a report addressing the comments from the CVM-issued letter has been prepared. UMESC, Rangen, Inc., and Maxxam Analytics will finalize the report and method standard operating procedure following a conference call with CVM. The report and revised procedure will be submitted to CVM along with a 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.

Outreach Overview
There is no specific outreach component to this study because it entails the study of an unapproved drug.

Targeted Audiences
The targeted audience is U.S. tilapia producers. Tilapia is the fifth most consumed seafood in the U.S.

Outputs
None to date.

Outcomes/Impacts
The results from this project will directly affect the potential for approval of MT by 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.

Impacts Summary
Relevance: 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.

Responses: Approval of MT is contingent on providing data that will fulfill CVM 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 trial has been completed. The development of responses to CVM criticisms is ongoing.

Results: 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.

Recap: Once the data from the method transfer trial are accepted by CVM, the approval of MT-medicated feed for use by U.S. tilapia producers should be imminent.

Students/Participants
None.

Partnerships

Partner	Type	Level	Nature of Partnership
Doug Ramsey			Drug sponsor
David Brock			Drug Sponsor
Philip Gobbi			Contract laboratory liaison
Nilmini Wijewickreme			Contract laboratory co-investigator
Jeff Meinertz			Final report co-author

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, 2013

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.
(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.

[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.

(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- Complete

OBJECTIVE 2 - Complete

OBJECTIVE 3 - Complete

OBJECTIVE 4 - Complete

OBJECTIVE 5

Pivotal effectiveness field studies were conducted at Spirit Lake Fish Hatchery, Spirit Lake IA. In 2011, a study following the approved protocol was completed with juvenile muskellunge. The protocol was again followed in 2012 with juvenile walleye at the same facility.

OBJECTIVE 6

Study reports are complete pending the generation of specific data on the aeromonas isolates collected during the 2011 and 2012 trials. Motile aeromonad isolates will have two gene targets amplified then sequenced to determine the specific species responsible for the infection and mortality. Once those sequences are compared to known species sequences, the information will be added to the study reports and submitted to CVM. The collection of these data was delayed because of personnel transitions at UMESC.

OBJECTIVE 7
Depends on completion of #6.

OBJECTIVE 8
Depends on completion of #7.

OBJECTIVE 9
Depends on completion of #8.

OBJECTIVE 10
Depends on completion of #9.

Outreach Overview
There is no specific outreach component to this study because it entails the study of an unapproved drug.

Targeted Audiences
The effectiveness studies of this project should lead to supplemental NADA approvals by CVM for either, or both, oxytetracyline dihydrate or florfenicol. This in turn would allow the use of an approved drug to control mortality associated with MAS in cool- and warm-water finfish.

Outputs
Other than the final reports, outputs may support supplemental NADA approvals for these two drugs.

Outcomes/Impacts
The control of mesophilic or motile *Aeromonas* infections is extremely relevant to the aquaculture industry as it has experienced income losses in food, sport and bait fish facilities due to MAS. The results from this project will directly affect the potential for approval of oxytetracycline dihydrate and/or florfenicol by CVM. The data from this study, if accepted by CVM, will support the potential approval of oxytetracycline dihydrate and/or florfenicol-medicated feed for use in cool- and warm-water fish to control mortality due to MAS.

Impacts Summary
Relevance: Supports supplemental NADA approvals of drugs needed in aquaculture to reduce mortalities due to MAS.

Responses: Pivotal studies were completed that showed effectiveness of oxytetracycline dihydrate and/or florfenicol to control mortality due to MAS in cool- and warm-water fish.

Results: Not completed at this time

Recap: Results from these two field studies demonstrated effectiveness of Terramycin 200 for Fish® and Aquaflor® to control mortality caused by *Aeromonas* spp in cool and warm water 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

Probiotics in Yellow Perch and Tilapia Culture

Project ***Progress Report*** for the Period
September 1, 2012 to August 31, 2013

NCRAC FUNDING: $240,000 (September 1, 2012 to August 31, 2014)

PARTICIPANTS:

Konrad Dabrowski	The Ohio State University	Ohio
Zhongtang Yu	The Ohio State University	Ohio
Timothy Johnson	University of Minnesota	Minnesota
Nicholas Phelps	University of Minnesota	Minnesota

Industry Advisory Council Liaison:

William Lynch	Millcreek Perch Farm	Ohio

PROJECT OBJECTIVES

(1) Characterize the microbial community of early ontogeny of yellow perch and tilapia during growout phase in control (laboratory) setting and compare to practical industry conditions (minimum of 2 farms for each species).

(2) Isolate bacteria that possess the characteristics resulting in inhibition of pathogenic *Vibrio* and *Aeromonas* species.

(3) Compare commercial probiotics to those isolates identified in Objective 2.

(4) Establish culture of axenic fish model to evaluate probiotics and inoculants which possess disease inhibition.

Anticipated Benefits

The proposed studies include comprehensive characterization of the microbiota of the yellow perch digestive tract and surrounding water in production facilities of the North Central Region (NCR). These results will be used to identify cultures of probiotic bacteria that are inhibitory to yellow perch pathogens.

It is expected that probiotic strains that can protect yellow perch juveniles from infection by at least two common pathogens, Aeromonas and Vibrio species without negative effects on the host fish, will be identified. Therefore, the probiotics identified in this study can potentially contribute to sustainable development of the aquaculture industry and securing an organic produce status for fish.

Project Progress

Objective 1.

The first experiment was conducted to compare the microbial communities of adult yellow perch raised outdoor in ponds in a commercial setting and indoor in laboratory conditions. Fecal matter

was sampled from six or seven fish from two ponds located in Marysville, Ohio (Bill Lynch, Northern Facility). Feces from 3 or 4 fish were combined into one vial and then the vial was filled with buffer. Anterior and posterior intestinal contents were taken by making an incision into the body cavity and cutting out the intestinal tract. The first half of the intestinal tract was considered the anterior and the second half the posterior. These sections were removed from the fish and all contents were pushed out of the intestine into the vial. These vials were filled with anaerobic buffer and then sealed. All vials were stored on ice and then transported back to the lab and stored at 4 C. The same procedure was then applied to sampling fecal material from Columbus laboratory fish. Fish (114-165 g; n=20) in Marysville and Columbus were fed the same diet (Aquamax Grower 550; PMI Nutrition International, Brentwood, MO).

In this experiment we found that the tank samples had a consistently high bacterial load throughout the intestinal tract while samples from pond fish showed a general increase in bacterial abundance from the anterior to the posterior end of the intestinal tract. This is likely a result of the timing of the last feeding prior to sampling as the yellow perch intestine is relatively short. Their bacterial load in tank raised fish throughout their intestines, including their anterior section was consistently high (up to 108 colonies forming units (CFU)/ml) and there was little variation between the two intetinal sample sites collected.

Alternatively, pond raised fish had a high variability between the two sites of intestine within the same pond. Additionally, they had a high fecal bacteria load, while their anterior gut section was relatively low (101- 103 CFU/ml).

The CFU/g of the MRS agar plates exhibited a similar trend as the tryptic soy agar (TSA) plates. Though MRS agar plates are a selective media often used to select for Lactobacillus, there were some higher counts on these plates compared to the non-selective TSA plates (up to 102 fold) particularly for the tank samples. This may be due to a particularly abundant species that prefer the growth conditions of the MRS plates.
The second experiment was conducted in two phases in order to evaluate how the microbial community of yellow perch changes throughout ontogeny. The 1st phase was carried out in a recirculating system with nine 50-L conical tanks, initially stocked at ~3000 larvae/tank. Live rotifers (Brachionus plicatilis) were provided at a density of 10 rotifers/mL for the first two days of feeding, then fish were transitioned to Artemia (4 nauplii/mL) for the remaining 8 days of the experimental phase.

Temperature was maintained at 23±1˚C and marine microalgae and evaporated salt were added to the system to maintain a turbidity of 12±4 NTU and a salinity of 3-4 ppt. The 2nd phase was carried out in 9 60-L cylindrical flow-through tanks, initially stocked with 500 perch/tank. During this phase, fish were fed Artemia for 3 days then gradually transitioned to a formulated starter diet (Otohime A1) over an 11 day period. Several sets of samples (larval and juvenile) were taken throughout both phases: after hatching, after feeding rotifers/artemia, and after receiving commercial diet. In addition, samples of live food and commercial diet were taken. All

the samples are currently stored at -80 C and will by analyzed for characterization of microbial communities.

The third experiment was conducted in order to determine if different sources of dietary protein affect the intestinal microbial community of juvenile yellow perch. This study was carried out in a 4,000-L system that consisted of 18, 50-L conical fiberglass tanks with partial water recirculation. A total of 270 juvenile perch (12.6 ± 0.3 g, initial mean weight) was randomly distributed into tanks in triplicate groups per treatment. The system consisted of an overhead tank with a temperature controlling device (22.4 ± 0.3 oC) and a biofilter. Fish biomass was measured every 10 days in order to monitor temporary fish growth and food utilization. Four experimental diets were formulated based on: 1) fishmeal (FM) as the major protein source (Control), 2) wheat gluten meal replacing 75% of the FM protein (WG), 3) the new improved variety of soybean meal protein replacing FM (Soy A), and 4) conventional soybean meal replacing FM (Soy B). Diets 1 and 3 were fed three times per day ad libitum and, for comparison purposes, all the experimental diets were provided in a pair-feeding ration adjusted to the level of the least consumed diet. Fish growth, survival, and feed conversion ratio (FCR) were monitored during the 10-week feeding trial.

The survival of fish from all the treatment groups was close to 99%. At the end of this experiment the fecal samples were taken from three fish per tank from all the groups and stored at -80 C for futher microbial analysis.

Objective 2.
Fish sampled from the tank exhibited a high abundance of Lactococcus raffinolactis which has been studied previously as a significant probiotic isolated in other fish species (Hagi 2009. Biosci.Biotechn. 73:1479). Alternatively, set 1 of fish isolated from pond 2 carried a high abundance of Lactococcus garvieae, which is considered a fish pathogen as well as a large number of Clostridium. This does not indicate the fish were diseased but indicates a potential for disease, if stressful conditions arise.

Another possibility is the presence of one diseased fish within the sampling set.
Overall, diversity was very low in terms of number of Operational Taxonomic Unit (OTUs) within and between samples. This low diversity is likely a result of sampling and/or culturing technique, as similar studies of fish intestinal bacteria show much higher diversity in the intestinal tract. There did not seem to be strong variation between samples from the ponds as both sets from each pond vary considerably. Samples from the tank were very similar between sets as well as within the intestinal tract which was expected given the standardized living conditions, limited outside contamination (filtered city water) and single feed type. Samples from pond fish varied greatly both between ponds and between sampling sites which gives us an idea of natural variation in fish farm conditions.

Objectives 3 and 4 will be addressed in the second year of this project.

Outreach Overview
This activity will be the focus of the project in the second year.

Target Audiences
Fish culture operations in the North Central Region (NCR) have all experienced disease outbreaks on occasion, resulting in significant monetary loss. Good husbandry practices can significantly reduce but not eliminate such outbreaks. Given that most aquaculture in the NCR occurs in ponds, administering chemotherapeutic drugs is not economically feasible because the large amount of water in individual ponds precludes treating the water and individual fish from many NCR species often cease or reduce feeding once infected by a pathogen. The industry has long recognized that feeding a nutrient complete diet is a good husbandry practice and that inclusion of probiotics that increase resistance to common pathogens would enhance the effectiveness of such a diet. A cost-effective reduction in fish losses will increase the economic viability of all culture operations within NCR.

Outputs
Presentations at professional meetings.
Aquaculture America WAS symposium, February 2014, Seattle, WA:
1. Improving yellow perch larvae culture by utilizing live food enrichment with PUFA.
2. Can replacement of 75% fish meal protein with soybean mealsupport optimal growth of yellow perch?

Outcomes/Impacts

Impacts Summary
Relevance: The use of chemotherapeutic drugs which may have severe negative impacts on fish, and lead to development of drug-resistant pathogens requires development of probiotics which may eliminate the use of drugs, thus contributing to sustainable development of the aquaculture industry.
Response: In order to better understand the mechanisms by which alteration of microbial communities affect host-fish growth and diet utilization, experiments focused on defining the microbial communities involved in fish health were conducted.
Results: We identified microbial communities in yellow perch obtained from different culture conditions which can be further used to isolate bacteria possesing characteristics of inhibition of pathogenic Vibrio and Aeromonas strains.
Reecap: A microbial community was characterized for yellow perch in control (laboratory) setting and compared with practical industrial fish farm pond population.

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, 2013

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 CO2 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.

(5) 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).

(6) 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.

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

(8) 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.

(9) 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.
(10) 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 alternatives to Rotenone for aquaculture operations where nondesirable fish species need to be controlled. 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.

Project Progress

Objective #1: (Complete)

A manuscript is in the advance stage of development. The first draft was prepared as introduction section to the dissertation by T. Parker which was defended in August 2013. The manuscript focuses on toxicity of ammonia nitrogen compounds at different oxygen levels in common carp and discusses, among other aspects, the changes to the gill tissues associated with the ammonia/hypoxia exposure. Data obtained for common carp is being finalized into a separate manuscript prepared for submission to “Aquatic Toxicology”. Toxicity of ammonia to carp, percids, salmonids, and catfish will be compiled and presented with a literature review to make a white paper to be reported to NCRAC.

The literature review for the use of CO_2 as a control agent for undesired fish species is currently under review by U.S. Geological Survey and U.S. Fish and Wildlife Service partners for

submission for publication to Fisheries. This literature review incorporates findings from preliminary experimental work with multiple fish species and gives a comprehensive description of CO_2, its use and efficacy.

Objective #2: (Complete)
The cost per acre foot for pond treatment was calculated the amount of CO_2 to produce mortality observed in pond trials (Objective 5). The cost of CO_2 gas was $0.34/kg ($0.16/lb) while liquid CO_2 was $0.23/kg ($0.11/lb). Therefore, the costs to treat an acre foot of pond with CO_2 gas was $361.35 and $244.16 if treated with liquid CO_2. The actual cost for fish producers will be dependent upon local distributors.

The cost per acre foot for pond treatment was calculated for the amount of ammonia chloride to produce mortality observed in pond trials (Objective 5). The cost of ammonia chloride was $1.36/kg ($0.62/lb). The cost to treat an acre foot of pond with this ammonia chloride was $105.95; however, 100% mortality was not achieved. A 2.5 times of the dose used in our trials (i.e. adding 62.5 kg [28.41 lb]rather than 25 kg [11.37 lb]) would be expected to produce 100% mortality. This would increase the costs to $264.88 for treating an acre foot of pond with ammonia chloride to produce 100% mortality. However, the actual cost will be dependent upon local distributors.

For comparison, the cost per acre foot for pond treatment was calculated for the amount of rotenone to produce 100% mortality. This piscicide would cost approximately $140.00 to treat an acre foot of pond. However, the cost of removal and disposal of rotenone-exposed fish are not included. These additional costs could substantially increase the total costs for use of this chemical and would be minimized in using CO_2 or ammonia chloride. Thus, both CO_2 and ammonia chloride may be viable alternatives to the use of rotenone for the removal of fish remaining in ponds during late winter.

Objective #3: (Complete)

Objective #4: (Complete)

CO_2 Trials
All laboratory trials were conducted at the Bozeman Fish Technology Center (BFTC; U.S. Fish and Wildlife Service), Bozeman, Montana, USA. Fish used in the trials were either wild caught or brought in from hatchery facilities. Upon arrival at the BFTC, all individuals were acclimated in holding tanks to the temperature range used in the trials (i.e., 10 - 11°C [50 - 52 °F). This temperature range represents the minimum achievable temperature at BFTC facilities, though it does not fully mimic minimum water temperatures frequently observed under winter kill conditions in natural rearing ponds (i.e., 2 - 4°C [36 - 39 °F).

Carbon dioxide was injected and mixed with BFTC water. The CO_2 enriched water was then added to experimental tanks at a rate of 7.6 L/min (28.8 gal/min) and adjusted to achieve desired

treatment levels. At this time, experimental tanks were then filled with BFTC water and fish were added. After a 30-min acclimation period, the preset CO_2 levels were then restored to the tanks.

Individuals of each species were exposed to either five (rainbow trout (RBT): control, 75, 150, 180, 225, ppm) or seven channel catfish (CCF), common carp (CAP): control, 75, 150, 225, 300, 380, 495 ppm) concentrations of CO_2 for a period of 24 h. We documented pre-treatment and post-treatment dissolved oxygen concentrations (DO; ppm), temperatures (T; °C [°F]), pH, and CO_2 concentrations (ppm). At the end of the 24-h exposure, mortality was documented in each tank, and any remaining live individuals were euthanized with an overdose of tricaine methanesulfonate (MS-222). Immediately following, lengths (mm) and weights (g) were recorded for each individual included in our trials.

Using the empirically derived lethal concentration for 100% kill (LC100) for RBT determined in the dose response trials, we exposed RBT to this concentration at four different rates of reaching the LC100. Treatment levels for these trials were as follows: control, 10 min, 30 min (positive control), and 98 min. Once at concentration, RBT were exposed to the LC100 for 24 h. Consistent with the dose response trials, we documented pre- and post-treatment water quality parameters and recorded post-treatment lengths and weights.

We determined an LC100 for each species and calculated average mortality (+ 95% CI) for each species at each treatment level. We used this information to generate treatment-mortality curves for each species in SigmaPlot (Version 10.0.1). We then determined average weight (± SE) and length (± SE) for each species and averages of the water quality parameters (± SE) collected during the trials.

We calculated average RBT mortality (+ 95% CI) at each treatment level, average weight (± SE) and length (± SE), and averages of the water quality parameters (± SE) collected during the trials. Similar to the dose response trials, we also used ANOVA to test for differences in water quality parameters between pre- and post-treatment readings and between CO2 treatments within each trial to confirm that water quality did not affect our results.

We also determined that there were no tank effects on mortality in any trial (all P-values > 0.05). Additionally, for each species in the dose response trials, fish weight and length were greater in the second trial than in the first, but, within a trial, these metrics did not differ significantly between tanks at any treatment level.

Rainbow trout had a 24-h LC100 of 225 ppm CO_2, while the 24-h LC100 for both CAP and CCF was 495 ppm CO_2. Mortality was not observed in RBT below the 150 ppm CO_2treatment level, whereas mortality was not observed in CAP and CCF below the 225 ppm CO_2treatment level. The average weight ± SE of fish used in the trials was 4.97 ± 0.03 g (0.175 ± 0.001 oz), 3.51 ± 0.02 g (0.124 ± 0.001 oz), and 6.89 ± 0.02 g (0.243 ± 0.001 oz) for RBT, CAP, and CCF,

respectively. The average length ± SE was 7.521 ± 0.011 cm (2.96 ± 0.004 in), 6.975 ± 0.02 cm (2.75 ± 0.008 in), and 9.661+ 0.013 cm (3.80 ± 0.005 in) for RBT, CAP, and CCF, respectively.

Both treatment and species were significant factors. Additionally, we documented a significant interaction between treatment and species. The interaction was a result of differences in mortality between species at specific treatment levels. In the CAP and CCF trials, mortality in both the 380 ppm CO_2 and 495 ppm CO_2treatment groups was significantly higher than in the control, 75, 150, 225, and 300 ppm CO_2 groups, but mortality in the 495 ppm CO_2treatment was higher than in the 380 ppm CO_2 treatment. There was no difference in mortality between the 225 ppm CO_2 and 300 ppm CO_2 treatment groups in the CCF trials. There were no differences in mortality in any treatment groups between CAP and CCF. In the RBT trials, mortality was significantly different between all treatment groups except between the control and 75 ppm CO_2 treatments and between the 180 ppm CO_2 and 225 ppm CO_2 treatment groups. Rainbow trout experienced higher mortality in the 150 ppm CO_2 treatment and 225 ppm CO_2 treatment group.

Water quality differed between pre-and post-treatment measurements and between treatment levels in specific instances. DO was slightly lower across treatment levels in post-treatment measurements than in pre-treatment measurements in the CCF trials. For all three species, pH was greater in pre-treatment readings than in post-treatment readings, except in the control group , and, when comparing treatment levels, post-treatment pH was significantly lower in treatments with higher levels of CO_2 and varied by 1.3 pH units between control and 495 ppm CO_2 treatments.

Regardless of the amount of time spent bringing a tank to the desired CO_2 concentration, RBT fingerlings experienced 100% mortality after 24 h of exposure to 225 ppm CO_2 (i.e., LC100 from dose response trials). The average weight of fish used in the trials was 6.63 ± 0.02 g (0.212 ± 0.0007 oz), and the average length was 8.24 ± 0.013 cm (3.24 ± 0.005 in). pH was greater in pre-treatment readings than in post-treatment readings, except in the control group.

Inorganic N Trials

Four experiments were conducted from November 2012 to April 2013 at temperatures between 10 - 15°C (50 - 59 ^{o}F). Trials were completed with yellow perch (a surrogate species for walleye) and rainbow trout. Yellow perch were exposed to two concentrations of ammonia (NH_3-N) which were 0.37 ± 0.01 and 0.23 ± 0.03 ppm NH_3-N. Fish in each experiment were exposed to one of the three levels of oxygen (normoxia 7.93 ± 0.45 and 8.90 ± 0.26 ppm O_2, moderate-hypoxia 3.19 ± 0.42 and 2.62 ± 0.13 ppm O_2, and severe hypoxia 1.97 ± 0.13 and 1.72 ± 0.11 ppm O_2). The oxygen levels were slowly lowered over a period of 24h and fish were given 3 d to acclimate to the hypoxic conditions before 24h ammonium exposure. The same methods were used for rainbow trout except ammonia concentrations were 0.15 ± 0.02 and 0.26 ± 0.02 ppm NH_3-N. Oxygen concretions were as follows: normoxia 9.45 ± 1.27 and 9.88 ± 0.85 ppm O_2, moderate-hypoxia 4.08 ± 0.23 and 5.01 ± 0.25 ppm O_2, and severe hypoxia 2.13 ± 0.09 and 2.98 ± 0.11 ppm O_2 for the treatments.

There was significant interaction between ammonia concentration and dissolved oxygen is significant in yellow perch which indicates that ammonia toxicity is increased by reduced oxygen content in the water for yellow perch. Yellow perch mortality for fish exposed to 0.37 ± 0.01 ppm NH_3-N was 6.25 ± 6.25%, 93.75 ± 6.25%, and 100.0 ± 0.0% in normoxia, moderate-hypoxia, and severe hypoxia, respectively. When exposed to 0.23 ± 0.03 ppm NH_3-N mortality for the three oxygen treatments, in the same order, were 2.50 ± 2.50%, 22.5 ± 13.1%, and 72.5 ± 24.3%. All rainbow trout survived during exposure to 0.15 ± 0.02 ppm NH_3-N. However, during exposure to 0.26 ± 0.02 ppm NH_3-N, rainbow trout experience significantly higher mortality in severe hypoxia. Rainbow trout experienced 6.25 ± 8.84%, 7.81 ± 7.86%, and 48.4 ± 25.7% in normoxia, moderate-hypoxia, and severe hypoxia, respectively.

These data established target concentrations of ammonia that would result in nearly total mortality of the examined fish species within 24h of exposure and provides practical guidelines for utilization of this substance to control undesired fish species in nursery ponds.

Objective #5. (Complete)
A controlled outdoor pond study to assess the use of CO_2 and ammonia chloride for enhancement of winter kill conditions was performed at the U.S. Geological Survey Upper Midwest Environmental Sciences in La Crosse, Wisconsin. In early October of 2012, eight fish-free 0.04-ha (0.1-acre) outdoor rectangular ponds were filled with well water to a mean depth (± SD) of 96 ± 4 cm (37.8 ± 1.57 in). On October 11, 2012, each pond was stocked with 100 common carp (5.0 - 7.5 cm [2.0 - 3.0 in]) and 100 channel catfish (7.5 - 20.3 cm [3.0 - 8.0 in]). Ponds were left to freeze under natural conditions.

On February 19, 2013, a 12.7 cm (5 in) diameter hole was drilled through the ice in the center of each pond. Temperature and dissolved oxygen were measured at the surface and bottom of each pond and pH was measured at the bottom of each pond. Carbon dioxide was measured with a commercially available titrant kit.

On March 7, 2013, one 12.7 cm (5 in) hole was drilled in the northeast corner and a similar size second hole in the northwest corner of each pond. One hole was used to pump water (approximately 0.264 L/min [30 gal/min]) from the bottom of the pond, treated with the chemical and then returned through the second hole just below the ice. Each of three randomly selected ponds was injected with ammonia chloride (Prince Agri Products, Inc., Quincy, IL). At the side of the pond, 55.12 lbs (25 kg) ammonia chloride was dissolved in approximately 208 L (55 gal) of pond water and injected into each of the at approximately 113 L/m flow (29.85 gal/min). This amount of ammonia chloride was used to achieve approximately 85 ppm ammonia, which is more than double the 31.7 ppm required to reach 0.85 ppm NH_3-N at a pH of approximately 9.0 at 4°C (39.2°F; Piper, 2010).

Between March 8 and March 10, 2013, three other randomly selected ponds were injected with 48.4 ± 2.9 kg (106.7 ± 6.39 lbs) of CO_2 delivered as a gas at a rate of 25 L (6.60 gal) at approximately 113 L/min flow (29.85 gal/min). This amount of amount of $CO_2$was used to

achieve a nominal concentration of 150 ppm CO_2, which was expected to overcome alkalinity of the pond water and result in toxic levels of free CO_2, greater than 25 ppm.

On March 11, 2013, a 12.7 cm (0.12 ft) diameter holes were drilled through the ice in the center of the pond, one on the north end and one on the south end of each pond. Temperature and dissolved oxygen were measured at the surface and bottom of each pond and pH was measured for each pond. Dissolved CO_2 was measured with a commercially available titrant kit (CHEMetrics, Inc., Midland, VA). Ponds were then left to thaw under ambient conditions. On April 11, 2013, all ponds were free of ice and immediately drained. All live fish were harvested from each pond and counted.

All water quality parameters (i.e. pH, temperature, dissolved oxygen, ammonia and CO_2) and the number of surviving carp and catfish are reported as means with standard deviations. Water quality parameters were compared between treatments pre- and post-treatment using ANOVA. Initial, pre-treatment, water qualities were similar among ponds. Water temperatures were 3.11 ± 0.67°C (37.60 ± 1.38°F). All ponds were slightly basic, 9.14 ± 0.34. Ponds treated with CO_2 had the greatest pH, 9.57 ± 0.19 and differed from that in ponds treated with ammonia (8.83 ± 0.08) or left untreated (8.96 ± 0.08). Dissolved oxygen was found to be super saturated in all ponds, 219.5 ± 35.9%. No dissolved CO_2 or ammonia was detected in any of the ponds prior to treatment.

Differences were observed in water quality parameters post-treatment. Water temperatures were lower in all ponds when measured following treatments on March 11, 2013 then when measured during pre-treatment. Water temperatures were lowest in ponds treated with CO_2 (0.47 ± 0.12°C [32.85 ± 0.21°F]), but similar between control ponds and ponds treated with ammonia 1.58 ± 0.21°C (34.84 ± 0.38°F) and 1.43 ± 0.23°C (34.57 ± 0.42°F) respectively. pH did not change in either the control ponds or ponds treated with ammonia. The pH in ponds treated with CO_2 dropped from 9.57 ± 0.19 to 6.13 ± 0.11, thus the CO_2-treated ponds had lower pH than either the control ponds or ponds treated with ammonia. DO did not change in control ponds or ponds treated with CO_2, but increased to 32.94 ± 3.13 ppm in ponds treated with ammonia. No differences in DO were found between any of the treatments on March 11, 2013. Total ammonia was greatest in ponds treated with ammonia chloride, 12.60 ± 11.18 ppm, but was only a fraction of the 31.7 ppm needed to produce 100% mortality. Total ammonia levels in ponds treated with CO_2 or untreated had very low levels of total ammonia, 0.22 ± 0.17 ppm and 0.13 ± 0.12 ppm respectively. Dissolved CO_2 was detected in only those ponds treated with CO_2.

The number of fish surviving varied among treatments. No live common carp were found in one of the control ponds, while 90 channel catfish were alive in that same pond. Thirty-five common carp and 9 channel catfish were alive in the other control pond. Survival ranged from 0 to 35 for common carp and from 1 to 36 for channel catfish in ponds treated with ammonia. Only a single common carp and no channel catfish survived in all ponds treated with CO_2. Ponds treated with CO_2 did have greater total fish mortality than the control ponds. Ammonia chloride had no effect on mortality at levels used in our study.

Objective #6: *Collect late winter water chemistry condition data in representative NCR natural rearing ponds.* (Complete)

A yellow perch aquaculture farm in Marysville, Ohio, with eight ponds was identified for the site to monitor late winter water quality. Water chemistry was monitored with an YSI Professional Plus with probes attached to monitor temperature, dissolved oxygen, and ammonia. Water conditions were monitored on November 20, 2012 for pre-ice water quality in the deepest portion of the pond. Ponds were began to freeze over on December 22, 2012 and covered with ice and snow on December 26, 2012. Water quality was then monitored on January 7, 2013 after ice was safe to walk on (12 cm [4.7 in] thick). Measurements for the water quality taken on November 20, 2012 at 1.5 meters (4.9 ft) were: temperature 7.0 ± 0.2°C (44.6 ± 0.36°F); dissolved oxygen 11.8 ± 0.2 ppm; pH 7.9 ± 0.1; NH4-N 0.12 ± 0.02 ppm. Water quality measurements taken on January 7, 2013 at 1.5 m (4.92 ft) were: temperature 4.2 ± 0.1°C (39.56 ± 0.18°F); dissolved oxygen 7.6 ± 0.2 ppm; pH 7.9 ± 0.1; NH_4-N 0.65 ± 0.07 ppm. Air temperatures increased after the measurements taken on January 7, 2013 causing the ice to melt. Long term ice cover was never present in central Ohio and therefore winterkill conditions were not observed.

Objective #7: (In Progress)

UMESC held an initial coordination meeting with EPA to discuss whether the use of CO_2 will require EUP to allow its use to enhance winterkill conditions. Depending on the size and scope of the application, it is likely that EUP would be required prior to registration. As part of its efforts to assess the use of CO_2 in barriers to deny aquatic invasive species access to critical habitat, UMESC will be evaluating the required registration information required to register CO_2 for use to control aquatic nuisance species.

Objective #8: (In Progress)

UMESC is coordinating with EPA to determine the information required to register CO_2 for uses to control aquatic nuisance species. UMESC and its collaborators on this project will compile data reports of project generated information to meet those registration requirements, where appropriate.

Objective #9: (In Progress)

Extension materials will be prepared pending coordination with EPA to determine registration requirements. Extension materials cannot be prepared and disseminated until a determination is made regarding the registration requirements of CO_2 or inorganic nitrogen compounds.

Outreach Overview
There is no specific outreach component at present because the compounds under investigation are not registered for the intended use.

Targeted Audiences
The targeted audience is public and private fish producers that use natural rearing ponds.

Outputs
None to date.

Outcomes/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)

Impacts Summary
Relevance: The approval of CO_2 and/or ammonia for the removal of unwanted fish in natural rearing ponds will be a significant benefit to the public and private aquaculture producers. Removal of these fish will enhance production in these ponds.

Responses: Approval of CO_2 and/or ammonia as a chemical for the control of unwanted fish species is contingent on providing evidence on the effectiveness of these chemicals to produce mortality in targeted fishes. Thus, an initial study required includes the establishment of efficacy of both CO_2 and ammonia. Trials have been completed.

Results: Both CO_2 and ammonia produced mortality in laboratory and field trials. Cost of both of these chemicals is greater than that required to treat ponds with the registered piscicide rotenone. However, the use of CO_2 or ammonia as a fish toxicant will not produce dead fish that will require special disposal like those killed with rotenone. The removal of these unwanted fish is critical to improvement of efficiency of public and private fish producers.

Recap: CO_2 infusion under ice was effective in eliminating channel catfish and common carp during under ice exposures.

Publications

Treanor, H.B., Ray, A.M., Amberg, J.J., Gaikowski, M.P., Ilgen, J.E., Gresswell, R.E. and Webb, M.A.H. Submitted. Assessing carbon dioxide as a tool for supplementing winter kill in natural rearing ponds. Aquaculture.

Students/Participants

None.

Partnerships

Partner	Type	Level	Nature of Partnership
Jeffrey L. Gunderson			Industry Council
Gregory Oswald			Industry Council
Barnaby Watten			Industry Council

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

[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.

PROGESS AND PRINCIPAL ACCOMPLISHMENTS
OBJECTIVE 1

Ohio
The 3rd generation of selected lines was created via marker-assisted cohort selection. More than 100 egg ribbons were produced from the improved line at the Piketon station. Twenty of them were delivered to Mill Creek (MC) Perch Farms in Ohio on March 16, 2011. At the same time, 30 and 18 families from the Ohio local strain were produced by mass spawning at MC Perch Farm and the Piketon Station, respectively. At both locations, nursery was done in 0.2- acre ponds and feeding-training was conducted in round tanks. For year 1 rearing, 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 and a local-strain were reared in separate ponds, each having two replicates, with a density of 69,300 fish/ha (28,000/acre); 2) at MC Perch Farm, the selected line and a local-strain were raised communally in two 0.2-acre ponds at a density of 232,900 fish/ha (94,300/acre). All of the ponds were harvested at the end of October and in early November, 2011. 150 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 fincliped. 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 perch grew significantly larger than local perch native to the farm in two communal ponds. The improved line outweighed the local strain by 32.00% on average at the end of the Year 1 test. Fingerling survival in the MC Perch Farm's communal ponds with improved fish was as high as they have ever experienced. In the Piketon 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) than the local Ohio strain (37.62 g).

Wisconsin
Approximately 207,000 OSU eggs were delivered to NADF of the UW-Stevens Point and 10 OSU egg strand to Coolwater Farms, LLC (CF) in Wisconsin in late March and early April 2012. At the same time, The NADF received approximately 160,000 local strain eggs from18 strands of eggs produced by mass spawning from CF Perch Farm. At the NADF, the two OSU improved fry ponds had 7.5% and 0% fry to fingerling survival due to poor transportation. The one local WI strain pond at the NADF had 20% fry to fingerling survival resulting in 28,000 fingerlings. Feed training rates for OSU improved and WI local fingerlings at the NADF was 96% and 82%, respectively. An additional 15,000 feed-trained fingerlings (average 2.0 g [0.07 oz]) were hauled from OSU Piketon Research Facility to the NADF on June 26, 2012. These fish arrived in very good condition with <1% mortality. 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. Since only 5 OSU fry were recovered for feed-training the experiment was terminated at CF Perch Farm. At the NADF, all ponds were harvested in early October

2012. Two hundred fish from each pond were sampled, with OSU perch from two ponds averaging 125.1mm (4.9 in) and 25.6 g (0.9 oz) and 120.3 mm (4.7 in) and 22.5 g (0.8 oz), respectively. WI strain perch from two ponds averaged 118.6 mm (4.7 in) and 20.1 g (0.7 oz), and 111.8 mm (4.4 in) and 17.9 g (0.6 oz), respectively. 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.

OBJECTIVE 2

Ohio

In October 2011, all the harvested fish in Year-1 ponds were re-stocked into Year-2 ponds in MC Perch Farms and Piketon Station in Ohio. 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 and a local-strain were re-stocked and reared in separate 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 Year-1 harvesting, commercial grading practice was performed. Each pond was size-graded and the largest fish with mean size of 40.2g and 40.9g were re-stocked into two 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). All of the ponds were harvested in October, 2012. Two hundred fish from each of the separate ponds at Piketon, and 250 fish from each of the communal ponds at MC Perch Farm were sampled, individually weighed and fincliped. Eight molecular markers were used to assign selected and local-strain perch to their family of origin for communal rearing. Fifty large fish from each of the ponds at both sites were sampled for dress-out percentage analysis. For MC Perch Farms, out of 240 fish that were family-origin identified from each of the two ponds, OSU improved fish accounted for 71.25% and 51.25% with an average of 61.25%, suggesting improved fish and MC perch had survival rates of 61.25% and 38.75%, respectively. OSU improved perch from two ponds averaged 218.1 mm and 142.7 g, and 212.0 mm and 129.9 g, respectively. MC local Ohio strain from two ponds averaged 204.0 mm and 105.6g, and 193.9 mm and 92.9 g, respectively. The improved line outweighed the local strain by 35.16% and 39.90%, respectively, with an average of 37.53%, at the end of the Year 2 test in two communal ponds. 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, the improved line still grew 25.50% faster than the unimproved fish. There was no significant difference in dress-out percentage between improved line and local Ohio strain.

Wisconsin

In early October, 2012, 3,202 and 3,301 OSU improved strain and 3,198 and 3,209 WI local strain yellow perch were graded and stocked back into four production ponds at the NADF. Fish were fed based on proposal protocol. All four ponds were harvested on September 9 –12, 2013.

A total of 29 and 40 OSU improved perch were harvested from two ponds and 40 and 21 WI local strain were harvested from two ponds. OSU perch averaged 198.17 mm (7.80 in) and 111.90 g (3.95 oz) and 202.0 mm (7.95 in) and 114.28 g (4.03 oz), respectively. WI strain perch from two ponds averaged 189.53 mm (7.46 in) and 89.85 g (3.17 oz) and 197.24 mm (7.77 in) and 100.91 g (3.56 oz), respectively. Length and weight were both significantly ($p<0.001$) greater for OSU improved strain yellow perch compared to WI local strain yellow perch. Fillet weight was significantly ($p<0.001$) greater for OSU strain perch, while percent fillet yield was significantly greater for WI local strain yellow perch. Northern WI experienced severe and prolonged winter weather in 2013 that resulted in pond ice cover that did not dissipate until early May. We believe that the extreme weather were a significant cause in the poor survival of both groups of fish. Due to the poor survival of all fish, total production and percent market size were unable to be calculated.

OBJECTIVE 3

Farm test progress and results have been disseminated to industry and to end-user customers via three articles published in newsletters, journals and website.

Outreach Overview
Farm test progress and results have been disseminated to industry and to end-user customers via three articles published in:

Newsletters (http://southcenters.osu.edu/sites/southc/files/site-library/site-documents/aquaTeam-research/Winter%202013%20Newsletter.pdf),

Magazine (http://ohioseagrant.osu.edu/_documents/twineline/v35i1.pdf) and

Website (http://southcenters.osu.edu/about-us/news/farm-tests-show-higher-production-growth-rate-and-survival-improved-perch).

Complete results will be disseminated to industry and to end-user customers via fact sheets, workshop and journal articles in 2014.

Targeted Audiences
Aquaculture industry and fish farkers and researchers

Outputs
Third generation of genetically improved yellow perch that grow ~35% faster than unimproved fish; Improved breeding technology;
Website (http://southcenters.osu.edu/about-us/news/farm-tests-show-higher-production-growth-rate-and-survival-improved-perch). Journal articles will be out in 2014.

Outcomes/Impacts
Genetically improved yellow perch that grow ~35% faster than unimproved fish would have a tremendous positive impact on the NCR yellow perch aquaculture industry. The impact of this project will be primarily through the delivery of the 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.

Impacts Summary
Relevence: The yellow perch is a particularly important aquacultural and ecological species in the Midwest, and has a unique and niche market in the NCR. One factor in particular constraining yellow perch aquaculture expansion has been the relatively slow growth of currently cultured strains

Responses: The Ohio State University has established selective breeding program for genetic improvement of growth in yellow perch. The third generation of genetically improved perch has been created. Farm tests of the genetically improved lines were conducted in three sites in NCR.

Results: Farm tests showed the improved fish had ~35% faster growth rate and ~20% higher survival rate than unimproved fish. Fish farmers could use the genetically improved perch to increase production efficiency of yellow perch aquaculture.

Recap: Fish farmers could use the genetically improved perch to significantly improve production efficiency of yellow perch aquaculture.

Publications:
Oral: From markers to markets: Genetically improved yellow perch show a higher production, growth rate and survival by farm tests. Regional Aquaculture Extension Conference, Feb. 2014

Extension factsheets: On-farm tests show higher production, growth rate and survival of improved yellow perch, OARDIP Newsletter, 2013 (1): 1-2 (http://southcenters.osu.edu/sites/southc/files/site-library/site-documents/aquaTeam-research/Winter%202013%20Newsletter.pdf).

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 (≤10 h) transport events. The Upper Midwest Environmental Sciences 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 effectiveness data. UMESC has submitted the protocols to CVM through the UMESC publicly-disclosable Investigational New Animal Drug (INAD) permits for AQUI-S®20E and requested an informal CVM review prior to conducting the study. The staff of CVM was uncertain about how to assess a potential label claim but considered that data generated through the proposed effectiveness trials would be important for development of a pivotal effectiveness study design. CVM concluded that official pivotal protocol concurrence would not be given until preliminary data was reviewed.

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.

OBJECTIVE 3 - UMESC interacted with CVM to determine whether a Categorical Exclusions from the requirement to complete an Environmental Assessment was 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 submitted draft protocols to CVM for an informal review to assess the effectiveness of AQUI-S®20E for transport. Data generated from these effectiveness studies has been compiled and is being submitted to CVM for further review. Pending CVM review of the data, a pivotal effectiveness protocol will be submitted based on input from CVM, UMESC and the drug sponsor (AQUI-S New Zealand Ltd.). The CVM concurrence letters will be reviewed and appropriate modifications made to the study protocols before initiation of the pivotal effectiveness trials.

OBJECTIVE 5 - Following concurrence in Objective 4, UMESC will conduct the pivotal effectiveness studies in the spring/summer of 2014.

OBJECTIVE 6 – A final study report will be prepared and its associated data 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 has a maximum of 60 days to provide review comments to UMESC before the complete final study report and all trial data were archived according to UMESC Standard Operating Procedures. The final study report will then 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 without increasing 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.

Accomplishments

Outreach Overview – Multiple presentations about the procedures involved and results from the effectiveness studies were given to state, regional, and national aquaculture groups. This included presentations to the Wisconsin Aquaculture Association, the North Central Regional Aquaculture Center, and the USFWS Aquatic Animal Drug Approval Partnership. Also, over the next 12 months, the final report for this project will be submitted to NCRAC for public distribution and parts of the effectiveness studies will be published in peer-reviewed scientific journals for broad public distribution.

Targeted Audiences – There are two targeted audiences: 1) directly, the manufacturer of AQUI-S®20E will gain knowledge in species-specific effects and applied concentration parameters for the commercial product of 10% eugenol. Also, the U.S. Food and Drug Administration Center for Veterinary Medicine will use this knowledge as they draft action regarding the use of AQUI-S®20E as an immediate release finfish sedative and 2) indirectly, fish haulers/transporters and

aquaculturists will benefit should AQUI-S®20E be approved for use when hauling yellow perch and tilapia as it could permit greater loading densities during transport.

Outputs – None to date

Outcomes/Impacts - Results of the effectiveness studies resulted in changes in knowledge based on the following:

1. Concentrations of AQUI-S®20E ranging from 200-300 mg/L resulted in light sedation and >95% mean survival 7 days post-transport for yellow perch at loading densities up to 360 g/L (three times the industry standard) in 17°C water. Tilapia held at 22°C showed signs of sedation for less than 4 h when exposed to AQUI-S®20E concentrations up to 300 mg/L, but had high mean survival (>90%) following a 10 h static exposure at a loading density of 480 g/L (two times the industry standard).
2. Concentrations of AQUI-S®20E ranging from 200-300 mg/L were effective at reducing metabolic rates for yellow perch in 17°C water relative to unsedated control fish. Tilapia exposed to 300 mg/L AQUI-S®20E at 22°C had significantly reduced metabolic rates relative to control fish at a loading density of 120 g/L. Results indicated that AQUI-S®20E sedation may benefit yellow perch at high loading densities during transport due to a reduction in metabolic rates, while further research is needed to assess the benefits of AQUI-S®20E sedation for tilapia at densities greater than 120 g/L.

Impacts Summary

Relevance: 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. Fish haulers/transporters are interested in any means by which to increase fish loading densities in hauling tanks to make deliveries more efficient.

Response: 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. Effectiveness studies were conducted to examine the sedation effect of AQUI-S®20E on yellow perch and tilapia as a means of decreasing fish respiration while increasing fish loading densities and thereby maximizing hauling efficiencies.

Results: Knowledge gained from the effectiveness studies included species-specific fish loading densities for yellow perch based on sedation effects and respirometry data. Results from the tilapia studies showed minimal changes in metabolic rates and sedation under simulated transport conditions and suggested that further studies are needed to characterize the response of tilapia to AQUI-S®20E during transport at high loading densities. These results are directly applicable to the manufacturer of AQUI-S®20E and the U.S. Food and Drug Administration Center for Veterinary Medicine as they evaluate AQUI-S®20E as an immediate release finfish sedative.

Recap: AQUI-S®20E has the potential to allow fish haulers to transport fish at increased loading densities though the sedation effects were more pronounced for yellow perch than tilapia.

Publications:

Presentations –

Oral

The effects of Aqui-S 20E (10% eugenol) sedation on yellow perch, *Perca flavescens*, and Nile tilapia, *Oreochromis niloticus*, during transport. Aaron Cupp M.S. Thesis Defense, UW-Stevens Point, Stevens Point, WI, June 27, 2013.

A.R. Cupp, M.P. Gaikowski, K.T. Fredricks and C.F. Hartleb. Effects of AQUI-S®20E sedation on yellow perch *Perca flavescens* and Nile tilapia *Oreochromis niloticus* during transport. Presented at the 19th Annual Drug Approval Coordination Workshop in Bozeman, MT. July 2013.

A.R. Cupp, J.R. Meinertz, C.F. Hartleb, K.T. Fredricks, S.T. Porcher, and J.R. Smerud. Effectiveness of AQUI-S® 20E for yellow perch *Perca flavescens* and tilapia *Oreochromis niloticus* for live transport. Presented at the 18th Annual Aquaculture Drug Approval Coordination Workshop. July 2012. La Crosse, WI.

Poster

A.R. Cupp, C.F. Hartleb, K.T. Fredricks and M.P. Gaikowski. Effects of AQUI-S® 20E sedation on yellow perch *Perca flavescens* and tilapia *Oreochromis niloticus*. Poster presented at the 2013 Wisconsin Aquaculture Conference in Oconomowoc, WI. March 2013.

Non-peer reviewed

The effects of Aqui-S 20E (10% eugenol) sedation on yellow perch *Perca flavescens* and tilapia *Oreochromis niloticus* for transport. Aaron Cupp M.S. Thesis. University of Wisconsin-Stevens Point. 102 p.

Students/Participants:

Name: Aaron Cupp
Whether Degree was completed during the reporting period (Aaron Cupp, yes)
Capstone/Thesis Title: The effects of AQUI-S®20E (10% Eugenol) sedation on yellow perch *Perca flavescens* and tilapia *Oreochromis niloticus* for transport
Date of Graduation: 8/9/13

Partnerships:

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.

Evaluate Phase II Production of Bluegill Sunfish Comparing a Least-Cost Diet Utilized in the Phase I Verification Study Compared to an "Industry Standard" for One Production Cycle

Project ***Progress Report*** for the Period
September 1, 2012 to August 31, 2013

NCRAC FUNDING: $75,000 (September 1, 2011 to August 31, 2013)

PARTICIPANT:

Paul Brown	Purdue University	Indiana
Chris Hartleb	University of Wisconsin-Stevens Point	Wisconsin
Charles Hicks	Lincoln University	Missouri
Robert Rode	Purdue University	Indiana
James E. Wetzel	Lincoln University	Missouri
Industry Advisory Council Liaison:		
Mark Willows	North American Fish Farmers Cooperative	North Dakota

PROJECT OBJECTIVES

1. Using consistent protocols, evaluate/determine performance of age-2 bluegill fed the diet (41% protein/<8.3% lipid) previously developed by a NCRAC funded project compared to an “industry standard” diet used in the on-going project at two distinct latitude locations in ponds for one growing season.

2. Coordinate dissemination of project results with the NCRAC Technical Committee/Extension Subcommittee. The expected deliverable will be a technical bulletin containing such detailed information as growth, production parameters, size composition, and survival using data collected over grow out to market size, i.e., the first year from the on-going plus this year’s project.

ANTICIPATED BENEFITS

Results garnered from this project will provide the aquaculture industry with relevant field-tested information related to the culture of Phase II bluegills using a least cost experimental diet. Project results will be coordinated with the NCRAC Technical Committee/Extension Subcommittee to provide deliverables such as technical bulletins containing such detailed information as growth, production parameters, size composition, and survival using data collected over grow-out to market size; i.e., Phase I to the end of Phase II.

PROJECT PROGRESS

OUTCOMES/IMPACTS

Results of the production field trials resulted in changes in knowledge based on the following: The cost differential between the two feeds would justify using the least cost diet to reduce the cost per pound of producing bluegill.

IMPACTS SUMMARY

Relevance.— Fish feed often accounts for >50% of the variable costs in aquaculture budgets. To reduce variable costs field trials using lower-cost foodstuffs, e.g. vegetable or animal by-products, need to be conducted. The open formula bluegill diet developed by Robert Hayward (University of Missouri-Columbia) is such a diet that needs to be tested on age-2 northern bluegill to record its effects on growth, production and survival.

Response.— Field trials of age-2 northern bluegill were conducted at two fish farming sites at geographically distinct locations in the NCR. Comparative studies were conducted examining the growth, production, and survival of bluegill fed an industry standard diet and the open formula diet.

Results.— The Phase II least cost diet test for bluegill sunfish indicated that feed formulated with lower cost formulations can show similar results as using a currently availabe diet developed for salmonids which currently dominate the market.

Recap. — The less-expensive-to-manufacture open formula bluegill diet produced similar growth, production and survival to the industry standard diet and may be a cost effective replacement for the industry.

PUBLICATIONS

Oral

Phase II production of Lepomis macrochirus comparing a “least-cost” diet to an “industry standard”. Wisconsin Aquaculture Conference, Pewaukee, WI March 1-2, 2013.

Sunfish Diet Comparison Studies in the North Central Region, Nutrition Workshop, Yellow Springs Ohio,

Summary of Sunfish Research, NCRAC Annual Planning Workshop, Ames, IA, February, 2012

EXTENSION FACTSHEETS

Phase II production of Lepomis macrochirus comparing a “least-cost” diet to an “industry standard”. B.S. Senior Thesis. University of Wisconsin-Stevens Point. 11 p.

Extension[4]

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

[4]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

The existing aquaculture industry members need relevant information on new techniques and technologies in aquaculture, as well as updated information related to changing state and federal regulations. Increasingly, a large number of individuals are interested in aquaculture as a means of agriculture diversification or urban development. The NCRAC Extension Work Group meets these diverse client needs through on-site advice, publications, and specialized workshops. As the industry matures, the advisory service needs will shift toward more pecialized and advanced knowledge than is currently provided at general introductory conferences and events. Entrepreneurs and prospective aquaculturists often require an enormous amount of time to educate and can benefit from the availability of the electronic media.

The NCRAC Extension Work Group will continue and expand its efforts to promote and advance commercial aquaculture in a responsible fashion through its organized education/training outreach programs and through educating the public on the health benefits of commercially raised fish. 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; improved lines of communication between interstate aquaculture extension specialists and associated industry contacts; and an enhanced legal and socioeconomic atmosphere for aquaculture in the NCR.

The development of aquaculture education programs for the NCR has provided "hands-on" opportunities for prospective and experienced producers. Approximately 6,000 individuals have attended workshops or conferences organized and delivered by the NCRAC Extension Work Group. Clientele attending regional workshops have gained information related to aquaculture development strategies in other areas of the country and acquired information which was of direct use to their own enterprises. Education programs also created situations where problems encounter ed by producers were expressed to extension personnel who later relayed them to researchers at NCRAC work group meetings for possible solutions through the research effort.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Examples follow for each of the objectives from the fourteen projects funded to date with emphasis on activities funded during the last project.

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 a non-funded collaborator on the Regional Aquaculture Extension Specialist;
- Met with industry representatives and university researchers involved with aquaculture to discuss how the aquaculture industry could grow in the NCR.
- A revised NCRAC Web site was developed in 2010 and 2011, revised in 2013 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.

OBJECTIVE 2

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. This extension network is critical to being able to match specific aquaculture questions with the best source of information.

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 assist the Kansas Aquaculture Association by developing, printing and distributing the Kansas Aquaculture Association Directory. In a similar fashion, Bauer distributed NCRAC information to the Nebraska aquaculture industry; 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

Hicks developed three aquaculture factsheets: Freshwater Prawn Production in Missouri, Bluegill Sunfish Production in Missouri and Swine Barn Production for Fish Culture and conducted In-Pond-Raceways tests with cooperating farmers. Regional extension cooperators for the eXtension project (including the 2012 eXtension Aquaculture Virtual Workshop), including include Jim Held, Allen Pattillo, Ron Kinnunen, and Laura Tiu who have presented on a variety of topics including aquaponics, species-specific culture (bluegill, freshwater prawn, yellow perch, and walleye), recirculating systems, prospective considerations of aquaculture operations, and HACCP training.

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). 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. For instance, Kinnunen has worked with Michigan Wholesale Baitfish Association on a possible 3rd party certification program for the AIS-HACCP program.

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. An AIS-HACCP plan has also been developed by Kinnunen and Gunderson 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.

More recently, Kinnunen participated in the Michigan DNR Lake Superior Citizen Advisory Committee meeting and presented how the AIS-HACCP program can be used to prevent the spread of aquatic invasive species in baitfish. The effectiveness of the AIS-HACCP is reflected in the fact that aquatic invasive species have not been identified in the baitfish trade according to surveys conducted by Michigan DNR staff. Nick Phelps (University of Minnesota Veterinary Diagnostic Lab) and Kinnunen also conducted two Aquaculture Biosecurity/AIS-HACCP Workshops in 2012.

Starting in 2010 and continuing to the present, 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. All fish processors, including those who handle aquaculture products, are now required by law to process their fish following HACCP guidelines.

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. In one example, 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.

The AT2 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 added a new aquaponics list serve this year and now hosts four electronic list serves, the most popular of which is the Aqua-Ohio list serve. Over 790 clients are subscribed to the list serves which allows for timely dissemination of aquaculture related news and resources. This information is further disseminated by the list subscribers to additional interested parties.

Outreach Overview

Enhancing state-wide and regional communication and training among those in the aquaculture industry is imperative for continued growth of aquaculture in the Midwest. 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 workshops were mainly hands-on, which enabled participants to acquire knowledge and skills in indoor recirculating aquaculture systems. Some workshop participants have started aquaculture operations after attending the workshops. Additional services include on-line educational materials, workshops, business planning assistance, facility tours and production training.

Target Audiences
Current and prospective fish farmers

Outputs
Ohio Aquaculture Feed and Nutrition Workshop, May 10th in Yellow Springs, Ohio
Over 70 existing fish farmers, new and beginning fish farmers, associated industries (feed mill, brewery, protein supplements), University folks and soybean industry representatives attended the workshop held at YSI in Ohio. A variety of speakers shared the scope of the fish feed and nutrition sector, why fish feed and nutrition is so important to them and the growth and survival of their industry, and what kind of research and development is being done, or is needed to move the aquaculture feed industry forward. Presentations available here: http://southcenters.osu.edu/aquaculture/boot-camp/intensive (click on May).

Over 139 people attended the February 2013 OAA Aquaculture Conference in Wooster, Ohio. Two special sessions were coordinated by OCARD staff: Introduction to Aquaculture and Aquaculture Business. These activities keep fish farmers in Ohio up to date on the latest aquaculture research and extension activities in the Midwest and recordings are available at: http://southcenters.osu.edu/aquaculture/presentations/oaa-workshop-presentations-2013. Also, video advice from existing farmers was recorded and can be viewed here: http://southcenters.osu.edu/aquaculture/extension/education/advice-from-the-experts

OUTCOMES/IMPACTS
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. List serves for the North Central Region and the Indiana Aquaculture Association have been started based on the success of aqua-Ohio.

Over 139 people attended the February 2013 OAA Aquaculture Conference in Wooster, Ohio. Two special sessions were coordinated by OCARD staff: Introduction to Aquaculture and Aquaculture Business. These activities keep fish farmers in Ohio up to date on the latest aquaculture research and extension activities in the Midwest and recordings are available at: http://southcenters.osu.edu/aquaculture/presentations/oaa-workshop-presentations-2013. Also,

video advice from existing farmers was recorded and can be viewed here: http://southcenters.osu.edu/aquaculture/extension/education/advice-from-the-experts

Impacts Summary

Relevance Fish farmers require some basic extension services including responding to various questions relating to fish production. Extension activities would include providing resources relating to addressing issues such as poor water quality, diseases, low oxygen levels, water temperature, and feeding strategies. Some prospective fish farmers need farm visits to assist with hands-on experiential learning on various fish production issues.
Response Workshops and on-line materials developed.
Results Participants to acquire knowledge and skills in indoor recirculating aquaculture systems.
Recap In response to industry need, workshops have been identified throughout the region to address indusry issues.

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

Oral
Kinnunen, R. E. 2012. Growing Power Conference, Fish Handling, Processing, Seafood HACCP, and Fish Marketing, Milwaukee, Wisconsin, September 8, 2012.

Kinnunen, R. E. 2012. Upper Midwest Invasive Species Conference, Aquatic and Terrestrial Invasive Species-Hazard Analysis Critical Control Point (HACCP) Workshop, La Crosse, Wisconsin, October 29, 2012..

Kinnunen, R. E. 2012. Seafood HACCP Training Workshop, Bay Mills, Michigan, December 4-6, 2012.

Kinnunen, R. E. and J. Gunderson. 2013. International Conference on Aquatic Invasive Species, The HACCP Approach to Prevent the Spread of Aquatic Invasive Species by Aquaculture and Baitfish Operations, Niagara Falls, Ontario, April 24, 2013.

Pattillo, D. A. Why Aquaponics? FarmTek's Fall 2013 Controlled Environment Agriculture School.

Pattillo, D. A. Why Aquaponics? FarmTek's Summer 2013 Controlled Environment Agriculture School.

Morris, J. E., D. A. Pattillo, C. Weeks. The role of the North Central Regional Aquaculture Center in Addressing Challenges Facing Regional Aquaculture Development Using Extension and Research Partnerships. Aquaculture 2013, Nashville, TN. February 24,2013

Morris, J. E., S. Brown and D. A. Pattillo. Logic Model and Impact Statements for NCRAC. 2013 NCRAC Annual Meeting. February 18, 2013

Pattillo, D. A. and C. Weeks. Deliverables for NCRAC Funded Projects. 2013 NCRAC Annual Meeting. February 18, 2013

Burden, D. and D. A. Pattillo. Marketing for Aquaculture. 2013 NCRAC Annual Meeting. February 18, 2013

Pattillo, D. A. Walleye Culture. 2012 eXtension Virtual Aquaculture Workshop. November 14, 2012 http://www.extension.org/sites/default/files/Walleye_Culture_Pattillo_eXtension_11-2012.pdf

Peer-Review
Hicks, C. 2012. Two-Year Growth of Sunfish Hybrids in Missouri. World Aquaculture Society Magazine.

Digital
2012 eXtension Virtual Aquaculture Workshop, Walleye Culture, Recorded November 12-15, 2012
http://www.extension.org/pages/66298/proceedings-of-the-2012-extension-virtual-aquaculture-workshop

Popular Articles
WHOtv – Agribusiness Report – Growing Crops in Water – Aired October 2, 2013.
http://whotv.com/2013/10/02/agribusiness-growing-crops-in-water/

Iowa Public Television – Market to Market – Closed-Loop Aquaponics Growing System Combines Land and Lake – Aired September 20, 2013.
http://www.iptv.org/mtom/story.cfm/feature/10981/mtom_20130920_3904_feature

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

NCRAC AND Marketmaker™

Project ***Termination Report*** for the Period
September 1, 2010 to August 31, 2013

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

REASON FOR TERMINATION

Project objectives completed and funds have been terminated.

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

Regional producers have been provided with the information portal needed to place their specific information into the MarketMaker system.

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

In the 2012 and 20133 NCRAC annual meetings, participants were provided with the training materials needed to be informed as to the potential of the MarketMaker system for their operations.

OBJECTIVE 3

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

A final online educational module http://www.ncrac.org/files/MarketMaker-Pub-0034.pdf that will assist in the registration of individual operations in the region will be placed onto the NCRAC web site. An association presentation on using MarketMaker is located at http://www.ncrac.org/files/Using%20Market%20Maker_Pattillo%202-8-13.pdf .

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.

Economic Impact Assessment

Project ***Progress Report*** for the Period
September 1, 2012 to August 31, 2013

NCRAC FUNDING: $115,000 (September 1, 2012 to August 31, 2013)

PARTICIPANTS:

Steven C. Deller	University of Wisconsin-Extension	Wisconsin
Christopher F. Hartleb	University of Wisconsin-Stevens Point	Wisconsin
Laura Tiu	Ohio State University	Ohio
Industry Advisory Council Liaison:		
Robert Baldwin		Michigan
Extension Liaison:		
James A. Held	University of Wisconsin-Stevens Point	Wisconsin

PROJECT OBJECTIVES

1. Characterize the aquaculture industry throughout the NCR (species, systems, purpose, size, sales, jobs, etc.).
2. Determine the direct, indirect and induced contributions of the aquaculture industry to regional and state – by -state economies.

ANTICIPATED BENEFITS

There is a need for information to educate the public, bureaucrats, and regulators on the value of the aquaculture industry's contribution to regional and state economies. This approach will maximize the applicability of our work by emphasizing high quality deliverables for immediate use by the industry. Educational materials will emphasize the value of local food production, environmental sustainability, and resource enhancement to elevate public awareness of the benefits of commercial aquaculture and dispel misinformation. The political effectiveness fact sheet will be particularly useful in leveraging support for the industry when presented to state and federal political entities as well as university and technical college administrators.

PROGESS AND PRINCIPAL ACCOMPLISHMENTS

Objectives 1 and 2. Progress for this project has focused on determination of the best sources of information characterizing the aquaculture industry in the NCR. It was the consensus of the work group that analysis based on the currently available 2005 data could lead to misrepresentations of the current state of the industry and surveys of the industry conducted by the work group would fail to capture adequate responses upon which to base meaningful analysis. Therefore work group discussions have resulted in agreement that the 2012 U.S. Census of Agriculture that will be published in early 2014 as well as the 2013 U.S. Census of Aquaculture (results expected in early 2015) will provide the most relevant databases for analysis. Characterization of the size, capacity and economic impact industry will begin in 2014 once data becomes available. Once the cost structure data has been reviewed it may become necessary to survey the industry for

expenditures specific to aquaculture. We expect this limited survey will be more successful in garnering responses due to the non-proprietary nature of the information requested.

Outreach Overview
Results of the analysis will be utilized to produce 13 separate educational brochures (one for each of the 12 states, and one for the entire NCR) that will characterize the industry and describe the existing economic impacts, as well as the predicted future impacts, of the aquaculture industry in the NCR. A summary report will be written detailing the methods used, completed data sets, and findings of the IMPLAN analysis. The summary report will also include an executive summary that condenses the information included in the summary report into bullet points and graphic representations to highlight the findings of the study. Copies of the educational brochures will be supplied to state aquaculture associations, regional and state extension programs, and university-based aquaculture research and education programs for promotional and educational activities.

Targeted Audiences
The target audiences will be state aquaculture associations, regional and state extension programs, and university-based aquaculture research and education programs for promotional and educational activities.

Outputs
None to date.

Outcomes/Impacts
None to date

Impacts Summary
None to date

SUPPORT
To date, NCRAC has provided $18,811 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 Economic/Marketing activities.

Regional Aquaculture Extension Specialist (RAES)

Project ***Progress Report*** for the Period
September 1, 2011 to August 31, 2013

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 Project activties are anticipated to a) increase awareness of sustainable aquaculture across the industry, regulatory agencies, and the general public, b) transfer important information to the NCR aquaculture community, c) increase support for NCR aquaculture on local, state, and national levels and d) gain a better understanding of the effectiveness of NCRAC extension, outreach, and research activities in terms of industry impact

PROGESS AND PRINCIPAL ACCOMPLISHMENTS

Objective 1: To date, the RAES has Objective 1: Continue RAES support to the NCR aquaculture community through ongoing activities in areas of liaison services, leadership, assessing and addressing industry needs, and information transfer.

Project PI has actively provided leadership and liaison services to the NCR aquaculture community as necessary to carry forward industry interests including:

Maintaining updates to the NCRAC regulation website;

Attend state association meetings and provide support, including updates and presentations at 2-3 state association meetings per year;
Maintaining an open door policy fielding frequent questions from potential and seasoned aquaculturists on a wide variety issues dealing with aquaculture development including systems, species, markets, regulations and fish health;
Participation with eXtension.org, Ask-an-Expert, a web-based outreach extension tool;
Continue information dissemination on the NCR fish culture list-serve (5 or more per week), including topical news, training opportunities and solicitations for aquaculture community involvement in regulatory processes such as comments to Federal Register posts.

Objective 2: Develop and implement strategies to address and promote aquaculture sustainability in the NCR.

RAES accomplishments towards addressing aquaculture sustainability are as follow:

RAES conducted a review of available materials from previous regional sustainability oriented projects. Examples include NCRAC's Environmental Strategies for Aquaculture Symposium (Kinnunen et al. 2000) and Best Management Practices for Aquaculture in Wisconsin and the Great Lakes Region (Malison and Hartleb 2005). After a thorough review, the project PI then developed a workshop style presentation on sustainable aquaculture, which he presented during state association meetings in Kentucky (tri-state conference with OH, IN and KY), Michigan, and Missouri from February - March 2012. Results from the workshops were published in journal article: Sustainable Aquaculture in the North Central Region US - A Review of perceptions and recommendations from the aquaculture community.

Objective 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.

In 2012, the project PI obtained voting memberships in NAA, Michigan Farm Bureau (MFB), MFB Aquaculture Advisory Committee, NSF Food Division Advisory Council Regulatory/Seafood sector, and Great Lakes Panel for Aquatic Nuisance Species. In 2013 the PI also became a member of the newly formed National Institute of Animal Agriculture Aquaculture Advisory Committee, and gave a presentation to this group entitled U.S. Aquaculture: the Past, Today and the Future. In all cases the RAES has represented NCR aquaculture industry interests towards sustainable aquaculture industry practices and program development. Reviews conducted personally by the RAES include: Draft Strategic Planning Documents U.S. Fish & Wildlife Service Aquatic Invasive Species Program, and Policy and Research Committee Priorities for Great Lakes Panel on Aquatic Nuisance Species.

The RAES has given 9 presentations in outreach and public education at state association meetings as well as various interest group and national meetings over the course of the project. These are listed in the section titled "Outputs" below.

Objective 4: Coordinate efforts for seeking non-NCRAC support for NCR aquaculture development.

The RAES initiated and was an integral part of the submission of 2 grant proposals for aquaculture industry development in the NCR:

Integrated Assessment: Expansion of Michigan's Existing Commercial Aquaculture Activities into a Major Sustainable Seafood Production Industry: author - Weeks; funding source: Michigan Sea Grant; amount: $267,000; status: awarded - expected completion February, 2014.

A Practical Approach to Rapid Aquaculture Development Across Midwestern US Rural Communities - Market Research, Value Chain Analysis and Production Expansion of Regional Seafood Production Systems: authors – Weeks, Hicks, Bouras, Quagrainie, Tiu, Held, Phelps; funding source: USDA AFRI; amount: $497,800; status: proposal rejected - missing 2 forms from submission package, currently under consideration for re-submittal under new grant RFP (to be determined).

The RAES has worked directly with a foundation which has provided support for aquaculture development in the state of Michigan, as well as agri-business organizations Indiana Soy Alliance, Michigan Soybean Association, and Soy Aquaculture Alliance. In addition, Michigan's Farm Bureau Aquaculture Advisory Committee provides comments and recommendations to the National Farm Bureau Aquaculture Advisory Committee.

Objective 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.

RAES continues to work with the NCRAC Director and IAC Chair on NCRAC Annual Program Planning Meeting procedures in order to more narrowly define key targeted priorities, allow for increased TC involvement, and improve the effectiveness of the NCRAC project selection. The procedure was modified in 2010 with support by the RAES. In its first 2 years, the revised process has shown to help streamline the program planning meeting project selection process. The RAES also helped provide support to the NCRAC Director in development of a NCRAC survey regarding guidelines for extension and research for NCRAC projects. Results of the survey were presented by the Director at a National Aquaculture Conference (see "Outputs")

Outreach Overview

The RAES project uses a variety of information outlet strategies as described in previous sections including open door communication policy, phone and email communications, eXtension.org Ask an Expert website, NCR Fish Culture List Serve, state association meeting attendance and presentations, and representation on various committees deemed important for NCR aquaculture industry development (example: Policy Council member of the Great Lakes Panel for Aquatic Nuisance Species - meets twice per year).

Targeted Audiences
The RAES strives to provide leadership for members of aquaculture and baitfish industry sectors in the NCR and enhance information transfer to help further development within these sectors. In addition to industry members, the RAES target audience includes all stakeholders, academicians, and government employees, especially those who may be impacted, directly or indirectly by fish production, consumption, aquatic animal and human health, animal rights, live aquatic animal trade and regulations thereof.

Outputs
The NCRAC regulation website is updated annually by the RAES with support from IT personnel at Iowa State University. This site has exceeded over 500 hits per month for over 2+ years running (count not currently available). Additional outputs include over 5+ posting per week by the PI to the NCR Aquaculture List Serve (currently 140+ subscribers), and participation to eXtension.org Ask an Expert program. The RAES has also given 3 Sustainable Aquaculture workshop-style presentations at the Tri-state conference with OH, IN and KY, Michigan, and Missouri from February - March 2012 with data and results obtain from these being published in an online extension journal. PI has also presented 6 additional times on various topics of NCR industry needs and interests (see oral publications).

Outcomes/Impacts
1) 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.
2) New partnerships for aquaculture support in the NCR.
3) Increase level of awareness regarding AIS impacts on the NCR aquaculture industry.
4) Increased non NCRAC funding support for NCR aquaculture industry development.

Impacts Summary
Relevance.— There are a limited number of extension full-time equivalents (FTEs) in the region to address the needs of aquaculture and baitfish industry sectors. To that end, regional support in the area of aquaculture extension has been requested by the IAC.
Response. — The RAES has continued support to the NCR aquaculture community through ongoing activities and strategic pathways established to date. New areas such as aquaculture sustainability and AIS regulations have been assessed and activities to address these issues are being implemented. Non-NCRAC partnerships and support have been explored, formed and initiated.
Results. — The NCR as a whole seems to be gaining interest and momentum in aquaculture development, especially in areas of aquaponics, sustainability, healthy seafood for consumption and locally grown products. RAES and other extension personnel across the region are helping to promote commercial fish production, further the industry, and facilitate outreach to interested, new and seasoned producers through communication and partnerships. State associations and

other non-NCRAC groups continually seek RAES support for funding opportunities, information and regulations.
Recap.— The RAES project appears to be making a positive impact on commercial aquaculture and baitfish industry sectors in the NCR through liaison, outreach, and regional coordination activities.

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

Weeks. 2013. Aquaculture trends from around the world to my back yard. Ohio Aquaculture Association Wooster, Ohio. February 8-9, 2013.

Weeks C. 2013. Regulation impacts on commercial aquaculture in the North Central Region. Wisconsin Aquaculture 2013. Pewaukee, Wisconsin. March 1-2, 2013.
Weeks C. 2013. Global aquaculture, where is it going and why – should I really be buying more seafood? Purdue Veterinary Medicine Conference. Purdue University, West Lafayette, Indiana. August 22, 2012.

Weeks C. 2013. Health maintenance in aquaculture. Purdue Veterinary Medicine Conference Purdue University, West Lafayette, Indiana. August 22, 2012.

Weeks C.*, N. Phelps and R. Johnson 2013. Are regulations the biggest obstacle to a growing aquaculture industry in the North Central Region US?. Aquaculture 2013. Nashville, Tennessee
February 22-25, 2013.

Weeks C. 2012. Sustainable aquaculture in the NCR (workshop–style presentations). Tri-state conference with OH, IN and KY, Michigan, and Missouri from February - March 2012.
Weeks. C. 2012. AIS impacts on regional aquaculture – the need for effective but fair regulations and a proactive industry. Upper Midwest Invasive Species Conference, La Crosse, Wisconsin October 29-31, 2012.

Morris, J.*, D. Pattillo and C. Weeks 2013. The Role of the North Central Regional Aquaculture Center in addressing challenges facing regional aquaculture development using extension and research partnerships. Aquaculture 2013. Nashville, Tennessee, February 22-25, 2013.

Peer-reviewed:

Weeks C.T. 2013. Sustainable Aquaculture in the North Central Region US - Review of perceptions and recommendations from the aquaculture community. Journal of Extension v51 no.2 - 2COM1.

SUPPORT
To date, NCRAC has provided $96,770 which is the total amount allocated for this objective.

Snail Management/Grub Control[5]

Project ***Progress Report*** for the Period
September 1, 2007 to August 31, 2013

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

(1) 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 completed 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 portale pfor fish producers to access and obtain information potentially relevant to their snail problems. Among the various options, information regarding effectiveness, legal implications, and potential for impact on pond general ecology, e.g.,

[5] 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.

zooplankton dynamics in fish fingerling ponds, will be listed. This Web page will be hosted on the revised North Central Regional Aquaculture Center (NCRAC) Web site.

WORK PLANNED

In 2014 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)[6]

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

ANTICIPATED BENEFITS

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

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

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

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

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

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 have been summarized and submitted to UMESC.

Personnel from Northern Aquaculture Demonstration Facility (NADF) applied one of four iodophor-disinfection levels to newly fertilized lake herring eggs taken from 3 separate matings of wild-caught adult herring. Fertilized control eggs were water hardened and rinsed with well water only whereas treated eggs were iodophor-disinfected at: 50 mg/L for 30 min, 100 mg/L for 30 min, 100 mg/L for 15 min. Eggs separated by disinfection and mating were transported to the U.S. Geological Survey Upper Midwest Environmental Sciences Center (UMESC) for rearing. Samples of ovarian fluid, fertilized eggs, and the adult herring were transported to the U.S. Fish and Wildlife Service La Crosse Fish Health Center (LFHC) to determine the presence/absence of the virus responsible for VHS; all samples were negative for VHS.
Mimicing current standard egg disinfection procedures, treated eggs received an iodophor disinfection of 100 mg/L for 10 min upon arrival at UMESC. Eggs were then distributed volumentrically to minature egg incubation chambers. Control eggs were processed last and received a sham disinfection by placing the eggs in well water for 10 min before transfer to the assigned incubaiton chamber. Samples were collected from each group for VHS testing.

Hyphae (presumably Saprolegnia sp.) were observed on a non-viable egg, subsequently 13 hydrogen peroxide treatments (15 min, nominal 500 mg/L, measured concentrations ranged from 510-544 mg/L) were applied once daily on alternate (first 3 treatments) then consecutive days. Eggs with adhering hyphae were removed daily and the number of eggs removed estimated.

The development of a definite eye-spot was first observed 19 d post-fertilization and by 24 d post-fertilization eggs in all incubation chambers. Percent of eggs that had a definite eye-spot was estimated and the incubation at UMESC stopped to return the survivors to NADF for use in other studies (NADF needed these embryos to make up for the minimal spawning and egg take). The percent of eggs with a definite eye-spot was determined by first transferring the eggs from an incubation chamber to a 100-mL cylinder then removing an aliquot of the eggs (~1 mL in volume). The number of viable eggs (i.e., eggs with a definite eye-spot) was determined. Viable lake herring eggs were then shipped overnight to NADF.
The percent of eggs with a definite eye-spot was: control – 76.2% (65.4 – 82.2%); 50 mg for 30 min – 69.3% (63.4 – 80.2%); 100 mg for 15 min – 69.23% (62.2 – 80.9%); 100 mg for 30 min – 80.43% (74.5 – 81.5%). Iodophor disinfection did not substantially alter the percent of eggs developing an eye-spot. Parental pairing did appear to contribute to the portion of eggs that developed an eye-spot: pairing A – 72%; pairing B – 81.1%; pairing C – 67.1%. Although the lack of VHS-positive eggs precluded assessment of the efficacy of iodophor disinfection to eliminate VHS, the results suggest that current iodophor disinfection procedures do not alter the development of lake herring eggs and that shipment of eyed lake herring eggs does not affect survival.

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.

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.

Objecitve 5 - Three model fish disease HACCP plans (one each for flow-through, pond, and recirculation systems), relying heavily on the specific plans developed under Objective 4 were developed and used in DVD produced in Objective 6.

Objective 6 - Production of the HACCP biosecurity video completed. Completed biosecurity workshop videos and model HACCP plans will be posted by ISU for free access on the CFSPH and Focus on Fish Health Web sites.

OUTREACH OVERVIEW

The work with lake herring will be summarized as part of a series of publications to be developed with NADF. A U.S. Geological Survey Fact Sheet was published in FY 2010 (see http://pubs.usgs.gov/fs/2009/3107/).

Evaluations of the biosecurity 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). 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.

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 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.

Targeted Audiences
This information represents a growing body of evidence that iodophor disinfection is a safe and effective technique to reduce the transfer of certain pathogens (e.g. VHS) during the collection of gametes from the wild. The information can be used by natural resource management agencies and the private aquaculture sector to ensure that eggs used for fish culture are free of disease.

Both private aquaculture and natural resource management agencies participated in the biosecurity workshops. From the workshops they came away with knowledge on how to develop biosecurity and and AIS-HACCP plans specific to their own facilities so that they can prevent the movement of fish diseases and aquatic invasive species.

OUTPUTS
The VHS "response" packet was developed by Iowa State University. 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. All of the materials were forwarded to Project Leaders (Malison and Kinnunen) and were incorporated into the eight biosecurity workshops along with the already developed AIS-HACCP curriculum. 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).

Three model fish disease HACCP plans (one each for flow-through, pond, and recirculation systems), relying heavily on the specific plans developed during the biosecurity workshops were developed and used in DVD. Completed biosecurity workshop videos and model HACCP plans will be posted by ISU for free access on the CFSPH and Focus on Fish Health Web sites.

OUTCOMES/IMPACTS

Egg iodophor disinfection appears to effectively eliminate VHSv (strain IVb) from the surface of walleye and northern pike eggs. The findings of this study indicate the lake herring eggs can be safely disinfected and that eyed eggs can be safely shipped overnight

Initial skepticism of the participants at the biosecurity workshops 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.

IMPACTS SUMMARY

Relevance.— Interest in raising lake herring to supplement the stocks in the Great Lakes has grown. However, wild brood stock may be VHS positive. Determining a way to safely eliminate the VHS virus from the fertilized eggs would prevent the spread of VHS.
Response.— Eggs from wild lake herring were taken from VHS postive waters (Lake Superior). Fertilized eggs were disinfected with iodine then reared until defined eye-spots were observed.

Results.— This work demonstrated that iodophor disinfection did not adversely impact the development of lake herring embryos and confirmed that lake herring eggs can be safely shipped after developing an eye-spot.

Recap.— Disinfection of fertilized lake herring eggs with iodine at a concentrationf of 50 mg/L for 30 min or 100 mg/L for 15 or 30 min does not adversely affect embryo development.

PUBLICATIONS

Presentations:
Oral

Held, J.A., J.A. Malison, R.E. Kinnunen, R.E. Johnson, and S.E. Kaatz. VHS biosecurity workshops and the development of fish disease HACCP plans in the North Central Region of the US. Aquaculture America 2009, Seattle, Washington, February 16-19, 2009.

Kinnunen R.E. 2010. Biosecurity and VHS virus update. NCRAC Baitfish Workshop. La Crosse, WI. September 21, 2010.

Kinnunen, R.E., M.P. Gaikowski, M.T. Tuttle-Lau, J.J. Rach, J.A. Malison, J.A. Held, R.E. Johnson, S.E. Kaatz, G.D. Dvorak. North Central Regional Aquaculture Center VHS Project. American Fisheries Society Fish Health Section Annual Conference, La Crosse, Wisconsin, August 1, 2012.

Kinnunen, R.E., M.P. Gaikowski, M.T. Tuttle-Lau, J.J. Rach, J.A. Malison, J.A. Held, R.E. Johnson, S.E. Kaatz, G.D. Dvorak. North Central Regional Aquaculture Center VHS Project. American Fisheries Society Annual Conference, St. Paul, Minnesota, August 20, 2012.

Kinnunen R.E. and J. Gunderson. The HACCP Approach to Prevent the Spread of Aquatic Invasive Species by Aquaculture and Baitfish Operations. International Conference on Aquatic Invasive Species. Niagara Falls, Ontario, April 24, 2013.

Tuttle-Lau, M.T., K.A. Phillips and M.P. Gaikowski. An Evaluation of the Efficacy of Iodophor Disinfection of Walleye Sander vitreum and Northern pike Esox lucius to Control Viral Hemorrhagic Septicemia virus. 34th Annual Eastern Fish Health Workshop, Shepherdstown, West Virginia, May 24-28, 2010.

- Non-Peer-reviewed:
    - Extension factsheets

Tuttle-Lau, M.T., K.A. Phillips, and M.P. Gaikowski. 2010. Evaluation of the efficacy of iodophor disinfection of walleye and northern pike eggs to eliminate viral hemorrhagic septicemia virus. U.S. Geological Survey Fact Sheet 2009-3107.

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

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