

ANNUAL PROGRESS REPORT

For the Period
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NORTH CENTRAL REGIONAL AQUACULTURE CENTER

A table of commonly used abbreviations and acronyms can be found inside the back cover.

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INTRODUCTION

The U.S. aquaculture industry generated nearly \$1.4 billion for over 3,000 producers in 2013. Though minor in a global context, accounting for 0.6% of total world value, the domestic impact of U.S. aquaculture is substantial, accounting for approximately 181,000 jobs and generating an estimated \$5.6 billion annually. 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 U.S. imports a majority of its fish and shellfish and, after Japan, is the world's second largest importer of seafood (valued at \$17.6 billion in 2012). Fisheries imports are the largest contributor to the U.S. trade deficit among agricultural products.

Landings for most U.S. commercial capture fisheries species and recreational fisheries have been relatively stable during the last decade, with many fish stocks being overexploited. In this situation, aquaculture provides an opportunity to reduce the trade deficit and meet the rising U.S. demand for fish products. This can be achieved by a partnership of the Federal government, State and local public institutions, and the private sector with expertise in aquaculture development.

The U.S. Congress has stressed the importance of a strong domestic aquaculture industry to: (1) increase American production of fish and shellfish, (2) reduce dependence on foreign suppliers, and (3) benefit rural America by the development of alternative agricultural crops and creation of new jobs. Recognizing that the aquaculture industry cannot achieve full potential without strong national leadership and direction, the U.S. Congress created an opportunity for making significant progress in aquaculture development in 1980 by passage of the National Aquaculture Act (P.L. 96-362). This act addressed the importance of a strong domestic aquaculture industry and established the Joint Subcommittee on Aquaculture (JSA). The JSA is an interagency body that is chaired by the Secretary of Agriculture. It has numerous responsibilities and is to provide coordination and recommendations for Federal aquaculture policy. The Congress also amended the National Agricultural Research, Extension, and Teaching Policy Act of 1977 in Title XIV of the Agriculture and Food Act of 1980 (P.L. 97-98) by granting authority to USDA 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, and western regions, and one in Hawaii. 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

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programs provided by 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 RACs is to support aquaculture research, development, demonstration, and extension education to enhance viable and profitable U.S. aquaculture which will benefit consumers, producers, service industries, and the American economy. The North Central Regional Aquaculture Center (NCRAC) 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. NCRAC also provides for coordination of interregional and national programs through USDA's National Coordinating Council for Aquaculture (NCCA). The council is composed of the RAC directors and USDA 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;
- Coordinate 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.

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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); Regional Extension Meetings;
- 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;
- Preparation of impact statements resulting from NCRAC-funded extension projects;
- Maintain the NCRAC video collection and distribution;
- Initial editing of “final” draft of new NCRAC publications;
- Review and prepare responses to e-mail requests sent to NCRAC@iastate.edu;
- NCRAC mailings;
- 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.

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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 24 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), FY2012 (2012-38500-19550), FY2013 (#2012-38500-19550 Amendment) and FY2014 (2014-38500-22138) with monies totaling \$19,616,906. Currently, five grants are active (FY10-14); the first 22 grants (FY88-09) have terminated.
- ▶ The Center annually coordinates a biannual program planning meeting which typically sets priorities for the next 2-year funding cycle and calls for

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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, 2014, the Center has funded or is funding 106 projects through 514 subcontracts from the first 24 grants received. Funding for these Center-supported projects is summarized in Table 1 below (pages 8-11). 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 on-line 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 NCA; numerous oral and written presentations to both professional and lay audiences; working with other fisheries and aquaculture programs throughout the North Central Region; and 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 21 separately funded projects in regard to Extension and 10 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, 2013 to August 31, 2014. Projects, or Project components, that terminated prior to September 1, 2013 have been reported on in earlier documents (e.g., 1989-1996 Compendium Report and other Annual Progress Reports). The following reports

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are placed in order of selected key word(s):
Aquaculture Drugs, Baitfish,
Conferences/Workshops, Crayfish,
Economics/Marketing, Extension, Hybrid
Striped Bass, Largemouth Bass, National
Coordinator for Aquaculture,
Nutrition/Diets, Other, Salmonids, Sunfish,
Tilapia, Viral Hemorrhagic Septicemia,
Walleye, Wastes/Effluents, and White
Papers. In addition, the format style of these

reports differs from previous years, e.g.,
inclusion of Project Summary and Impacts
Summary.

A cumulative list of all publications,
manuscripts, papers presented, or other
outputs for all funded NCRAC project areas
is located at <http://ncrac.org> .

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Table 1. North Central Regional Aquaculture Center-Funded Projects.

Project Area	Project Number	Proposed Duration Period	Funding Level	Grant Number
Aquaculture Drugs	1	7/1/96-6/30/97	\$27,000	95-38500-1410
	2	12/1/96-11/30/97	\$950	95-38500-1410
	3	10/1/99-9/30/00	\$8,415	97-38500-3957
	4	6/1/04-11/30/05	\$223,677	2003-38500-12995
	5	7/15/04-7/14/05	\$60,000	2003-38500-12995
	6	11/1/04-10/31/06	\$50,000	2002-38500-11752
	7	1/1/06-12/31/06	\$129,936	2005-38500-15847
	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	9/1/11-8/31/11	\$100,000	2010-38500-20929
	11	9/1/12-8/31/14	<u>\$240,000</u>	2012-38500-19550
			\$1,017,858	
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
			<u>\$88,003</u>	2005-38500-18547
			\$261,973	
Conferences/Workshops/Symposia				
Environmental Strategies Symposium	1	9/1/00-5/31/01	\$5,000	96-38500-2631
Nat'l. Aquaculture Exten. Workshop/Conference	1	10/1/91-9/30/92	\$3,005	89-38500-4319
	2	12/1/96-11/30/97	\$3,700	95-38500-1410
	3	11/1/02-10/31/03	\$4,500	00-38500-8984
	4	1/1/06-12/31/06	\$5,000	2005-38500-18547
	5	9/1/10-8/31/11	<u>\$5,000</u>	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	<u>\$3,000</u>	96-38500-2631
			\$10,000	
Percis III	1	11/1/02-10/31/03	\$4,000	00-38500-8984
Crayfish	1	9/1/92-8/31/94	\$49,677	92-38500-6916
Economics/Marketing	1	5/1/89-12/31/91	\$127,338	88-38500-3885
			\$34,350	89-38500-4319
	2	9/1/91-8/31/92	\$53,300	91-38500-5900
	3	9/1/93-8/31/95	\$40,000	93-38500-8392
	4	9/1/99-8/31/01	\$47,916	97-38500-3957
	5	9/1/03-8/31/04	\$50,000	2002-38500-11752
	6	9/1/10-8/31/11	\$23,565	2010-38500-20929
	7	9/1/12-8/31/14	<u>\$115,000</u>	2012-38500-19550
			\$491,469	
Extension	1	5/1/89-4/30/91	\$39,221	88-38500-3885
("Base" Extension—Project			\$37,089	89-38500-4319
Nos. 1-15; Aquaculture	2	3/17/90-8/31/91	\$31,300	89-38500-4319
Regional Extension Facilitator	3	9/1/91-8/31/93	\$94,109	91-38500-5900
[AREF]—Project No. 16; and	4	9/1/93-8/31/95	\$110,129	91-38500-5900

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Project Area	Project Number	Proposed Duration Period	Funding Level	Grant Number
Regional Aquaculture Extension Specialist [RAES]— Project Nos. 18, 19 and 20	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
	15	9/1/13-8/31/15	\$45,000	2012-38500-19550
	16	9/1/03-8/31/05	\$100,000	2002-38500-11752
	17	9/1/05-5/31/09	\$199,624	2004-38500-14269
	18	9/1/09-8/31/11	\$150,000	2008-38500-19157
	19	9/1/11-8/31/13	\$196,612	2010-38500-20929
	20	9/1/13-8/31/14	\$101,280	2012-38500-19550
21	9/1/14-8/31/15	\$103,347	2014-38500-22138	
		\$1,904,373		
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
	2	9/1/14-8/31/16	\$155,000	2014-38500-22138
		\$325,000		
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
	2	5/15/99-5/14/00	\$10,000	95-38500-1410
		7/15/04-7/14/05	\$9,000	2003-38500-12995
		9/15/05-8/31/06	\$15,000	2004-38500-14269
		9/1/06-8/31/08	\$40,000	2006-38500-16900
		5/15/08-5/14/09	\$25,000	2007-28500-18469
		\$144,241		
Nutrition/Diets	1	9/1/04-8/31/06	\$200,000	2002-38500-11752
	2	9/1/07-8/31/09	\$80,000	2006-38500-16900
	3	9/1/09-8/31/11	\$80,000	2008-38500-19157
	4	9/1/10-8/31/12	\$43,363	2008-38500-19157
	5	9/1/12-8/31/13	\$124,400	2010-28500-20929

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Project Area	Project Number	Proposed Duration Period	Funding Level	Grant Number
			\$75,000 \$559,400	2012-38500-20929
Other				
Feed Training Carnivorous Fish	1	9/1/06-8/31/08	\$165,446 <u>\$134,554</u> \$300,000	2005-38500-15847 2006-38500-16900
Snail Management/Grub Control	1	9/1/07-8/31/09	\$212,495	2007-38500-18469
RAS Microbial Communities	1	9/1/09-8/31/10	\$65,000	2008-38500-19157
Assessment of Winter Kill in Ponds	1	9/1/11-8/31/13	\$175,000	2008-38500-19157
Salmonids	1	6/1/90-8/31/92	\$9,000	89-38500-4319
	2	9/1/92-8/31/94	\$120,799	90-38500-5008
	3	9/1/94-8/31/96	\$149,997	92-38500-6916
	4	9/1/97-8/31/99	\$199,290 <u>\$158,656</u> \$637,742	94-38500-0048 97-38500-3957
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
	6	9/1/13-8/31/15	<u>\$160,000</u> \$1,013,788	2012-38500-19550
Tilapia	1	9/1/96-8/31/98	\$118,791	96-38500-2631
	2	9/1/98-8/31/00	<u>\$150,000</u> \$268,791	98-38500-5863
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 \$59,835	94-38500-0048 95-38500-1410
	7	9/1/99-6/30/02	<u>\$127,000</u> \$927,627	98-38500-5863
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 <u>\$88,814</u> \$448,300	00-38500-8984 2001-38500-10369
White Papers	1	7/1/98-12/31/98	\$4,999	96-38500-2631
	2	9/1/99-12/31/99	<u>\$17,495</u> \$22,494	97-38500-3957
Yellow Perch	1	5/1/89-8/31/91	\$76,957	88-38500-3885

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Project Area	Project Number	Proposed Duration Period	Funding Level	Grant Number
	2	6/1/90-8/31/92	\$85,723	89-38500-4319
	3	9/1/91-8/31/93	\$92,108	90-38500-5008
	4	9/1/93-8/31/95	\$99,997	91-38500-5900
	5	9/1/95-8/31/97	\$150,000	93-38500-8392
	6	9/1/97-8/31/99	\$199,507	95-38500-1410
	7	9/1/98-8/31/00	\$185,458	97-38500-3957
	8	9/1/01-5/31/04	\$92,370	98-38500-5863
	9	9/1/10-8/31/13	\$326,730	00-38500-8984
	10	9/1/13-8/31/15	\$125,016	2001-38500-10369
			\$150,000	2010-38500-20929
			<u>\$190,000</u>	2012-38500-19550
			\$1,773,866	
TOTAL			\$11,814,219	

PROJECT REPORTS

NORTH CENTRAL REGIONAL AQUACULTURE CENTER

NORTH CENTRAL REGIONAL AQUACULTURE CENTER

PROGRESS REPORT

Project Title: Drug Approval Research on 17 α -Methyltestosterone (Official Transfer of 17 α -Methyltestosterone [MT] Analytical Method for Feed) ¹

Key Word(s): Aquaculture Drugs

Total Funds Committed: \$54,615

Initial Project Schedule: September 1, 2019 to August 31, 2010

Current Project Year: September 1, 2013 to August 31, 2014

PARTICIPANTS:

Mark P. Gaikowski, USGS, Upper Midwest Environmental Sciences Center, Wisconsin
Nilmini Wijewickreme, Reference Laboratory, CANTEST Ltd., British Columbia

Extension Liaison: Kevin Fitzsimmons, University of Arizona

Industry Liaison: Mark Willows, Binford Eagle Fisheries, North Dakota

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).

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.

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8. Determine whether any changes are needed to the MT analytical feed method developed UWMadison 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.
-

Project Summary

The approval of 17 α -Methyltestosterone (MT) medicated feed for use in tilapia to produce male fish would be of significant benefit to the industry. Tilapia is now the fifth most consumed seafood in the United States. Male fish grow faster than do their female counterparts, and by using all male fish, reproduction can be minimized or eliminated in grow-out systems, further benefiting growers. Approval of MT will allow all tilapia producers to have legal access to MT without an investigational new animal drug permit and will provide them with a legal means to yield increased biomass, thus resulting in more revenue for those producers. The production of male populations of tilapia is important to the U.S. tilapia industry if they are to remain competitive with foreign producers of tilapia. The remaining data requirements necessary for MT approval include a method transfer trial where naive and experienced laboratories process MT medicated feed. Data from the sources must match for the transfer trial to be successful, and the method to be accepted by CVM.

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.

Project Progress

Objectives 1 – 10 —Completed

Objective 11. —The following statement was issued by CVM in a letter to UMESC (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 discussed with CVM the comments from the CVM-issued non-concurrence letter.

The comments included: 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

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method recovery discrepancies between the reference and participating laboratories; and 4) a reference made to a confidential INAD belonging to Rangen, Inc. UMESC efforts, in collaboration with Rangen, Inc. and Maxxam, to address the review responses are ongoing.

Objective 12. —During the week of March 24, 2014, UMESC with representatives from Rangen, Inc. participated in a conference call with CVM. The outcome of the call was a clear definition of what needed to be included in a response to CVM concerning their non-concurrence letter of December 17, 2010. A response was prepared along with a detailed standard operating procedure titled “Procedure for determining concentrations of 17 α -Methyltestosterone in MASCULINIZING FEED FOR TILAPIA®” to CVM on May 5, 2014. A request was made to accept the method transfer study data described in the final study report. If in CVM’s response to the most recent submission there is a requirement for substantial work to be completed by UMESC then additional resources would be required

Target Audiences

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

Outreach Overview

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

Deliverables (Outputs)

A comprehensive standard operating procedure for determining MT

concentrations in fish feed was developed and submitted to CVM. Upon approval of MT, the standard operating procedure will become the official method for determining MT concentrations in fish feed for dose verification.

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 17 α -Methyltestosterone (MT) medicated feed for use in tilapia to produce male fish would be of significant benefit to the industry. The remaining data requirements necessary for MT approval include a method transfer trial where naive and experienced laboratories process MT medicated feed. Data from the sources must match for the transfer trial to be successful, and the method to be accepted by CVM.

Response — A method transfer trial was coordinated and conducted at Maxxam Analytics (formerly CANTEST Ltd.) in British Columbia, Canada. The trial has been completed and the data from the trial submitted to CVM. CVM provided criticisms of the data. After negotiations with CVM,

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responses to those criticisms were developed and submitted to CVM.

Results — A comprehensive standard operating procedure for determining MT concentrations in fish feed was developed and submitted to CVM. Upon approval of MT, the standard operating procedure will become the official method for determining MT concentrations in fish feed for dose verification.

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.

Publications, Manuscripts, Workshops, and Conferences

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

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PROGRESS REPORT

Project Title: Effectiveness Research Leading to Approvals for Controlling Mortality in Coolwater and Warmwater Finfish due to Aeromonad Infections with Terramycin 200 for Fish® (oxytetracycline dehydrate) and Aquaflor® (florfenicol)²

Key Word(s): Aquaculture Drugs

Total Funds Committed: \$150,000

Initial Project Schedule: September 1, 2008 to July 31, 2012

Current Project Year: September 1, 2013 to August 31, 2014

PARTICIPANTS:

Mark P. Gaikowski, USGS, Upper Midwest Environmental Sciences Center, Wisconsin

Extension Liaison: Joseph E. Morris, Iowa State University

Industry Liaison: Mark Willows, Binford Eagle Fisheries, 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 the CVM before beginning the effectiveness studies.

² ²NCRAC has funded nine Aquaculture Drugs projects. This Progress Report is for the eighth Aquaculture Drugs project. It is a 2-year funded project that began January 1, 2008. A Termination Report for the first project is contained in the 1997-98 Annual Progress Report; a Termination Report for the second project is contained in the 1996-97 Annual Progress Report, a Termination Report for the third project is contained in the 2001-02 Annual Progress Report, a Termination Report for the fourth project is contained in the 2006-07 Annual Progress Report, and Termination Reports for the sixth and seventh projects are contained in the 2007-08 Annual Progress Report. A fifth project, which provided \$60,000 for a portion of the funds required to purchase sufficient radiolabeled AQUI-S[®] for use in a total residue depletion study in rainbow trout, is reported on under the Termination Report for the National Coordinator for Aquaculture New Animal Drug Applications (NADAs) in the 2008-09 Annual Progress Report. A Progress Report for the ninth project is contained elsewhere in this report.

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5. Conduct pivotal effectiveness studies on Terramycin 200 for Fish® and Aquaflor® according to Good Clinical Practice and the CVM concurred protocols.
6. Analyze the effectiveness data and prepare draft final study reports for Terramycin 200 for Fish® and Aquaflor® no more than four months after the studies are completed.
7. Submit the respective draft study reports to PAH and SPAH for their review.
8. Submit the final study reports through established INADs for Terramycin 200 for Fish® and Aquaflor® to CVM for acceptance no more than two months after PAH and SPAH have completed their reviews of the draft study reports.
9. Ensure that all questions and concerns about the final study reports are answered no more than one month after receiving comments from CVM.
10. If CVM accepts the data as proving effectiveness for the aeromonad infections encountered in the NCR, provide the acceptance letter and effectiveness studies to PAH and SPAH so that they can pursue supplemental NADA approvals for their respective drug products.

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Project Summary

The efficacy of Aquaflor®-medicated feed therapy to control mortality associated with motile aeromonad infections was evaluated in muskellunge and walleye under field conditions at Spirit Lake Fish Hatchery, a state walleye, northern pike, and muskellunge hatching and rearing station production facility in Spirit Lake, Iowa. The hatchery historically experiences rising mortality rates due to motile aeromonad septicemia as the water temperature rises in early July. Parameters evaluated included daily mortality, clinical observations, feed consumption, and water chemistry observations.

Anticipated Benefits

The data from this study, if accepted by CVM, will support the potential approval of oxytetracycline dehydrate (OTC) and florfenicol (FFC) medicated feed for use in cool- and warm-water fish to prevent mortality due to motile *Aeromonas septicemia*.

Project Progress

Objectives 1-5. — Complete

Objective 6.— Final reports under development.

Objectives 7-10.— No Progress.

Target Audiences Aquaculturists rearing cool and warm water fish species who have the need to control mortality associated with mesophilic or motile *Aeromonas* infections (MAI).

Outreach Overview

UMESC will complete final reports when the *Aeromonas* species in the remaining

isolates are identified and confirmed. Final reports will be disseminated to CVM, Phibro Animal Health and Merck Animal Health.

Deliverables (Outputs)

None.

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 MAI. The results from this project will directly affect the potential for approval of oxytetracycline dihydrate and florfenicol by the U.S. Food and Drug Administration's Center for Veterinary Medicine (CVM). The data from this study, if accepted by CVM, will support the potential approval of OTC and FFC medicated feed for use in cool- and warm-water fish to prevent mortality due to motile *Aeromonas septicemia*.

Impacts Summary

Relevance — 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

Response — Pivotal studies were completed to assess the effectiveness of OTC and FFC in reducing mortality due to MAS in juvenile muskellunge and walleye.

Results— Results are being compiled.

Recap — Results are pending.

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Publications, Manuscripts, Workshops, and Conferences

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

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PROGRESS REPORT

Project Title: Efficacy of Eugenol (AQUI-S®20E) to Reduce Transport Stress and Mortality of Tilapia and Yellow Perch

Key Word(s): Aquaculture Drugs

Total Funds Committed: \$100,000

Initial Project Schedule: September 1, 2011 to August 31, 2013

Current Project Year: September 1, 2013 to August 31, 2014

PARTICIPANTS:

Mark P. Gaikowski, USGS Upper Midwest Environmental Sciences Center, Wisconsin
Christopher F. Hartleb, University of Wisconsin – Stevens Point, Wisconsin

Industry Liaison:

Mark Willows, Binford Eagle Fisheries, 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.

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Project Summary

Fish transport costs are a substantial portion of the operational expenses in 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.

Anticipated Benefits

Transporting more fish per gallon of fuel would directly reduce operational costs resulting from less fuel being purchased and consumed. Additionally, fewer staff days would 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 maintaining acceptable post-transport survival.

Project Progress

Objective 1. — Upper Midwest Environmental Science Center (UMESC) collaborated with Center for Veterinary Medicine (CVM) and developed an acceptable protocol and study design for generating non-pivotal effectiveness data. UMESC submitted the protocol 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 and data generated through non-pivotal effectiveness trials would be important for development of a pivotal effectiveness study. Data and reports from non-pivotal effectiveness trials and a draft pivotal effectiveness protocol were submitted to CVM in January of 2014. Responses from CVM regarding these submissions were received May 2014. Briefly, CVM response indicated that non-pivotal data informed them on both effectiveness and target animal safety. Suggestions concerning future studies under these conditions were to use fewer response variables. Itemized comments for revisions of the pivotal effectiveness protocol are currently being addressed to work toward protocol concurrence from CVM and a revised pivotal effectiveness protocol should be resubmitted in early 2015.

Objective 2. — All protocols, data, and final study reports submitted to CVM will be submitted by UMESC to INAD 011-766.

Objective 3. — Work within this objective is dependent on progress made by the drug sponsor on completion of an original Environmental Assessment for the use of AQUI-S® 20E.

Objective 4. — A revised 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 2015.

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Objective 6. — A final study report will be prepared and its associated data audited by the UMESC QA Officer before review and acceptance by UMESC management. The drug sponsor has a maximum of 60 days to provide review comments to UMESC before the complete final study report and all trial data are archived and the FSR will then be submitted to CVM through the UMESC (INAD 011-766).

Objective 7. — UMESC will submit the FSRs and associated data to CVM. 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 address specific study related issues identified by CVM 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 and will provide draft FOI summaries to the drug sponsors 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.

Target 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 CVM 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 potentially support greater loading densities during transport.

Outreach Overview

Multiple presentations about the methods and results from the effectiveness studies were given to state, regional, and national aquaculture groups. This included presentations to the Wisconsin Aquaculture Association, MidContinent Warmwater Fish Culture Workshop, the North Central Regional Aquaculture Center, and the USFWS Aquatic Animal Drug Approval Partnership. Also, 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 (one manuscript currently accepted by journal).

Deliverables (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 multiple levels of sedation (including 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 (63 °F) water. Tilapia held at 22°C (72 °F) 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 (63 °F) water relative to unsedated control fish.

Tilapia exposed to 300 mg/L AQUI-S®20E at 22°C (72 °F) had significantly reduced metabolic rates relative to control fish at a loading density of 120 g/L (1 lb/gal). 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 (1 lb/gal).

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 that 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 CVM 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, Manuscripts, Workshops, and Conferences

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

Technical Update:

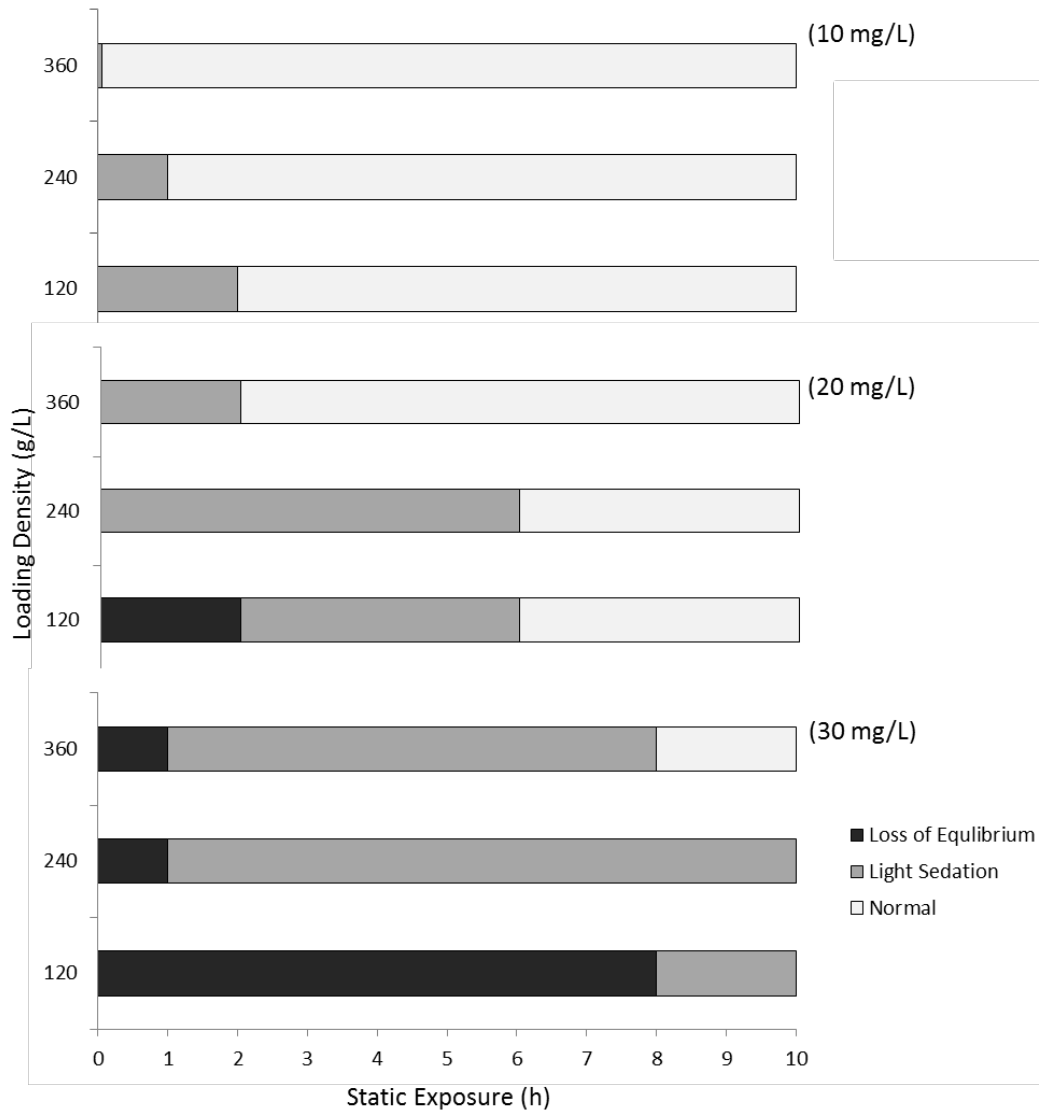


Figure 1. Observed behavior of yellow perch throughout static exposure in response to three concentrations of AQUI-S®20E (10, 20 and 30 mg/L eugenol) at three loading densities (120, 240 and 360 g/L [1, 2 and 3 lb/gal]). [Figure is from manuscript currently being prepared for journal submission]

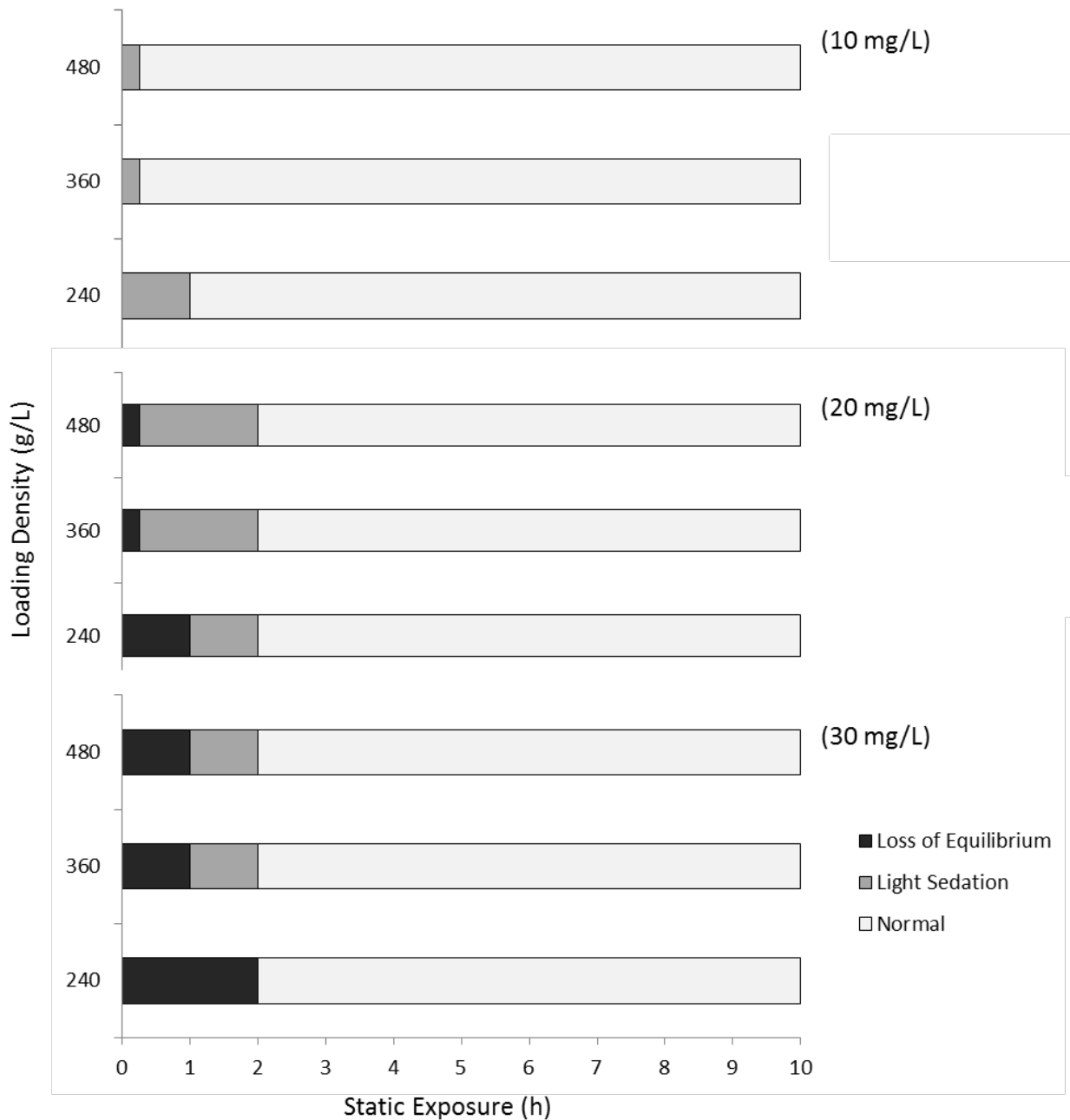


Figure 2. Observed behavior of Nile tilapia throughout static exposure in response to three concentrations of AQUI-S®20E (10, 20 and 30 mg/L eugenol) at three loading densities (240, 360 and 480 g/L [1, 2 and 3 lb/gal]). [Figure is from manuscript currently being prepared for journal submission]

PROGRESS REPORT

Project Title: Probiotics in Yellow Perch and Tilapia Culture

Key Word(s): Aquaculture Drugs

Total Funds Committed: \$240,000

Initial Project Schedule: September 1, 2012 to August 31, 2014

Current Project Year: September 1, 2013 to August 31, 2014

PARTICIPANTS:

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

Extension Liaison: Nicholas Phelps, University of Minnesota, Minnesota

Industry Liaison: William Lynch, Millcreek Aquaculture. Marysville, 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.

Project Summary

Yellow perch larvae were cultured in high density (30-40 per L; 7.9-10.6 per gal) using live zooplankton for 17 days. The average rate of survival through the entire experimental period was $32.0 \pm 7.6\%$ and the swim bladder inflation rate was $35.8 \pm 20.6\%$. The average juvenile weight was 24.5 ± 5.0 mg (0.86 ± 0.18 oz) and average growth rate $29.4 \pm 1.6\%$ day⁻¹. Fish were then subjected to treatments with isolated probiotic strains of bacteria and potential pathogenic bacteria isolates. Isolates from adult yellow perch were used to further characterize by heat shock challenge and determine their inhibitory potential against common fish pathogens, *Vibrio anguillarum* and *Aeromonas salmonicida*. Of the eight isolates tested all but three isolates showed inhibition of *Vibrio*, while there appears to be only a weak inhibition to *Aeromonas*.

A feeding experiment was performed with yellow perch juveniles that included dietary treatments with yellow perch isolated probiotic, commercial probiotic, and control. *Flavobacterium columnare* challenge was performed by adding final bacterial density of 108/ml. The final survival rates for treatments did not differ significantly (93-100%). There were no mortalities during columnaris exposure and no disease symptoms were observed in the following 17 days.

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. — Yellow Perch larvae used in 2014 experiments were bred from several 5-6 year old females from the OSU aquaculture facility and males either from the same source or from Millcreek Perch Farm (Marysville, Ohio). The batch produced for intensive rearing in the OSU aquaculture greenhouse facility originated from egg ribbons that were released and fertilized within the broodstock tank on April 23rd and 25th, 2014.

For Phase I, 50-L (13.2 gal) conical tanks were initially stocked with 1628+ 340 (n=9) larvae/tank. This phase began with the first feeding of larvae at 3 days-post-hatching (dph) and continued throughout the first 10 days of exogenous feeding. The system was equipped with a constant inflow of evaporated sea salt and Nannochloropsis algae paste. After 10 days of feeding, 300 larvae were randomly sampled from each tank and moved to the indoor laboratory facility.

Phase II lasted for 7 days fish were reared in nine 60-L cylindrical tanks with constant inflow of water. Temperature remained at 17.2 ± 0.2 °C (63 ± 32 °F) throughout this phase. The rotifers *Brachionus*, a continuous culture maintained at aquaculture lab, and *Artemia* nauplii were hatched from cysts prior to enrichment. During the second phase, fish were initially provided with *Artemia*, then transitioned to Otohime A® diet. The average rate of survival was $32.0 \pm 7.6\%$. Swim bladder inflation rate was $35.8 \pm 20.6\%$ at the end of the second phase. The average juvenile weight was 24.5 ± 5.0 mg (0.86 ± 0.18 oz). The results suggest that the growth of yellow perch larvae/juveniles

is greater in the EE-enriched groups than the TAG-enriched groups, especially during the first 10 days of exogenous feeding.

Objective 2.— Potential probiotic bacteria were isolated from the intestinal tract of yellow perch collected in OSU aquaculture laboratory. Isolates were challenged by heat shock to further determine their inhibitory potential against common fish pathogens, *Vibrio anguillarum* and *Aeromonas salmonicida*.

To test their direct inhibitory abilities to the two pathogens, we first streak plated on agar with our isolates, heat shocked and cross streaked with the pathogenic species. Of the eight isolates tested all but three isolates showed inhibition of *V. anguillarum* but only weak inhibition to *A. salmonicida*. Once we have determined that our isolates have probiotic potential in-vitro to the selected pathogens, the 16S rRNA gene of the isolates was sequenced. Results indicated that five of the six isolates are strains of *Lactococcus lactis* and one isolate was classified to *Pseudomonas*.

Objective 3.— Preparation of isolates for *in vivo* experiment included isolate V9 and commercial probiotic 2B. Volume of each culture was adjusted to yield 10^9 cfu/ml and cultures were freeze dried prior to processing into the fish feed. The test consisted of two sets of 12 aquaria that were open (challenge) or semi-recirculating (control). Feeding experiment was performed with yellow perch juveniles (0.08 g). The following dietary treatments were included in the study: commercial diet (control), diet with yellow perch isolated probiotic, diet with commercial probiotic, and yeast and krill based diet. One day before the bacterial challenge fish were divided into 30 fish per tank (designated for the challenge) and the remaining fish were distributed into a parallel system (no challenge).

Objective 4.— *Flavobacterium columnare* isolation was performed using infected fish (approx. 50% of the external body area infected). Samples were transferred onto a plate with beef extract/agar medium. The colonies were identified (yellowish with not-defined ragged edges). One plate was used to confirm bacterial strain by DNA sequencing method. The bacterial culture from the vial from which the plate had been streaked was used to inoculate additional cultures. In order to carry out columnaris challenge, bacterial culture was added to each tank to provide final bacterial density of 10^8 /ml. The desired colonies were found in the challenged group but not in the uninfected group. The density of bacterial colonies in challenged tanks was estimated as 8.7×10^5 CFU/ml. The results at the completion of the feeding experiment indicated the largest weight was observed in fish that were fed the control diet (0.57 ± 0.02 g) followed by probiotic supplemented groups, 0.51 ± 0.13 , 0.42 ± 0.03 , and 0.23 ± 0.02 g in experimental diet. The final survival rates for treatments after the challenge were 100, 98, 96, and 93%, respectively. There were no mortalities during the 24-h columnaris incubation period. No disease symptoms were observed due to introduction of columnaris bacteria.

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.

Outreach Overview

Oral presentation to the joint annual meeting of the Ohio and Michigan Aquaculture Associations in Toledo, February 22, 2014.

Deliverables (Outputs)

Nothing to report

Outcomes/Impacts

Nothing to report

Impacts Summary

Nothing to report

Publications, Manuscripts, Workshops, and Conferences

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

PROGRESS REPORT

Project Title: Economic Impact Assessment³

Key Word(s): Economics/Marketing

Total Funds Committed: \$75,279

Initial Project Schedule: September 1, 2012 to August 31, 2014

Current Project Year: September 1, 2013 to August 31, 2014

Participants:

Steven G. Deller, University of Wisconsin Extension, Wisconsin

Christopher F. Hartleb, University of Wisconsin-Stevens Point, Wisconsin

Laura G. Tiu, The Ohio State University, Ohio

Extension Liaison: James A. Held, University of Wisconsin Extension, 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.

³ NCRAC has funded eight Economics/Marketing projects. Termination Reports for the first two projects are contained in the 1989-1996 Compendium Report; a Termination Report for the 3rd project is contained in the 1996-97 Annual Progress Report; a Termination Report for the 4th project is contained in the 2002-03 Annual Progress Report; and a Termination Report for the 5th project is contained in the 2003-04 Annual Progress Report. This Progress Report is for the 8th project. It is a 2-year project that began September 1, 2012.

Project Summary

The aquaculture industry in the NCR suffers from a lack of clearly documented information that describes the industry (e.g., species, systems, purpose, size, sales, jobs, etc.) and its economic value to the region. This information is critical to assist NCR producers to inform and leverage the support of political, regulatory and educational decision-makers on issues that impact aquaculture production and expansion of the industry.

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.

Project Progress

Objectives 1 & 2.— Analysis of the recently released USDA Census of Aquaculture (CoA) has been initiated and discussions of relevant information that is not included in the CoA have been undertaken by the project team. Alternative data sets to augment the CoA information are being investigated and a preliminary version of an information-gathering survey to accumulate supplemental economic information has been developed. The survey is intended to

be distributed to a select number of aquaculture producers in each state to help determine important economic parameters such as cost of operation for a variety of aquaculture operations and to more closely define categories of vendors and customers that will assist in upstream and downstream economic impact of the industry. The survey is currently being prepared for Institutional Review Board (IRB) review to allow publication of the results. A list of potential recipients for the survey is being compiled.

Target 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.

Outreach Overview

Results will be made available through a series of educational pamphlets that characterize the industry and describe the economic impact for each of the states within the NCR as well as a document that does the same for the entire region.

Deliverables (Outputs)

We will prepare 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.

Outcomes/Impacts

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.

Impacts Summary

Relevance: 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.

Publications, Manuscripts, Workshops, and Conferences

See the Appendix for a cumulative output for all NCRAC-Funded Economics/Marketing activities.

TERMINATION REPORT

Project Title: NCRAC and Marketmaker™ Collaboration⁴

Key Word(s): Economics/Marketing

Dates of Work: September 1, 2010 to August 31, 2014

Total Funds Committed: \$23,565

Participants:

Daniel J. Burdan, Iowa State University, Iowa

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.

⁴ NCRAC has funded seven Economics/Marketing projects. Termination Reports for the first two projects are contained in the 1989-1996 Compendium Report; a Termination Report for the 3rd project is contained in the 1996-97 Annual Progress Report; and a Termination Report for the 5th project is contained in the 2003-04 Annual Progress Report. This Termination Report is for the 6th project that began September 1, 2010.

Project Summary:

This project resulted in improved producer to consumer value-chain visibility that addressed 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.

Technical Summary and Analysis:

Market Maker™ is a computer - based multi-state agricultural - producer and agriculture - product buyer assistance tool developed by the University of Illinois Extension Service and currently co - coordinated with the ISU Extension Value Added Agriculture Program (VAAP). The national MarketMaker™ link at www.agmrc.org is hosted by the national Agricultural Resource Center (AgMRC.org) at Iowa State University.

Market Maker™ is also a coordinated GPS - mapping and product – sales - data system that allows producers, buyers, distributors, and end – users to find one another, as well

as to conduct pre-venture market research, identify potential customers, as well as potential competitors and cooperators. Each state has a unique site, but all the data from other MarketMaker™ states can be accessed from any state MarketMaker™ location.

Currently, Market Maker™ is one of the most innovative and extensive collections of searchable food – industry - related data in the country. The site continues to grow and adapt to meet the needs of all sectors of agriculture including aquaculture. Market Maker™ provides producers with the ability to identify processors, wholesalers, and retailers of fish and fish products, and buyers with the ability to query nearly two - dozen types of fish and fish products by an almost unlimited number of product attributes. The Market Maker™ site continues to being updated to give higher visibility and easier access to both aquaculture producers and products (processors and retailers). The aquaculture products include both food and non-food fish.

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 MarketMaker training. In the 2012 and 2013 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. A video depicting the actual use of MarketMaker™ for Iowa's aquaculturists can be viewed at <https://www.youtube.com/watch?v=59fcTVE6WHc&feature=youtu.be>.

Impacts:

- Aquaculture industry can now link their products with consumer base including retail and wholesale outlets.
- Materials developed in this project can inform both consumers and producers potential aquaculture products in the North Central Region (NCR).
- In December 2013 website hits noted in the five NCR states (Illinois, Indiana, Iowa, Nebraska and Ohio) ranged from 7,506 to 171,552.

Recommended Follow-Up Activities:

Given the importance of marketing to the success of aquaculture operations and the information available from the MarketMaker™ network, future projects should use information garnered from this network to enable the NCRAC Extension specialists to develop a region-wide approach to aquaculture association development.

Publications, Manuscripts, or Papers Presented:

See the Appendix for a cumulative output for all NCRAC-funded Economics/Marketing activities.

PROGRESS REPORT

Project Title: Extension⁵

Key Word(S): Extension

Total Funds Committed: \$971,625

Initial Project Schedule: May 1, 1989 to August 31, 2014

Current Project Year: September 1, 2013 to August 31, 2014

Participants:

Dennis E. Bauer, University of Nebraska-Lincoln, Nebraska
Mark E. Clark, North Dakota State University, North Dakota
James A. Held, University of Wisconsin-Stevens Point, Wisconsin
Charles E. Hicks, Lincoln University, Missouri
Paul Hitchens, Southern IL University – Carbondale, Illinois
Ronald E. Kinnunen, Michigan State University, Michigan
Charles D. Lee, Kansas State University, Kansas
D. Allen Pattillo, Iowa State University, Iowa
Burton F. Pflueger, South Dakota State University, South Dakota
Nicholas Phelps, University of Minnesota, Minnesota
Kwamena K. Quagraine, Purdue University, Illinois/Indiana
Laura G. Tiu, Ohio State University, Ohio
Christopher Weeks, Michigan State University, Michigan

Industry Liaison: Mark Willows, Binford Eagle Fisheries, North Dakota

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)

⁵NCRAC has funded a number of Extension activities, both as stand-alone projects or as components of species-or topical-specific projects, including 14 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, projects 5-13 were chaired by Joseph E. Morris and the 14 project chaired by D. Allen Pattillo. 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.

Project Summary

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 specialized 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.

Anticipated Benefits

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 encountered 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.

Project Progress

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; and
- Met with industry representatives and university researchers involved with aquaculture to discuss how the aquaculture industry could grow in the NCR.

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; and 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 on freshwater prawn and bluegill production while also conducting In-Pond-Raceways tests with cooperating farmers. Regional extension cooperators for the eXtension project (including the 20 12 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. 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.

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.

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.

Deliverables (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/oa-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>

Deliverables (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.

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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 industry issues.

Publications, Manuscripts, Workshops, and Conferences

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

PROGRESS REPORT

Project Title: Regional Aquaculture Extension Specialist (RAES)

Key Word: Extension

Total Funds Committed: \$101,820

Initial Project Schedule: September 1, 2013 to August 31, 2014

Current Project Year: September 1, 2013 to August 31, 2014

Participant(s):

Christopher Weeks, Michigan State University, Michigan

Extension Liaison: K. Quagrainnie, Purdue University, Indiana

Industry Liaison: William Lynch, Mill Creek Perch Farms, Marysville, Ohio

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.

Project Summary

Globally, aquaculture production for food fish has grown at a rate of 8.3% per year since 1970, and now meets half the world's seafood demand. However, according to USDA Census of Aquaculture, the number of aquaculture facilities in the US dropped from 4,309 in 2005 to 3,093 in 2013. Over this same time frame, the actual value of aquaculture products sold in the US increased from \$1.09 billion to \$1.37 billion, even though production volume was dramatically reduced. Gains in the North Central Region (NCR), especially for seafood production, have been slow to develop.

Support for regional baitfish, recreational stocking, and interstate live fish hauler sectors appears to be falling across the region as well. The NCR aquaculture industry is currently grossly under-funded and economic development opportunities need to be greatly enhanced. Capital investment for new aquaculture ventures is needed, but regulations are considered by many to be restrictive and negatively impacting commercial development.

Collaboration among current producers, academia and regulatory authorities needs to be improved. Continued coordinated effort is severely needed across the region in support for a growing aquaculture industry.

Anticipated Benefits

- Higher degree of information transfer on sustainable aquaculture development to the industry, the public, and state, federal and tribal agencies through list serve activity, remote learning outlets, websites and news articles.
- Continued updates on the NCRAC regulation website for easy access of interstate transport regulation

information in the NCR and surrounding states.

- A regional workshop tied in with the 2014 NCRAC Annual Program Planning Meeting.
- AIS BMPs specific for the baitfish industry. Evaluation and potential development towards a 3rd party AIS certification program for commercial aquaculture and baitfish sectors in the NCR.
- Increased partnership building for aquaculture expansion support.

Project Progress

Objective 1. —

- Continue RAES support to the North Central Region (NCR) aquaculture community through ongoing activities in areas of liaison services, leadership, assessing and addressing industry needs, and information transfer. Activities to date:
- Conducted full update to NCRAC regulation website •
- Attended MI, Tri-state (OH), and WI Association Meetings • Member of planning steering committee for Michigan Aquaculture Association meeting
- Member of planning steering committee for the NCR Aquaculture Conference, Toledo, OH
- RAES supported presentations for the NCR Aquaculture Conference included: - Should Your Farm Be Certified? (N. Stone) - The Impact of Regulations on U.S. (N. Stone) - Potential of a U.S. Aquaculture Check-off (D. Vogler) •
- Continued general outreach and information transfer efforts via phone, emails, and eXtension.org, Ask-an-Expert
- Continued information dissemination on the NCR fish culture list-serve (5

or more per week examples: news, training Federal Register post notifications and summaries).

- Conducted 2014 NCR Aquaculture Industry Needs Survey - presented results to NCRAC. Journal article in draft.
- Working with Director on potential updates to NCRAC Strategic Plan 1999 and current Problem Statement List (PSL).

Objective 2.—

- Develop and implement strategies to address aquatic invasive species (AIS) in aquaculture and baitfish in the NCR. Activities conducted:
- Serving on the Great Lakes Panel (GLP), Policy Committee, for ANS as industry representative, attended bi-annual meetings
 - Conducted 2 AIS HACCP workshops with Ron Kinnunen (NCRAC, Michigan Sea Grant)
- Recent award of \$80,000 grant through state of Michigan to explore expanding current AIS HACCP into a recognized certification/certification program. This is a NCRAC initiated program with C. Weeks as lead PI, and R. Kinnunen and N. Phelps (NCRAC, UMN) as project collaborators.
- Collaboration with Ron Johnson, Nathan Stone (National ANS Task Force), and Mike Freeze (NAA President) on current national ANS issues
- Dissemination of pertinent information to industry and supporters via NCR list serve.

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. Activities conducted:

- Serving on panels and committees of: Michigan Farm Bureau Aquaculture Advisory Committee, NSF Food Division Advisory Council Regulatory/Seafood sector, Great Lakes Panel for Aquatic Nuisance Species, Coalition of US Seafood Production (CUSP), various state and federal funding review panels
- Have established good working relationships with Soy Aquaculture Alliance, Indiana Soybean Alliance, Michigan Soybean Association
- Working with members of the newly formed National Institute of Animal Agriculture Aquaculture Advisory Committee, and in 2013 gave a presentation to this group entitled U.S. Aquaculture: the Past, Today and the Future.
- Have conducted reviews and participated in discussion on policy drafts that include: Draft Strategic Planning Documents U.S. Fish & Wildlife Service Aquatic Invasive Species Program, Policy and Research Committee Priorities for Great Lakes Panel on Aquatic Nuisance Species, and Grass Carp Policy Recommendations to GLP
- Have provided support for Vogler (MAA president) to give presentations and generate dialogue on establishing an aquaculture industry check-off program.
- In July of 2014, the RAES was offered and accepted a 50% appointment with Michigan State University Extension. In agreement

with the NCRAC Director, the RAES will continue work on regional aquaculture development, but at a 50% effort (and salary) reduction.

Target Audiences

The RAES ultimately serves the aquaculture industry in the North Central Region. However, goals and objectives require working with stakeholders from across a wide background and include the general public, state, federal and tribal agencies, legislators, NGO's, industry and social development groups, and environmental protection groups.

Outreach Overview

The RAES has had direct interaction with over 1,000 individuals over the 2013-2014 project period through various activities that include the following: Through state association meetings and conferences, the RAES has either personally presented, or provided support for presenters covering 8 different topics, to an audience count of approximately 400 individuals. Phone calls and emails – more than 300 Meetings – more than 300 indirectly, the RAES program has reached an estimated 2-3 times the above amount through activities that include, but are not limited to the NCRAC Regulation website, the NCR Fish Culture List Serve, eXtension Ask the Expert, as well as highly publicized review, including press, related to the release of the Strategic Plan for Michigan Aquaculture.

Deliverables (Outputs)

1. Information transfer to the NCR aquaculture community through 15 or more meetings and conferences, the NCR Fish Culture list serve (141 subscribers) and the NCRAC regulation website.
2. Two AIS HACCP workshops.

3. Support in planning, effort, funding and presentations for the North Central Region Aquaculture Conference, Toledo OH, Feb 2014.
4. North Central Region Aquaculture Needs Survey results disseminated to NCRAC members and used for 2014 and 2015 program planning activities. Potential extension article is in draft form and planned for submission to JOE in winter 2014-2015.
5. A Strategic Plan for a Thriving & Sustainable Michigan Aquaculture. The final report has been submitted to Michigan Sea Grant and will be made available on their website in the near future. A draft of this report is available at:
<http://michiganaquaculture.org/strategic-plan/strategic-plan-for-michigan-aquaculture/>

Outcomes/Impacts

Due to complexities associated with advancing finfish aquaculture in the US, measurable outcomes from RAES activities are difficult to assess. Examination of the 2013 aquaculture census (USDA 2014) shows the value of NCR aquaculture increased from \$35.4 million in 2005 to \$36.7 million in 2013 (3.74%), although correlation to the RAES project is not possible. The 2014 NCR aquaculture survey received a good response rate (37%) suggesting that respondents believe NCR aquaculture development is important. Results of the survey were presented at the 2014 annual NCRAC meeting and utilized in discussions regarding NCRAC priority selection and strategic plan revision. Regulations were considered a top impediment to industry development according to the survey. NCRAC's State Import Regulations website (<http://www.ncrac.org/node/378>) continues to be utilized to a great extent by private and

public aquaculture related entities. Two AIS HACCP workshops were presented in Michigan in 2014 with each receiving good to excellent evaluations. Currently AIS policy issues do not appear to be a priority for the majority of aquaculture producers, although implications could be significant in the near future. The AIS HACCP verification project underway is expected to elevate discussion on AIS issues among industry and agency personnel and partner agency and industry in AIS prevention. Through great combined effort, the 2014 North Central Aquaculture Conference was considered a great success with over 170 in attendance, providing an average overall evaluation score of 3.3 out of 4.0. Presentations received an average score of 3.6.

Impacts Summary

Relevance— Production in the NCR has been slow to develop, and support for regional baitfish, recreational stocking, and interstate live fish hauler sectors appears to be falling. Current and pending state and federal regulations are not stable and severely impede profitable aquaculture trade within the region. Participation among current producers, academia and regulatory authorities needs to be improved, and the general public remains relatively uneducated and often misinformed of quality, availability and safety of seafood products raised in the NCR. Continued coordinated effort is severely needed across the region in support for a growing aquaculture industry.

Response.— The RAES project provides support directly to the aquaculture industry in areas including but not limited to: regulation clarification, liaison between regulators and industry, workshops and training, and information dissemination. As a liaison, the RAES also sits on a variety of committees and panels, whose decisions and

actions have direct or indirect impacts on the industry.

Results— The summarization of all NCR state regulations and provisional aquaculture contacts for each state allows for rapid clarification and improved efficiency for routine operations within the industry. Through coordinated effort we have been successful in providing information and training to practicing and potential aquaculturists. This should in turn result in greater economic returns from aquaculture, a lower failure rate in industry startups, and reduce the risk (real and perceived) associated with obtaining capital for aquaculture businesses.

Recap— The RAES project is helping to coordinate research and outreach activity within NCRAC (and the NCR) and provide a wide range of stakeholders important information necessary to further the development of the aquaculture industry in the region and nation.

Publications, Manuscripts, Workshops, and Conferences

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

Technical Update:

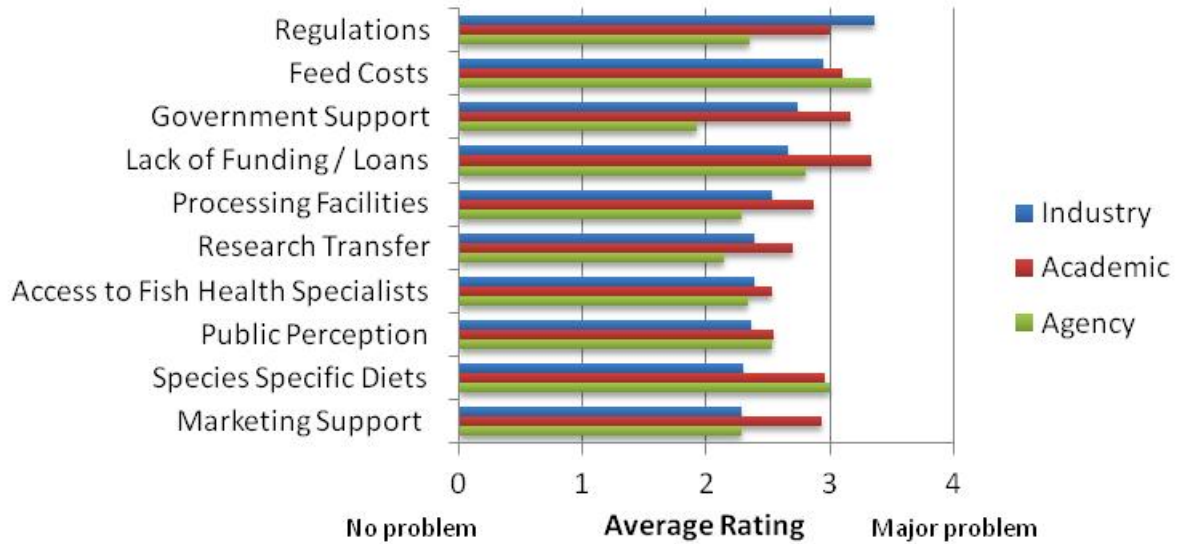


Figure 1. Average impediment ratings to aquaculture development in the North Central Region by industry, academic and state and federal agency groups (data from 2014 NCR aquaculture survey).

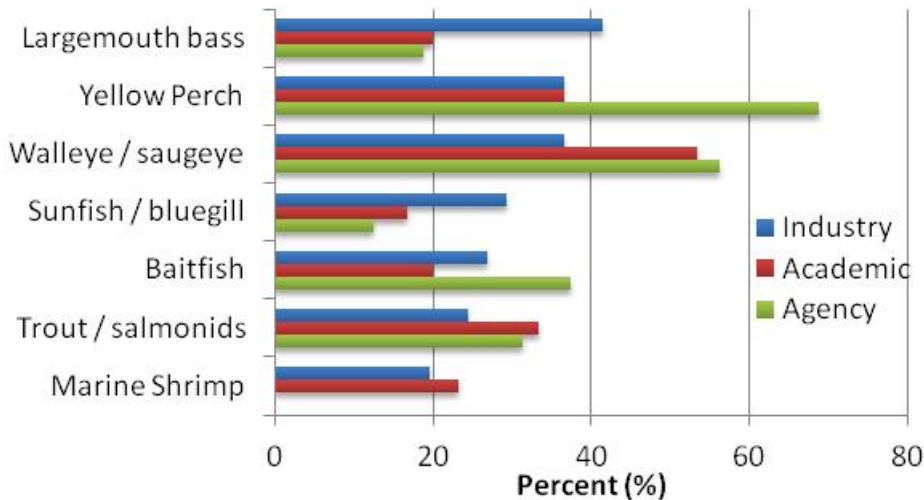


Figure 2. Species according to respondents having best potential to substantially increase NCR aquaculture in the next five years (data from 2014 NCR Aquaculture survey).

PROGRESS REPORT

Project Title: 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

Key Word(s): Nutrition/Diets

Total Funds Committed: \$75,000

Initial Project Schedule: September 1, 2012 to August 31, 2013

Current Project Year: September 1, 2013 to August 31, 2014

PARTICIPANTS:

Christopher F. Hartleb, University of Wisconsin-Stevens Point, Wisconsin
Charles E. Hicks, Lincoln University, Missouri
James Wetzel, Lincoln University, Missouri

Extension Liaison: Charles E. Hicks, Lincoln University, Missouri

Industry Liaison: Paula Moore, Jones & Eaker Farms, Missouri

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.

Project Summary

Growth in the North Central Region's (NCR) aquaculture industry mirrors, and is driven by, broader U.S. and worldwide changes in the seafood industry. However domestic aquaculture production has remained about the same for the last five years (NMFS 2009). Aquaculture-related business in the NCR continues to be an "emerging" industry in that selection of appropriate species and associated culture practices including feed selection is evolving. As with any animal industry, feed costs can be a considerable component. Feeds often account for $\geq 50\%$ of the variable costs in aquaculture budgets. To reduce variable costs there have been numerous research efforts in the NCR as well as nationally addressing the possible uses of lower-cost foodstuffs, e.g., vegetable or animal by-product as a major component of fish feeds. Clearly, substantial need exists to reduce costs and develop nutritionally adequate diets for Sunfish and other species cultured in the NCR.

Anticipated Benefits

Feed costs often represent 55% of fish farmers' indirect costs and as the cost of fish meal increases there is a need to develop and verify feeds that have alternate and less costly animal protein sources. This study used a feed (Hayward) in which 90% of the protein was supplied by poultry by product meal. The cost of poultry by product meal is much less than fish meal and will represent reduced indirect costs to the fish farmer.

Project Progress

Researchers from two NCR universities, Lincoln University of Missouri (LU) and the University of Wisconsin- Stevens

Point (UW-Stevens Point) compared age-2 bluegill production at densities of 7,674 sunfish/ha (2,800/acre) using two diets, the recently developed open formula versus an industry standard diet (40% crude protein and 10% lipids); both diets were produced by one common facility and distributed among the two locations. The standard diet is a commercial trout chow and the test diet is the open formula diet (Appendix 1) developed by Robert Hayward, University of Missouri-Columbia.

Earthen ponds at LU and UW-Stevens Point (0.10-ha; 0.25-acre) were used for part or all of the study described below. Fish were stocked in (LU) ponds (6) the last week of March and feeding commenced 1 April 2012. Feeding rings were placed in each pond and fish were fed by hand twice daily except once on Saturday and no feeding on Sunday. Dissolved oxygen and water temperature were recorded daily and water quality measurements were conducted weekly.

All fish at (LU) were harvested 10/25-26/2012. Total pond fish weights and numbers were determined and 105 fish from each pond were individually weighed and measured. Fillet weights, liver, viscera, and gills and viscera weights were determined for each fish. Thirty (30) fillet samples were taken from pond and pooled. Eighty(80) gram subsamples of fillet tissue from each pond and submitted to a Laboratory at the University of Missouri for moisture, crude protein, lipid and ash analysis. A summary of the information is included in Table 1.

Target Audiences

The target audience is active fish farmers or aquaculture producers in the North Central

Region producing sunfish and sunfish hybrids.

Outreach Overview

A presentation about the diets, procedures involved and results from Wisconsin portion of the study was given at the annual Wisconsin Aquaculture Conference. Also, over the next 12 months, the final report for this project will be submitted to NCRAC for public distribution and a technical bulletin along with a publication will be submitted to a peer-reviewed scientific journal for broad public distribution. eXtension Webinar-Nov. 2012 Bluegill Sunfish Nutrition workshop-Comparison of Sunfish Diets-Yellow Springs, Ohio NCRAC Planning Meeting-Summary of Sunfish Research in North Central Region.

Deliverables (Outputs)

Results of the production field trials resulted in changes in knowledge based on the following: Overall, significant differences in growth, production and survival were not great enough or consistent enough to recommend either of the two diets as being superior to the other based on production only. This is important to note since the open formula diet was designed to be less expensive to manufacture because of its lower fishmeal content.

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 available 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, Manuscripts, Workshops, and Conferences

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

Technical Update:

Table 1. Summary of Phase II bluegill production and dressout data.

Location	Pond	TRT	Stocked Wt.	End Wt. (kg/ha)	Gain	Stocked (#/ha)	Harvest (#/ha)	% Surv.	Mn Individual Wt (0.1g)	TL mm	% On-Round	% Fillet
LU	4	IS	188.4	1263.4	65.2	7672	5502	71.7	238.5	207	89.5	23.5
LU	7	IS	189.9	1353.1	81.7	7672	5973	77.9	229.0	204	88.7	22.6
LU	12	IS	202.1	1316.2	62.1	7672	5677	74.0	241.2	207	88.9	20.6
LU	8	LC	184.6	1539.4	124.4	7672	6751	88.0	226.2	203	89.2	24.6
LU	9	LC	190.7	861.9	-17.7	7672	3650	47.6	228.2	203	89.7	21.3
LU	11	LC	171.6	1027.7	34.7	7672	4680	61.0	221.1	204	89.2	24.3
WI	PB	LC		753.7	41.3	7672	3498	45.6	214.7	201		38.6
WI	PR	IS		753.1	39.8	7672	3314	43.2	229.5	201		35.5
WI	PF	LC		1124.8	56.0	7672	6537	85.2	171.5	225		37.4
WI	B3	LC		599.3	45.0	7672	6475	84.4	185.3	196		36.5
WI	B4	IS		561.8	38.5	7672	5171	67.4	212.8	201		36.4
WI	B5	IS		603.8	39.3	7672	4135	53.9	208.9	200		36.7

Table 2. Summary of bluegill fillet proximate composition.

Location	Pond	TRT	Moisture	%CP	%LIPID	%ASH	LSI	VSI
LU	4	IS	76.6	20.9	1.9	1.3	1.8	8.1
LU	7	IS	78.0	20.1	1.3	1.3	1.7	8.5
LU	12	IS	76.5	21.0	1.7	1.7	1.7	8.9
LU	8	LC	77.4	20.7	1.3	1.3	1.9	7.9
LU	9	LC	77.7	20.3	1.5	1.4	1.9	7.9
LU	11	LC	76.5	20.8	2.1	1.5	1.9	8.1
WI	PB	LC	78.3	18.7	2.7	1.1		11.8
WI	PR	IS	78.1	18.3	3.1	1.1		11.9
WI	PF	LC	79.2	18.2	2.0	1.2		12.4
WI	B3	LC	77.8	18.7	3.0	1.2		11.9
WI	B4	IS	77.9	18.9	2.8	1.2		11.9
WI	B5	IS	77.3	19.3	3.1	1.2		11.8

VSI= Visceral Somatic Index LSI= Liver Somatic Index

PROGRESS REPORT

Project Title: Assessment of Carbon Dioxide (CO₂) and Inorganic Nitrogen Compounds To Enhance Winter Kill In Natural Rearing Ponds Used For Fish Production In The North Central Region

Key Word(s): Other

Total Funds Committed: \$175,000

Initial Project Schedule: September 1, 2011 to August 31, 2013

Current Project Year: September 1, 2013 to August 31, 2014

PARTICIPANTS:

Konrad Dabrowski, The Ohio State University, Ohio

Mark Gaikowski, USGS Upper Midwest Environmental Science Center, Wisconsin

Jason Gross, UUSGS, Northern Rocky Mountain Science Center (NOROCK),
Colorado

Extension Liaison: Jeffrey Gunderson, Minnesota Sea Grant, Minnesota

Industry Liaison: Gregory Oswald, Ellendale, Minnesota

Project Objectives

1. Conduct a literature review to summarize the toxic effects of carbon dioxide (CO₂) and inorganic nitrogen compounds (e.g. N₂, NO₂⁻, NH₃, etc.) 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 CO₂ or inorganic nitrogen compounds to enhance winter kill conditions.
4. Determine, through laboratory study, application rates required of CO₂ or inorganic nitrogen compounds to enhance winter kill conditions to remove unwanted fish from natural rearing ponds. Studies required for the registration of CO₂ or inorganic nitrogen compounds to enhance winter kill conditions will be conducted according to GLP regulations (40CFR160).
5. Evaluate, through laboratory pond experiments, the efficacy of laboratory-derived application rate data for CO₂ or inorganic nitrogen compounds to enhance winter kill conditions.
6. Collect late winter water chemistry condition data in representative NCR natural rearing ponds

7. Obtain an experimental use permit (EUP) from the EPA and appropriate state regulatory agencies to conduct experimental applications of CO₂ or inorganic nitrogen compounds, singularly or in combination, to enhance winter kill conditions in natural rearing ponds to remove populations of unwanted fish.
8. Compile data into final study reports suitable for submission to the EPA to support potential approval of CO₂ or inorganic nitrogen compounds to enhance winter kill conditions.
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. Summarize results into appropriate extension materials for dissemination to NCR aquaculturists.

Project Summary

Natural winter kills effectively eliminate unwanted fish species from commercial rearing ponds, saving producers time and money. However, winter kill conditions occur sporadically and are difficult to predict. Eliminating unwanted species (e.g., bullhead, carp) by enhancing natural winter kill conditions can increase harvest and productivity of desired cultured species. Identification of methods that consistently and inexpensively enhance natural winter kill conditions is needed to enhance this natural process and ensure winter kill conditions are reliably achieved in natural rearing ponds.

Anticipated Benefits

The proposed studies include determining thresholds of the combined effects of CO₂/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 (O₂ and N; CO₂ 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 undesirable 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.

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. — The literature review for the use of CO₂ 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 CO₂, its use and efficacy.

Objective 2.— Calculations completed and both CO₂ 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.— This has been completed.

Objective 4. — CO₂ - Studies have been completed. A significant interaction between treatment and species was observed. The interaction was a result of differences in mortality between species at specific treatment levels. In the common carp (CAP) and channel catfish (CCF) trials, mortality in both the 380 ppm CO₂ and 495 ppm CO₂ treatment groups was significantly higher than in the control, 75, 150, 225, and 300 ppm CO₂ groups, but mortality in the 495 ppm CO₂ treatment was higher than in the 380 ppm CO₂ treatment. There was no difference in mortality between the 225 ppm CO₂ and 300 ppm CO₂ treatment groups in the CCF trials. There were no differences in mortality in any treatment groups between CAP and CCF. Inorganic

N - Studies have been completed. 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 NH₃-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 NH₃-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 NH₃-N. However, during exposure to 0.26 ± 0.02 ppm NH₃-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. Objective

Objective 5. — Studies have been completed. 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 nine 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 CO₂. Ponds treated with CO₂ 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.—Completed - Long term ice cover was never present in central Ohio and therefore winterkill conditions were not observed.

Objective 7.— Completed - No experimental use permit will be obtained.

Objective 8.— Completed - Data is currently archived and being held by USGS for potential support for registration.

Objective 9.— Completed - No extension material made since neither compound will be registered.

Target Audiences

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

Outreach Overview

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

Deliverables (Outputs)

A simple method for delivering CO₂ or ammonia below ice was developed.

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

Issue— The approval of CO₂ 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.

Response — Approval of CO₂ 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 CO₂ and ammonia. Trials have been completed.

Results— CO₂ infusion under ice was effective in eliminating channel catfish and common carp during under ice exposures.

Recap— CO₂ may be viable alternatives to the use of rotenone for the removal of fish remaining in ponds during late winter

Publications, Manuscripts, Workshops, and Conferences

See the Appendix for a cumulative output for all NCRAC-Funded Other activities

PROGRESS REPORT

Project Title: Snail Management/Grub Control⁶

Key Word(s): Other

Total Funds Committed: \$20,500

Initial Project Schedule: September 1, 2007 to August 31, 2009

Current Project Year: September 1, 2013 to August 21, 2014

Participants:

Richard D. Clayton, Iowa State University, Iowa

Christopher F. Hartleb, University of Wisconsin-Stevens Point, Wisconsin

Todd Huspeni, University of Wisconsin-Stevens Point, Wisconsin

Joseph E. Morris, Iowa State University, Iowa State University

Gregory W. Whitley, Southern Illinois University-Carbondale, Illinois

Extension Liaison: Joseph E. Morris, Iowa State University, Iowa

Industry Liaison: Rex Ostrum, Nebraska

Project Objectives

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

⁶ 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. Whitley. It began September 1, 2007.

Project Summary

With the long history of trying to control snails in fish ponds and types of control mechanism being used (mechanical/chemical/predatory), a literature review was undertaken to compile the information to date. This list of references is from journal articles only. The references are sub-divided by the type of control that the researcher's evaluated or commented on: mechanical, chemical, or biological control. This information was then combined with information garnered from this project to produce a detailed presentation that is now available to North Central Region (NCR) aquaculture community.

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.

Project Progress

Objective 2. — 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 was combined with information garnered from this research project to develop detailed on-line presentation for 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., zooplankton dynamics in fish fingerling ponds, will be listed. The detailed presentation 'Snail Management in Culture Ponds' is hosted on the revised North Central Regional Aquaculture Center (NCRAC) Web site – Aquatic Biological Management

<http://www.ncrac.org/node/627> . The literature review 'Review of Snail Control in Fish Ponds' is also available on the same web site. Additional information on grubs in freshwater fish is available in the NCRAC Technical Bulletin #115 'Biology, Prevention, and Effects of Common Grubs (Digenetic trematodes) in Freshwater Fish', <http://www.ncrac.org/node/354> .

Since the initiation of this project, an updated factsheet on snails and associated grubs has been developed at Texas A&M University and is available at <http://fisheries.tamu.edu/files/2013/09/My-fish-have-grubs-final.pdf> .

Target Audiences

NCR pond culturists.

Outreach Overview

To achieve the goal of this objective, information on snail control methods has been provided by presentations (2014 North Central Regional Aquaculture Conference, Toledo, Ohio) and materials available on the NCRAC web site.

Deliverables (Outputs)

- Literature review of past and current literature on snail control methods
- PowerPoint presentation on snail management
- Web-based information portal on snail identification and control methods

Outcomes/Impacts

Results from this project have been used to provide an updated review of snail control methods available to the aquaculture community. Although to date there is no one ideal management solution to controlling the snail population in aquaculture ponds, aquaculturists should consider the following:

- Prevention of snail infestation when possible.
 - Use of approved aquatic herbicides or grass carp (consult state-specific regulations to decrease the amount of submerged vegetation including filamentous algae.
 - Drying pond bottoms between crops.
 - Use of flow in side tanks to limit infestations.
- Use care in the use of chemical controls as many will affect the cultured fish directly.
 - Chemical treatments often effective along pond margins but small fish may be susceptible.
 - Use of lime to modify water pH can affect both adult fish and their offspring through direct and indirect effects as well as low oxygen levels associated with decaying vegetation.
 - Applications copper sulfate (combined with citric acid) are effective control measures but can direct or indirect effects on cultured fishes; previous research notes the effect of 0.25 mg/L of copper sulfate can kill desired zooplankton prey for larval fishes.
- Prior to chemical control use, culturists need to check total alkalinity of the culture ponds to help decrease deleterious effects from lime or copper sulfate applications.
- Consider use of biological controls for long-term controls.
 - Redear Sunfish are effective in controlling Physa but not rams-horn snails until they are fully matured.
 - Use of hybrid Redear Sunfish can help to reduce snail populations over the culture period.
 - Other possible predators include crawfish, blue catfish, freshwater drum and freshwater prawns.

Impacts Summary

Issue— There is a need for current information for controlling snails in culture ponds given their role in grub infestation in fish.

Response— A literature review of snail control methods was conducted. Information from this review was combined with information garnered from this project was combined into a summary report.

Results— Information is now available on the NCRAC web site for public information.

Recap—In response to a need to inform the aquaculture community, a literature review combined with a detail presentation was developed on snail management.

**Publications, Manuscripts, Workshops,
and Conferences**

See the Appendix for a cumulative output
for all NCRAC-Funded Other activities.

PROGRESS REPORT

Project Title: Developing Genetically Fast-Growing Monosex Populations in Bluegill

Key Word(s): Sunfish

Total Funds Committed: \$160,000

Initial Project Schedule: September 1, 2013 to August 31, 2015

Current Project Year: September 1, 2014 to August 31, 2014

PARTICIPANTS:

Charles E. Hicks, Lincoln University, Missouri
Han-Ping Wang, The Ohio State University, Ohio
James Wetzell II, Lincoln University, Missouri

Extension Liaison:

Charles E. Hicks, Lincoln University

Industry Liaison:

Curtis Harrison, Harrison Fisheries, Inc., MO

Project Objectives

1. Identify additional super - males and performance - selected females from existing populations.
2. Create all - male bluegill populations by crossing super - males with females of selected and non - selected stocks.
3. Rear populations at two or more locations in the NCR.
4. Compare sex ratios and production characteristics of sub - populations as based on maternal stocks.

Deliverables

1. Characterize the performance characteristics and sex ratios of super - male/performance - selected cross.
2. Characterize the economic cost benefits of culturing the super - male/performance - selected cross.
3. Publication of results in journal article, and extension publications (i.e., factsheets, research tours).

Project Summary

Improving the growth rate and broodstock of bluegill and its hybrids has been ranked as one of the top priorities in USDA-NCRAC. The proposed research will specifically address the needs identified by that agency. The results of this research can be expected to advance our understanding of sex-determining mechanisms in fish. Further, using this information, we expect to be able to obtain super male broodfish. By the completion of the proposed research, we expect to generate genetically fast-growing all-male populations by crossing super males with genetically improved females. Not only will a monosex culture be expected to produce the greatest biomass in a given period of time, but also all male bluegill culture may promote growth by reducing the metabolic cost of sexual growth and reproduction. This will benefit fish farmers by increasing the efficiency and profitability of sunfish aquaculture production in the U.S.

Anticipated Benefits

Improving the growth rate and broodstock of bluegill and its hybrids has been ranked as one of the top priorities in USDA-NCRAC. The proposed research will specifically address the needs identified by that agency. By the completion of the proposed research, we expect to generate genetically fast-growing all-male populations by crossing super- males with genetically improved females. These outcomes will enable us to develop GMB-producing broodstock and mass production of monosex populations. Not only will a monosex culture be expected to produce the greatest biomass in a given period of time, but also an all-male bluegill culture may promote

growth by reducing the metabolic cost of sexual growth and reproduction.

Therefore, this will benefit fish farmers by increasing the efficiency and profitability of sunfish aquaculture production in the U.S. The impact of this project will be primarily via the delivery of fast-growing all-male bluegill population to fish farmers in Ohio, the Midwest, and other states. The greatest return on investment for this project is the ultimate reduction in production costs due to increased growth rate and reduced feed costs. A successful creation of genetically male bluegill strains would have a tremendous impact on the sunfish aquaculture industry by increasing growth rate of 30-35% (Wang and Hayward, 2006) and saving energy expenditure of 20-30% for sex growth.

Project Progress

Objective 1. — Progeny test for all-male populations using improved fish from Lincoln University of Missouri was tried; temperature effects on sex ratio have been found in some geographic populations: producing more males in high temperature, more females in low temperature. The findings were published in AQUACULTURE. Follow-up investigation using four different geographic populations strongly suggests that both temperature-dependent sex determination (TSD) and genetic sex determination (GSD) exist in bluegill.

Target Audiences

Aquaculture Farmers.

Outreach Overview

Nothing to report yet

Deliverables (Outputs)

Nothing to report yet

Outcomes/Impacts

The impact of this project will be primarily via the delivery of fast-growing all-male bluegill populations to fish farmers in Ohio, the Midwest, and other states. The greatest return on investment for this project is the ultimate reduction in production costs due to increased growth rate and reduced feed costs. A successful creation of genetically male bluegill strains would have a tremendous impact on the sunfish aquaculture industry by increasing growth rate of 30- 35% and saving energy expenditure of 20-30% for sex growth

Impacts Summary

Relevance —Despite this opportunity, rapid expansion of the bluegill aquaculture industry has not occurred in this country. One reason in particular hindering expansion has been the relatively slow growth of currently cultured populations of this species.

Response — Monosex culture would hold considerable potential as a method to increase the efficiency and profitability of bluegill food aquaculture by improving growth rate, and eliminating the problem of prolific reproduction, precocious maturity and their consequences. We have started a project to create all-male bluegill populations by crossing super-males with females of selected and non-selected stocks.

Results— A successful creation of genetically male bluegill strains would have a tremendous impact on the sunfish aquaculture industry by increasing growth rate of 30-35% and saving energy expenditure of 20-30% for sex growth.

Recap — The greatest return on investment for this project is the ultimate reduction in production costs due to increased growth rate and reduced feed costs.

Publications, Manuscripts, Workshops, and Conferences

See the Appendix for a cumulative output for all NCRAC-Funded Sunfish activities.

TERMINATION REPORT⁷

Project: Viral Hemorrhagic Septicemia (VHS)

Key Word(s): Viral Hemorrhagic Septicemia (VHS)

Dates of Work: September 1, 2008 to August 31, 2014

Total Funds Committed: \$197,960

Participants:

Glenda D. Dvorak, Iowa State University, Iowa State University

Ronald R. Kinnunen, Michigan State University, Michigan

Jeffrey A. Malison, University of Wisconsin-Stevens Point Northern Aquaculture Demonstration Facility, Wisconsin

Jeffrey J. Rach, USGS Upper Midwest Environmental Sciences Center, Wisconsin

Extension Liaison: Ronald E. Kinnunen, Michigan State University, Michigan

Industry Liaison: Christopher Weeks, Rives Junction, Michigan

Reason for Termination: Project objectives completed and funds have been terminated.

⁷This 2-year funded project is chaired by Jeffrey A. Malison and it began September 1, 2008.

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.

Project Summary

Cool and warmwater fish eggs are cultured in large numbers at private, state, and federal hatcheries. The introduction of VHS in natural waters has caused extensive fish mortalities and the virus may be spread to other fish throughout the U.S. by the exchange of fish or their eggs among commercial fish suppliers or state and federal hatcheries. Fish culturists want to avoid introducing diseases from wild brood stock to their captive fish populations. If VHS would infect fish in an aquaculture facility, all fish would probably have to be destroyed and the facility would have to be disinfected.

This project evaluated the use and safety of iodophor disinfection of fish eggs for 3 species of fish. In the first study, VHS-challenged walleye and northern pike eggs were disinfected with iodophor for different lengths of time, and then checked for the presence or absence of the VHS virus. In the second trial, wild caught lake herring were spawned and their eggs disinfected with iodophor. They were cultured to determine if the disinfection process affected survival.

These trials were important since the threat of VHS to the aquaculture industry is potentially devastating. Following detection of VHS, direct losses can occur from facility closures, the restriction of movement orders and quarantines (Bebak 1998). Although some fish may survive an outbreak, they may have reduced growth rates, lower yields, or reduced product quality, which also impacts market value. A facility's reputation may also be damaged following identification of a disease on the premises.

The threat of VHS, as well as other aquatic animal diseases, to the aquaculture industry is potentially devastating. Direct losses can occur following the death of fish from the disease, facility closures, and possible

restriction of movement orders or quarantines following detection of a disease.

One approach to this problem is to apply the Hazard Analysis and Critical Control Point (HACCP) concept similar to that used by the seafood industry to minimize seafood consumption health risks. The advantages of this system are that it can effectively deal with a diverse industry, it has proven to be a good partnership between industry and government regulators, and it is effective when properly applied. The HACCP approach concentrates on the points in the process that are critical to the safety of the product, minimizes risks, and stresses communication between regulators and the industry.

Technical Summary and Analysis

Objective 1.— Tests with walleye and northern pike were conducted at the Upper Midwest Environmental Sciences Center (UMESC). Adult walleye and northern pike collected by the Genoa National Fish Hatchery from the Upper Mississippi River, transported to UMESC where eggs and semen were collected. Fertilization occurred in a secure lab where eggs were divided into two equal groups and exposed to the VHSV (105 or 108 PFU/mL) immediately concurrent with sperm activation. VHSV (strain IVb) used was isolated by the USFWS La Crosse Fish Health Center (LFHC) from emerald shiners, *Notropis atherinoides*, collected from Lake Erie in 2006. Eggs were challenged with VHSV for 30 min. Walleye egg adhesion was reduced by immersing the eggs in a bentonite solution for approximately 2 minutes during VHSV challenge then rinsed with well water and then placed back into the virus for the remainder of the challenge.

Fertilized VHSV-challenged eggs (~25 mL/jar) were divided among 48 egg jars from each of the four treatment groups, of which six jars were from each of the two

virus titer. One of four treatments was assigned to one side of each of the four systems (two VHSV virus titers per system). Eggs were equally divided into four disinfection treatment groups: (1) a VHSV-challenged nontreated control group; 2) a VHSV-challenged group which received a 30 min-100 mg/L iodophor disinfection 30 min after fertilization; 3) a VHSV-challenged group which received a 60 min-100 mg/L iodophor disinfection 30 min after fertilization; or 4) a VHSV-challenged group which received a 10 min-100 mg/L iodophor disinfection 90 min after fertilization. Eggs were maintained in egg jars until hatch with no other chemical treatments applied. Egg and fry samples were collected and the presence or absence of VHSV determined using the USFWS Standard Procedures for Aquatic Animal Health Inspections/American Fisheries Society Fish Health Section Blue Book (2007) procedures.

VHSV was not isolated from any iodophor-disinfected treatment groups. However, VHSV was isolated from control eggs immediately after challenge and for up to four days after challenge in northern pike eggs challenged at 108 PFU/mL. The virus was not detected in positive control eggs one day post-challenge for either northern pike or walleye eggs challenged at 105 PFU/mL and was not detected in fry of either control or iodophor-disinfected treatment groups. Though some iodophor treatments reduced hatch, eggs and fry appeared to develop normally. Iodophor disinfection did not substantially reduce northern pike egg hatch but walleye egg hatch was reduced when eggs were held for 30 or 60 min in the iodophor disinfection solution. 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), iodophor disinfection did not alter egg hatch. Incorporation of iodophor disinfection at 100 ppm during gamete collection from non-salmonid fishes immediately post-fertilization (<5 min) for 30 min or at 90 min after fertilization for 10 min may reduce VHSV (strain IVb) transmission without affecting egg hatch.

Personnel from Northern Aquaculture Demonstration Facility (NADF) spawned wild lake herring from Lake Superior and then disinfected the eggs on the boat. Fertilized eggs from three separate matings were split into four disinfection schemes, resulting in three groups of eggs for each disinfection assignment. Control eggs were water hardened and rinsed with well water. Treated eggs were disinfected with iodine as follows: 50 mg/L for 30 min or 100 mg/L for 30 min or 100 mg/L for 15 min. Eggs were transported in separate labeled containers in coolers to UMESC. Samples of ovarian fluid, fertilized eggs, and the spawned fish themselves were collected by NADF personnel, separately bagged and transported to LFHC for VHS testing; all samples tested were negative for VHS.

Treated eggs received a second iodophor disinfection (100 mg/L for 10 min) upon arrival at UMESC before placement in egg jars (~25 mL of eggs measured by volume displacement per jar. A sample of eggs for VHS testing was taken from each group. The control eggs, processed last, were sham treated (placed in rearing water for 10 min) then placed in assigned jars.

Saprolegnia hyphae were observed on dead eggs 7 d after receipt at UMESC, subsequently seven once-daily hydrogen peroxide treatments (500 mg/L for 15 min) were administered to all egg jars (including controls) over a 15-d period. Eye spots were visible in viable embryos at 19 d post fertilization; the percentage of embryos with

a visible eye spot was determined at 24 d post fertilization. All viable embryos (i.e., those with a visible eye spot) were transferred back to NADF at 25 d post fertilization.

The mean percent of embryos with a visible eye spot at 24 d post fertilization were: control - 76.2% (65.4 – 82.2%); iodophor disinfection at 50 mg for 30 min - 69.3% (63.4 – 80.2%); iodophor disinfection at 100 mg for 15 min - 69.2% (62.2 – 80.9%); iodophor disinfection at 100 mg for 30 min - 80.43% (74.5 – 81.5%). Iodine disinfection of lake herring eggs did not adversely affect eye-up of the eggs. Shipment of eyed lake herring eggs did 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 were 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.

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.

Objective 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 video and model HACCP plans will be posted by ISU for free access on the CFSPH and Focus on Fish Health Web sites as well as the NCRAC web site.

Principal Accomplishments

Objective 1.— Incorporation of iodophor disinfection at 100 ppm during gamete collection from northern pike and walleye immediately post-fertilization (<5 min) for 30 min or at 90 min after fertilization for 10 min may reduce VHSv (strain IVb) transmission without affecting egg hatch.

Iodine disinfection of lake herring eggs for at 50 mg/L or 100 mg/L for 30 min did not affect eye-up of the eggs. This trial also showed that shipment of eyed lake herring eggs did not affect survival.

Objectives 2-6.— The combination of workshops combined with HACCP Plans have resulted in increased awareness of and associated strategies in dealing with current and future diseases as well as improvement in biosecurity.

Impacts

- The project demonstrated that iodophor disinfection may safely and effectively reduce the risk associated with VHSV exposure during spawning/egg take operations from wild brood fish.
- The project demonstrated that coolwater fish eggs retain VHSV for up to 4 days following immersion challenge but that eggs may not retain VHSV through egg hatch (all fry, including controls, were VHSV negative).
- Caution should be used when disinfecting eggs of species whose eggs have not been evaluated for potential sensitivity to iodophor disinfection.
- 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.
- Through workshops and educational materials on biosecurity, farmers have become aware of the risks and potential hazards diseases from outside sources bring.
- 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.

Recommended Follow-Up Activities

Information garnered from this project should be used to guide future disease related projects. The combination of a basic research approach to address specific diseases and extension projects that address biosecurity issues can result in improved industry responses for managing future diseases.

Publications, Manuscripts, or Papers Presented

See the Appendix for a cumulative output for all NCRAC-funded Viral Hemorrhagic Septicemia (VHS) activities.

PROGRESS REPORT

Project Title: Determination of Production Parameters of Selected Yellow Perch Lines at Commercial Densities in Ponds at Two or More Facilities in the North Central Region

Key Word(s): Yellow Perch

Total Funds Committed: \$150,000

Initial Project Schedule: September 1, 2010 to August 31, 2013

Current Project Year: September 1, 2013 to August 31, 2014

Participants:

Christopher F. Hartleb, University of Wisconsin – Stevens Point, Wisconsin

Laura G. Tiu, The Ohio State University, Ohio

Geoffrey K. Wallat, The Ohio State University, Ohio

Han-Ping Wang, The Ohio State University, Ohio

Extension Liaison: Geoffrey K. Wallat, Ohio State University, Ohio

Industry Liaison: Charles E. Hicks, New Bloomfield, Missouri

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.

Project Summary

Yellow perch has its unique niche market in the Great Lake Region and the North Central Region (NCR). Despite this opportunity, rapid expansion of the yellow perch aquaculture industry has not occurred in these regions. One reason in particular hindering expansion has been relatively slow growth of currently cultured populations of this species. Using current yellow perch strains, only 60% of the fish cultured in aquaculture operations reach market size in a normal growth cycle (16 months), with the rest being below market size. This is an inefficient use of resources, feed, and operational costs, and leads to marginal profits at best. Therefore, improving and promoting yellow perch growth and aquaculture using new technology will significantly improve the profitability of fish farmers. Genetic improvement of aquaculture species offers a substantial opportunity for increasing production efficiency, health, production quality and, ultimately, profitability in aquaculture industries. The Ohio State University has developed genetically improved yellow perch. On-station and on-farm tests are important steps for commercialization of genetically improved strains.

Anticipated Benefits

The impact of this proposed project will be primarily through the delivery of superior yellow perch strains to farmers for use in a wide range of culture and exposure conditions across the NCR. The greatest return on investment for this project is the ultimate reduction in production costs due to increased growth rate and reduced feed costs by using genetically improved strains. 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.

Project Progress

Objective 1.—

Ohio: Researchers conducted replicated tests of the selected line of fish vs. the local-strain using two types of rearing tests: 1) at the Piketon Station (selected line and a local-strain were reared in separate ponds, each having two replicates) and at Mill Creek (MC) Perch Farm (selected line and a local-strain were raised communally). Eight molecular markers were used to assign selected and local-strain yellow perch to their family of origin for communal rearing.

In the first year the selected line of fish grew significantly larger than local perch native to the farm in two communal ponds at MC Perch Farm. The selected line fish 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, the selected line 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 selected line fish resulted in a significantly higher density and lower feed rations, these fish still had a higher mean body weight than the local Ohio strain. Wisconsin: Approximately 15,000 feed-trained selected line fingerlings were hauled from OSU Piketon Research Facility to the WI NADF with <1% mortality. All ponds were harvested in October of year 1. The selected line fish from two ponds averaged 125.1mm (4.93 in) and 25.6 g (0.90 oz) and 120.3 mm (4.74 in) and 22.5 g (0.79 oz),

respectively. The WI-strain perch from two ponds averaged 118.6 mm (4.67 in) and 20.1 g (0.71 oz), and 111.8 mm (4.65 in) and 17.9 g (0.63 oz), respectively. Average fingerling survival was 92.0% for selected line and 72.0% for WI local strain. Although the 20.0% higher survival rate of the selected line 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), they still grew 26.60% faster than the unimproved fish.

Objective 2.—

Ohio: For MC Perch Farms, out of 240 fish that were family-origin identified from each of the two ponds, selected line fish accounted for 71.25% and 51.25% in each of the individual ponds with an average of 61.25% which suggests that selected line fish and MC perch had survival rates of 61.25% and 38.75%, respectively. The local Ohio strain at MC Perch farms from two ponds averaged 204.0 mm (8.03 in) and 105.6g (3.72 oz), and 193.9 mm (7.63 in) and 92.9 g (3.28 oz), respectively. The selective line fish from two ponds averaged 218.1 mm (8.59 in) and 142.7 g (5.03 oz), and 212.0 mm (8.35 in) and 129.9 g (4.58 oz), respectively. The selected line fish 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, selected line 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: All four ponds were harvested in September, 2013. A total of 29 and 40 selected line perch were harvested from two ponds, and 40 and 21 WI local strain fish were harvested from two ponds. The selected line 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 (3.16 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 selected line yellow perch compared to WI local strain yellow perch. Fillet weight was significantly ($p < 0.001$) greater for selected line perch, while percent fillet yield was significantly greater for WI local strain yellow perch.

Northern Wisconsin experienced severe and prolonged winter weather in 2013 that resulted in pond ice cover that did not dissipate until early May. This extreme weather conditions in turn was like the 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.— The original intent was to host a field day in Wisconsin but northern Wisconsin experienced severe and prolonged winter weather in 2013 that resulted in pond ice cover that did not dissipate until early May. This extreme weather conditions in turn resulted 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, and researchers were not able to conduct the farm field day. Since then, farm test progress and results have been disseminated to industry and to end-user customers via three articles published in newsletters, journals and website. A scientific publication and an associated factsheet are scheduled for completion.

Target Audiences

Aquaculture industry, fish farmers and researchers.

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 peer-review journal articles in two years.

Deliverables (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>).

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

Relevance— 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

Response— 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, Manuscripts, Workshops, and Conferences

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

PROGRESS REPORT

Project Title: Develop Systems and Diet Strategies to Reduce Yellow Perch Larval Mortality Burst in Indoor Recirculating Aquaculture Systems

Key Word(s): Yellow Perch

Total Funds Committed: \$190,000

Initial Project Schedule: September 1, 2013 to August 31, 2015

Current Project Year: September 1, 2013 to August 31, 2014

PARTICIPANTS:

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Project Objectives

1. Develop system(s) to address physical and behavioral barriers to enhance mass production and survival of yellow perch (YP) from onset of first feeding up to 70 days.
2. Develop strategies to increase survival of fry and larvae of yellow perch reared indoors using different feeding regimens.

Deliverables

1. Develop modules for self/group training for YP aquaculturists. Modules should be prepared at the initiation of the project and updated to include new procedures/protocols learned from the project.
2. Prepare an overall report of the findings including an executive summary

Project Summary

In culture conditions, there are several critical factors affecting survival of larval yellow perch, including small mouth gape, dependence on live food organisms, non-feeding behavior, non-inflation of the gas bladder, clinging behavior, and cannibalism. Despite the availability of high quality feeds for small larvae, mainly formulated for marine species, the acceptance, growth and survival of larval yellow perch fed formulated diets as starting food are still highly variable and rather unsatisfactory. This project is investigating the development of systems and strategies to enhance mass production and survival of yellow perch from onset of first feeding up to 70 d, the critical period for yellow perch in recirculating aquaculture systems.

Anticipated Benefits

Results garnered from this commercial-scale research will be incorporated into an overall report, including executive summary, about culture strategies and protocols that can be used to increase the survival of larval (fry) yellow perch in indoor recirculating systems using culture methods and feeding regimens that maximize mass production. These new methodologies will greatly improve larval yellow perch survival and help feed the growing RAS production of yellow perch food fish, an important aquaculture species in the NCR.

Project Progress

Objective 1. (UWSP-NADF). UWSP: Non-feeding behavior, non-inflation of the gas bladder, clinging behavior, and cannibalism were examined using a series of methodical experiments evaluating the effects of:

- Turbid water – three treatments of clear (0 NTU), slightly (50 NTU), and turbid (100 NTU) water.
- Water surface spray – three treatments of no flow (0 L/min, 0 gal/min), moderate (0.4 L/min, 0.11 gal/min), and high (0.8 L/min, 0.21 gal/min) water flow.
- Tank color – three treatments of white, blue, and black interior colored tanks.

A random sample of 25 fry per tank was collected every two weeks and measured for length and weight gain; mortalities were recorded as observed mortality and removed daily. Mortalities were examined microscopically for swim bladder inflation and food in the alimentary canal (as a proxy of feed acceptance). At the end of the 70-day study, remaining fish were counted and measured for total length and an aggregate weight of 100 fry which was used as an estimate of mean fry weight along with 100 fry/tank examined for the presence of food in the gut and gas bladder inflation. Percentages of unobserved mortality (cannibalism) and observed mortality (death) were calculated. No differences ($p > 0.05$) in growth, length and weight, were observed every two weeks nor were differences observed after 70 days. Ten days post hatch (dph) food was found in the gut of >90% of the fry. Twenty-three dph swim bladder inflation was observed in 100% of the fry. Biweekly mortality rates were very high for all treatments and fewer than 10% of larval yellow perch survived 70 days. High rates of deformities were observed in spinal, jaw, gill plate, and caudal regions. We hypothesize that the extended winter, ice-out on broodstock ponds occurred the first week in June nearly a month later than usual, may have been responsible for poorly developed and smaller than usual fry that ultimately resulted in deformities and high mortality

rates. We recommend repeating year 1 objectives before advancing to year 2 refinements of culture systems. To improve repeated year 1 methods, we have moved broodstock indoors so that environmental variability can be controlled.

Objective 2. — Objective 2.1 - Develop and improve marine rotifer production and feeding systems for freshwater RAS to enhance survival of yellow perch. Two commercial-scale algae auto-feeders and rotifer production systems were constructed that allow us to culture and concentrate the needed number of rotifers to feed at a rate and concentration deemed necessary for the amount of fry in each tank. This allows us to feed rotifers 24 hours per day using a peristaltic pump, and digital repeat cycle timer. These systems that are able to produce high density and mass production, and deliver them to the fish frequently are critical to enhance survival of yellow perch, because marine rotifers can only survive for 15 – 20 minutes in freshwater. Sixteen rotifer auto-feeders were constructed and paired with sixteen yellow perch production tanks (1 m diameter). The feed reservoirs holding the rotifers in the feeders were placed in campus-style refrigerators that allow us to “cold bank” the rotifers, slowing their metabolism and maintaining their nutritional value above that which could be achieved through storing them at room temperature. The sixteen sets of systems will be used and tested with 16 feeding regimens in Objective 2.2 to select the two best feeding protocols. Objective 2.2 - Develop diet strategies and protocols to increase survival of fry and larvae of yellow perch reared indoors using marine rotifers, microdiets and various feeding regimens Five feeding regimes or diets

(rotifers, Licalife®, rotifers + Licalife®, rotifers + Microalgae (HUFA) N-Rich Ultra PL and Copepods) were applied for newly hatched yellow perch larvae starting from zero dph up to 80 dph. Newly hatched fry were stocked into 400-L experimental tanks at a density of 8000 – 10000 fry/tank at water temperature of 14 ± 1 °C. Fry were fed the five different larval feeding diets using the automatic feeding system. The mortality rates were high with the five different feeding diets during the first 10 days of feeding. The groups fed with Rotifers and rotifers plus HUFA showed ~20% survival rates after 30 dph and continued in good survivability. Other feeding groups had almost 100 % mortality rates.

Deliverables.— Modules have been postponed until 2015 due to program delays.

Target Audiences

Current and future yellow perch culturists; culturists of other Percid fishes

Outreach Overview

Results will be made available at the end of year 2; both as modules and in a report.

Deliverables (Outputs)

Modules for self/group training will include new procedures/protocols learned from the project. In 2015 an overall report of the findings including an executive summary will be written at conclusion of project.

Outcomes/Impacts

The impact of this proposed project will be primarily through the development of systems and strategies to enhance mass production and survival of yellow perch from onset of first feeding up to 70 days, the critical period for yellow perch in recirculating aquaculture systems. The

greatest return on investment for this project is the ultimate reduction in production costs due to increased growth rate and survival of larval yellow perch by using tank systems and feeding regimes optimized for the early-life stage. At the completion of this project, we expect to not only have information available about recirculating system components, feeding strategies, and feed types for successfully reducing the early-life stage bottlenecks, but to have online training modules available through the NCRAC website for aquaculturists in the NCR. The online training modules will provide direct information to yellow perch culturists on current and updated procedures and protocols for reducing yellow perch larval mortality burst in indoor recirculating aquaculture systems.

Impacts Summary

Relevance— One roadblock hindering expansion of yellow perch aquaculture has been low survival and availability of fry and fingerlings. The survival rate of pond nursed fry is dependent on weather and late winter storms can kill all the fry in ponds overnight. Developing the indoor culture of yellow perch has significant advantages over pond culture. Limiting this possibility has been the poor indoor survival of newly hatched fry to the stage where they are completely feed- trained.

Response — We have started a project to address the roadblock hindering expansion of yellow perch aquaculture.

Results— Two commercial-scale algae auto-feeders and rotifer production systems were constructed that allow us to culture and concentrate the needed number of rotifers to feed at a rate and concentration deemed necessary for the amount of fry in each tank. Five feeding regimes or diets were tested for newly hatched yellow perch larvae starting from zero dph up to 80 dph. Non-feeding behavior, non-inflation of the gas bladder, clinging behavior, and cannibalism were examined using a series of methodical experiments. The results have played foundation for completing this project.

Recap— The greatest return on investment for this project is the ultimate reduction in production costs due to increased growth rate and survival of larval yellow perch by using tank systems and feeding regimes optimized for the early-life stage.

Publications, Manuscripts, Workshops, and Conferences

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

SOME COMMONLY USED ABBREVIATIONS AND ACRONYMS

×	cross, by, or times
AIS	aquatic invasive species
anamnox	anaerobic ammonium oxidizing bacteria
AOA	ammonia oxidizing archaea
AOB	ammonia oxidizing bacteria
APHIS	Animal and Plant Health Inspection Service
AREF	Aquaculture Regional Extension Facilitator
AquaNIC	Aquaculture Network Information Center
BOD	Board of Directors
BW	body weight
°C	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
°F	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 <i>Aeromonas</i> infection
MAS	motile <i>Aeromonas</i> 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

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