NORTH CENTRAL
REGIONAL AQUACULTURE CENTER

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TWENTY-SIXTH ANNUAL PROGRESS REPORT

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NCRAC

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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 -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 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. 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, Industry Advisory Council (IAC), and Technical Committee/Research and Extension (TC/R and E);
- Monitor research and extension activities;
- Recruit other Administrative Center staff as authorized by the Board of Directors;
- Serve as an additional source of technical information for the regional aquaculture community;
- Maintain liaison with other RACs; and
- Serve on USDA's National Coordinating Council for Aquaculture.

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

- Give regional presentations;
- Develop and distribute (including posting on the Web) news releases for new NCRAC publications;
- Supervise technical editors for NCRAC publications;
- Oversee the development of extension projects;
- 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 (Michigan State University), 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/E 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.

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 each from the North Central Regional Association of State Agricultural Experiment Station Directors and the North Central Cooperative Extension Association, a member from a non-land grant university, representative from the university (Iowa State University) responsible for the Center, a member from a 1890 institution, 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 and Experiment Station Directors within the North Central Region appoint representatives to the TC/E and TC/R, respectively. 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 28 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), FY2014 (2014-38500-22138) and FY2015 (#2014-38500-19550 Amendment) with monies totaling $21,321,056. Currently, three grants are active (FY12-16); the first 25 grants (FY88-10) have terminated, and the 2012 grant will end June 30, 2017.

- The Center annually coordinates a biannual program planning meeting which typically sets priorities for the next 2-year funding cycle and calls for development of project outlines to address priority problem areas.
- Work Groups are formed which submit project outlines to the Center. The projects are peer reviewed by experts from both within and outside the region and a Project Review Committee.
- The BOD, using the Project Review Committee's recommendation and reviewers' responses, decides which projects are to be approved and funding levels. The Center conveys BOD decisions to all Project Work Groups. Those that are approved for funding are asked to submit revised project outlines incorporating BOD, Project Review Committee, and reviewers' comments.
- The Center then submits the revised project outlines as a Plan of Work (POW) to USDA for approval.
- Once a POW is approved by USDA, the Center then prepares subcontracts for each participating institution. The Center receives all invoices for subcontractual agreements and prepares payment vouchers for reimbursement. Thus, Center staff serve as fiscal agents for both receiving and disbursing funds in accordance with all terms and provisions of the grants.

Through August 31, 2016, the Center has funded or is funding 114 projects through 522 subcontracts from the first 27 grants received. Funding for these Center- supported projects is summarized in Table 1 below (pages 9-12). 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 22 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, 2015 to August 31, 2016. 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 are placed in order of selected key word(s): Aquaculture Drugs, Aquaponics, 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

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

Project Area	Project Number	Proposed Duration Period	Funding Level	Grant Number
Aquaculture Drugs	1	7/1/96-6/30/97	$27,000	95-38500-1410
	2	12/1/96-11/30/97	$950	95-38500-1410
	3	10/1/99-9/30/00	$8,415	97-38500-3957
	4	6/1/04-11/30/05	$223,677	2003-38500-12995
	5	7/15/04-7/14/05	$60,000	2003-38500-12995
	6	11/1/04-10/31/06	$50,000	2002-38500-11752
	7	1/1/06-12/31/06	$129,936	2005-38500-15847
	8	9/1/08-8/31/10	$150,000	2008-38500-19157
	9	9/1/09-8/31/10	$27,880	2008-38500-19157
	10	9/1/11-8/31/31	$100,000	2010-38500-20929
	11	9/1/12-8/31/14	$240,000	2012-38500-19550
			$1,017,858	
Aquaponics	1	7/1/16-6/30/17	$24,596	2014-38500-22138
			$24,596	
Baitfish	1	9/1/92-8/31/94	$61,973	92-38500-6916
	2	9/1/06-8/31/08	$111,997	2006-38500-16900
			$88,003	2005-38500-18547
			$261,973	
Conferences/Workshops/Symposia				
Environmental Strategies Symposium	1	9/1/00-5/31/01	$5,000	96-38500-2631
Nat'l. Aquaculture Ext. Workshop/Conference	1	10/1/91-9/30/92	$3,005	89-38500-4319
	2	12/1/96-11/30/97	$3,700	95-38500-1410
	3	11/1/02-10/31/03	$4,500	00-38500-8984
	4	1/1/06-12/31/06	$5,000	2005-38500-18547
	5	9/1/10-8/31/11	$5,000	2008-38500-19157
			$21,205	
NCR Aquaculture Conference	1	6/1/90-3/31/91	$7,000	90-38500-5008
	2	12/9/98-6/30/99	$3,000	96-38500-2631
			$10,000	
Percis III	1	11/1/02-10/31/03	$4,000	00-38500-8984
Crayfish	1	9/1/92-8/31/94	$49,677	92-38500-6916
Economics/Marketing	1	5/1/89-12/31/91	$127,338	88-38500-3885
			$34,350	89-38500-4319
	2	9/1/91-8/31/92	$53,300	91-38500-5900
	3	9/1/93-8/31/95	$40,000	93-38500-8392
	4	9/1/99-8/31/01	$47,916	97-38500-3957
	5	9/1/03-8/31/04	$50,000	2002-38500-11752
	6	9/1/10-8/31/11	$23,565	2010-38500-20929
	7	9/1/12-8/31/14	$75,276	2012-38500-19550
			$451,745	
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

Project Area	Project Number	Proposed Duration Period	Funding Level	Grant Number
Regional Aquaculture	5	9/1/95-8/31/97	$10,813	92-38500-6916
Extension Specialist [RAES]—			$20,391	95-38500-1410
Project Nos. 18, 19 and 20	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	9-1-15-8-31-16	$23,175	2012-38500-19550
	17	9/1/16-8/31/18	$50,000	2014-38500-22138
	18	9/1/03-8/31/05	$100,000	2002-38500-11752
	19	9/1/05-5/31/09	$199,624	2004-38500-14269
	20	9/1/09-8/31/11	$150,000	2008-38500-19157
	21	9/1/11-8/31/13	$196,612	2010-38500-20929
	22	9/1/13-8/31/14	$101,820	2012-38500-19550
	23	9/1/14-8/31/16	$103,347	2014-38500-22138
	24	9/1/16-8/31/18	$124,993	2014-38500-22138
	25	7/1/16-6/30/17	$34,950	2014-38500-22138
	26	7/1/16-6/30/17	$34,977	2014-38500-22138
	27	9/1/16-8/31/18	$70,000	2014-38500-22138
			2,243,008	
Hybrid Striped Bass	1	5/1/89-8/31/91	$68,296	88-38500-3885
			$68,1141	89-38500-4319
			$101,000	90-38500-5008
	2	6/1/90-8/31/92	$96,550	90-38500-5008
	3	9/1/91-8/31/93	$168,000	91-38500-5900
	4	9/1/93-8/31/95	$150,000	93-38500-8392
	5	9/1/95-8/31/97	$15,000	95-38500-1410
	6	6/1/99-5/31/00	$98,043	96-38500-2631
	7	9/1/01-5/31/04	$211,957	98-38500-5863
			$976,960	2001-38500-10369
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	1	9/1/93-8/31/94	$2,000	89-38500-4319
Aquaculture INADs/NADAs		5/15/95-5/14/96	$5,000	94-38500-0048
		5/15/96-5/14/97	$6,669	92-38500-6916
			$3,331	95-38500-1410
		5/15/97-5/14/98	$15,000	96-38500-2631
		5/15/98-5/14/99	$13,241	94-38500-0048
		5/15/99-5/14/00	$10,000	95-38500-1410
	2	7/15/04-7/14/05	$9,000	2003-38500-12995
		9/15/05-8/31/06	$15,000	2004-38500-14269
		9/1/06-8/31/08	$40,000	2006-38500-16900
		5/15/08-5/14/09	$25,000	2007-28500-18469
			$144,241	

Project Area	Project Number	Proposed Duration Period	Funding Level	Grant Number
Nutrition/Diets	1	9/1/04-8/31/06	$200,000	2002-38500-11752
	2	9/1/07-8/31/09	$80,000	2006-38500-16900
	3	9/1/09-8/31/11	$80,000	2008-38500-19157
	4	9/1/10-8/31/12	$124,400	2008-38500-19157
	5	9/1/12-8/31/13	$75,000	2010-28500-20929
			$559,400	
Other				
Feed Training Carnivorous Fish	1	9/1/06-8/31/08	$165,446	2005-38500-15847
			$134,554	2006-38500-16900
			$300,000	
Snail Management/Grub Control	1	9/1/07-8/31/09	$225,000	2007-38500-18469
RAS Microbial Communities	1	9/1/09-8/31/10	$65,000	2008-38500-19157
Assessment of Winter Kill in Ponds	1	9/1/11-8/31/13	$175,000	2008-38500-19157
Field Assessment of Design and Operation of Midwestern Aquaculture Buildings	1	7/1/16-6/30/17	$34,998	2014-38500-22138
			$799,998	
Salmonids	1	6/1/90-8/31/92	$9,000	89-38500-4319
			$120,799	90-38500-5008
	2	9/1/92-8/31/94	$149,997	92-38500-6916
	3	9/1/94-8/31/96	$199,290	94-38500-0048
	4	9/1/97-8/31/99	$158,656	97-38500-3957
			$637,742	
Sunfish	1	6/1/90-8/31/92	$130,758	90-38500-5008
	2	9/1/92-8/31/94	$149,799	92-38500-6916
	3	9/1/94-8/31/96	$173,562	94-38500-0048
	4	9/1/96-9/31/98	$199,921	96-38500-2631
	5	9/1/99-8/31/01	$199,748	99-38500-7376
	6	9/1/13-8/31/15	$160,000	2012-38500-19550
			$1,013,788	
Tilapia	1	9/1/96-8/31/98	$118,791	96-38500-2631
	2	9/1/98-8/31/00	$150,000	98-38500-5863
			$268,791	
Viral Hemorrhagic Septicemia (VHS)	1	9/1/08-8/31/10	$197,960	2008-38500-19157
Walleye	1	5/1/89-8/31/91	$177,517	89-38500-4319
	2	6/1/90-8/31/92	$111,657	90-38500-5008
	3	9/1/91-8/31/92	$109,223	91-38500-5900
	4	9/1/92-8/31/93	$75,000	89-38500-4319
	5	9/1/93-8/31/95	$150,000	93-38500-8392
	6	9/1/95-8/31/97	$117,395	94-38500-0048
			$59,835	95-38500-1410
	7	9/1/99-6/30/02	$127,000	98-38500-5863
			$927,627	
Wastes/Effluents	1	9/1/92-8/31/94	$153,300	92-38500-6916
	2	9/1/96-8/31/98	$100,000	96-38500-2631
	3	9/1/01-8/31/04	$106,186	00-38500-8984
			$88,814	2001-38500-10369
			$448,300	

Project Area	Project Number	Proposed Duration Period	Funding Level	Grant Number
White Papers	1	7/1/98-12/31/98	$4,999	96-38500-2631
	2	9/1/99-12/31/99	$17,495	97-38500-3957
			$22,494	
Yellow Perch	1	5/1/89-8/31/91	$76,957	88-38500-3885
			$85,723	89-38500-4319
	2	6/1/90-8/31/92	$92,108	90-38500-5008
	3	9/1/91-8/31/93	$99,997	91-38500-5900
	4	9/1/93-8/31/95	$150,000	93-38500-8392
	5	9/1/95-8/31/97	$199,507	95-38500-1410
	6	9/1/97-8/31/99	$185,458	97-38500-3957
	7	9/1/98-8/31/00	$92,370	98-38500-5863
	8	9/1/01-5/31/04	$326,730	00-38500-8984
			$125,016	2001-38500-10369
	9	9/1/10-8/31/13	$150,000	2010-38500-20929
	10	9/1/13-8/31/15	$190,000	2012-38500-19550
			$1,773,866	
TOTAL			$12,262,186	

PROJECT REPORTS

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) [Termination Report]
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, 2015 to August 31, 2016
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
Reason for Termination: Project objectives completed and funds have been terminated.

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.
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.

Project Summary

The efficacy of Terramycin 200 for Fish®- or 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, IA. 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 measurements..

Technical Summary and Analysis

Field efficacy trials were initiated at Spirit Lake Fish Hatchery following presumptive diagnosis of motile aeromonad infection in muskellunge (*Esox masquinongy*) fingerlings in 2011 and in walleye (*Sander vitreus*) fingerlings in 2012. In both trials, fingerlings were indiscriminately removed from the culture facility source tanks and randomly transferred to test system tanks. Fifty-eight fingerlings were transferred to each of 18 tanks in the test system consisting of 38 L (10 gal) fiberglass tanks. The test tanks were individually plumbed and received the same culture water as the source tanks. Fingerlings were offered non-mediated control diet, Aquaflor®-medicated diet (equivalent to 15 mg per kg of body weight per day [mg/kg BW/d] florfenicol), or Terramycin 200®-medicated diet (equivalent to 87.5 mg/kg BW/d oxytetracycline dihydrate). Each treatment was given to six test tanks for 10 days followed by 14 days of observation during which only the non-medicated diet was offered.

Survival of muskellunge fingerlings fed Aquaflor®-medicated feed (less than 17%) was not significantly different at 14 days post-treatment than that of non-medicated controls (16.2%). Survival of muskellunge fingerlings fed Terramycin 200®-medicated feed (greater than 32%) was significantly greater than that of non-medicated controls (figure 1; $p<0.05$). Efficacy of either medicated feed for walleye could not be determined, because all walleye tanks received a DIQUAT concurrent treatment wrongly and inadvertently administered by hatchery personnel.

The etiological agents were identified as mostly *Aeromonas allosaccharophila* in the muskellunge and mostly *A. veronii* in the walleye with 1 occurrence of A. media, 1 occurrence of *A. salmonicida*, 2 occurrences of *A. sobria*, 1 occurrence of *Actinobacter* sp., 1 occurrence of *KIebsiella oxytoca*, 3 occurrences of *Pseudomonas* sp., and 12 occurrences of *Plesiomonas shigelloid*es. This work was conducted under INAD numbers 11-902 and 11-366. The protocols had concurrence with the two drug sponsors and CVM, and the study was conducted following good clinical practices. Study reports were not submitted to CVM or the drug sponsors, because (1) efficacy for either drug could not be determined during the walleye trials for the reasons previously mentioned, (2) Aquaflor® was not efficacious reducing mortality in infected muskellunge, and (3) although the reduction in muskellunge mortality with Terramycin 200® was statistically significant, the reduction (~15%) was considered marginal. These data would not support the supplemental NADA approvals for either drug.

Principal Accomplishments
Efficacy could not be evaluated in walleye because of the inadvertent concurrent DIQUAT treatment. Although the reduction in muskellunge mortality with Terramycin 200® was statistically significant, the reduction (~15%) was considered marginal.

Impacts
Because of the reason listed previously, these data will not support the supplemental NADA approvals for Terramycin 200® (oxytetracycline) or Aquaflor® (florfenicol) either drug.

Recommended Follow-Up Activities
None.

Technical Update

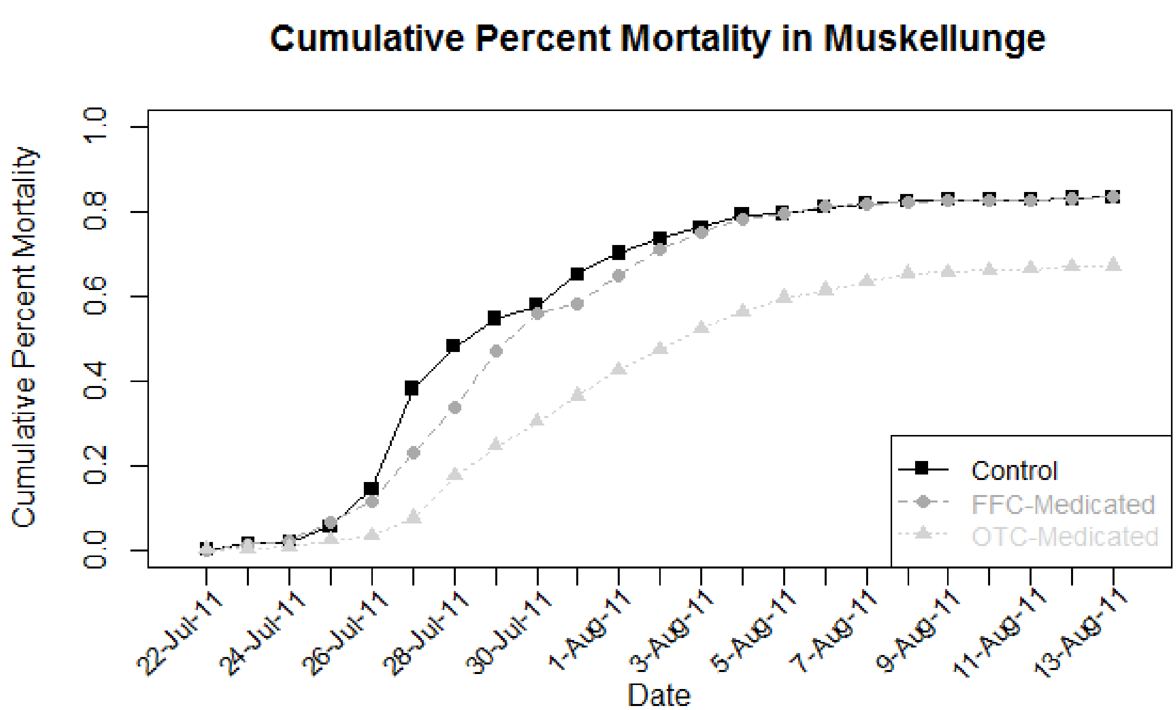

Figure 1. Cumulative percent mortality in muskellunge. Cumulative percent mortality of muskellunge offered non-medicated control feed, FFC-medicated feed at a nominal dose of 15 mg FFC/kg bodyweight/d, or OTC-medicated feed at a nominal dose of 82.5 mg OTC/kg BW/d for 10 days. Mortality was associated with motile aeromonad infection.

Project Title: Efficacy of Eugenol (AQUI-S®20E) to Reduce Transport Stress and Mortality of Tilapia and Yellow Perch [Termination Report]
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, 2015 to August 31, 2016
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
Reason for Termination: Project objectives completed and funds have been terminated.

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.

Project Summary

Fish transport costs are a substantial portion of the operational expenses in the aquaculture industry in the North Central Region (NCR). Increasing fish loading density during transport could substantially increase the efficiency of NCR aquaculture operations by enabling the transport of more fish per unit of fuel. This project was undertaken to determine if eugenol sedation would benefit fish transport procedures to improve fish welfare and post-transport survival on two economically important fish in the NCR.

Technical Summary and Analysis

This project was completed through a series of three studies: (Study #1) to determine the physiological effects of eugenol on yellow perch (*Perca flavescens*) and tilapia, (Study #2) to determine the anesthetic (behavioral) effects of eugenol on yellow perch and tilapia, and (Study #3) to determine the survival of yellow perch and tilapia transported under eugenol sedation. Results from Study #1 are published in Cupp et al. (2016a). This study evaluated the effects of AQUI-S®20E (10% eugenol) on the mass specific metabolic rates of yellow perch and Nile tilapia using static respirometry. In 17°C (62.6^{O}F) water and loading densities of 60, 120, and 240 g/L (0.5, 1.0, and 2 lb/gal), yellow perch control groups (0 mg/L eugenol) had metabolic rates of 329.6-400.0 mg O2/kg/h, while yellow perch exposed to 20 and 30 mg/L eugenol had significantly reduced metabolic rates of 258.4-325.6 and 189.1-271.0 mg O2/kg/h, respectively. Nile tilapia immersed in 30 mg/L eugenol had significantly reduced metabolic rates (424.5±42.3 mg O2/kg/h) relative to control fish (546.6±53.5 mg O2/kg/h) at a loading density of 120 g/L in 22°C water. Metabolic rates at 240 and 360 g/L loading densities were similar across all sedation levels for Nile tilapia. Results from this study demonstrated that eugenol reduced the metabolic rates of yellow perch at high loading densities, but Nile tilapia showed only minor suppression of metabolic rates in response to eugenol sedation. Results from

Study #2 is published in Cupp et al. (2016b). This range finding study evaluated combinations of loading densities, eugenol concentrations, and exposure durations to determine the anesthetic effects on yellow perch and Nile tilapia using static exposures. Yellow perch were immersed in 0, 10, 20, and 30 mg/L eugenol for 2, 6, and 10 h at 120, 240, and 360 g/L (1, 2, and 3 lb/gal) loading densities. Nile tilapia were immersed in 0, 10, 20, and 30 mg/L eugenol for 2, 6, and 10 h at 240, 360, and 480 g/L (2, 3, and 4 lb/gal) loading densities. In general, eugenol depleted rapidly from static exposure tanks regardless of starting concentration, while sedation levels were highly varied. Yellow perch immersed in 20 and 30 mg/L eugenol were lightly sedated (i.e. reduced swimming and startle responses with equilibrium maintained) for up to 7h during static exposure. However, immersion in 30 mg/L also induced loss of equilibrium in yellow perch; an unwanted endpoint during live transport due to the risk of suffocation. Nile tilapia sedation was modest at these same concentrations and all fish were fully recovered within 2 h of static exposure.

Collectively, this study found that eugenol is effective to sedate high loading densities of yellow perch and Nile tilapia, but sedation levels vary with species, loading density, and starting eugenol concentration. Results from Study #3 are currently in journal review. Briefly, this study determined the effectiveness of eugenol sedation during live transport to enhance post-transport survival relative to unsedated fish. Fish were transported in 0, 10, and 20 mg/L eugenol for 6 h at 240 g/L (yellow perch) and 480 g/L (Nile tilapia) across various roads, highways, and interstates. Similar to Study #2, eugenol depleted rapidly from transport tanks and the behavioral effects of sedation were short-lived for both species. Survival was >98% for both species up to 14-d post-transport. No differences in survival between sedated fish and unsedated fish were found.

Principal Accomplishments

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. CVM staff were 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 were addressed to work toward protocol concurrence from CVM and a revised pivotal effectiveness protocol was submitted in February 4, 2015. On March 27, 2015, CVM did not concur with the revised protocol submission. Although CVM agreed with overall experimental design, protocol concurrence because no official label claim was put forth by AQUI-S New Zealand Ltd. (product sponsor) specifying the use of AQUI-S20E® for fish transport. No further protocol concurrence will be issued by CVM until a label claim is developed; regardless of their agreement with study design.

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 was submitted based on input from CVM, UMESC and the drug sponsor (AQUI-S New Zealand Ltd.). The CVM non-concurrence letters were received. See description in Objective 1.

Objective 5. — Pivotal effectiveness study was completed in Summer 2015. However, protocol concurrence with study design was not attained prior to study initiation for reasons described in Objective 1.

Objective 6. — FSR has been developed and is currently under peer-review with a scientific journal. Data archiving will take place upon acceptance of FSR.

Objective 7. — MESC will submit the FSR to CVM for review. As previously described, the effectiveness technical section will not be completed from this work because no label claim has been made by the drug sponsor.

Objective 8. — See line item 7 above.

Objective 9. — See line item 7 above.

Impacts

Results from this project describe considerations with sedating yellow perch and tilapia for live transport.

Recommended Follow-Up Activities

Further collaboration between industry and drug sponsors will be important for describing the needs for eugenol sedation during live transport and will be necessary to develop a label claim for FDA. Although this project determined that yellow perch and tilapia can be effectively sedated using 10-30 mg/L eugenol, no differences in post-transport survival between fish that were sedated or not sedated during live transport. Future research should also consider transporting fish under different circumstances (e.g. extreme temperatures, long transport durations, low dissolved oxygen), as the benefits of sedation on fish welfare are likely to be realized under more adverse conditions.

Publications, Manuscripts, Workshops, and Conferences

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

Project Title: Probiotics in Yellow Perch and Tilapia Culture [Termination Report]
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, 2015 to August 31, 2016
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
Reason for Termination: Project objectives completed and funds have been terminated.

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±7.6% and the swim bladder inflation rate was 35.8±20.6%. The average juvenile weight was 24.5±5.0 mg (0.86±0.18 oz) and average growth rate 29.4±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 anguillarium* 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*.

University of Minnesota investigators validated 16S rRNA surveying in yellow perch. Significant technical effort was put towards finding a DNA extraction procedure and PCR conditions suitable for analysis of intestinal contents from farmed yellow perch and tilapia, and compared them to several other fish species from fish farms and wild. To address isolated probiotic from Ohio State University (OSU) yellow perch and commercial probiotics a feeding experiment was performed with yellow perch juveniles that included dietary treatments with yellow perch isolates and controls, and unchallenged fish. *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. An affordable axenic apparatus was constructed for use in the culture of fish larvae and juveniles by modification of existing flaws in equipment described in the literature. Check valves were added to all incoming and outgoing water lines to

limit backflow, reducing the risk of contamination through exposure to the external environment. All chambers have been placed on a 6-position magnetic stir plate to keep water mixed within the chambers. This prevented “hypoxic” zones, maintained live zooplankton (rotifers) in suspension, as well as allow for more efficient removal of metabolites and detritus from the system. The system was tested with hybrid cichlid (*Cichlasoma synspilum*, female x *Amphilophus citrinellum* male) and performance (survival and growth) was comparable to fish reared in open system.

Technical Summary and Analysis

The results of the yellow perch larvae/juveniles rearing are indicative of the highest performance. 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, *Aeromona*s 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.

Principal Accomplishments

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 23 and 25, 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 *Brachio*nus, 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 *Artemi*a, then transitioned to Otohime A® diet. The average rate of survival was 32.0±7.6%. Swim bladder inflation rate was 35.8±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 ethyl ester fatty acids (EE)-enriched groups than the triglyceride fatty acids (TAG)-enriched groups, especially during the first 10 days of exogenous feeding.

Findings of DNA extraction and PCR analysis of intestinal content from yellow perch are presented in Technical Update section (Figure 1). The image displays 16S amplicon sequencing from homogenized intestinal tissue from three fish, and a corresponding water tank sample. With the exception of one distal intestinal sample, intestinal samples were predominantly phyla

Fusobacteria and Proteobacteria, respectively. While the predominant genus in these samples was *Cetobacterium* spp., the Proteobacteria were substantially more diverse, including genera such as *Plesiomonas*, *Ralstonia*, and *Aeromonas* among many others. Actinobacteria were mostly classified as *Mycobacterium* spp. The water tank sample predominantly contained sequences classified as Mycobacterium, Azospira, Pedobacter, and Cetobacterium.

Objective 2. — Bacterial community profiling of wild and farmed fish in the upper Midwest. The analysis was performed using hierarchical clustering which takes into account species presence and abundance, then performs unsupervised clustering based on these parameters (Figure 2). Clear trends emerge which showed association of perch intestinal microbiota with farmed walleye but not with other farmed fishes.

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 anguillarium* and *Aeromonas salmonicid*a. To test their direct inhibitory abilities to the two pathogens, investigators 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. anguillarium* but only weak inhibition to *A. salmonicida*. Once it was determined that the 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). *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 8 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 $8.7*^{105}$ 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 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. To date, construction and refinement of the axenic fish model needs to be completed for this objective.

Objective 4. — Investigators have constructed an affordable axenic apparatus (Figure 3) for use in the culture of fish larvae and juveniles by modification of existing flaws in equipment described in the literature. Check valves were added to all incoming and outgoing water lines to limit backflow, reducing the risk of contamination through exposure to the external environment. All chambers have been placed on a 6-position magnetic stir plate to keep water mixed within the chambers. This prevented "hypoxic" zones, maintained live zooplankton (rotifers) in suspension, as well as allow for more efficient removal of metabolites and detritus from the system. The system was tested with zebrafish (*Danio rerio*) larvae (size comparable to yellow perch larvae) and hybrid cichlid (*Cichlasoma synspilum*, female x *Amphilophus citrinellum* male). Zebrafish embryos were injected into the system and hatched in the chambers. However, survival was compromised and experiments were terminated at this point. Hybrid cichlid, the size of newly hatched fish comparable to Nile tilapia acclimated well in chambers and performance (survival and growth) was comparable to fish reared in open system. Survival of fish in axenic system was 81.2+10% and fish increased weight from 2.3 mg (prior to first feeding) to 14.5+2.8 mg within 7 days at 28^{o}C (82.4 ^{o}F).

Impacts

Yellow perch are often stocked at high densities under environmentally stressed conditions that often result in increased number of diseases. 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.

The proposed studies included 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. Potential probiotic bacteria were isolated from the intestinal tract of yellow perch collected in OSU aquaculture laboratory. The probiotics identified in this study can potentially contribute to sustainable development of the aquaculture industry and securing an organic produce status for fish.

Recommended Follow-Up Activities

Intensive sampling is underway using the established protocols to better understand the succession of bacterial species during fish maturation, and the relationship of bacterial succession in the intestines with the surrounding water environment.

Technical Update

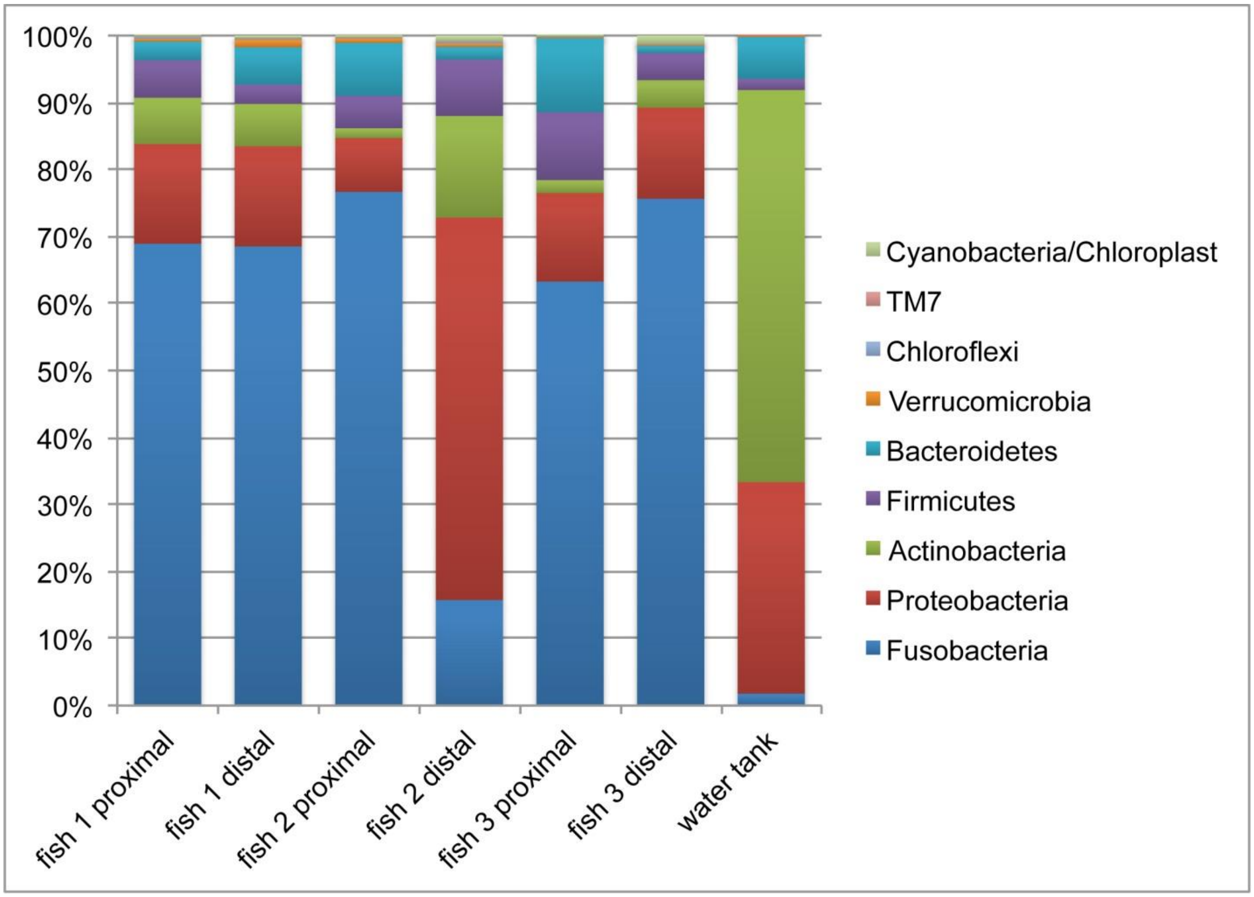

Figure. 1 Identification of microbiota based on amplicon sequencing of 16S fragment from intestinal content from farmed yellow perch and the water from the tank the fish were in.

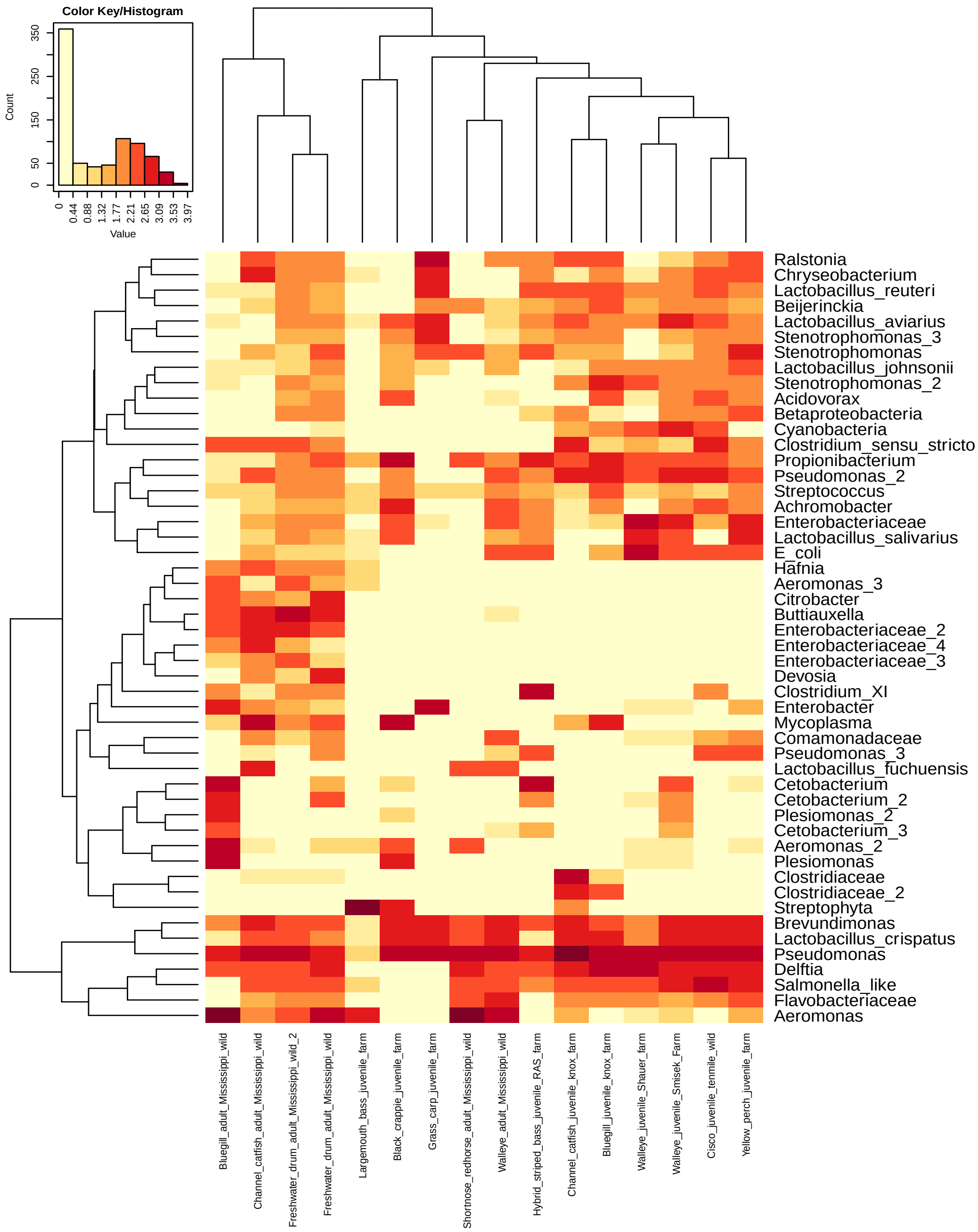

Figure 2 Bacterial community profiling of wild and farmed fish in the upper Midwest. The plot is generated using the presence or absence of all OTUs among all samples tested, and samples that are more closely related to one another are closer to each other on the plot. Some trends were observed. For example, bluegill separated from the rest of the fish, which were mostly intermingled. In particular wild bluegill were responsible for this separation while farm bluegill clustered with other fish types. Except for bluegill, it was difficult to distinguish between species of fish, location of fish, or even farm versus wild fish.

Figure 3. Axenic apparatus after modifications that followed preliminary experiments with zebrafish larvae. A stainless steel "air-stone" was added to each water reservoir (not shown) so that oxygenation with pure oxygen gas is more efficient. Also, AV fistula with 16 gauge syringe needles were added to the rubber septa in order to limit punctures when feeding (zooplankton suspension) or injecting embryos. This reduces the risk of damaging the septa caps due to the use of a large syringe for multiple injections per day. A water pump was added to the outflow manifold to suck water from the system. It was observed that gravity did not provide enough force to remove water from each chamber. Adding a pump, increases the flow rate so that water changes are more efficient and the system is easier to operate.

Project Title: Extension [Progress Report]
Key Word(S): Extension
Total Funds Committed: $1,176,685
Initial Project Schedule: May 1, 1989 to August 31, 2016
Current Project Year: September 1, 2015 to August 31, 2016
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; Allen Pattillo, Iowa State University, Iowa; Alvaro Garcia, South Dakota State University, South Dakota; Nicholas Phelps, University of Minnesota, Minnesota; Kwamena K. Quagrainie, Purdue University, Illinois/Indiana; Matthew Smith, Ohio State University, Ohio; Christopher Weeks, Michigan State University, Michigan
Industry Liaison: Dan Vogler, Harrietta Hills Trout Farm, Michigan

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)

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.

Weeks (Michigan):

- Served as a lead coordinator on the Steering Committee for the 2016 NCR Aquaculture Conference in Milwaukee, WI.
- Served on steering committees for: 2015 Michigan Seafood Summit (East Lansing, MI), 2015 Dialogue on Open Water Aquaculture (St. Ignace, MI), 2016 Michigan Seafood Summit (Traverse City, MI), 2016 Great Lakes Regional Seafood Workshop (Milwaukee, WI).
- Principal investigator for project to examine feasibility of an AIS HACCP verification program.
- Liaison between NCR aquaculture and baitfish industry members and regulatory agencies.

- Disseminate information to stakeholders through presentations, list serves, the NCRAC regulation website

Lee has continued to assist the Kansas Aquaculture Association by developing, printing and distributing the Kansas Aquaculture Association Directory. Bauer distributed NCRAC information to the Nebraska aquaculture industry. 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. Pierce recently assumed Hicks' role in developing factsheets on pond aquaculture and sportfish management.

Pattillo developed two NCR-centered fact sheets covering aquaculture and hydroponic components of aquaponic systems. Pattillo also led the development of an aquaculture webinar series that is currently underway. This webinar series is a partnership between NCRAC, the National Aquaculture Association and the United States Aquaculture Society and has covered a range of important and timely topics. Pattillo, Phelps, Smith, Wiermaa, and Quagrainie delivered extension presentations at the 2016 NCRAC/Wisconsin Aquaculture Association Conference in Milwaukee, WI.

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 Phelps to address the growing concern of biosecurity, particularly in regard to diseases such as viral hemorrhagic septicemia (VHS). Kinnunen and Phelps 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.

Several states have on-site facilities that are used for extension programming, e.g. The Piketon facilities operated by Ohio State University are used to inform the public about aquaculture as well as foster grass root support for this agriculture enterprise. The Aquaculture Boot Camp (ABC) program by Ohio State is a successful ongoing aquaculture immersion program.

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)

Pattillo developed two NCR-centered fact sheet s covering aquaculture and hydroponic component s of aquaponics systems. These have gone through peer-review and are scheduled to be uploaded to www.ncrac.org in Spring 2017 . Pattillo also led the development of an aquaculture webinar series that is currently underway. This webinar series is a partnership between NCRAC, the National Aquaculture Association and the United States Aquaculture Society and has covered a range of important and timely topics. Pattillo, Kinnunen, and Phelps all contributed talks to the webinar series. Topics included aquaponics, biosecurity, economic cost of regulations, seafood facts for retailers, seafood benefits for dieticians, use of social media in aquaculture, branding opportunities for aquaculture producers, new Food Safety Inspection Service information, veterinary feed directive updates, recreational pond management, AIS-HACCP issues, fish health, and indoor marine shrimp production techniques. Archived webinars can be accessed at http://www.ncrac.org/video or https://vimeo.com/channels/958980 , as well as through www.thenaa.net and www.usaquaculture.org .

Hitchens, Pattillo, Phelps, Quagrainie, and Weeks delivered extension presentations at the 2016 NCRAC/Wisconsin Aquaculture Association Conference in Milwaukee, WI. Topics included live hauling and marketing of fish, aquaponic system design and management, fish health, and aquaculture economics, and regulatory issues associated with net pen aquaculture. Presentations are available at http://www.ncrac.org/presentation/2016-north-central-aquaculture-conference .

Outcomes/Impacts

Wisconsin Aquaculture Association (OAA) collaborated with NCRAC to host the 2016 NCR Aquaculture Conference in Milwaukee, WI. Over 250 fish farmers and industry experts took part in the 2016 North Central Regional Aquaculture Conference held in Milwaukee March 2016. Presentations are available at http://www.ncrac.org/presentation/2016-north-central-aquaculture-conference.

Collaborations with the Regional Aquaculture Extension Specialist (Weeks) led to additional direct outreach to 600 attendees at conferences, meetings and summits. Personal verbal communications estimated at over 50 per month, over 100 postings to 150 subscribers on the NCR Fish Culture List, information dissemination through email contacts (several per week), and 11 presentations.

The 2016 Aquaculture Webinar Series fostered a partnership between NCRAC, the National Aquaculture Association, and the United States Aquaculture Society. This partnership broadened the scope and participation in these webinars nationwide. This 12-part series covered timely and relevant aquaculture topics for the NCR and the overall US aquaculture industry. Thus far 11 webinars have been recorded and uploaded to the NCRAC website, 3 more are planned. Current viewership of these webinars is over 1,400 views. Plans are in place to boost viewership further through use of YouTube and other social media outlets.

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. Fish farmers need basic and advanced aquaculture information in an easy to understand format that is readily accessible to them to improve their operations. Web-based training opportunities fit this need.

Response — Pattillo led the development of an aquaculture webinar series that is currently underway. Topics included aquaponics, biosecurity, economic cost of regulations, seafood facts for retailers, seafood benefits for dieticians, use of social media in aquaculture, branding opportunities for aquaculture producers, new Food Safety Inspection Service information, veterinary feed directive updates, recreational pond management, AIS-HACCP issues, fish health, and indoor marine shrimp production techniques.

Results — Current viewership of these webinars is over 1,400 views. Recording can be accessed at https://vimeo.com/channels/958980.

Recap — In response to industry concerns, webinars, workshops, publications, videos, and other web-based resources have been developed throughout the region to address industry needs.

Publications, Manuscripts, Workshops, and Conferences
See the Appendix for a cumulative output for all NCRAC-funded Extension activities.

Project Title: Regional Aquaculture Extension Specialist (RAES) [Termination Report]
Key Word: Extension
Total Funds Committed: $205,165
Initial Project Schedule: September 1, 2013 to August 31, 2016
Current Project Year: September 1, 2015 to August 31, 2016
Participant(s): Christopher Weeks, Michigan State University, Michigan
Extension Liaison: K. Quagrainie, Purdue University, Indiana
Industry Liaison: William Lynch, Mill Creek Perch Farms, Marysville, Ohio
Reason for Termination: Project objectives completed and funds have been terminated.

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

According to the USDA aquaculture censuses, aquaculture in the North Central Region (NCR) experienced a 19% drop in registered farms, and a 4% increase in production value between 2005 and 2013. Over this same period, aquaculture extension FTEs in the region dropped from approximately ten down to five. A 2014 NCRAC needs survey (Weeks, Lynch, and Morris unpublished data) found that the majority of survey participants perceived regulations, feed costs and lack of available funding to be top impediments to industry expansion. There are growing concerns within the industry of rising regulatory constraints including the Lacey Act, incidental possession of aquatic invasive species, effluent discharge requirements, and problems obtaining permits for expansion of traditional, outdoor, open water systems. Current public perception and policy appears to favor development of indoor recirculating aquaculture systems (RAS). A review conducted by the RAES showed close to 80% failure rate of RAS startups in the US, and 90% failure in the NCR. The RAES continues to assess these patterns, and develop partnerships and strategies to address barriers holding back regional aquaculture expansion.

Technical Summary and Analysis

Objective 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. Aquaculture Needs Survey The RAES, along with the Chair of the Industry Advisory Council (IAC; Bill Lynch), and NCRAC Director (Joe Morris) conducted a NCR Aquaculture Needs survey in 2014 administered in Survey Monkey™ using the NCRAC email contact list as an initial population pool. Listed were 703 individuals from industry, academic institution and government agency backgrounds who have expressed interest in aquaculture.

Twenty-five individuals were randomly selected from each state for survey participation. North Dakota was the only exception with 19 contacts listed. Invitations were also extended to all NCRAC members (50) and aquaculture state association officers (52) in the NCR, whose email addresses investigators were able to obtain.

Respondents perceived regulations, feed costs and lack of funding and loans were rated as the top three impediments facing regional aquaculture growth by all groups collectively. There were apparent differences; however, in how impediments were perceived across career groups (Figure 1). When asked if NCRAC was to focus on three species or groups with best potential to substantially increase NCR aquaculture in the next five years, the collective group responded with walleye/ saugeye as number one (44%), followed by yellow perch (43%), and a tie between largemouth bass and trout/salmonids (29%) to finish the top four. However, there were rather wide differences in respondent perceptions towards species priorities when broken down by state (Table 1). Additional activities undertaken by the RAES from 2013-2016 under objective one include: • 2014 and 2016 updates to the NCRAC Regulation website "State Importation and Transportation Requirements for Cultured Aquatic Animals". • Steering committee action and conference facilitation for: 2014 NCR Aquaculture Conference (Toledo, OH), 2015 Michigan Seafood Summit (East Lansing, MI), 2015 Dialogue on Open Water Aquaculture (St. Ignace, MI), 2016 NCR Aquaculture Conference (Milwaukee, WI), 2016 Michigan Seafood Summit (Traverse City, MI), 2016 Great Lakes Regional Seafood Workshop (Milwaukee, WI). • Twenty presentations at conferences and association meetings. • Extension support through phone, email, list serves, eXtension Ask-an-Expert, site visits and direct personal contact at numerous venues across the region.

Public education related to open water aquaculture at various speaking and comment forums. • Industry representation at public meetings likely to impact aquaculture and baitfish industry sectors (e.g., Great Lakes Panel for ANS), identifying contacts for industry consultation, and leading case by case discussions on regulatory issues for NCR producers.

Objective 2. Develop and implement strategies to address and promote aquaculture sustainability in the NCR. In a paper published by Journal of Extension, the RAES summarizes results from a series of open forum discussions on sustainable aquaculture development in the NCR (Weeks2013). Specifically, that paper discusses the concept of sustainable aquaculture, how it is perceived regionally, and presents a model that allows for increased focus towards three principle components of sustainability: environmental conservation, social benefits, and economic viability.

The RAES obtained outside (non-NCRAC) funding and was principal investigator on a project that produced a strategic plan for a thriving and sustainable aquaculture industry in Michigan (Colyn et al. 2014). In a Sea Grant published report stemming from that project, the authors stated that Michigan has high potential to increase sustainable aquaculture through indoor, flow through, and net pen systems in the Great Lakes. The release of that report generated a flurry of media, discussion and legislation activity due in large part to a strong opposition base against open water aquaculture in the Great Lakes. An important lesson learned from these events was that social carrying capacity is a critical component to aquaculture expansion in the US. Most of the opposing views seem to concede that aquaculture, especially seafood production, is important, but that aquaculture can be done indoors in urban settings. In response, the RAES completed an ad hoc review examining success rates of recirculating aquaculture systems in the US and presented results at four regional meetings. Successes were defined as facilities operating more than five years. Overall the success rate was estimated to be approximately 20% in the US, and 11% in the NCR, with nearly all production going to live fish premium markets. Indoor RAS have higher capital costs, greater carbon footprint, and require premium market prices compared to traditional systems.

The RAES continues to further discussion aimed at finding ways to overcome these obstacles. An additional focus area is aquatic invasive species (AIS). Currently the RAES, with fellow NCRAC researchers from Michigan Sea Grant and University of Minnesota, are examining the feasibility of a verification program designed to reduce risk of AIS movement in aquaculture and baitfish practices (Weeks et al. 2016a; Weeks et al. 2016b). This project also is supported by non-NCRAC funding and is expected to be completed by the end of this year.

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. The RAES provided voice regarding industry interests at various private and public forums and has standing appointments to the Committee for the Right to Farm Generally Accepted Agriculture and Management Practices, Michigan Commission of Agriculture; Great Lakes Panel on Aquatic Nuisance Species; National Aquaculture Association ANS Committee, Aquaculture in Michigan (AIM), and NSF International Global Food Division Advisory Council.

In 2014 the RAES was voted into the NCRAC Board as an ex-officio member, working closely with NCRAC Director and others on issues such as improving NCRAC's project selection protocols and updating the NCRAC Strategic Plan. The RAES also helped to build and strengthen partnerships with a number of other organizations including, Michigan Sea Grant, Indiana and Michigan Soybean Associations, Soy Aquaculture Alliance, Coalition for U.S. Seafood Production, National Institute for Sustainable Aquaculture, initiative for Ohio Seafood co-op, and private agricultural business startup groups such as Originz, and Engle-Stone Aquatic $ LLC. In addition, the RAES continued to work with nonprofit groups including Aquaculture Research Corporation on Great Lakes open water aquaculture, and the Nature Conservancy on aquatic invasive species issues.

Objective 4. Coordinate efforts for seeking non-NCRAC support for NCR aquaculture development. From 2013-2016, the RAES obtained as $80,000 of outside funding as a principal investigator, and an additional $460,000 as a co-investigator for projects to support NCR aquaculture. Additionally, the RAES has supplied a number of support letters for other regional projects and is a project collaborator on the recently awarded Aquaculture Boot Camp 2 project awarded to The Ohio State University and other partners by USDA.

Principal Accomplishments

The RAES program has continued to provide important contributions towards aquaculture development over a time frame when aquaculture extension effort in the region was drastically reduced. Potential for aquaculture to expand centers on sustainability and social carrying capacity. The RAES has built a reputation that continually strives to message this as an important principle, and that sustainable development must address the environment, society, and economics at the same time. Regulations, permitting, and funding continue to be problematic for current and potential producers. The RAES, through the NCRAC regulation website, presentations, liaison services, list serves and workshops, has helped bring clarity, awareness, and knowledge of regulations across the NCR. For example, the NCRAC regulation website received 393 page views with 234 hits to specific state regulation web pages in September of 2016. Over the past two years the RAES has helped coordinate direct outreach activity to 600 attendees at conferences, meetings and summits. Additional outputs include personal verbal communications (over 50 per month), NCR Fish Culture List serve postings (100 postings to 159 subscribers on the annually), email inquiries (several per week), and over 20 presentations since 2013.

The RAES along with two other NCRAC members secured funding through the 2015-2016 State of Michigan Comprehensive Aquatic Invasive Species State Management Plan to examine the potential of establishing an AIS prevention verification program. That project is nearing completion and receiving interest in both industry and regulatory agencies. An initial report on AIS management in Great Lakes aquaculture and baitfish sec tors, and a feasibility assessment have been completed. A case study is now underway. Access to funding remains a challenge and is due in large part to the amount of risk associated with high costs and failure rates associated with RAS. Additional risk and burden is associated with regulatory costs and pressure arising from various groups opposing expansion of traditional, open water systems.

The RAES has continued to provide sound science based information to stakeholders and legislators, and seek partnerships and opportunities to improve viability of all aquaculture systems through a platform that c enters on sustainability. The Michigan Aquaculture Strategic Plan has been an instrument of change and discussion for aquaculture across the region. It has also been a good exercise on social carrying capacity in aquaculture development attempts. The state of Michigan has reportedly doubled its commercial fish production over since the release of the plan. The RAES has been working to build new partnerships and strengthen existing ones. Investigator Weeks is frequently asked for industry viewpoint in national and international program meetings such as the Great Lakes Panel on aquatic nuisance species and the NSF International Global Food Division Advisory Council. The RAES is currently co-investigator on two additional NCRAC projects with Dr. Carol Engle, Engle-Stone Aquatic $ LLC, designed to build leadership skills to aquaculture association members in the NCR. The RAES is also co-investigator on a $456,000 grant for education and outreach for sustainable aquaculture in Michigan.

Impacts

Impacts are difficult to measure because of current trends in US aquaculture as a whole. Overall fish production is down, mainly due to dramatic reductions in US catfish production from southern US states. With that said, there are some indications that production is stable or increasing in the NCR. The state of Michigan went from one to three facilities operating under NPDES permits that raise rainbow trout for the food market. According to a recent farm Bureau article, one of those facilities is currently producing up to one million pounds per year. Aquaponics systems are rising up across the NCR, mainly at hobby scale level, but there are also reports of facilities achieving positive cash flow, mainly in areas with close access to white table cloth restaurants. RAS economics remain questionable; however, there are a number of tilapia facilities operating in Ohio and Indiana, a large scale barramundi startup operation in Iowa, and a number of new small marine shrimp production facilities across the NCR. It is noted that the RAES has no direct ties to some of these facilities, but the regional extension network as a whole continues to provide support and information in effort with the overarching goal of helping them become successful.

Recommended Follow-Up Activities

Additional recourses and effort are necessary to address how aquaculture and bait harvest practices are accepted by society at local and regional scale (social carrying capacity). Specifically, more work is needed to educate all stakeholders on aquaculture sustainability comprehensively across aspects of human health, economics, food and water demands, nutrient utilization, and resource management potential. In addition, we must continue to strive, through research and extension, ways to reduce aquaculture associated risk, expand markets, and provide training and tools to help entrepreneurs develop, and operate, economically viable RAS and aquaponics business models.

Publications, Manuscripts, Workshops, and Conferences

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

Technical Update

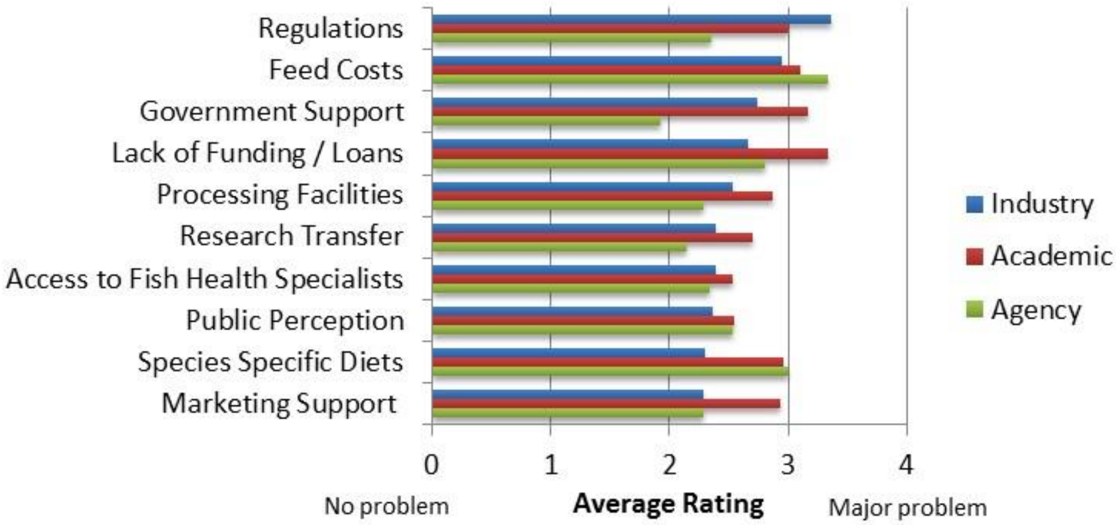

Figure 1. Average Impediment Ratings to Aquaculture Development in the North Central Region by Industry, Academic and State and Federal Agency Groups.

Table 1. The top three species with greatest potential for commercial aquaculture as perceived collectively by state in the North Central Region.

State	#1 Species	#2 Species	#3 Species
IL	Tilapia	Largemouth Bass	Hybrid Striped Bass
IN	Largemouth Bass	Yellow Perch	Shrimp (Marine)
IA	Shrimp (Marine)	Yellow Perch	Walleye / Saugeye
KS	Catfish	Sunfish / Bluegill	Largemouth Bass
MI	Trout / Salmonids	Walleye / Saugeye	Yellow Perch
MN	Baitfish	Yellow Perch	Walleye / Saugeye
MO	Sunfish / Bluegill	Largemouth Bass	Crappie
NE	Walleye / Saugeye	Yellow Perch	Trout / Salmonids
OH	Largemouth Bass	Yellow Perch	Walleye / Saugeye
SD	Baitfish	Yellow Perch	Marine / New
WI	Walleye / Saugeye	Trout / Salmonids	Yellow Perch

Project Title: Establishing Largemouth Bass Strains for Rapid Growth to 1.5 Pounds in the North Central Region [Progress Report]
Key Word(s): Largemouth Bass
Total Funds Committed: $155,000
Initial Project Schedule: September 1, 2014 to August 31, 2016
Current Project Year: September 1, 2015 to August 31, 2016
Participants: Brian Small, Southern Illinois University- Carbondale; Han-Ping Wang, The Ohio State University, Ohio; D. Glover, The Ohio State University, Ohio
Extension Liaison: Paul Hitchens, Southern Illinois University, Carbondale

Project Objectives

1. Identify the best genetically distinct largemouth bass populations for fast growth in the NCR.
2. Conduct a meta-analysis using all appropriate data for largemouth bass from both published and non-published sources to identify at minimum three populations of LMB with the potential to exhibit rapid growth to target weight in the NCR.
3. Evaluate the identified populations at two or more latitudes in the NCR to identify the optimal source population

Deliverables

1. Publication of results in journal articles(s).
2. Extension products, including a selection mix.

Project Summary

Largemouth bass (LMB) is an important aquaculture species. Interest in improving commercial culture efficiency has grown due t o t he great demand and high value compared to other cultured species. While this fish has been extensively investigated for management of the fisheries and hatchery production, little research has been conducted to maximize growth for commercial foodfish production. A NCRAC Priority is to increase the efficiency of LMB growth to market size through means beyond dietary modification. One impediment beyond nutritional insufficiency is the rearing of LMB stocks with little to no domestication or selective breeding for efficient product ion. Therefore, strain evaluation and identification of the best largemouth bass populations for fast growth would result in an immediate impact on the economic ret urn of many small aquaculture operations in the North Central Region (NCR). At the completion of this project , anticipated out comes include a descript ion of LMB genetic diversity among commercial and public stocks available to NCR, the development of a select ion markers and a matrix for fast growing LMB, and the identification of fast growing populations verified in product ion systems.

Anticipated Benefits

The great demand for largemouth bass and t heir high selling price and growth rate (compared to other cultured species) have raised interest in t heir commercial culture. Differential performance of genetic strains of largemouth bass is an import ant management consideration for bot h recreational fisheries and aquaculture. Therefore, strain evaluation and identification of the best genetically distinct largemouth bass populations for fast growth and the optimal source population would result in an immediate impact on the economic ret urn of many small aquaculture operations in the North Central region. A NCRAC Priority is to increase the efficiency of LMB growth to 0.45-0.68 kg (1–1.5 pounds) through means beyond dietary modification. At the completion of this project, we expect to identify the best populations of LMB with the potential to exhibit rapid growth to target weight for the NCR aquaculture industry. Furthermore, a descript ion of diversity among commercial and public stocks available to NCR producers will be available. At present there is little consensus regarding the stocks currently produced, with opinions that all the LMB produced are of similar genetic background, coming primarily from a single fingerling producer, to the opinion that many producers have already selected for fast growth on their own.

If stock improvement is to be made, a thorough investigation of the genetics and the management must first be assessed. Objectives 1 and 2 will address these issues. Specifically, Objective 2 will take the growth information obtained from the literature, surveys, and public databases to predict stocks of fast growing fish for the NCR. As a result, a select ion matrix will be made available to commercial producers. The third objective will validate the result s of Objective 1, used for selecting fast growing stocks, by conducting product ion studies during the first year of growth. Although this project does not provide funding beyond year 2, it is anticipated that the PIs will be able to find funding to continue the product ion studies to market weight and ultimately present conclusive evidence t o t he NCR producer. Regardless, workshop materials will be developed to discriminate the result s and t rain producers how to use the genetic data and select ion matrix for t their own breeding programs. Result s will also be published as fact sheet s and research papers.

Project Progress
Most of work has been completed on objective 1. In Year 2, The Ohio State University genotyped 200 additional largemouth bass from 20 wild populations across the United States using eight microsatellite loci, which are standard genetic markers for population genetic analysis. The data are been analyzed together with previous data to confirm the major findings resulted from previous data: (1) Allelic richness was lower among cultured populations than among wild populations; (2) Effective population size in hatcheries could promote high levels of genetic variation among individuals and minimize loss of genetic diversity; (3) The majority of largemouth bass populations had a significant heterozygosity excess, which is likely to indicate a previous population bottleneck; (4) The phylogeny based on eight microsatellites revealed a clear distinct ion bet ween northern and southern populations, although samples from 25 populations were different (Figure 1). The information provides a valuable basis for development of aquaculture genetic breeding programs in largemouth bass.

Southern Illinois University Carbondale (SIUC) collected DNA from 30 LMB populations across the NCR in year 1. DNA from each fish was sent t o t he Ohio Stat e University for analysis of genetic diversity, to be determined in year 2 and added t o t he results discussed above. Analysis of putative genetic growth markers associated with the IGF-I and IGF-II genes was conducted on these samples at SIUC. Unique alleles for these genes were identified NRC fish and share some similarities t o t hose published for increased growth in Chinese LMB populations (Li et al. 2009, 2012). A unique allele for IGF-II in some of the populations was identified. Based on these results and those of Li et al. (2009, 20012), four LMB stocks were selected for a juvenile growth study to verify predicted growth associations.

Objective 2 was completed in year 1 by collecting largemouth bass weight - and length-at -age data from LMB populations throughout the NCR. Meta-analysis results by state suggested Kansas had the fastest growing LMB with an estimated time to market size of 1.05 years. However, Kansas had data from only one reservoir. States with large data sets ranked as follows: Illinois (1.38 years), Iowa (1.56), South Dakota (1.81), Wisconsin (2.22), Minnesota (2.40), Ohio (2.53), and Nebraska (4.19). Remaining NCR states either did not send data or it was insufficient for use with the statistical model, as many hatcheries and fish farms do not collect and keep records of data or were unwilling to share information.

Objective 3: Based on result s from Objectives 1 and 2. A 12-week growth trial was performed at SIUC using largemouth bass fingerlings from four different LMB stocks picked based on results from genetic analysis. The growth trial showed no definitive correlation between growth rate and IGF I or II alleles. The growth trial did show that largemouth bass that came from JM Malone & Sons farm showed faster growth by weight and length as well as showing better body condition than fish from the other three sources. Malone's fingerlings finished with the most weight (68.18 g; 2.4 oz) followed by Farm Cat Inc. (60.25 g; 2.12 oz), Logan Hollow Fish Farm (57.44; 2.0), and Arkansas Pond Stockers (45.24; 1.60 oz). Based on these results it is suggested that Malone's fish be used for a full scale growth trial to complete objective 3. SIUC delivered ~120 identified LMB broodfish to Piketon aquaculture facility in June 2016. Around 60 females and 60 males from the identified group were stocked in 0.10 ha (0.25 acre) pond at Piketon, and a similar numbers of fish from Ohio control group were stocked in another 0.40 ha (0.25 acre) pond. The control group produced around 10,000 fingerlings, but the identified group produced only about 800 fingerlings. Investigators believed that most fish in the identified group had already spawned before arriving Ohio, since the spawning season is May to June in Arkansas. Investigators will repeat this task to produce enough fingerlings for Objective 3 pond trials next year.

Target Audiences

Largemouth bass growers and breeders

Outreach Overview

Workshop materials will be developed to disseminate the results and train producers how to use the genetic data and selection matrix for their own breeding programs. Results will also be published as fact-sheets and research papers. We anticipate results of Objectives 1 and 2 being made available in year 2 and objective 3 within a year of study completion.

Deliverables (Outputs)

Selection matrix and genotyping data will be compiled into a user-friendly format for largemouth bass growers and breeders, and presented through extension presentations and through the NCRAC website. Planned outputs also include publications of genetic diversity results, growth associated genotypes results, selection matrix results, and growth trials in both journal articles and extension products.

Outcomes/Impacts

- Predictions of which populations of LMB will grow the fastest to market size in the NCR have been made which have the potential to be used by largemouth bass breeders and growers in deciding what fish to acquire, breed, or purchase.

- New knowledge of genetic diversity in largemouth bass population will contribute toward the development of selective breeding programs.
- Discovery of genetic markers associated with largemouth bass growth will accelerate the rate of selective breeding for growth to market weight.

Impacts Summary

Relevance. — The great demand for largemouth bass (LMB) and their high selling price and growth rate (compared to other cultured species) have raised interest in their commercial culture. Slow growth has led to a call for increased efficiency of LMB growth to 1–1.5 pounds through means beyond dietary modification.

Response. — Strain evaluation and identification of the best genetically distinct largemouth bass populations was assessed by microsatellite and SNP genetic analysis for genetic diversity and growth. A complementary meta-analysis of growth data from NCR LMB population was conducted to suggested fast growing populations for broodstock development.

Results. — Predictive measures of fast growth and the identification of optimal source populations would result in an immediate impact on the economic return for many small aquaculture operations in the North Central region.

Recap. — At the completion of this project, anticipated outcomes include a description of LMB genetic diversity among commercial and public stocks available to NCR, the development of selection markers and a matrix for fast growing LMB, and the identification of fast growing populations verified in production systems. Improved growth efficiency and shorter-time to a market size of 1.5 pounds will improve overall survival and the economics of production for NCR LMB producers.

Publications, Manuscripts, Workshops, and Conferences

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

Project Title: Developing Genetically Fast-Growing Monosex Populations in Bluegill [Progress Report]
Key Word(s): Sunfish
Total Funds Committed: $160,000
Initial Project Schedule: September 1, 2013 to August 31, 2015
Current Project Year: September 1, 2015 to August 31, 2016
Participants: Charles E. Hicks, Lincoln University, Missouri; Han-Ping Wang, The Ohio State University, Ohio; James Wetzel II, Lincoln University, Missouri
Extension Liaison: Charles E. Hicks, Lincoln University
Industry Liaison: Curtis Harrison, Harrison Fisheries, Inc., Missouri

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 (*Lepomis macrochirus)* 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 continued. Temperature effects on sex ratio were investigated using four different geographic populations and results strongly suggest s that both temperature-dependent sex determination (TSD) and genetic sex determination (GSD) exist in bluegill.

Objective 2 - Three batches of Bluegill that were a product of Lincoln University's (LU's) selective breeding program for food-fish production were transported to Ohio State University's (OSU's) South Center Wet Lab. Twenty-four selected females were single-mated to 24 of OSU's super – males with each pair in one of 24 round flow - through tanks with each tank equipped with an artificial spawning nest. Water temperature and photoperiod were set at 25°C (77^{o}F) and 16 h light: 8 h dark. Fish were checked twice daily and when nests were found with eggs, the nests were singly placed in the bottom of aerated 400-L (106-gal) tanks similar to those used for mating pairs. Fifteen pairs spawned yielding 12 useable batches of expected all-male Bluegill stock.

Objective 3 - The all – male Bluegill were sent to LU for comparison of growth, sex ratios and production characteristics with unselected Northern Bluegill and Coppernose Bluegill stocks. Initial intent was to rear the all – male and reference stocks in separate tanks (3 tanks per stock) of the same recirculating aquaculture system (RAS) with similar stocking densities although the number of fish provided by OSU was too low to allow for stocking densities likely to be employed in a tank setting. Adjustment was made to rear the three stocks commingled in multiple tanks (common gardens). Each common garden contained similar numbers of fish of all stocks at time of stocking. The common gardens were started in April 2016 and monitored for growth performance.

Growth was less for all three stocks than realized for similar sized Bluegill used in other trials of the same RAS. In an effort to promote faster growth the three common gardens were split into six in August 2016 using tanks of the same size roughly halving stocking density. Estimated growth at that time caused LU to request an additional no-cost extension from October 31, 2016 through April 30, 2017. Additionally, the increased number of tanks is allowing the comparison of manual-feeding (hand-feeding) and automatic feeding. The former is most prevalent with commercial production while the latter has been shown to produce better results for Bluegill up to the average size fish used at the beginning of this trial. The extended common garden trial will be terminated in February 2017, at which time fish will be processed for determination of sex based on gonads and dress out.

Two additional trials have completed using excess Bluegill generated at LU for intended use as objective 3 reference fish. The first trial compared current commercially available diets hand-fed to Bluegill in tanks. Higher protein and energy diets generally produced better growth and feed conversion efficiency although production cost as a function of feed cost supported use of intermediate diets in terms of crude protein and energy level. Where feed conversion is more important, such as with fish reared under conditions that are more intensive or other management issues are more costly, then the higher protein and energy diets are likely to be preferable.

A second additional trial is looking at sex within a brood as a function of size well before fish can be sexed externally or based on appearance of gonads. Three broods where each sorted into three groups based on size. Seventy-five fish for largest and smallest group of each brood were reared to the point where they could be sexed with certainty based on appearance of gonads. Sex ratio was not a correlated with size of fry at the time of sorting before sex could be determined by gross observation. Additionally sex ratio can vary markedly between broods that differ only in the parents used to conceive them.

At Piketon facility, an experiment on comparison of growth, sex ratios and production characteristics of mostly-male (~95%) and regular mixed population was started in late November 2016. The preliminary data showed: 1) Size was much uniform for near-all-male group and coefficient of variation (CV) for body weight (100*standard deviation / mean) was significantly lower in the male groups comparing control groups (53.1 vs.76.0 on average); 2) Survival of near-all-male groups was significant higher than that of mixed sex groups (25.0% v.s. 3.4% on average); CV for body weight is the most important determinant for survival because we found a few number of large size fish chased and bite small size fish and resulted in mass mortality just; 3) So far, no significant difference in body weight of juvenile has been detected between the two treatments. The comparison experiment will be continued for at least 6 months.

Objective 4 - At the end of the above experiments, sex ratios of the two groups at two locations will be determined and compared. At the end of the above experiments of growth evaluation, production data including survival, growth rate, and total production, FCR (for separate rearing experiment) will be compared between the selected all or mostly-male bluegill and the control

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.

Project Title: Develop Systems and Diet Strategies to Reduce Yellow Perch Larval Mortality Burst in Indoor Recirculating Aquaculture Systems [Progress Report]
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, 2015 to August 31, 2016
Participants: Gregory Fisher, University of Wisconsin-Stevens Point, Wisconsin; Christopher F. Hartleb, University of Wisconsin-Stevens Point, Wisconsin; D. Allen Pattillo, Iowa State University, Iowa; Han-ping Wang, The Ohio State University, Ohio
Extension Liaison: Allen Pattillo, Iowa State University
Industry Liaison: Rich Lackaff, V - Bar Aquaculture, NE

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 or 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 North Central Region (NCR).

Project Progress

Objective 1. (UWSP-NADF). UWSP: Based on year 1 results, culture conditions were optimized into a 3x3x1 factorial design with three replicates per treatment. The interior of all tanks were painted black, turbidity was narrowly defined as either 12.5, 25, or 37.5 NTU, and water surface spray was limited to 0.6 L/min (0.16 gal/min),0.8 L/min (0.21 gal/min), or 1.0 L/min (0.26 gal/min). A random sample of 25 fry per tank was collected every 2 weeks and measured for length and weight gain; mortalities were recorded as observed mortality and removed daily. At the end of the 70-day study, remaining fish were counted and measured for tot al length and an aggregate weight of 100 fry along with 100 fry/tank examined for the presence of food in the gut and gas bladder inflation. Percent ages of unobserved mortality (cannibalism) and observed mortality were calculated. No statistical differences ($p>0.05$) in growth, length and wet weight, were observed every two weeks nor were differences observed after 70 days for larval yellow perch raised in any of the treatments. Both length and weight gain were greatest, though minimal gain, for larval yellow perch raised with water surface spray of 1.0 L/min (0.26 gal/min) with turbidity set at 25 and 37.5 NTU.

After the first week, 100% of the larval yellow perch sampled contained food in their gut (feed acceptance), yet after three weeks only 50% of the fish had food in their gut. From week five to eight, 75-100% of the remaining larval yellow perch had food in their gut. From week one through week eight, 96% of the larval yellow perch had inflated their swim bladder. Mortality rates were high throughout the experiment and among all treatments. Approximately 3% of the larval yellow perch survived until the end of the 70-day experiment regardless of treatment. Minimal cannibalism was observed but developmental deformities were common including poor opercula development, spinal deformities, and mouth structural abnormalities.

Objective 2 (OSU).
Objective 2.1 – Commercial-scale rotifer and *Artemi*a product ion system was further improved, and the 24/7 auto-feeders were optimized. Rotifer was harvested and fed twice rather than once in consideration of its rapid life cycle. Live feed product ion systems were improved either through reducing feeding times for rotifer or reducing production cost, e.g. decreasing salinity for *Artemia* hatching from 25-30% to 12-15%, without any compensation of output. The regular air stones in the stocking buckets of live feeds (rotifer/*Artemia)* in the auto-feeders were replaced by round, flat air stones because we found a considerable proportion of live feed sink on the bottom and were not pumped out to fry tanks last year. This improvement will allow more than 90% of live feed to be delivered to fry tanks. Feeding interval for 24/7 auto-feeders was adjusted to 1 hour because we found that *Artemia* would survive up to about 1 hour when they were dispersed into freshwater rearing tanks. In addition, investigators found that contamination between two production systems, rotifer and *Artemi*a, should be avoided because a few number of *Artemia* will considerably reduce rotifer product ion.

Objective 2.2 – The two best feeding regimes based on 2015 result s were used. Through co-feeding strategy, mixing rotifer and *Artemi*a in stocking bucket, feeding formula feed before hand-feeding live feed, gradually reducing live feed, significantly increased larvae survival to about 25% at 31 days post-hatching (DPH) for the following regime: 3-7 DPH, rotifer (160 µm); 5-10 DPH, small Artemia (428 µm); 8-20 DPH, regular *Artemi*a (486 µm) + Otohime B1 (200-360 µm) + Zeigler AP100-150 µm; 21-30 DPH, regular *Artemia* (486 µm) + Otohime B2 (360-600 µm) + Zeigler AP100-150 µm + Zeigler AP150-250 µm; 30-45 DPH, regular *Artemia* (486 µm) + Otohime B2 (360-600 µm) + Zeigler AP250-450 µm + Purina® AquaMax® Fry Starter 100 (800 µm). This feeding regime provided yellow perch larvae with combination of live feed and formula feed, a wide range of feed size, as larvae grow.

During 25 to 31 DPH, perch fry were aggressive to formula feed and could digest formula feed without any observable problem. Unfortunately, a technique issue (heavy aeration caused violent agitation of water for about an hour) resulted in totally loss of fry. It is suggested that live feed should not be completely replaced until fry reach 40 DPH. Several related studies was completed: 1) by monitoring egg size produced by different strains/families, we have identified some strains/families that produced significantly larger-mouth gape progeny and larger eggs than others; 2) variation of egg size is dramatically different among strains of our genetically improved fish, indicating there is a large range of selection for large eggs; 3) we found predation and ingest ion of prey at the beginning of feeding is limited by the mouth gape in fish larvae which determine larvae survival.

Target Audiences

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

Outreach Overview

Results will be made available in Spring 2017 both as modules and in a report.

Deliverables (Outputs)

Two videos were produced on "Feeding Yellow Perch Fry" and "Growing & Maintaining Natural Feeds for Larval Fish" by Iowa State University. They are available on the NCRAC website under the "videos" tab and on the NCRAC vimeo channel at https://vimeo.com/127717048 and https://vimeo.com/127639646 . Modules for self/group training will include new procedures/protocols learned from the project. There was a no-cost extension for this project so that an additional training video can be developed out of the University of Wisconsin-Stevens Point-Northern Aquaculture Demonstration Facility (UWSP-NADF). This video foot age has mostly been collected and a rough draft of the video has been edited together. A script has been drafted, but the NADF needed more time to analyze their results from this year before they could be added into the video.

Outcomes/Impacts

The impacts of this proposed project will be primarily through the development of systems and strategies to enhance mass product ion 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 product ion costs due to increased growth rat e and survival of larval yellow perch by using tank systems and feeding regimes optimized for the early-life stage. At the completion of the 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
anammox	anaerobic ammonium oxidizing bacteria
AOA	ammonia oxidizing archaea
AOB	ammonia oxidizing bacteria
APHIS	Animal and Plant Health Inspection Service
AREF	Aquaculture Regional Extension Facilitator
AquaNIC	Aquaculture Network Information Center
BOD	Board of Directors
BW	body weight
□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 *Aeromonas* infection
MAS	motile *Aeromonas* septicemia
MDNRE	Michigan Department of Natural Resources and Environment
µg	microgram(s)
mg	milligram(s)
MC	Mill Creek
min	minute(s)
mL	milliliter(s)

mm	millimeter(s)
MSU	Michigan State University
MT	methyltestosterone
N	number
NAA	National Aquaculture Association
NADA	new animal drug application
NASAC	National Association of State Aquaculture Coordinators
NCC	National Coordinating Council
NCR	North Central Region
NCRAC	North Central Regional Aquaculture Center
NIFA	National Institute of Food and Agriculture
NOB	nitrite oxidizing bacterial
OCARD	Ohio Center for Aquaculture Research and Development
OSU	Ohio State University
oz	ounce(s)
PAH	Phibro Animal Health
PCR	polymerase chain reaction
PFU	plaque-forming units
POW	Plan of Work
ppm, ppt	parts per million, parts per trillion
Purdue	Purdue University
RAC(s)	Regional Aquaculture Center(s)
RAES	Regional Aquaculture Extension Specialist
RAET	Regional Aquaculture Extension Team
RAS	recirculating aquaculture system
RS	Rimler-Stotts
SPAH	Schering-Plough Animal Health
TC	Technical Committee (TC/E = Technical Committee/Extension; TC/R =Technical Committee/Research
™	trademark
TSA	Tryptic Soy Agar
UMESC	Upper Midwest Environmental Sciences Center
USDA	U.S. Department of Agriculture
USFWS	U.S. Fish and Wildlife Service
UW-Madison	University of Wisconsin-Madison
UW-Milwaukee	University of Wisconsin-Milwaukee
VHS	viral hemorrhagic septicemia
VHSv	viral hemorrhagic septicemia virus
WATER	Wisconsin Aquatic Technology and Environmental Research

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