

**NORTH CENTRAL
REGIONAL AQUACULTURE CENTER**



ANNUAL PROGRESS REPORT 2016-17

January 2018

TWENTY-SEVENTH ANNUAL PROGRESS REPORT

For the Period
September 1, 2016 to August 31, 2017



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January 2018

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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.

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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.

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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

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(#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 \$22,967,663. Currently, two 2-year grants are active (FY14-17); the first 26 grants (FY88-10) have terminated. The Center annually coordinates a biannual program planning meeting which typically sets priorities for the next 2-year funding cycle and calls for 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.
- ▶ In 2016, the Center developed a new grant development process that includes RFA for Pre-Proposal, Instructions for Submission of the full proposals, and Rapid Response Proposals for short-term projects.
- ▶ All project outlines are peer reviewed by individuals who are well qualified for a particular project because of their expertise and interests. Full project outlines are mailed to five or six reviewers within the twelve-state North Central Region as well as to five or six reviewers from outside of the region, including representatives from the aquaculture industry. Reviewers from outside of the region are selected from lists of experts that have been generated by solicitation of reviewers from the other four Regional Aquaculture Centers. Please see the attached Confidential Peer Review form for the review criteria.
- ▶ The Rapid Response Proposals are reviewed by the Executive Committees of the Industry Advisory Committee and Technical Committee of Research and Extension (10 members); outside reviewers can be done if directed by the Executive Committee. 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 January 1, 2018, the Center has funded or is funding 117 projects through 530 subcontracts from the first 28 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.

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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, 2016 to August 31, 2017. 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>

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

Project Area	Project Number	Proposed Duration Period	Funding Level	Grant Number
Aquaculture Drugs	1	7/1/96-6/30/97	\$27,000	95-38500-1410
	2	12/1/96-11/30/97	\$950	95-38500-1410
	3	10/1/99-9/30/00	\$8,415	97-38500-3957
	4	6/1/04-11/30/05	\$223,677	2003-38500-12995
	5	7/15/04-7/14/05	\$60,000	2003-38500-12995
	6	11/1/04-10/31/06	\$50,000	2002-38500-11752
	7	1/1/06-12/31/06	\$129,936	2005-38500-15847
	8	9/1/08-8/31/10	\$150,000	2008-38500-19157
	9	9/1/09-8/31/10	\$27,880	2008-38500-19157
	10	9/1/11-8/31/11	\$100,000	2010-38500-20929
	11	9/1/12-8/31/14	<u>\$240,000</u> \$1,017,858	2012-38500-19550
Aquaponics	1	7/1/16-6/30/17	<u>\$24,596</u> \$24,596	2014-38500-22138
Baitfish	1	9/1/92-8/31/94	\$61,973	92-38500-6916
	2	9/1/06-8/31/08	\$111,997	2006-38500-16900
			<u>\$88,003</u> \$261,973	2005-38500-18547
Conferences/Workshops/Symposia				
Environmental Strategies Symposium	1	9/1/00-5/31/01	\$5,000	96-38500-2631
Nat'l. Aquaculture 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	<u>\$5,000</u> \$21,205	2008-38500-19157
NCR Aquaculture Conference	1	6/1/90-3/31/91	\$7,000	90-38500-5008
	2	12/9/98-6/30/99	<u>\$3,000</u> \$10,000	96-38500-2631
Percis III	1	11/1/02-10/31/03	\$4,000	00-38500-8984
Crayfish	1	9/1/92-8/31/94	\$49,677	92-38500-6916
Economics/Marketing	1	5/1/89-12/31/91	\$127,338 \$34,350	88-38500-3885 89-38500-4319
	2	9/1/91-8/31/92	\$53,300	91-38500-5900
	3	9/1/93-8/31/95	\$40,000	93-38500-8392
	4	9/1/99-8/31/01	\$47,916	97-38500-3957
	5	9/1/03-8/31/04	\$50,000	2002-38500-11752
	6	9/1/10-8/31/11	\$23,565	2010-38500-20929
	7	9/1/12-8/31/14	<u>\$75,276</u> \$451,745	2012-38500-19550
Extension ("Base" Extension—Project Nos. 1-15; Aquaculture Regional Extension Facilitator [AREF]—Project No. 16; and	1	5/1/89-4/30/91	\$39,221 \$37,089	88-38500-3885 89-38500-4319
	2	3/17/90-8/31/91	\$31,300	89-38500-4319
	3	9/1/91-8/31/93	\$94,109	91-38500-5900
	4	9/1/93-8/31/95	\$110,129	91-38500-5900

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Project Area	Project Number	Proposed Duration Period	Funding Level	Grant Number
Regional Aquaculture Extension Specialist [RAES]— Project Nos. 18, 19, 20, 21,22,23 and 24.	5	9/1/95-8/31/97	\$10,813	92-38500-6916
			\$20,391	95-38500-1410
	6	9/1/97-8/31/99	\$38,000	97-38500-3957
	7	9/1/99-8/31/01	\$94,000	99-38500-7376
	8	9/1/01-8/31/03	\$28,500	99-38500-7376
			\$18,154	2001-38500-10369
	9	9/1/03-8/31/05	\$28,000	2002-38500-11752
	10	9/1/05-8/31/07	\$211,545	2003-38500-12995
			\$7,735	2005-38500-15847
	11	9/1/07-8/31/09	\$21,850	2006-38500-16900
			\$92,469	2007-38500-18469
	12	9/1/08-8/31/10	\$37,966	2007-38500-18469
			\$22,539	2008-38500-19157
	13	9/1/09-8/31/11	\$29,000	2008-38500-19157
	14	9/1/11-8/31/13	\$35,700	2010-35800-20929
	15	9/1/13-8/31/15	\$45,000	2012-38500-19550
	16	9-1-15-8-31-16	\$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
	191	9/1/05-5/31/09	\$199,624	2004-38500-14269
	201	9/1/09-8/31/11	\$150,000	2008-38500-19157
	21	9/1/11-8/31/13	\$196,612	2010-38500-20929
	222	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
	28	7/1/17-6/30/19	\$188,036	2016-38500-22138
	29	7/1/17-6/30/19	<u>\$158,963</u> <u>2,590,007</u>	2016-38500-22138
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	<u>\$211,957</u> \$976,960	98-38500-5863 2001-38500-10369
Largemouth Bass	1	9/1/05-8/31/07	\$170,000	2004-38500-14269
	2	9/1/14-8/31/16	<u>\$155,000</u> \$325,000	2014-38500-22138
National Coordinator for Aquaculture INADs/NADAs	1	9/1/93-8/31/94	\$2,000	89-38500-4319
		5/15/95-5/14/96	\$5,000	94-38500-0048
		5/15/96-5/14/97	\$6,669	92-38500-6916
			\$3,331	95-38500-1410
		5/15/97-5/14/98	\$15,000	96-38500-2631
		5/15/98-5/14/99	\$13,241	94-38500-0048
		5/15/99-5/14/00	\$10,000	95-38500-1410

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	2	7/15/04-7/14/05 9/15/05-8/31/06 9/1/06-8/31/08 5/15/08-5/14/09	\$9,000 \$15,000 \$40,000 <u>\$25,000</u> \$144,241	2003-38500-12995 2004-38500-14269 2006-38500-16900 2007-28500-18469
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	<u>\$75,000</u> \$559,400	2010-28500-20929
Other	1	9/1/06-8/31/08	Feed Training Carnivorous Fish \$165,446	2005-38500-15847
			<u>\$134,554</u> \$300,000	2006-38500-16900
	1	9/1/07-8/31/09	\$225,000	2007-38500-18469
	1	9/1/09-8/31/10	\$65,000	2008-38500-19157
	1	9/1/11-8/31/13	\$175,000	2008-38500-19157
1	7/1/16-6/30/17	<u>\$34,998</u> \$799,998	2014-38500-22138	
Salmonids	1	6/1/90-8/31/92	\$9,000	89-38500-4319
	2	9/1/92-8/31/94	\$120,799	90-38500-5008
	3	9/1/94-8/31/96	\$149,997	92-38500-6916
	4	9/1/97-8/31/99	\$199,290 <u>\$158,656</u> \$637,742	94-38500-0048 97-38500-3957
Sunfish	1	6/1/90-8/31/92	\$130,758	90-38500-5008
	2	9/1/92-8/31/94	\$149,799	92-38500-6916
	3	9/1/94-8/31/96	\$173,562	94-38500-0048
	4	9/1/96-9/31/98	\$199,921	96-38500-2631
	5	9/1/99-8/31/01	\$199,748	99-38500-7376
	6	9/1/13-8/31/15	<u>\$160,000</u> \$1,013,788	2012-38500-19550
Tilapia	1	9/1/96-8/31/98	\$118,791	96-38500-2631
	2	9/1/98-8/31/00	<u>\$150,000</u> \$268,791	98-38500-5863
Viral Hemorrhagic Septicemia (VHS)	1	9/1/08-8/31/10	\$197,960	2008-38500-19157
Walleye	1	5/1/89-8/31/91	\$177,517	89-38500-4319
	2	6/1/90-8/31/92	\$111,657	90-38500-5008
	3	9/1/91-8/31/92	\$109,223	91-38500-5900
	4	9/1/92-8/31/93	\$75,000	89-38500-4319
	5	9/1/93-8/31/95	\$150,000	93-38500-8392
	6	9/1/95-8/31/97	\$117,395	94-38500-0048

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	7	9/1/99-6/30/02	\$59,835 \$127,000 \$927,627	95-38500-1410 98-38500-5863
Project Area	Project Number	Proposed Duration Period	Funding Level	Grant Number
Wastes/Effluents	1	9/1/92-8/31/94	\$153,300	92-38500-6916
	2	9/1/96-8/31/98	\$100,000	96-38500-2631
	3	9/1/01-8/31/04	\$106,186	00-38500-8984
			\$88,814 \$448,300	2001-38500-10369
White Papers	1	7/1/98-12/31/98	\$4,999	96-38500-2631
	2	9/1/99-12/31/99	\$17,495 \$22,494	97-38500-3957
Yellow Perch	1	5/1/89-8/31/91	\$76,957	88-38500-3885
	2	6/1/90-8/31/92	\$85,723	89-38500-4319
	3	9/1/91-8/31/93	\$92,108	90-38500-5008
	4	9/1/93-8/31/95	\$99,997	91-38500-5900
	5	9/1/95-8/31/97	\$150,000	93-38500-8392
	6	9/1/97-8/31/99	\$199,507	95-38500-1410
	7	9/1/97-8/31/99	\$185,458	97-38500-3957
	8	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
	11	7/1/17-6-30-19	\$162,261 \$1,936,127	2016-38500-25753
TOTAL			\$12,694,489	

PROJECT REPORTS

Project Title: Extension [Progress Report]

Key Word(S): Extension

Total Funds Committed: \$1,243,437

Initial Project Schedule: May 1, 1989 to August 31, 2017

Current Project Year: September 1, 2016 to August 31, 2017

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. Quagraine, 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.

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 and led the development of an aquaculture webinar in 2016 and 2017. This webinar series was a partnership between NCRAC, the National Aquaculture Association and the United States Aquaculture Society and covered a range of important and timely topics.

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. One such an example is the AIS-HACCP plan developed by Kinnunen and Phelps to address 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.

In 2017 Pattillo coordinate a 2-day meeting of the NCRAC Publications Review Team in Des Moines, Iowa. This team of Extension and IAC members reviewed current NCRAC publications for content and whether or not they were still relevant to current aquaculture practices. Authors of past publications will be contacted for identified updates in 2018. New publications have been identified and developed in 2018.

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 completed two NCR-centered fact sheets covering aquaculture and hydroponic components of aquaponics systems. Pattillo also led the development of an aquaculture webinar series that was a partnership among 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.

Outcomes/Impacts

The 2016-17 Aquaculture Webinar Series fostered a partnership among NCRAC, the National Aquaculture Association, and the United States Aquaculture Society. This partnership broadened the scope and participation in these webinars nationwide. This 18-part series covered timely and relevant aquaculture topics for the NCR and the overall US aquaculture industry.

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 ca. 17,000 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.

NORTH CENTRAL REGIONAL AQUACULTURE CENTER

Project Title: Regional Aquaculture Extension Specialist (RAES) [Progress Report]

Key Word: Extension

Total Funds Committed: \$124,993

Initial Project Schedule: September 1, 2016 to August 31, 2018

Current Project Year: September 1, 2016 to August 31, 2017

Participant(s): Christopher Weeks, Michigan State University, Michigan

Extension Liaison: K. Quagraine, Purdue University, Indiana

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

Project Objectives

1. Continue RAES support to the NCRAC Aquaculture Community through ongoing activities in areas of liaison services, leadership, assessing and addressing industry needs and information transfer.
2. 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.
3. Coordinate efforts for seeking non-NCRAC support for NCR aquaculture development.

Deliverables:

1. Open door liaison services to the NCR aquaculture community
2. Serve on 3 or more committees and panels as an industry representative
3. Support for, and interaction with, all NCR state aquaculture associations; attendance at 3 or more state association meetings, regional and/or national conferences per year
4. Direct information exchange to over 500 individuals per year through personal communications and site visits
5. Continue information outlet and topical news on the NCR fish culture list -serve and Xtension Ask-an-Expert
6. Annual updates to the NCRAC regulation website
7. Dialogue and information exchange on policy issues (e.g. Federal Register posts, legislation and regulation)
8. Regional aquaculture needs survey (once every 3 years)
9. Establish partnerships for NCR aquaculture industry development, submitting at least one grant proposal per year as a team member for NCR industry support.
10. Assist the Directors office in strategic planning and project selection protocol effectiveness.

Project Summary

Commercial aquaculture in the North central region (NCR) could be characterized as an industry trying to sustain itself over a period of difficult economic and regulatory constraints. The number of farms in the region dropped from 417 in 2005 to 336 in 2012, yet annual value of production increased from \$35.4 to \$36.7 million (USDA 2013). Efforts to remedy this situation are the primary focus of aquaculture extension personnel; however, less than five state extension FTEs serve over the 12-state region. This includes the current effort provided by the RAES project. Permitting and regulatory issues appear to be a barrier to expansion and entry of facilities employing traditional aquaculture systems. In addition, the NCR has been experiencing a high turnover rate of indoor recycling aquaculture systems (RAS), mainly aquaponics and marine shrimp. This situation has increased information request loading on extension personnel. Economic viability is a primary problem with RAS and aquaponics startups, and finding ways to improve successful entry into the sector is a major concern. Collaboration among current producers, academicians, regulatory authorities, and other relevant entities at local, regional and national levels is vitally important in addressing these needs. The RAES project has demonstrated an ability to provide a coordinated effort in meeting these types of challenges.

Anticipated Benefits

Anticipated benefits include:

- Information transfer to the aquaculture community via list serve, websites, state association events and other direct contact methods
- Continued updates on the NCRAC regulation website
- Coordinated regional effort towards industry development
- An industry voice on state, regional and national regulatory issues such as AIS
- Strengthened partnerships for NCR aquaculture development

- Non-NCRAC support (funding and partnerships) for NCR aquaculture sector

Project Progress

Objective 1.— RAES activities over the 2016-17 project period include: NCRAC regulation website update, a region wide survey on concerns regarding risk of aquatic invasive species (AIS), phone and direct personal contact with stakeholders, attendance and presentations at state association and aquaculture development meetings, interviews, and postings to the NCR Fish culture List Serve. Additionally the RAES obtained funding and was PI on a team to develop a model AIS HACCP verification program for aquaculture and baitfish sectors. The RAES also took lead coordinating roles on steering committees of the 2016 and 2018 North Central Aquaculture Conferences.

Objective 2.— The PI has continued memberships with the National Aquaculture Association, Michigan Farm Bureau, and Coalition for Sustainable Seafood Production (CUSP); served on the Michigan Farm Bureau Aquaculture Advisory Committee, Great Lakes Panel for Aquatic Nuisance Species, NSF Food Division Advisory Council, and various funding review panels; worked with MN and MI DNR agencies, and the Nature Conservancy on AIS HACCP verification programs; and provided interaction with, and presentations to, the National Soybean Council, 4H, and Michigan Environmental Health Association.

The RAES also currently serves as co-PI on NCRAC projects including leadership training for NCR state aquaculture associations, NCRAC Base Extension, and as extension liaison for the NCRAC Comprehensive Outreach and Training project. Additionally the RAES has provided support to regional programs such as Aquaculture Boot Camp (Ohio), the Minnesota Aquaculture Workshop, and Coalition to Support Iowa's Farmers (CSIF).

Objective 3. — The RAES, over the course of the project, has been awarded \$347,000 from non-NCRAC sources to support regional aquaculture growth as PI and an additional \$456,840 as co-PI. He recently submitted a pre-proposal as PI for \$260,000 for a project of regional development from non-NCRAC funding source. Unfortunately, this project was not selected for a full proposal; however, additional funding opportunities for this project are anticipated.

Targeted Audiences

Target stakeholders of the RAES project are ultimately those who would benefit from sustainable aquaculture development in the NCR. Direct beneficiaries include existing producers as well as those in a beginning, or startup phase that have pulled information from the RAES project network. Those receiving potential indirect benefits include future producers, state, federal and tribal agencies, and the general public, through RAES efforts to expand sustainable food production and promotion of seafood health.

Outreach Overview

RAES results are extended to target recipients in a variety of ways. These include NCRAC regulation website update, written and verbal communications, site visits, program (e.g., conferences) facilitation, presentations, NCR List Serve, interviews, technical reports, and publications.

Deliverables (outputs)

Deliverables arising from RAES 2016-2017 activities include personal written and verbal communications directly related to NCR aquaculture estimated at over 50 per week, 90 postings to 167 direct subscribers on the NCR Fish Culture List Serve since 1/1/17, 12 presentations across the region, two interviews, two technical reports on AIS Management and HACCP in the Great Lakes, one technical bulletin submitted for peer review (ongoing), and a journal article draft on AIS HACCP verification. The PI has had discussions on matters related to NCR development with National Aquaculture Program managers and directors including NOAA, USDA, and the National Aquaculture Association. In addition the RAES has made significant contribution to the knowledge base of aquaculture development in the NCR, as well as the corresponding difficulties thereof.

Outcomes-Impacts

The focus of the RAES project centers on regional coordination towards sustainable aquaculture development. Obtaining direct impacts such as economic and production values from the industry is very difficult, and best served through the USDA Aquaculture Census. Unfortunately the census was last conducted in 2012 and is in need of an

update. Conversations with current producers suggest that production levels appear to be holding steady, or in some states increasing, and value is rising uniformly.

The RAES has gained a reputation as a go to information source, and as an industry liaison on regulatory and AIS matters. The RAES is often asked to help clarify legal, biological, environmental, business development and facility design questions, and asked repeatedly to present on these types of issues at meetings and conferences.

Entries to the sector have been primarily in RAS and aquaponics facilities, and a multitude of small operations have come and gone over the past several years. To date, economic viability with indoor systems in the NCR appears questionable, with failure rates between 80-90% based on inquiries and surveys conducted by the RAES. While a few small producers currently serve white tablecloth restaurants in the region, the vast majority of the product from indoor systems is sold into live ethnic markets. Several large-scale indoor facilities (>100,000 lbs) have started up and failed over the last 15 years in the NCR, and two more facilities are just beginning production. Extension personnel and the aquaculture community in general remain hopeful that success rates of indoor aquaculture systems will improve; however, individuals considering RAS and aquaponics should be fully aware of economic constraints, available markets and business development potential. The RAES is noted as an entity to bring this point into mainframe discussion.

In the region, and nation as a whole, awareness towards the importance of sustainable seafood production seems to be taking hold. Unfortunately, with minor exception, US aquaculture expansion is currently limited to sectors producing marine shellfish. This reaffirms that the US continues to struggle with aquaculture development, and remains a key focus area of the RAES.

Impact Summary

Impacts Summary #1. Relevance: Issue-what was the problem?

Expansion of sustainable fish production by existing production facilities in the NCR is being constrained by regulations and permitting. Currently, the majority of entries into the sector are indoor systems, mainly aquaponics, which are experiencing high failure rates. As a result, the risk level associated with aquaculture startups is going to continue to increase. In addition increased information request loading for indoor system development is requiring additional effort for a limited number of aquaculture extension personnel in the NCR.

Impacts Summary #2. Response: What was done?

The RAES has developed over time a series of ongoing and new activities designed to identify the needs of existing and potential producers, help them negotiate and work in the current regulatory environment, and develop and strengthen partnerships and support for NCR aquaculture expansion. These include direct liaison services, needs assessments, information dissemination, program facilitation, serving on panels and committees as an industry representative, and activity seeking and obtaining outside support.

Impacts Summary #3. Results: How did your work make a difference (change in knowledge, actions, or conditions) to the target audiences?

The RAES project continually strives to build a regional network in support of sustainable aquaculture expansion. Working closely with the NCRAC Director, industry, NCR researchers and extension personnel, NGO's, and State, Federal and Tribal agencies, the RAES works to identify industry and public needs, disseminate information, build partnerships, and develop outreach strategies and activities to maintain, and improve, sustainable aquaculture development in the NCR.

Impacts Summary #4. Recap: One-sentence summary.

The RAES has established a reputation as a source to obtain local, state, regional and national information, needs assessment, and teamwork coordination to aquaculture and baitfish industry sectors in the NCR.

Publications, Manuscripts, Workshops, and Conferences

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

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, 2016 to August 31, 2017

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 to the 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 production. Therefore, strain evaluation and identification of the best largemouth bass populations for fast growth would result in an immediate impact on the economic return of many small aquaculture operations in the North Central Region (NCR). 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 a selection markers and a matrix for fast growing LMB, and the identification of fast growing populations verified in production systems.

Anticipated Benefits

The great demand for largemouth bass and their high selling price and growth rate (compared to other cultured species) have raised interest in their commercial culture. Differential performance of genetic strains of largemouth bass is an important management consideration for both 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 return of many small aquaculture operations in the North Central region. A NCRAC Priority is to increase the efficiency of LMB growth to 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 description 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 selection matrix will be made available to commercial producers. The third objective will validate the results of Objective 1, used for selecting fast growing stocks, by conducting production 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 production studies to

market weight and ultimately present conclusive evidence to the NCR producer. Regardless, workshop materials will be developed to discriminate 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 factsheets and research papers.

Project Progress

Objective 1. — Objective has been completed. The Ohio State University genotyped ~1250 largemouth bass in total from 25 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 distinction between northern and southern populations, although samples from 25 populations were different. The information provides a valuable basis for development of aquaculture genetic breeding programs in largemouth bass. A manuscript has been completed based on the results.

Southern Illinois University Carbondale (SIUC) collected DNA from 30 LMB populations across the NCR in year 1. DNA from each fish was sent to the Ohio State University for analysis of genetic diversity, to be determined in year 2 and added to the 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 to those published for increased growth in Chinese LMB populations (Li et al. 2009, 2012). We also identified a unique allele for IGF-II in some of the populations. Based on our 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. — Objective 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 results 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 (68.18g) followed by Farm Cat Inc. (60.25g), Logan Hollow Fish Farm (57.44g), and Arkansas Pond Stockers (45.24g). 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 0.10-ha (0.25-acre) pond. In 2017, due to cannibalism in ponds only ca.2,000 fingerlings were collected from control group and 1,000 fingerlings from the identified group. Using the fish, OSU staff are conducting experiment to compare growth performance of the identified group vs. Ohio control group in an indoor system. The fish were stocked and are being cultured communally in two replicate tanks. As of November, fish from the identified group grew 126.6% faster than control group.

Targeted 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 next year 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 #1. Relevance: Issue-what was the problem?

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.

Impacts Summary #2. Response: What was done?

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 suggest fast growing populations for broodstock development. Growth experiment showed that the identified group grew 126.6% faster than control group so far.

Impacts Summary #3. Results: How did your work make a difference (change in knowledge, actions, or conditions) to the target audiences?

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.

Impacts Summary #4. Recap: One-sentence summary.

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 a 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 Largemouth Bass activities.

Project Title: Developing Genetically Fast-Growing Monosex Populations in Bluegill [Termination 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, 2016 to August 31, 2017

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 (*Lepomis macrochirus*) and its hybrids has been ranked as one of the top priorities in USDA-NCRAC. This research specifically addresses the needs identified by NCRAC. The results of this research will advance our understanding of sex-determining mechanisms in fish. Further, using this information, we expect to be able to obtain super male broodfish using data garnered from this project. The result will be 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.

In this project, we found that water temperatures and genetics affect the number of male Bluegills. Although we are not able to produce all-male offspring due to the nature of the complicated sex determination by both genetics and water temperature in bluegill, the progeny nonetheless were primarily males and the near-all-male population grew substantially greater and larger than unselected Northern Bluegill and Coppernose Bluegill stocks.

Technical Summary and Analysis:

Objective 1. — Progeny test for all-male populations using improved fish from Lincoln University of Missouri was conducted. Temperature effects on sex ratio have been found in some geographic populations, i.e., local conditions with higher water temperatures yielded larger numbers of males and more females in low temperatures. These findings were published in *Aquaculture*. Follow-up investigation using four different geographic populations strongly suggests that both temperature-dependent sex determination (TSD) and genetic sex determination (GSD) exist in bluegill. This paper was published by *The Biological Bulletin*. In addition, an article on the effects of *Astragalus membranaceus* (AM) on growth performance and stress profiles in bluegill sunfish was published in *Fish Physiology and Biochemistry*.

Objective 2 - Three batches of Bluegill that were a product of Lincoln University's selective breeding program for food-fish production was transported to OSU's South Center Wet Lab. Twenty-four selected females were single-mated to 24 of OSU's 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 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 tanks similar to those used for mating pairs. Fifteen pairs spawned yielding 12 useable batches of expected all-male Bluegill stock.

The number of offspring was limited due to the challenging nature of this research in a limited time span. In Spring 2017, OSU researchers produced ~5,000 this type of fish, and next year they should have enough broodfish to produce large number of all-male or mostly-male fingerling for large scale pond test.

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 (three tanks per stock) of the same recirculating aquaculture system (RAS) with similar stocking densities. However, the number of fish provided by OSU was too low to allow for stocking densities needed for the tank study. 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 started in April 2016 and fish monitored for growth performance.

Growth was less for all three stocks than realized for similar sized (initial TL 5 to 10 cm) Northern Bluegill used in other trials of the same RAS at the same time. 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 terminated in February 2017, at which time fish processed for determination of sex based on gonads and dress out.

Two additional trials were 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 more intensive conditions or other management issues are more costly, then the higher protein and energy diets are likely to be preferable.

A second additional trial was looking at sex within a brood as a function of size well before fish could be sexed externally or based on appearance of gonads. Three broods were 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 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 completed under Objective 4.

Objective 4 - At the end of the above experiments of growth evaluation, sex ratios and production data including survival, growth rate, FCR (for separate rearing experiment) were compared between the selected mostly-male bluegill and the control groups at both locations. At LU facility, the large mostly-male and small mostly-male stocks in terms of weight started smaller and grew significantly more than control group in terms of weight increase. Percent weight increase of the mostly-male stocks was 3 to 5 times ($P < 0.0001$) than observed for the reference Northern Bluegill and Coppernose Bluegill stocks; the large (mean 29.4g) and small (15.1g) mostly-male stocks increased in weight by 571% and 900% (Table 1), respectively. Specific growth rate was highest for the smaller mostly-male stock (1.43%) followed by the large mostly-male stock (1.20%) where the values for the Northern Bluegill and Coppernose Bluegill were lowest and not significantly different from each other ($P < 0.0001$; Table 1).

The sex ratio (male: female) for Northern Bluegill, Coppernose Bluegill, large mostly-male and small mostly-male groups are: 50%:50%, 60%:40%, 89%:11%, and 82%:18%, respectively. At OSU Piketon facility, the experimental results showed: 1) Size was much uniform for near-all-male group and coefficient of variation (CV) for body weight ($100 \times \text{standard deviation} / \text{mean}$) was lower in the male groups comparing control groups (53.1 vs.76.0, and 51.8 vs. 76.9 at the beginning and end of experiment, respectively); 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; and 3) mostly-males grew 16.7% faster than mixed population in body weight during juvenile phase. The experiment is continuing beyond NCRAC-funded period

Table 1. Results of 249-day combined common garden growth trials comparing stocks of commercially sourced Northern Bluegill (NBG) and Coppernose Bluegill (CBG) to large all-male and small all-male with feeding treatments by hand for 93 days followed by 156 days of hand-feeding 3x daily and 12-h belt feeders.

Stock	Mean Weight _{initial} (0.1 g)	Mean Weight _{final} (0.1 g)	Weight Increase (0.1g)	Weight Increase (%)	SGR	Standard Error for Stock*Tank
NBG	43.4 ^a	121.2 ^b	77.8 ^b	180 ^c	0.67 ^c	0.26 ^a
CBG	42.0 ^a	109.7 ^b	67.6 ^b	161 ^c	0.59 ^c	0.33 ^a
Mostly-male _{large}	29.4 ^b	188.6 ^a	159.2 ^a	571 ^b	1.20 ^b	0.24 ^a
Mostly-male _{small}	15.1 ^c	139.3 ^b	124.3 ^a	900 ^a	1.43 ^a	0.28 ^a

Principal Accomplishments

Genetically improved fast-growing mostly-male populations were created by cross of LU and OSU improved fish. Characteristics, performance and sex ratios of fast-growing male population were characterized and compared with mixed control population. At LU facility, the large mostly-male and small mostly-male stocks in terms of weight started smaller and grew significantly more than control group in terms of weight increase. Percent weight increase of the mostly-male stocks was 3 to 5 times than observed for the reference Northern Bluegill and Coppernose Bluegill stocks; large and small mostly-male stocks increased in weight by 571% and 900%, respectively. Specific growth rate was highest for the smaller mostly-male stock (1.43%) followed by the large mostly-male stock (1.20%); values for the Northern Bluegill and Coppernose Bluegill were lowest and not significantly different from each other. The sex ratio (male:female) for Northern Bluegill, Coppernose Bluegill, large mostly-male and small mostly-male groups are: 50%:50%, 60%:40%, 89%:11%, and 82%:18%, respectively.

At OSU Piketon facility, the experimental results showed: 1) Size was much uniform for near-all-male group and coefficient of variation (CV) for body weight was significantly lower in the male groups comparing control groups (53.1 vs.76.0, and 51.8 vs. 76.9 at the beginning and end of experiment, respectively); 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; 3) mostly-males grew 16.7% faster than mixed population in body weight during juvenile phase.

This rearing experiment is continuing beyond funded period using funds from state grants. Three journal articles on related results have been published in journals *Aquaculture*, *The Biological Bulletin*, and *Fish Physiology and Biochemistry*. One journal article is under review by journal *Aquaculture Research* (see publication list). Two papers have been accepted by a Wiley book “Sex Control in Aquaculture”, which will be published by Wiley & Sons at the end of 2017 (see publication list). Four papers/abstracts were presented and published in Conference Proceedings of World Aquaculture Society after oral presentations. Two additional oral presentations were made in other conferences (see publication list). Extension publications (i.e., factsheets) are in preparation. The next step is to do commercial-scale on-farm/on-station test of all or mostly-male populations vs regular mixed populations. Once this have been done and the results confirmed, OSU can deliver all or mostly-male seeds to fish farmers. Distribution of stocks will be based on the standard (OSU) procedure for commercialize genetically improved animals.

Impacts

The impact of this project will be primarily via the delivery of fast-growing mostly-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 can 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

Recommended Follow-Up Activities:

Results of experiments at two locations confirmed that genetically improved fast-growing mostly-male populations grew significantly faster than mixed-sex regular populations. Percent weight increase of the mostly-male stocks was 3 to 5 times than observed for the reference Northern Bluegill and Coppernose Bluegill stocks at LU aquaculture facility. To best examine genotype by environmental interactions and obtain commercial-scale results applicable across the North Central Region, we recommend conducting both commercial-scale on-farm and on-station tests of fast-growing mostly-male or all-male (once developed) strains with local mixed-sex regular populations at four locations using both separate rearing and communal rearing methods. Ponds at four geographic locations at different latitudes, including two demonstration/research stations and two commercial farms. We can develop and adhere to consistent rearing protocols at all of the selected sites. We can evaluate all of the key production parameters of fingerling production (e.g., survival, growth, feed conversion) in Year 1, and evaluate all of the key production parameters for raising the two groups of fish to market size (e.g., survival, growth, feed conversion for separate rearing, and fillet yield) in Year 2. It is at this time that economic cost benefits of these animals can best be estimated.

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 [Termination 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, 2016 to August 31, 2017

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.

Reason for Termination: Objectives completed

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 days post hatch (dph), the critical period for yellow perch in recirculating aquaculture systems.

Technical Summary and Analysis:

Objective 1.1. (UWSP-NADF)— In year 1, non-feeding behavior, non-inflation of the gas bladder, clinging behavior, and cannibalism were examined using a series of methodical experiments evaluating the effects of: 1) Turbid water – three treatments of clear (0 NTU), slightly (50 NTU), and turbid (100 NTU) water using clay (L&R Specialties, Missouri); 2) Water surface spray – three treatments of no flow (0 L/min, 0 gal/min), moderate (0.4 L/min, 0.11 gal/min), and high (0.8 L/min, 0.21 gal/min) water flow (horticultural hanging basket flex misters; Hummert International, Missouri); 3) Tank color – three treatments of white, blue, and black interior colored tanks. Newly hatched fry were fed initially fed high quality dry diets and then trained to standard commercial yellow perch diets.

Differences ($p < 0.05$) in growth, length and weight, were observed after 70 days for larval yellow perch raised in different color tanks (blue, white and black) with fish reared in black tanks showing the best growth. Significant differences ($p < 0.05$) in growth, length and weight, were observed for larval yellow perch raised under different water surface spray. High spray velocity (0.8 L/min; 0.21 gal/min) resulted in the greatest growth, both length and weight, especially when comparing no flow, poorest length gain, to high flow. No flow and moderate flow (0.4 L/min; 0.11 gal/min) showed similar weight gain with high flow having the greatest weight gain. Significant differences ($p < 0.05$) in growth, length and weight, were observed for larval yellow perch raised in different turbidity levels. Slightly turbid water (50 NTU) resulted in the greatest growth, both length and weight, when compared with clear water (0 NTU) and turbid water (100 NTU). There were no differences in growth of larval yellow perch between clear water and turbid water conditions.

Objective 1.2 (UWSP-NADF) — 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 two 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 total 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. Percentages 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 2.1 (OSU). – Commercial-scale marine rotifer and *Artemia* production systems were further improved and the 24/7 auto-feeders were optimized starting the first year of the project. Marine rotifers (hereafter referred to as simply rotifers) were harvested and fed twice rather than once in consideration of their rapid life cycle. Live feed production systems were improved either through reducing feeding times for rotifers 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 researchers found a considerable proportion of live feed sink on the bottom and were not pumped out to fry tanks last year. This improvement allows 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 *Artemia* survive up to about 1 hour when they were dispersed into freshwater rearing tanks. In addition, researchers found that contamination between two production systems, rotifers and *Artemia*, should be avoided because even a few *Artemia* will considerably reduce rotifer production.

Objective 2.2 (OSU).— Researchers tested and identified the two best feeding regimes (Table 1) The co-feeding strategy that consists of mixing rotifers and *Artemia* into one container, feeding formula feed before hand-feeding live feed, and gradually reducing live feed, significantly increased larvae survival to about 25% at 31 days post-hatching (dph) These two feeding regimes provided yellow perch larvae with a combination of live and formula feeds, a wide range of feed sizes that , as larvae grow. During 25 to 31 dph, OSU researchers found that perch fry were aggressive to formula feeds 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 not be completely replaced until fry reach 40 dph.

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Table 1. Two best feeding regimes for culturing yellow perch larvae using marine rotifers, *Artemia*, and commercial diets (Otohime, B1 200-360 µm and B2 360-600 µm; Zeigler 150-250 µm and 250-400 µm; and Purina® AquaMax® Fry Starter 100), Ohio State University.

Feeding Regime	Schedule (days post hatch – dph)						
	3-7 dph	5-10 dph	8-20 dph	21-30 dph	30-45 dph	45-55 dph	55 dph-
1	Rotifer	small <i>Artemia</i>	Regular <i>Artemia</i> +	Regular <i>Artemia</i> +	Regular <i>Artemia</i> + B2 + AP250-450 + Starter	B2 + AP250-450 + Starter	Starter
	160 µm	428 µm	Otohime B1 + AP100-150	Otohime B2 + AP100-150 + AP150-250			
2	Small <i>Artemia</i> 428 µm	Regular <i>Artemia</i> + Otohime B1 + AP100-150	Regular <i>Artemia</i> + Otohime B2 + AP100-150 + AP150-250	Regular <i>Artemia</i> + B2 + AP250-450 + Starter	B2 + AP250-450 + Starter	Starter	

Several related studies were 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; and 3) we found predation and ingestion of prey at the beginning of feeding is limited by the mouth gape in fish larvae which determine larvae survival.

Examination of food in digestive tract, combining with mouth size as well as total length data proved that the yellow perch larvae were able to ingest live feed, either rotifers or *Artemia*, when only total length reached 6 mm and mouth gape reached 0.6 mm, regardless the age of larvae, which was usual from 1 to 7 dph. Swim bladders started to inflate as early as 4 dph, and were correlated with the size of fish. When pooling all families together, we found that the swim bladders inflate when larvae the size reached 6.7 - 9.7 mm, regardless the age of fish. Yolk disappeared between 7 and 11 dph for most of larvae. For one family, yolk was still observed in 70% of larvae at 11 dph.

Principal Accomplishments

Non-feeding behavior, non-inflation of the gas bladder, clinging behavior, and cannibalism were examined using combinations of selected turbidity, water surface spray and tank color in husbandry tanks. Differences ($p < 0.05$) in growth, length and weight, were observed after 70 days for larval yellow perch raised in different color tanks (blue, white and black) with fish reared in black tanks showing the best growth. Significant differences ($p < 0.05$) in growth, length and weight, were observed for larval yellow perch raised under different water surface spray. High spray velocity (0.8 L/min; 0.21 gal/min) resulted in the greatest growth, both length and weight, especially when comparing no flow, poorest length gain, to high flow. No flow and moderate flow (0.4 L/min; 0.11 gal/min) showed similar weight gain with high flow having the greatest weight gain. Significant differences ($p < 0.05$) in growth, length and weight, were observed for larval yellow perch raised in different turbidity levels. Slightly turbid water (50 NTU) resulted in the greatest growth, both length and weight, when compared with clear water (0 NTU) and turbid water (100 NTU).

Based on the broad husbandry conditions a second year of larval yellow perch culture was completed with narrower tank conditions that included only black tanks and water surface spray and turbidity that centered on the optimal conditions identified in the first year. No differences in growth, length and weight, were observed every two weeks nor were differences observed after 70 days for larval yellow perch raised under these more stringently defined husbandry conditions. In contrast to similar percoid studies culturing Walleye (*Sander vitrus*) larvae, modifications in culture operations to address similar physical and behavioral barriers were not effective in improving yellow

perch larvae survival. Approximately 3% of the larval yellow perch survived until the end of the 70-day experiment regardless of treatment.

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. Eight rotifer/*Artemia* auto-feeders were constructed and paired with sixteen yellow perch fry production tanks. Twelve feeding regimes or diets were tested for newly hatched yellow perch larvae starting from day 3 dph for improve survival rate. Two effective regimes were identified.

Several other 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 ingestion of prey at the beginning of feeding is limited by the mouth gape in fish larvae which determine larvae survival. Examination of food in digestive tract, combining with mouth size as well as total length data proved that the yellow perch larvae were able to ingest live feed, either rotifer or *Artemia*, when only total length reached 6 mm and mouth gape reached 0.6 mm, regardless the age of larvae, which was usual from 1 to 7 dph. Swim bladders started to inflate as early as 4 dph, and were correlated with the size of fish. When pooling all families together, we found that the swim bladder would inflate when larvae the size reached 6.7 - 9.7 mm, regardless the age of fish. Yolk disappeared between 7 and 11 dph for most of larvae. For one family, yolk was still observed in 70% of larvae at 11 dph.

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/channels/958980>. Associated with this project is the video “Develop systems and diet strategies to reduce yellow perch larval mortality burst in indoor RAS” is at <https://www.ncrac.org/video/develop-systems-and-diet-strategies-reduce-yellow-perch-larval-mortality-burst-indoor-ras>. An addition video entitled “Developing Systems and Diet Strategies to Reduce Larval Yellow Perch Burst Mortality in Indoor RAS” is also available on the NCRAC website <https://www.ncrac.org/video/develop-systems-and-diet-strategies-reduce-yellow-perch-larval-mortality-burst-indoor-ras>. These materials discuss the results and conclusions drawn from the 2-year study on husbandry and culture conditions that resulted in the best survival of larval yellow perch under variable culture conditions including intensive tank rearing at the UW-Stevens Point Northern Aquaculture Demonstration Facility.

Impacts

The impacts of this proposed project will be primarily through the development of systems and strategies to enhance mass production and survival of yellow perch from onset of first feeding up to 70 days. However, data garnered from this project indicates that early rearing of yellow perch using modified culture conditions or feeding regimes using commercial diets is not yet practical to the commercial yellow perch aquaculture community. Information garnered from this include recirculating system components, feeding strategies and feed types for successfully reducing the early-life stage bottlenecks, and 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.

Recommended Follow-Up Activities

NCRAC-funded researchers have concluded that it is extremely difficult to improve survival rate through either culture conditions modification, i.e., tank color, water turbidity or surface spray, or different feeding regimes. One key reason for poor indoor survival of yellow perch larvae is the mouth gape limitation of newly hatched. Predation and ingestion of prey at the beginning of feeding is limited by the mouth gape in larvae, which determine larvae survival. Yellow perch larvae are not able to ingest small size *Artemia* (~428 μ m) until their total length reaches >6 mm and mouth gape >0.6 mm. Even though researchers provided an adequate number of rotifers small enough to be consumed by the fish, there were still high levels of deformities and larvae survival rates were still poor, as reported by others. These findings suggest that digestive enzymes may not establish in perch larvae until they reach >6 mm in length.

Future projects should investigate the role of digestive enzymes and their relation with larvae survival of yellow perch. As with other fish species with small larvae, there continues to be a need to further develop larval diets that are both small enough to be consumed and processed by the limited digestive enzymes present early in the development of the larvae.

In addition to studies investigating the perceived limited number of digestive enzymes in yellow perch larvae, a project to select yellow perch strains that have a larger mouth gape to improve egg quality and survival is suggested. To date, researchers have identified some strains/families that produced significantly larger-mouth gape progeny and larger eggs than others, indicating there is a large range of selection for larger-mouth gape traits. Therefore, developing yellow perch broodstock (using identified strains/families) with larger-mouth gape and larger egg size to increase indoor survival of larvae and fry has the potential to enhance yellow perch industry development.

At the same time, there is a need to identify more strains and families that genetically produce larger-mouth gape progeny and larger eggs, using genetically improved broodfish from different families/strains with different weights and ages. For both parts, broodfish can be selected, tagged and genotyped. Molecular pedigrees can be determined and a genetic relatedness chart developed. At least 60 pairs of related fish, with the highest breeding value can be selected for production of progeny. Newly hatched larvae (1st day dph) from each family can be subject to measurement of total length and the size of mouth gape. The parents that produced the largest mouth and fastest growing progeny can be selected as next generation of large-mouth broodfish,

Publications, Manuscripts, Workshops, and Conferences

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

NORTH CENTRAL REGIONAL AQUACULTURE CENTER

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Project Title: Professional Development Training in the North Central Region [Annual Report]

Chair: Carole Engle

Extension Liaison: Chris Weeks

Key Words: Other, Professional Development Training

Total Funds Committed: \$70,000

Initial Project Schedule: 9/1/16-8/31/18

Current Project Year: 9/1/16 – 8/31/17

Participants: Zajicek, P., National Aquaculture Association; Engle, C., Engle-Stone Aquatic\$ LLC; Weeks, C., Michigan State University; Phelps, N., University of Minnesota

Industry Liaison: West, B., Blue Iris Fish Farm, Black Creek, Wisconsin

Project Objectives:

1. Assess the status of aquaculture associations in the NCR.
2. Develop strategies to enhance association leadership and participation (e.g., workshops, mentoring, messaging, communication, member satisfaction, improve communication, incentivizing member participation).
3. Conduct training programs for individuals from state and multistate association(s) that support regional aquaculture development.
4. Prepare educational materials which facilitate the association development processes.

Project Summary

Strong industry associations provide support for industries to grow and develop. In the North Central Region, only four states have active state aquaculture associations. Of these, four have associations that meet infrequently or have minimal activity, and four others no longer have active associations. This decline coincided with administrative decisions to not fill vacancies when extension specialists retired or re-located. Strengthening state aquaculture associations would contribute to revitalization of the aquaculture industries in these states. Effective leadership skills are essential for associations to flourish, but are not inborn. Thus, development of leadership skills in the North Central Region is essential to revitalize state aquaculture associations that support growth of aquaculture. This project will design and implement a leadership development and training program. The immediate impact will be on the individual participants who will learn to prioritize projects and engage members in the association to solve industry issues. Other producers are likelier to join and become active when the association works on topics of perceived value to their farms. The deliverables from this project will provide a framework for extension specialists in the region to continue leadership development and training to strengthen state aquaculture associations which, in turn, will support a viable aquaculture industry.

Anticipated Benefits

The leadership training program to be developed and implemented in this project is expected to increase knowledge among industry participants in skills essential to effective leadership that include: 1) how to effectively engage association members in association initiatives and 2) how to develop effective media relationships (See Logic Model, p.3). As a result of these short-term knowledge gains, this project is expected to result in changed behaviors in the medium-term. These would include: 1) increased activities of state aquaculture associations; 2) increased membership in state aquaculture associations; and 3) increased positive media related to aquaculture in the North Central Region. The individual participants will be expected to serve as liaisons to continue to work towards enhancing state aquaculture associations and growth of aquaculture in the region.

The leadership development and training program will serve as a prototype model. The educational deliverables from this project will be made available to both industry and extension aquaculture personnel in the region through the North Central Aquaculture Center. These deliverables (including training packets, the recorded webinar, and fact sheet) will provide the basis for continuing the leadership training and development by extending it to include other participants and other states in the region. This model will be expected to be used to continue building strong state aquaculture associations.

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Accomplishments

Objective 1 has been completed, through the Rapid Response project entitled “Assessing the Status of Aquaculture Associations in the North Central Region.” A survey was conducted of producers in the North Central Region to improve understanding of the value of various association activities and services. The two most common reasons for not belonging were: 1) “Do not know how membership would benefit my business”; and 2) “Have never been asked to join”. More than half indicated that the following benefits/services would entice them to join: newsletters, networking opportunities (i.e., meetings, workshops), web sites, industry alerts, and representation with regulators. Current association members indicated strong loyalty to their associations. The strength of state extension support showed a strong positive effect on the value of membership.

Objective 2 has been completed. Strategies identified to support and strengthen aquaculture associations in the North Central Region include: 1) develop and maintain industry directory; 2) enhance member benefits and services identified; 3) implement recommended recruitment and retention strategies; 4) expand extension support in region; and 5) implement leadership training programs.

Objective 3 will be completed in the second year of this project. Project participants were identified and preliminary design and planning for the professional development training were completed this past year.

Objective 4 will be completed in the second year of the project.

Targeted Audiences

The primary targeted audiences for this project are aquaculture producers and their suppliers in the North Central Region followed by Extension personnel and others who are engaged with the aquaculture industry.

Outreach Overview

A critical part of the initial outreach on this project was to both notify aquaculture producers in the North Central Region about the upcoming professional development training and then to recruit participants. Notices were sent out through all extension and NCRAC communications means, and Extension personnel in the region assisted with recruitment of participants for the leadership development training.

Deliverables (outputs)

Project outputs delivered to date include:

Engle, C.R., Phelps, N., Quagraine, K., Smith, M.A., Weeks, C.W., and Zajicek, P. 2016. Striving to improve aquaculture businesses through strong state aquaculture associations. *Buckeye Aquafarming*. 1(2):4.

Engle, C.R. 2017. Strengthening Aquaculture Associations. North Central Regional Aquaculture Center Board of Directors and 2017 Program Planning Meeting, Des Moines, Iowa, February 4, 2017.

Engle, C.R. 2017. Strengthening Aquaculture Associations. National Webinar, United States Aquaculture Association, North Central Regional Aquaculture Center, and the National Aquaculture Association, April 5, 2017.

Outcomes-Impacts

Aquaculture producers have become more aware of the status of aquaculture associations in their respective states, and the project has resulted in increased discussion of the reasons for the current status and increased interest in strengthening associations.

Impact Summary

Impact Summary

It is too early to have measured impacts. Impacts will occur as we implement the leadership training workshops.

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Technical Update

There are not enough results to date from this project. Once we get the leadership development workshops going, we will have material to include under this heading.

Publications, Manuscripts, Workshops, and Conferences

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

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Rapid Response Projects

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Project Title: Assessing the Status of Aquaculture Associations in the North Central Region [Termination Report]

Key Words: Aquaculture associations, Extension

Dates of Work: September 1, 2016 to August 31, 2017

NCRAC Funding Level: \$34,977

Participants: Chris Weeks, Michigan State University, Michigan; Nicholas Phelps, University of Minnesota, Minnesota; Kwamena Quagraine, Purdue University, Indiana; Matthew Smith, The Ohio State University, Ohio; Carole Engle, Engle-Stone Aquatic\$ LLC, National Aquaculture Association; Paul Zajicek, National Aquaculture Association

Extension Liaison: Matthew A. Smith

Industry Advisory Council Liaison: Amy Stinton

Reason for Termination: Project objectives completed.

Project Objectives:

1. To design and implement a survey of aquaculture producers throughout the NCR to identify reasons why they have either never joined a state association, joined but then did not renew their membership, or are current members.
2. To measure how producers value the various types of activities undertaken by state associations, identify the types of services or programs that would attract membership, and identify barriers and challenges to organizing and sustaining state aquaculture associations.
3. To develop strategies likely to increase membership and strengthen state associations.

Project Summary

Strengthening state aquaculture associations will contribute to revitalization of aquaculture industries in the North Central Region. Better understanding of the most important reasons for membership will help associations focus on high priority activities and services.

A survey was conducted of producers in the North Central Region to improve understanding of the value of various association activities and services. The two most common reasons for not belonging were: 1) "Do not know how membership would benefit my business"; and 2) "Have never been asked to join". More than half indicated that the following benefits/services would entice them to join: newsletters, networking opportunities (i.e., meetings, workshops), web sites, industry alerts, and representation with regulators. Current association members indicated strong loyalty to their associations. The strength of state extension support showed a strong positive effect on the value of membership.

Project recommendations include:

1. Develop and maintain industry directory.
2. Enhance member benefits and services identified.
3. Implement recommended recruitment and retention strategies.
4. Expand extension support in region.
5. Implement leadership training programs.

Specific follow-up activities were identified.

Technical Summary and Analysis

A survey instrument was designed to obtain information on current membership in aquaculture associations, to assess the value of membership, and the loyalty of existing members to their associations. Additional questions explored the perceived value of various association benefits and services by current members, those who ceased their membership, and those who have never joined an aquaculture association. A list frame of all known producers (aquaculture, aquaponics, and baitfish harvesters) in the 12 states of the North Central Region (NCR) with E-mail addresses was compiled and hard copies were also distributed at the Ohio Aquaculture Association. The list frame included a total of 279 potential respondents (Table 1).

The electronic survey was launched January 22, 2017, with follow up reminders to those who had not responded on February 1, 2017, February 15, 2017, and February 25, 2017. In addition to the electronic survey, hard copy versions

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of the questionnaire were distributed at the Ohio Aquaculture Association annual meeting, with 17 completed hard copy questionnaires returned.

Results

Objectives 1-2. — Table 1 presents the number of respondents by each state in the NCR. Of respondents, 38% were from Ohio, 16% from Iowa, 11% from Indiana, 10% from Wisconsin, and fewer than 10% from the other states. No aquaculture producers were identified in North Dakota. Table 1 also includes the number of farms listed for each NCR state from the 2012 data collected in the 2013 Census of Aquaculture (USDA-NASS 2014). While the data from the Census of Aquaculture is somewhat dated, it appears that the response rates obtained reflect a somewhat over-representation of producers in Ohio and Indiana and under-representation of producers in Wisconsin and Minnesota.

Characteristics of Respondents

Eighty-four percent of respondents indicated that aquaculture was their primary type of business, followed by 6% who were not yet in business but planned to start up, 5% aquaponics, 4% baitfish harvesting, and 1% did not respond to this question (Table 2). A few respondents indicated secondary or tertiary types of businesses, all of which were baitfish harvesters or aquaponics in addition to their aquaculture businesses.

Twenty-six different types of aquatic animals were reported to be raised by respondents. The most frequently mentioned types of aquatic animals were: bass (13%), bream (13%), yellow perch (12%), baitfish (11%), catfish (9%), tilapia (7%), trout (7%), walleye (6%), and ornamentals (6%) (Table 3). In comparison with the 2012 data collected in the 2013 Census of Aquaculture (USDA-NASS 2014), survey respondents appeared to be similar in terms of the distribution of types of species raised with two exceptions: trout and the “other sportfish” categories appeared to be under-represented in the survey.

Overall, respondents reported raising from one to 15 different types of aquatic animals. More (38%) respondents raised only one species; 25% raised two to five species; 28% raised six to ten different species; and only 6% raised 11 to 15 species (Table 4). Respondents, on average, raised four different types of aquatic animals.

More respondents indicated that trout or tilapia (12%) were the most important species raised in terms of sales, followed by yellow perch (10%), marine shrimp (10%), bass (9%), baitfish (7%), bluegill/bream (5%), and catfish (5%) (Table 5). The other species were mentioned less frequently, in terms of the importance to overall business sales.

Nearly one-third of respondents (31%) reported using ponds for aquaculture production, followed by indoor tanks (27%), raceways (8%), cages (8%), and outdoor tanks (8%) (Table 6). Aquaponics producers reported using mostly indoor or greenhouse tanks, while baitfish harvesters tended to use either ponds or indoor tanks primarily. Respondents used a variety of different production systems. Table 7 shows that more than half of respondents used more than one production system on their farm; 46% used only one production system with 31% using two, and 23% using from three to six different production systems.

The greatest percentage of respondents (31%) reported sales of aquatic animals less than \$25,000 per year, followed by those with sales of from \$100,000 to \$500,000 (14%), \$50,000 to \$100,000 a year and \$1 million or more (11%), \$25,000 to \$50,000 (10%), and \$500,000 to \$1 million (9%) (Table 8).

Five respondents from three different states (Illinois, Michigan, Ohio) reported use of aquaponics systems. Of these, four indicated that aquaponics was their major business and one indicated that they also were engaged in aquaculture and baitfish harvesting. This fifth respondent indicated that aquaponics was his/her tertiary type of business, after aquaculture and baitfish harvesting. Moreover, this last respondent raised 13 different species, recording “ornamentals” as the type of species that generated the greatest amount of sales, overall. There was no indication as to which species was raised in aquaponics.

Of the four respondents who indicated that aquaponics was their major business, three raised tilapia, one bluegill/bream, catfish, and tilapia, and the fourth raised yellow perch in aquaponics. These four indicated that they used indoor tanks as the production system. Three of the four aquaponics respondents indicated that their sales of

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aquatic animals were less than \$25,000; in terms of plant sales, two indicated that sales of plants were from \$25,000 to \$50,000 and one respondent mentioned sales of plants, at \$50,000 to \$100,000.

Association Membership

Seventy-eight percent of respondents indicated that they had belonged to an association related to their business at one time, while 23% of respondents had never belonged to an association (Table 9). By way of comparison, in the National Aquaculture Association (NAA) survey, only 5% of respondents to that national survey had never belonged to an aquaculture association. Thus, there were few responses from those who had never chosen to join an aquaculture association. The average number of aquaculture association memberships in this NCR survey was 1.5 per respondent. In the NAA survey, the average number of association memberships per respondent was 2.7. In this current NCRAC survey, nearly two-thirds (62%) of those currently a member of an aquaculture association at the time of the survey belonged to only one association; 32% belonged to two different aquaculture associations, and 6% belonged to more (three to five). Of those who had at one time belonged to an association, 84% had continued to be a member of an association (although not always the same association), and 16% were no longer a member of any association. Table 10 provides some primary reasons given for never having joined an association and included: 1) "Do not know how membership would benefit my business" (29%); 2) "Have never been asked to join" (24%); 3) "There are none in my state" (18%); 4) New farm (18%); and 5) "Do not believe it is worth the membership fee (12%)".

Recruiting Members

When asked what types of benefits/services would entice them to join an association, respondents who had never joined an association selected the following (percentages of respondents in parentheses): 1) newsletter (71%); 2) networking opportunities (71%); 3) workshops on the latest research (71%); 4) an annual or biannual meeting (62%); 5) industry alerts (52%); 6) representation with regulators (57%); 7) web site (52%); 8) public relations campaigns (33%); 9) webinars (33%); 10) media materials (29%); and 11) other (10%) (Figure 1).

Retaining Members

Table 11 reports comments by respondents in terms of why they discontinued their membership. In some cases, individuals moved or changed the species that they were raising. However, the greatest number of reasons for discontinuing membership was related to: 1) declining effectiveness of the association; 2) declining membership; 3) not ever hearing anything from the association/little communication from the association; and 4) failure to add real value to the business. Lack of relevance to the business, expressed as not being helpful or applicable to their business, was also cited as a reason for discontinuing membership. Other reasons appeared to be due to personal disagreement with policies and/or individuals involved in associations, feeling un-welcome due to cliques, or being unable to attend meetings.

Figure 1 also indicates the types of benefits and services that would most entice someone who had once belonged to an association but who had discontinued membership in all associations. The most frequently mentioned activity was industry alerts (67%) and workshops on the latest research (67%). The activities mentioned as next most important were: annual or biannual meetings (44%); web site (33%); webinars (33%), and networking opportunities (33%). Newsletters, representation with regulators, and workshops on policies and regulatory issues were each mentioned by 22% of this category of respondents, whereas media materials, public relations campaigns, and "Other" each were only mentioned by 11% of this category of respondent (Figure 1).

Value of Membership and Loyalty of Members

Generally, respondents who currently were members of associations rated the benefits received from membership as "very good", with an average rating of 3.7 (in which 5 indicated "excellent;" 4 "very good;" 3 "good;" 2 "marginal;" and 1 "poor") (Table 12). Ratings of the likelihood of renewing membership, on average, were even higher, at 4.5, and of the likelihood of recommending membership to others averaged 4.3. These ratings indicate strong loyalty to their associations (by rating likelihood to renew and recommend membership to others at a score greater than 4). However, Table 12 also shows that there is room for improvement in services offered to members. The score of the value of their membership (3.7) was noticeably lower than that associated with their loyalty to the association.

Table 13 presents ratings of the value of benefits/services that can be provided by an aquaculture association. Overall, the benefit with the greatest mean score was that of holding an annual/biannual meeting in the respondent's

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state (4.3) and was followed closely by the value of networking opportunities (4.1). Media materials (3.9) and representation with regulators (3.9) were rated as the third and fourth highest and were followed closely by newsletters (3.7) and industry alerts (3.7). Workshops on the latest research (3.6) and the latest policies and regulations (3.6) followed, along with a web site (3.5). The average score for public relations campaigns (3.0) was only “Good” and that of webinars fell below the “Good” rating, at 2.7.

Response ratings from current members on association loyalty and the value of association benefits/services were sorted by gross sales categories (Table 14). Sales categories are a measure of the size of a business. Regardless of the size of the business (sales categories), the ratings of the value of membership were lower (ranging from 3.0 to 4.2) than the ratings of loyalty to the association (likelihood of renewal, 4.25 to 4.8, and recommending membership to others, 3.9 to 4.6). While ratings were similar across business sizes, rating scores of respondents in the highest sales category were most similar to those of respondents in the lowest sales category. The outlier category in terms of membership value was that of the \$500,000 to \$1 million sales category (Table 14). Respondents in this next-to-largest sales category scored the value of membership at 3.0 (compared to 3.5 to 4.2 for the other sales categories). Further examination of the data revealed that this sales category was the only one with a majority of respondents raising the same species. All other sales categories included a wide range of species produced. The majority of respondents in the \$500,000 to \$1 million sales category raised trout and tended to rate the value of membership lower than did other groups of respondents. Responses related to the value of specific benefits/services varied greatly but there were no clear differences due to income category.

Response data from current members of an aquaculture association were sorted by type of aquaculture product produced. Given the diversity of species in the dataset, responses were grouped across several types of the species indicated by respondents to be the main species raised, so that individual categories would have a reasonable number of responses (Table 15). Thus, ornamentals were combined with baitfish; bass, bluegill, and catfish were combined; tilapia, shrimp, and prawn were combined, and walleye were combined with yellow perch. Across species groups, there was little difference in the ratings, other than that trout producers tended to rate the value of industry alerts lower than did other species groups.

The strength of Extension support clearly showed an effect on increasing membership association values as well as increased value of various member services (Table 16). Firstly, there were more than twice as many respondents from states with “high” Extension support than “medium” and nearly six times more than those from states with “low” Extension support. The overall rating of the value of association membership was greater for respondents from states with “high” Extension support as were the ratings of individual benefits/services. These higher ratings may reflect greater value from benefits/services actually received, due to Extension support. For example, in states with greater Extension support, there may be more frequent and higher-quality newsletters, meetings, and other activities that result in greater value to association members.

Building Strong Associations

Table 17 also lists the barriers and constraints identified by those who had never belonged to an association. Of these, lack of participation or insufficient numbers of producers were mentioned by more than one respondent. One respondent pointed out that more frequent interaction outside the annual meetings would be important.

Table 17 also presents barriers and constraints identified by respondents who had once been a member of an association but were no longer members. Responses included those that represented a decline in effectiveness of the association (i.e. lack of interest), disagreement among members of the association (i.e. bad leadership, lack of involvement due to sense of competition and not acting for the common good), or personal reasons such as lack of time to attend meetings or the distance to attend meetings.

Figure 1 shows that there are some clear differences in the types of benefits and services that were deemed important to respondents who had never joined an association and those who ceased their membership in one. The majority of those who had never joined were interested in a variety of benefits and services including: meetings, newsletters, industry alerts, a web site, networking opportunities, workshops on the latest research and policies/regulations, and representation with regulators. However, respondents who had ceased membership in an association were primarily interested in industry alerts and workshops on the latest research. These individuals were more likely to be

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experienced fish farmers who had already developed strong networks, but who saw the need to stay current with new research results and with events that might impact their farms and industry.

Sustaining Strong Associations

Table 18 lists several comments by respondents in terms of what happened to state aquaculture associations that once existed in their states. While one respondent indicated that their state association appeared to be doing well, others mentioned the lack of participation, aging members, and individual agendas rather than looking out for the industry as a whole as reasons why their state association had declined.

Reasons offered by respondents who had ceased their association membership for the decline in activity of an association included lack of interest, fear of information sharing, and the existence of too many factions within the association (Table 19).

Survey Limitations

One of the challenges to conducting any survey is the development of a comprehensive and accurate list frame (contact information of all those in the study population). Entities that conduct routine surveys (e.g., USDA, US Census Bureau) have full-time personnel that work on list frame development. In the NCR, some states had more complete and up-to-date contact information than did others. Moreover, the lack of Extension personnel in some states exacerbated problems related to accurate list frame development. In addition, while the survey was geared more towards producers, not all respondents were actively in production. To evaluate the quality of the overall list frame, Table 1 lists the list frame numbers by state as compared to those reported in the 2013 Census of Aquaculture (USDA 2014). It is, of course, mandatory for producers to respond to the census. However, the census data are now 5 years old, and there is no way to determine whether the differences in the list frame numbers by state are due to individuals entering or exiting aquaculture production, or to inaccuracies in the list frame. We do not know to what extent the limitations of the list frame developed may have resulted in non-response bias and, without more complete listings of contact information, cannot test for non-response bias. We did compare responses from those who completed the hard copy questionnaires from the Ohio Aquaculture Association with those from Ohio who completed the electronic version. Responses were found to be similar and no evidence of non-response bias was found in terms of the method of survey administration.

Table 3 reports the percent of respondents by species/type as compared to the percentage of reports of individuals raising that species/type in the Census of Aquaculture. In general, the percentages of respondents by species more or less tracked those reported in the Census. The main exception appeared to be that of trout, for which the Census reported 18% of aquaculture producers in the NCR, but trout farmers responding to the survey constituted only 7% of respondents. A similar difference can be observed in the “other sportfish” category. These two exceptions may reflect under-representation of responses by trout farmers and those who raise the species included in the “other sportfish” category (muskellunge, northern pike, crappie, and grass carp).

Barriers and Challenges to Association Growth and Development

One of the barriers to the growth and development of associations in the NCR has been the decline in Extension specialist support in the region. Respondents from states with stronger Extension support tended to express a greater sense of value from their association membership. Since Extension specialists tend to provide support to associations in terms of identifying and locating speakers, compiling research reports or articles for newsletters, or otherwise assisting with meeting logistics, such results from the survey are not surprising.

A second barrier to association growth and development is the lack of up-to-date lists of producers in each state. Without such a list, it can be difficult to identify and locate prospective association members and to contact them to invite them to join.

Association growth and development is also constrained and challenged by declining interest, concern of information sharing, and the development of factions among members that result from disagreement within the association as noted in Tables 17 to 19. These largely reflect the need to both provide more frequent communication and services to members and to enhance leadership skills that include membership engagement and conflict resolution. Increased membership engagement can increase the sense of value obtained from membership when their input results in increased benefits and services and can maintain interest in the association. Effective conflict

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resolution skills and actions that develop trust between elected officers/board members and the general membership can lead to more rapid resolution of conflicts among members. Such conflicts often begin with small disagreements that, if un-resolved, can escalate into larger conflicts that become detrimental to an association.

The aquaculture industry in the NCR is generally smaller than those in other regions of the U.S. Nevertheless, several individual states in the NCR are important nationally with respect to certain species. Wisconsin, for example, ranks second nationally in the total number of trout farms and ranks 6th in terms of trout sales. Missouri ranks 5th in trout production sales in the U.S. Wisconsin ranks 12th overall in the number of aquaculture farms in the U.S. and Missouri ranks 19th in terms of sales nationally. Ohio ranks 14th nationally in terms of the number of farms, more than half of which are sportfish farms. In the U.S. baitfish industry, the NCR includes the #2 (Minnesota), #3 (Ohio), and #4 (Wisconsin) states in terms of value of sales. Thus, the NCR region is important nationally in the trout, sportfish, and baitfish industries, even though none of its states leads the nation in any of these industry segments. The lack of a large, dominant industry in any individual state in the NCR may have contributed to the decline in Extension support in several states.

The challenge for the NCR is to effectively meet the needs of industries that are important (i.e., trout, sportfish, and baitfish) even though none are dominant in any one state, given the decline in Extension support. A regional approach is likely the most pragmatic approach, but to be effective and to address the issues raised by potential members, will also need to provide value by offering meetings, alerts, communications, and networking opportunities relevant to the specific species that are raised in each state and to address the issues that are faced in their respective states.

Respondents commented that the distance to attend meetings is a constraint to participation. Baitfish and sportfish producers, as well as trout producers who sell into recreational markets (including many trout producers in Nebraska, Missouri, Ohio, Michigan, and Wisconsin), often load out fish nearly every day in the early morning. Thus, these producers are not able to travel much distance and may have difficulty attending meetings that require overnight stays. To provide value to such producers requires smaller, local meetings that last perhaps only half a day. Thus, leveraging Extension support by networking across states and associations may be effective given declining extension support, but will need to focus on providing greater numbers of smaller, shorter meetings that focus on needs of specific industry segments in locations where there is some concentration of farmers.

The effectiveness of an association depends on the dynamism of the leadership. In some cases, association leadership tends to change every couple of years, which might require frequent leadership training. In other situations, there are some individuals who have been in leadership positions for many years who would like to step down. In other cases, there have been conflicts of a more personal nature that have led to declining membership. These situations all point to a strong need to develop a leadership training program that likely would be done most effectively at the regional level. It is important to have an on-going process of leadership development to create new leaders who can assist with membership engagement, bring fresh new ideas, and to respond to the dynamic nature of issues faced by aquaculture industries.

Certain aspects of a regional training program can likely be incorporated into NCRAC meetings, already attended by existing association leadership (who attend the NCRAC Industry Advisory Council meetings). However, there is a strong need to cultivate and develop new leadership through on-going growth and development of younger, emerging industry leaders.

Objective 3.—

Recommendations and Strategies

Industry Directory

- Create a structure through the North Central Regional Aquaculture Center to annually update contact lists for aquaculture, aquaponics, and baitfish harvesters in each state.

The first barrier to the development and strengthening of state associations is the lack of comprehensive, up-to-date contact lists for major growers in each state. This problem became evident very early in the process of developing

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the survey. Serious thought needs to be given to develop an effective system to compile an up-to-date listing for each state each year. An example of such a system might be to task a regionally-appointed coordinator in the NCR to ensure that the list is updated each year. The list used in this survey will be provided to NCRAC and could be used as a starting point. An institution in each state would then need to commit to updating their state's list each year to submit to the NCR coordinator annually. This will require telephone or e-mail contacts with each individual on the existing list and those on lists of permittees to identify those who are not producers, i.e., those who have gone out of business, raising fish only as a hobby, and that simply maintain a permit because they might want to do something with fish one day. The only way to ensure an accurate list is to contact each individual on each state's list each year to verify their status. Without up-to-date lists, it is not possible to provide the benefits and services desired by prospective association members, to recruit effectively, or to provide effective Extension services.

Member Benefits and Services

- Improve the value of membership by enhancing the benefits/services offered.

The ratings measured in this survey indicate participants have a strong loyalty to their associations (by rating likelihood to renew and recommend membership to others with a score greater than 4). However, these same respondents who were loyal to their associations scored the value of their membership (3.7) noticeably lower than their own loyalty. This indicates that there is room for improvement in the benefits/services offered to members.

The survey data showed that face-to-face meetings were rated (4.3) as the top benefit of membership and was followed closely by networking opportunities (4.1) (clearly related to attending meetings). Other important member benefits and services that contributed to the value of their membership included: media materials (3.9), representation with regulators (3.9), newsletters (3.7), industry alerts (3.7), workshops (3.6) (on latest research and on policies and regulations), and a web site (3.6).

Recruitment

- Enlist current association members to recruit new members.

Current members of associations rated the value of their membership as positive. In addition to valuing their membership, their likelihood of renewal was high as was their willingness to recommend membership to others. Such results indicate a strong loyalty to the association. One of the primary reasons provided by respondents who had never joined an association was that they had never been asked to join. Current association members could contact some of these individuals personally to invite them to join, to attend a meeting, or to accompany them to an association event. For this strategy to be effective, however, there needs to be regularly scheduled association activities underway, such as the workshops, newsletters, etc. mentioned above.

- Develop materials that articulate the benefits/services and value of membership.

A number of respondents who had never joined an association indicated that they did not know how membership would benefit their business. Survey results showed that face-to-face meetings, networking opportunities, media materials, representation with regulators, newsletters, industry alerts, workshops on the latest research and on policies and regulations, and a web site were the types of activities that association members valued most highly.

For state associations that already have these types of activities in place, then materials summarizing the specific activities of that state's association should be developed and disseminated on web sites, in newsletters, and via all means available.

For state associations that do not yet have regularly scheduled activities, the first step is to develop the types of activities that create value for their members. The survey results showed that the most frequently mentioned enticements by those who had never joined an association were: newsletters, meetings, workshops (both on the latest research and policies/regulations), and other networking opportunities. A good starting point for an association that has not been very active is to begin to publish a newsletter. Someone will need to take responsibility to pull together information to do so. The second step is to begin to organize and hold meetings. For states with little Extension support, it may be difficult to organize an annual conference. However, smaller workshops offered once

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or twice a year on focused and critical topics held in venues where there are groups of producers raising a given species, would provide the same networking opportunities that are likely to entice new members to join. NCRAC could provide regional Extension support to help identify the most appropriate speakers. Often, other organizations such as Farm Bureau or those who provide supplies to aquaculture producers are willing to provide meeting spaces at no cost. Effective meetings can also be held on individual farms, in a shop on the farm, for example, and include some hands-on activities.

- Update association web sites and include examples of the value of membership in a prominent location on the home page.

Web sites are important sources of information and news about an association and were scored at a “very good” rating in terms of their value to association members. A web site is a good starting point to highlight and communicate the value of membership in that specific association. Each state association’s web site should be updated and maintained with a listing of the types of benefits and services that provide value to membership in that association. Providing continual updates of examples of specific activities of that association will contribute to member satisfaction and to a sense of value for their membership. Recruitment efforts can drive traffic to the web site for additional detail.

- Develop state association presence at major agriculture events to communicate what the association is about, why it exists, and what the value is of membership.

The value of membership in a state aquaculture association is also tied to its relevance on the state level. There are important opportunities for a state aquaculture association to engage in larger conversations about the importance and value of aquaculture and to identify common ground with other sectors of agriculture. Such opportunities can include livestock conventions, county and state fairs, state Farm Bureau activities, state Agriculture Days, state departments of agriculture activities, and many others. Setting up a booth to talk with attendees at these events, offering to speak at such meetings, nominating aquaculture producers for farming awards (eg., “Farmer-of-the-Year”) may require producers to spend some time outside their comfort zone, but can be very beneficial in building support for the association and increasing its overall effectiveness. Individual aquaculture producers who agree to attend such meetings can be briefed by others with experience in doing so in terms of talking points and key messages.

- Actively communicate the value of membership and activities of the state’s aquaculture association.

Active and effective communications strategies are critical for associations to recruit and maintain their membership and support. Each association needs to plan for frequent communications to their membership, to the broader agricultural community, to research and extension personnel, and to the broader public. Communications from the association lets current and prospective members know what activities are occurring, encourages participation by both members and non-members, and enhances the value of membership by providing on-going reminders of what that value is. Examples of communications activities include: periodic newsletters, annual reports, flyers of recent activities and events, industry alerts, announcements of meetings, and press releases announcing honors received by the state’s aquaculture producers.

Retention

- Provide support to inform state associations and non-member producers about current research, outreach and business development opportunities for aquaculture expansion.

To retain members, the key benefits/services desired seem to be industry alerts and workshops on the latest research results. The survey results indicated that the value of membership would be enhanced by providing: 1) regular, possibly quarterly, newsletters either in each state or by combining efforts across multiple states (while still addressing local issues for each with a section of each newsletter devoted to each participating state); 2) periodic industry alerts; and 3) periodic meetings (at least one meeting a year should focus on new research of interest in that state, bringing in out-of-state experts whenever necessary).

NCRAC funding for workshops on the latest research should be prioritized. Such workshops should be organized in conjunction with state associations, with input from industry in each state to identify the most important topics. It will be critical to ensure that all major species priorities are addressed in these workshops and it likely will be

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important to provide funding to bring in experts from out-of-state. Program planning meetings prior to workshops and conferences will be essential to engage association members to identify top priorities. One example might be to provide the latest research information on trout through workshops in the major trout-producing states in the NCR by bringing in lead researchers on trout production from Idaho, the Cool and Coldwater Aquaculture Research Center in West Virginia (USDA Agricultural Research Service), and others. To be effective in revitalizing state associations, an effort needs to be made to reach out to all segments of industry in each state, including members and non-members of state associations, for input on the most important topics.

- Obtain feedback from each association activity to inform future activities.

Each association activity provides an opportunity to engage members and other participants to identify ideas for future activities. Each workshop and conference should include a short evaluation form that provides an opportunity to identify which aspects of the meeting met the needs of participants and to gather information on new topics or changes to improve future meetings. Suggestions for future speakers, venues, times of the year, and other scheduling ideas can be collected as well as new ideas for future association activities.

Extension Support

- Expand Extension support in the North Central Region.

Develop and implement meetings by NCRAC/association representatives (industry, state associations, research, Extension personnel) with the appropriate individuals in states with little Extension support for aquaculture to request funding to increase Extension support for aquaculture.

- Develop and disseminate educational information on aquaculture in the state.

The NCR includes a wide range of aquatic animals and plants that are raised on aquaculture farms using a wide variety of production systems and targeting a wide variety of markets. Yet, there is little understanding on the part of the general public of these fascinating businesses and how they contribute to local economies and communities. Extension materials should be developed that provide such background information on aquaculture in the region. Specific deliverable extension materials should include: 1) videos, 2) PowerPoint presentations with voice-over recordings, 3) infographics, and 4) brochures/flyers. Materials should be designed to emphasize the most important aquaculture sectors in the region (eg., trout, baitfish, sportfish, yellow perch). States that play important roles nationally in specific segments of aquaculture should be emphasized (i.e., trout in Wisconsin and Missouri; baitfish in Minnesota, Ohio, and Wisconsin).

Leadership Training for Aquaculture Producers

- Develop and implement an on-going leadership training program for aquaculture association leadership in the North Central Region.

Effective leadership is critical to successful associations. An on-going leadership training program is needed in the NCR that includes training for the existing leadership as well as cultivates and engages with young producers for their development into future industry leadership positions. Leadership training should address the nuts and bolts of running associations (adequate and updated bylaws, legal status, appropriate accounting methods), ways to improve member engagement to develop loyalty and improve retention, and ways to improve the frequency and effectiveness of communications both internally with members and externally with media and the general public. NCRAC should take the lead to develop and implement such a leadership training program.

- Develop conflict resolution training modules as part of the leadership training program.

Conflict among members of any group of people is inevitable. Successful associations are those that are able to resolve conflicts when they arise among members. Survey responses revealed that such unresolved differences among members have led to some members ceasing to renew their membership in associations in the region. Conflict resolution techniques are readily available and should be adapted to aquaculture association leadership by NCRAC.

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- Develop a “Young Farmers” program in the North Central Region.

Developing young farmers to become future leaders of an industry is vital to the long-term success of associations and of industries. All too often, an effective association led by a strong leader suffers when that leader steps down or retires from the industry. Transition planning for individual associations is critical, but needs to include an active leadership training component that targets young farmers. A model program is the Young Catfish Farmers program organized each year by the Catfish Farmers of America. Many “graduates” of this program are now elected officers in state associations. One of the key benefits of this program is that it involves young farmers from across several states who get to know each other and provide mutual support to each other over the succeeding years.

References

USDA-NASS (United States Department of Agriculture-National Agricultural Statistics Service). 2014. Census of Aquaculture (2012). National Agricultural Statistics Service, USDA, Washington, District of Columbia, USA. Accessed July 25, 2017 at www.agcensus.usda.gov.

Principal Accomplishments

The principal accomplishment of this project was to complete a survey of aquaculture producers in the NCR that identified: 1) why some aquaculture producers choose to join associations and others do not; 2) how producers value the various types of activities undertaken by state associations; 3) the types of services or programs that would attract membership; and 4) barriers and challenges to organizing and sustaining state aquaculture associations. Such information will be used to inform the leadership training program that has been approved by the NCRAC Board. The recommendations from the project form a basis for developing effective strategies to strengthen state associations in the NCR.

Impacts

The leadership training and development project will benefit from information obtained through the survey of state association members and non-members. The short-term impact has been the greater knowledge obtained about how aquaculture producers in the NCR value their membership in associations and what types of benefits/services are valued the most by current members, non-members, and those who have ceased to maintain their memberships.

Expected long-term impacts from this project are that state aquaculture associations in the NCR will be revitalized. The end result of this initiative would be a greater number of active associations in the region with increased numbers of more engaged members.

Recommended Follow-up Activities

1. Create a structure through the North Central Regional Aquaculture Center to annually update contact lists for aquaculture, aquaponics, and baitfish harvesters in each state.
2. Improve the value of membership by enhancing the benefits/services offered.
3. Enlist current association members to recruit new members.
4. Develop materials that articulate the benefits/services and value of membership.
5. Update association web sites and include examples of the value of membership in a prominent location on the home page.
6. Develop state association presence at major agriculture events to communicate what the association is about, why it exists, and what the value of its membership.
7. Actively communicate the value of membership and activities of the state’s aquaculture association.
8. Provide support to inform state associations and non-member producers about current research, outreach and business development opportunities for aquaculture expansion.
9. Obtain feedback from each association activity to inform future activities.
10. Expand extension support in the North Central Region.
11. Develop and disseminate educational information on aquaculture in the state.
12. Develop and implement an on-going leadership training program for aquaculture association leadership in the North Central Region.

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13. Develop conflict resolution training modules as part of the leadership training program.
14. Develop a “Young Farmers” program in the North Central Region.

Table 1. Responses by state.

State	Number in list frame	Survey responses by state		Percentage of total responses received by state	Number of farms reported in 2013 Census of Aquaculture (data for 2012)	
		No.	%		No. of farms	% in NCR
Illinois	15	4	27%	5%	23	7%
Indiana*	33	9	27%	11%	7	2%
Iowa	53	13	25%	16%	31	9%
Kansas	6	2	33%	3%	4	1%
Michigan*	17	7	41%	9%	32	10%
Minnesota	5	1	20%	1%	35	10%
Missouri	14	5	36%	6%	35	10%
Nebraska	8	0	0%	0	21	6%
North Dakota	0	0	n.a.	0	0	0
Ohio*	82	31	38%	38%	61	18%
South Dakota	1	1	100%	1%	7	2%
Wisconsin	45	8	18%	10%	80	24%
TOTAL	279	81	29%	100%	336	99%

*Includes paper and electronic copy.

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Table 2. Number and percentage of respondents by type of business.

Type of business	Number of respondents	Percent of respondents
Primary type of business		
Aquaculture	67	84
Aquaponics	4	5
Baitfish harvesting	3	4
Not currently a producer	5	6
No response	1	1
TOTAL		100
Secondary type of business		
Baitfish harvesting	4	5
Tertiary type of business		
Aquaponics	1	1

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Table 3. Number of species raised by respondents to NCRAC Association Survey

Species raised	Number of respondents raising this species	Percent of respondents raising this species	Census of aquaculture (% of reports in region)
Baitfish (fathead minnows, golden shiners, spotfin shiners, “all types of baitfish”)	34	11%	14
Bass (largemouth, hybrid striped bass, striped bass)	38	13%	18
Bluegill/bream/redear	37	13%	15
Catfish	26	9%	12
Crayfish	12	4%	1
Ornamentals (goldfish, koi, snails)	19	6%	8
Marine shrimp/freshwater prawns	15	5%	2.5
Tilapia	22	7%	7
Trout	21	7%	18
Walleye	19	6%	9
Yellow perch	35	12%	11
Other food species (barramundi, paddlefish, salmon, turtles)	8	3%	4
Other sportfish (muskie, northern pike, black crappie, grass carp)	11	4%	13
TOTAL NUMBER OF REPORTS	297	100%	132

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Table 4. Number and percent of respondents by number of species raised.

Number of species raised	No. of respondents	Percent
1	30	38
2 to 5	20	25
6 to 10	22	28
11 to 15	5	6
No response	3	4
Total	80	101

Table 5. Number and percent of respondents by the type of aquatic animal raised that was most important in terms of sales.

Name of Species	Number of respondents reporting it to be most important in terms of sales	Percent
Tilapia	10	12
Trout	10	12
Yellow perch	8	10
Marine shrimp	8	10
Bass	7	9
Baitfish	6	7
Bluegill/bream	4	5
Catfish	4	5
Walleye	2	3
Turtles	2	3
Barramundi	1	1
Caviar	1	1
Crayfish	1	1
Freshwater prawn	1	1
Goldfish	1	1
Koi	1	1
Ornamentals	1	1
Grass carp	1	1
Tuna	1	1
No response	11	14
TOTAL	80	99

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Table 6. Types of production systems reported by respondents.

Production System	Number of respondents using this production system	Percent
Aquaculture		
Cages	12	8
Ponds	49	31
Raceways	13	8
Indoor tanks	42	27
Greenhouse tanks	3	2
Outdoor tanks	12	8
Tanks	4	3
Aquaponics		
Raceways	1	1
Indoor tanks	6	4
Greenhouse tanks	5	3
Outdoor tanks	2	1
Baitfish harvesting		
Cages	2	1
Ponds	3	2
Raceways	1	1
Indoor tanks	2	1
Outdoor tanks	1	1
TOTAL	158	102

Table 7. Numbers of different production systems on same farms

Type of production	Number of production systems on individual farms		
	1	2	3-6
Aquaculture	34	22	16
Aquaponics	2	4	1
Baitfish harvesting	2	0	2
All respondents	38	26	19
Percent	46%	31%	23%

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Table 8. Income categories reported by respondents.

Income category	Number of respondents using this production system	Percent
Aquatic animals		
< \$25,000	25	31
\$25,000 to \$50,000	8	10
\$50,000 to \$100,000	9	11
\$100,000 to \$500,000	12	14
\$500,000 to \$1 million	7	9
\$1 million & above	9	11
No response	10	10
TOTAL	80	100

Table 9. Membership in associations.

	n	Number of respondents	Percent of respondents
Ever belonged to association	80	62	78%
Never belonged to association	80	18	23%
Ended membership in an association	64	10	16%
Currently member of an association	64	54	84%

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Table 10. Reasons for not joining an association.

Category	Number of respondents	Percent of respondents
There are none in my state	3	18
Do not know how membership would benefit my business	5	29
Have never been asked to join	4	24
Do not believe it is worth the membership fee	2	12
Just beginning/new to this industry; only have small, pilot program now/new farm; will join	3	18
TOTAL	17	101

Table 11. Reasons why respondents ended their membership in an association.

Reason given
Not relevant to business
No longer doing that species
Not helpful or relevant
Not applicable to my business
Not pertinent to my career
Moved
Moved to another state
Shut down facility and moved.
More involved in a different state
Disagreement
Bad policy priorities
This organization is not for the industry, but for one family
Personal
Didn't feel welcome. It's too clickish
Unable to attend meetings
Decline in effectiveness of association
No longer exists
Lost interest and its failure to add real value to our business
Little communication, or resources in my immediate region
Never heard anything from them
Membership decline

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Table 12. Ratings of benefits received, likelihood to renew membership, and likelihood of recommending membership to others.

Category	Rating (scale of 1 to 5 where 5 = excellent)
Benefits from membership in association	3.7
Likelihood of renewing membership in association	4.5
Likelihood of recommending membership to others	4.3

Table 13. Mean ratings of value of benefits/services of membership in an association. A score of 5 is “Excellent”, 4 is “Very good,” 3 is “Good,” 2 is “Fair,” and 1 is “Poor.”

Benefit/service received from aquaculture association	Average score
Annual/biannual meeting in state	4.3
Networking opportunities	4.1
Media materials	3.9
Representation with regulators	3.9
Newsletter	3.7
Industry alerts	3.7
Workshops on latest research	3.6
Workshops on latest policies & regulations	3.6
Website	3.5
Public relations campaigns	3.0
Webinars	2.7

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Table 14. Gross sales/business scale effects on ratings of the value and benefits/services of association membership.

	< \$25 K (n=14)	\$25 to \$50 K (n=7)	\$50 to \$100 K (n=9)	\$100 to \$500 K (n=7)	\$500 K to \$1 million (n=7)	> \$1 million (n=7)
Value of membership	4.1	3.5	3.8	3.7	3.0	4.2
Renewal likelihood	4.6	4.25	4.5	4.3	4.4	4.8
Recommendation likelihood	4.6	3.9	4.2	4.3	4.0	4.4
Meetings	4.4	3.9	4.6	3.7	4.1	4.9
Newsletter	4.3	2.9	4.4	3.3	3.0	4.1
Webinar	2.4	2.6	3.4	3.3	2.3	3.4
Alerts	4.2	3.5	3.5	4.0	2.6	4.1
Website	3.9	3.3	3.7	4.2	3.3	3.0
Network	4.4	4.1	3.8	4.0	4.1	4.6
Workshops-research	4.1	2.8	4.0	3.7	3.4	3.6
Media materials	4.0	4.0	3.3	4.3	4.0	4.0
Workshops-policy/regs.	3.8	3.2	3.3	3.5	3.6	3.7
Public relations	3.2	2.7	3.0	3.3	2.6	3.7
Representation w/regulators	4.1	3.5	3.5	3.7	3.9	4.1

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Table 15. Species type effects on ratings of the value and benefits/services of association membership.

	Baitfish/ ornamentals (n=8)	Bass, bluegill, catfish (n=11)	Tilapia, shrimp, prawn (n=8)	Trout (n=9)	Yellow perch, walleye (n=9)	Other, including no report of primary species (n=8)
Value of membership	4.0	3.7	3.9	3.6	3.7	3.8
Renewal likelihood	4.5	4.6	4.3	4.6	4.4	4.5
Recommendation likelihood	4.3	4.4	4.1	4.4	4.0	4.4
Meetings	4.1	4.0	4.4	4.1	4.7	4.4
Newsletter	4.1	3.2	3.7	3.4	4.1	3.8
Webinar	2.1	2.3	3.0	2.5	3.4	3.4
Alerts	4.0	4.2	3.9	2.2	3.9	4.1
Website	3.4	3.0	4.1	3.4	3.8	3.5
Network	4.2	4.0	4.5	3.9	3.9	4.2
Workshops-research	3.8	3.0	4.0	3.2	4.3	3.9
Media materials	4.0	3.7	4.0	4.0	3.5	4.0
Workshops-policy/regs.	3.5	3.4	3.5	3.5	3.9	4.0
Public relations	3.2	2.5	3.3	3.1	3.0	3.2
Representation w/regulators	4.1	3.6	4.2	3.8	3.7	3.9

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Table 16. Effect of the degree of Extension support on ratings of the value and benefits/services of association membership.

	Low Extension support (n = 6)	Medium Extension support (n = 16)	High Extension support (n = 32)
Value of membership	3.3	3.6	3.9
Renewal likelihood	4.0	4.6	4.5
Recommendation likelihood	3.5	4.5	4.3
Meetings	2.4	4.4	4.5
Newsletter	2.2	3.1	4.2
Webinar	2.6	2.2	3.0
Alerts	3.5	3.7	3.8
Website	2.3	3.3	3.8
Network	3.7	3.9	4.3
Workshops-research	2.7	3.1	4.1
Media materials	3.0	-	3.9
Workshops-policy/regulations	3.7	3.4	3.7
Public relations	2.2	2.9	3.2
Representation w/regulators	3.0	3.9	4.0

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Table 17. Barriers and constraints to the development of an effective association that would provide benefits and services to their business identified by respondents.

Barriers and Constraints Identified by Those Who Had Never Joined an Association

Decline in effectiveness of association

Low membership; need to help build and support the industry.

Lack of participation

Lack of membership.

There is a little lack in cohesion when it comes to the aquaculture community within the state because there is limited interaction between members unless (1) they already knew each other before joining or (2) they only are forced to interact once a year at the annual conference. More events and a monthly newsletter would help bridge the gap significantly.

Size of industry

Not enough producers of any decent size.

Not enough interest in Aquaculture. Not enough producers to make it viable.

Specific association activities needed

Reduce regulations!

Practical objectives and adequate use of time.

Help get pcr testing approved for use

Other

We have NCRAC but they are only interested in intensive large-scale operations.

None

Barriers and Constraints Identified by Those Who Ceased Membership in an Association

Decline in effectiveness of association

Lack of interest

Disagreement

Bad leadership

Lack of response by other businesses because of a sense of competition instead of common ground

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Personal

Time to get away to go to meetings

Name of specific person^a

Distance to attend meetings, meeting dates and times

Governmental regulations

Lack of funding to grow and create new farms

^a Response referred to a specific name that has been omitted due to confidentiality.

Table 18. Thoughts by respondents related to aquaculture associations that no longer exist and what happened.

Lack of participation.

The association in my state appears to be doing well.

Active here in my state but they only care about the big intensive operations going in.

I have seen these type of associations try before; they never seem to work. Just like today's labor unions, nobody worries about the group, just about themselves.

Old members

Table 19. Reasons for decline in activity of aquaculture association by respondents who had once been a member of an association but ceased their membership.

Decline in effectiveness of association

Lack of interest

Lack of interest or fear of information sharing

Disagreement

Too many factions

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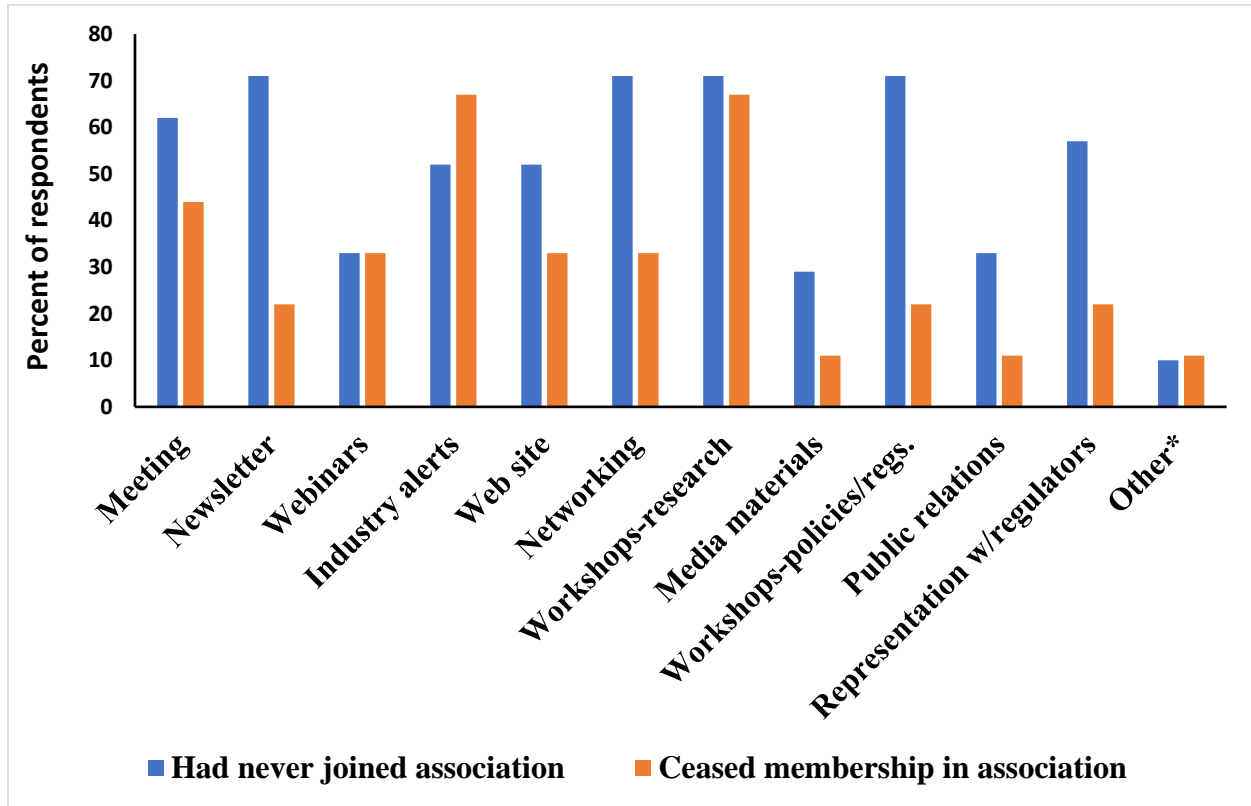


Figure 1. Frequency of responses of interest in various benefits/services by respondents who had never joined an association and those who had ceased membership.

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Project Title: Youth Education in Aquaculture [Termination Report]

Key Word(s): Extension, Education

Dates of Work: September 1, 2016- August 31, 2017

NCRAC Funding Level: \$34,907.16

Participants: Barbara I. Evans, Lake Superior State University; Christopher Smith, Lake Superior State University

Extension Liaison: (Dr. Ronald Kinnunen, Michigan Sea Grant, MSU, MI; Ms. Emma Wiermaa, University Wisconsin Steven's Point NADF, WI)

Industry Liaison: (Mr. Bill West, Blue Iris Fish Farm, LLC, WI)

Reason for Termination: Project objectives completed.

Project Objectives:

- 1) To assess the level of aquaculture curricula/programs in high schools throughout Michigan and Wisconsin
- 2) Develop a web based platform that a) allows assessment of the level and distribution of aquaculture curricula throughout the region; b) will be accessible by the schools to link the programs and c) is expandable to all states in NCRAC and possibly to other RACs.
- 3) Develop incentives for the students to solve aquaculture problems such as expansion of the "Aquaculture Challenge". Competitions should be scalable to other states within NCRAC.

Project summary

Advancement of the aquaculture industry can be augmented by engagement of youth throughout the K-12 system. Many schools do incorporate aquaculture into the curriculum, but there is a need to integrate these activities. We have developed an online forum for Youth Education in Aquaculture (YEA) to inter-connect high schools throughout Michigan and Wisconsin. By identifying the level of aquaculture engagement that currently exists, we will be able to coordinate these efforts via the online forum. This will allow us see what resources are needed to support current programs, and where we need to initiate/encourage new activities. Aquaculture is growing rapidly, but the projected workforce in the US is insufficient to meet future demands. High school training in aquaculture will provide students with the skills needed for this industry. In addition, aquaculture involves advanced concepts in math and science, and leads to potential careers in business, engineering and the life sciences. Currently in the US, 91% of seafood consumed is imported, contributing to a \$14 billion seafood trade deficit. When we rely on imports, we lose control of food quality, as foreign regulations on production are less stringent than in the US. Food security can be obtained with a viable US aquaculture industry, but we need to develop an educated workforce. We have begun the process of identifying schools that engage youth in aquaculture, throughout Michigan (MI) and Wisconsin (WI).

Technical Summary and Analysis:

Objective 1. — We gathered data on approximately 1500 grade schools in Michigan and Wisconsin. This data includes: school name, grade range, latitude/longitude, school website, and any current participation in an aquaculture based curriculum. We are still in the process of gathering school data regarding participation in aquaculture curricula, CTE programs related to aquaculture, and participation in Salmon/Trout in the Classroom. Two full-time employees, and later four part-time employees have accomplished this work originally. The process primarily utilized the internet as a resource. We would search for the school, make sure it is the right one in the correct location, and peruse their website. We were looking for any pertinent contact info we could use to inquire about the participation in aquaculture. In addition to the contact information, we also searched for any sign of potential aquaculture involvement, and we have found participating schools this way. To further assess the level of aquaculture curricula, we have created a newsletter describing our goals with the NCRAC-YEA project and a survey with the intent to gather more information from the schools. The newsletter and link to the Survey Monkey survey were distributed via email and will continue to be distributed (amended for the new grant) in hopes to gather more responses and uncover more schools which participate in aquaculture.

Objective 2. — To accomplish this Objective #2, we created an html and php website. It additionally uses some Javascript and utilizes Google Fusion Tables to visualize the data we found. We used a third party hosting company to host the website for five years. The website contains multiple pages that help accomplish this goal. The pages include a home page to describe our objectives, an outreach page to show the schools in the area, a resources page to view links to Arduino configuration pages and general aquaculture resources, a forum page where teachers can register, log in,

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and discuss topics regarding aquaculture and the Aquaculture Challenge, and an Aquaculture Challenge page outlining the competition and material provided by the participants. The sub-objectives were accomplished with these pages.

Objective 3. — Aquaculture Challenge: We are continuing to develop the aquaculture challenge competition. Travel costs and time conflicts made it difficult for many schools to attend the physical showcase, so we are working to transfer this to a virtual, online venue. We have been working with Elliot Nelson from Michigan Sea Grant on this project, and have developed a competition manual for students in the current competition.

Principal Accomplishments

High schools (1500) throughout Wisconsin and Michigan were identified, and many were contacted for their aquaculture-related interest and activity. 2) A website was constructed for Youth Education in Aquaculture (YEA) (www.ncrac-yea.org), and this information was uploaded to the site. 3) The YEA website is also being used to host an aquaculture competition, as an incentive to engage youth in aquaculture.

Impacts

The project has initiated a process to interconnect fish farmers with educational entities to increase the visibility of aquaculture as a career option for youth. By directing interest towards aquaculture education, we expect to see increases in the skilled aquaculture workforce, as well as recruiting entrepreneurs into the aquaculture industry

Recommended Follow-Up Activities

We have received follow-up funding to expand the scope, and extend this project to all states of NCRAC.

Future Objectives

1. Assess the workforce needs for the aquaculture industry throughout the 12 states of the North Central Region (NCR). Identify which skills are needed in the workforce to promote industry growth.
2. Assess the level of youth focused aquaculture curricula/programs in the NCR. Identify schools with a Career and Technical Education (CTE) certification in the Agriculture, Food and Natural Resources career cluster that includes aquaculture. Identify other youth aquaculture related activities.
3. Integrate industry and high school/youth career center information through a web-based platform that:
4. creates awareness of the aquaculture industry skill needs
5. allows assessment of the level and distribution of aquaculture curricula
6. utilizes the web-based platform (ncrac-yea.org) to co-develop curricula to address industry needs
7. creates incentives for youth to pursue aquaculture skills training
8. Create and promote aquaculture workshops for educators and Extension professionals and provide access funding for them to attend existing workshops.
9. Identify community colleges or universities with aquaculture courses/programs, and create opportunities for interested students to be dual enrolled in existing college classes, or "less than class size" internship opportunities at fish farms and hatcheries.

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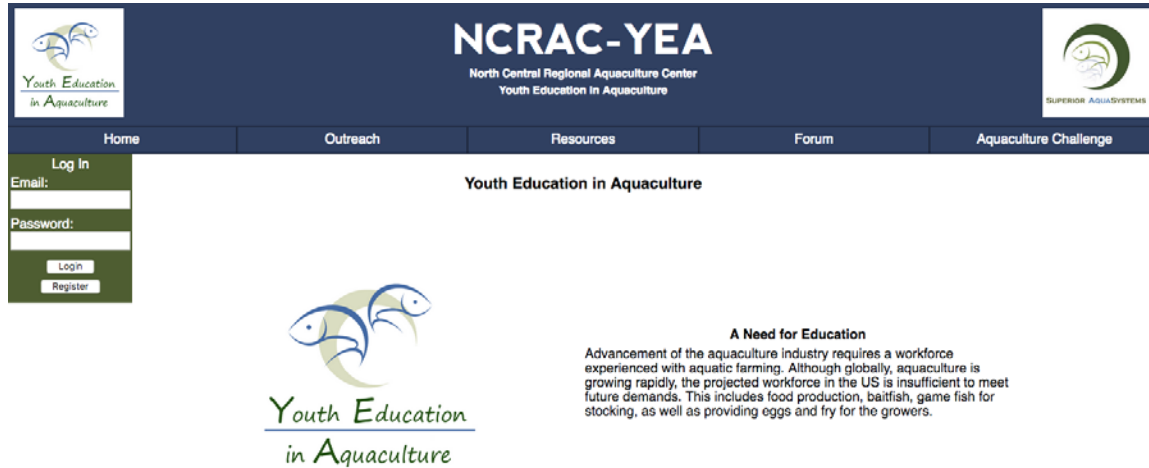


Figure 1. Screenshot of secure home page of website www.ncrac-yea.org

Aquaculture Outreach

One of the primary objectives of Youth Education in Aquaculture is to identify and quantify the level of aquaculture engagement that exists in high schools in the North Central Regional Aquaculture Center region, and potentially in other Regional Aquaculture Centers in the future. The interactive map below displays the known level of aquaculture participation of contacted high schools.

Aquaculture Involvement

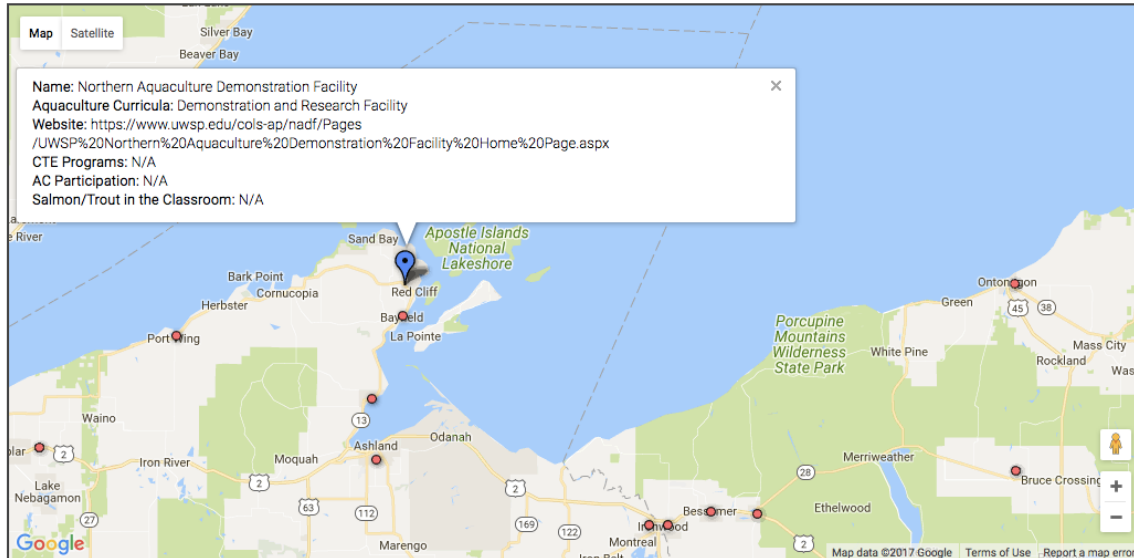


Figure 2. Interactive map showing locations of aquaculture outreach facilities and nearby high schools. If you wish to review your answers at a later date, DO NOT click the forward button now. Your information should be automatically saved at this point. Clicking forward will submit your report.

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Project Title: Metagenomic Analysis of Microbial Populations in Aquaponic Systems [Termination Report]

Key Words: Aquaponics, metagenomics, bacteria, nitrogen cycle

Project Period: 1 September 2016 to 31 August 2017

NCRAC Funding Level: \$24,596

Participants: Matthew Rogge, University of Wisconsin-Stevens Point, Wisconsin; Nicholas Phelps, University of Minnesota, Minnesota; Daniel Graf, University of Wisconsin-Stevens Point, Wisconsin.

Extension Liaison: Nicholas Phelps, University of Minnesota

Industry Liaison: Rebecca Nelson, Nelson and Pade, Inc., Wisconsin

Reason for Termination: Project objectives completed.

Project Objectives:

1. Collect microbial samples from multiple sites within an aquaponics system and from various systems across a geographic range and extract bacterial DNA using commercially-available kits.
2. Submit DNA samples to the UW-Madison Biotechnology Center for PCR amplification, Illumina next-generation sequencing, and bioinformatics analyses to compare microbial populations among sampling sites within a system, among systems at a single production site, and among production sites in the North-Central Region (NCR).
3. Develop a fact sheet explaining the importance of microbial populations in an aquaponics system, including the risk that certain organisms can cause (e.g., plant, fish, and human pathogens), develop a recorded presentation for dissemination using the NCRAC website, and produce oral presentations and a peer-reviewed manuscript.

Project Summary

Aquaponic food production is dependent upon the microbial populations present in the system.

Microbes play a role in nitrogen conversion from animal waste to plant fertilizer and mineralization of organics in the system, but may also cause fish, plant, or human diseases. Although the importance of microbes is recognized, little is known about the specific types of microbes found in an aquaponics system or where those microbes are found. Without this knowledge, it is difficult to manage those populations to best enhance fish and plant growth while reducing the risk of plant, fish, and human pathogens in the system. This study used a metagenomic approach to studying microbial populations in aquaponics systems. Microbial 16S RNA was sequenced using Illumina next generation sequencing and microbes were identified to taxonomic levels. Analyses were performed to determine the relative abundance and richness of taxa at each site, compare among different sites within a system, compare replicate systems, and to compare the microbial diversity between the two facilities.

Technical Summary and Analysis

Objective 1.—The University of Wisconsin-Stevens Point Aquaponics Innovation Center (UWSP-AIC) houses six replicate commercially-available systems (Nelson and Pade, Inc.). Each system contained two 416-L fish tanks that flowed by gravity into 416-L settling tanks, then to a mineralization tank containing plastic netting to provide surface area for microbial colonization. Water flowed to a degassing tank, then to four 4'W×6'L×1'D raft tanks. Water was then pumped back to the fish tanks. The fish stocked into the tanks were hybrid walleye at low, medium, or high density, and the plants were butterhead lettuce, romaine lettuce, kale, and pak choi.

To sample microbial populations in these systems, samplers were placed into the system on 28 October 2016. For microbial populations that grow on tank wall surfaces, four 7.5cm × 7.5cm pieces of tank wall material were suspended in each system. Two samplers were placed in the fish tanks, and two were placed in the raft tanks. To sample the microbial populations that colonize the netting of the mineralization tank, eight layers of 30cm × 30cm netting material were attached to a 30cm × 30cm PVC frame and placed into the mineralization tank of each system. To sample the microbial populations that colonize the underside of the foam rafts, 7.5cm × 7.5cm pieces of foam material were placed into raft tanks. Two foam samplers were placed into each system. The samples were undisturbed in the systems for 80 days before being removed 19 January 2017. Water and tank detritus samples were also collected 19 January 2017.

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Accumulation of material on each tank wall and foam sampler was scraped from the samplers using a sterile cell lifter and placed into sterile 2ml microcentrifuge tubes containing 500 μ l sterile deionized water. From each netting sampler, a 7.5cm \times 7.5cm portion of the eight-layer sampler was cut using sterile scissors and placed into a sterile 50ml conical-bottom centrifuge tube with 25ml of sterile deionized water. Water samples (25ml) were taken from the mineralization tank and raft tanks using a 25ml serological pipet and placed into a sterile 50ml conical-bottom centrifuge tube. Detritus samples were taken near the inflow pipe of the raft tank using a 25ml pipet. The tip of the pipet was placed into the detritus and the pipet was filled to the 25ml mark. The detritus sample was placed into a sterile 50ml conical-bottom centrifuge tube. Root cuttings were taken from random plants in the system using sterilized forceps and scissors and placed into sterile 2ml microcentrifuge tubes. All samples were stored on ice or refrigerated until DNA extraction.

The University of Minnesota aquaponics facility houses six replicate systems representative of “homemade” systems in which the foam rafts are in the same tank as the fish. Water is circulated through a 208-L gravel filter bed. Tank wall and foam raft surfaces were sampled in duplicate from each system using sterile swab samplers. Water samples (50ml) were taken in duplicate from the fish/raft tank and water exiting the gravel filter using sterile 50ml conical-bottom centrifuge tubes. Root cuttings were taken in duplicate using a sterile blade and placed into Whirl-Pak bags. Duplicate samples of gravel bed medium (approximately 20 pieces of gravel) were collected from each system and placed into 50ml conical-bottom centrifuge tubes. Pump filter material (approximately 2.5cm \times 2.5cm \times 10cm) was collected in duplicate from each system and placed into sterile 50ml conical-bottom centrifuge tubes. All samples were stored on ice and sent to UW-Stevens Point for DNA extraction.

Due to the different substrates and methods of sample collection, the samples required different methods of preparation for DNA extraction. Water samples from WI and MN were prepared by centrifuging 25ml of the sample at 10,000 \times g for 5min. Pellets were resuspended in 250 μ l sterile deionized (DI) water. Tank wall and foam swabs from MN were resuspended in 250 μ l sterile DI water by pressing and turning the swab against the wall of the microcentrifuge tube. Tank wall and foam scrapings from WI were centrifuged at 10,000 \times g for 5min, and pellets were resuspended in 250 μ l sterile DI water. Twenty-five grams of each MN gravel sample was added to 25ml of sterile DI water vortexed vigorously to dislodge material from the gravel. The gravel pieces were aseptically removed, and the suspensions were centrifuged at 10,000 \times g for 5min. The pellet was resuspended in 250 μ l sterile DI water. The MN pump filter material was placed into 25ml of sterile DI water and vortexed vigorously to dislodge material from the filter. The material was removed, and the suspension was centrifuged at 10,000 \times g for 5min, and the pellet was resuspended in 250 μ l sterile DI water. The MN root samples were placed into 25ml of sterile DI water and vortexed vigorously. Samples were centrifuged at 10,000 \times g for 5min, and the pellet was resuspended in 250 μ l sterile DI water. The suspension was transferred to a new microcentrifuge tube to reduce plant root material in the sample. The WI root cutting samples were suspended in 1ml sterile DI water and vortexed vigorously. The suspension was centrifuged at 10,000 \times g for 5min, and the pellet was resuspended in 250 μ l sterile DI water. The suspension was transferred to new microcentrifuge tube to reduce plant root material in the sample. The WI netting samples were vortexed vigorously to dislodge material from the plastic. The netting was aseptically removed, and the suspension was centrifuged at 10,000 \times g for 5min. The pellet was resuspended in 250 μ l sterile DI water. The WI detritus samples were vortexed vigorously to suspend the detritus. A 250 μ l sample of the resuspended material was removed for DNA extraction protocols.

DNA extraction was performed on 250 μ l of each sample by using the DNeasy PowerSoil Kit (Qiagen, Inc., Germantown, MD) per the manufacturer’s protocol. DNA concentrations were determined fluorometrically using the Qubit dsDNA BR Assay Kit (Thermo-Fisher Scientific, Waltham, MA) per manufacturer’s instructions for 10 μ l of each sample. Samples that had a concentration below the detectable limit (< 0.20ng/ μ l) were recorded as zero.

The six WI systems were stocked with high, medium, or low densities of hybrid walleye at two systems per stocking density. Fish stocking density had no significant effect on the DNA concentrations extracted from samples throughout the system. The six MN systems have red wiggler worms in the gravel biofilter of three systems and no worms in the other three systems. The DNA concentrations purified from samples were significantly lower in the pump filter medium ($P = 0.002$, Mann-Whitney Test) and gravel samples ($P = 0.002$, Mann-Whitney Test) in the systems containing worms (Fig. 1). This correlated with the observance of smaller pellets after sample centrifugation, although pellet weights were not measured. This suggests that the presence of the worms is reducing

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the amount of organic material buildup in the gravel filters and on the pump filter.

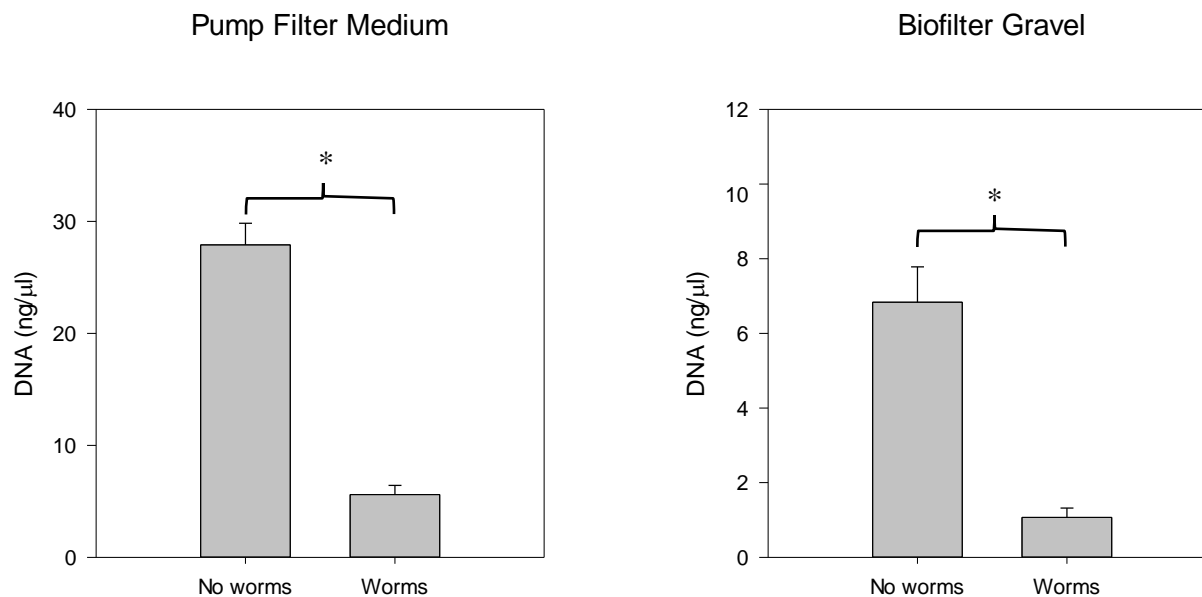


Figure 1. DNA concentrations of samples taken from pump filter medium and biofilter gravel used in the University of Minnesota aquaponics systems. Bars represent the mean DNA concentration (\pm standard error). Asterisks indicate a significant difference ($P < 0.05$) using a Mann-Whitney Rank Sum Test.

When comparing samples longitudinally in the system, there were significant differences in the concentration of DNA extracted from the samples in both the WI and MN systems (Tables 1 and 2). At both facilities, the water samples had relatively low DNA concentrations, suggesting fewer microbes present than on surfaces within the system. When comparing all DNA concentrations between the MN and WI facilities, the WI samples have a significantly higher value ($P < 0.001$) concentration. In pairwise comparisons, only the MN root cuttings have a higher average DNA concentration than the WI root cuttings. For samples of surfaces such as tank walls and foam, this difference is likely due to using samplers in the WI systems that were removed and scraped to acquire organic material compared to using a swab to sample a smaller area in the MN systems.

The amount of DNA extracted from samples may affect the ability to acquire quality DNA sequencing results and an accurate snapshot of the microbial community of the area sampled. In both the MN and WI systems, sampling 25mls of water resulted in low DNA concentrations, which may lead to microbial populations being underrepresented or unidentified in the water samples. Similarly, swabbing fixed surfaces within the MN systems resulted in lower DNA yields than surfaces from uniformly sized, removable samplers from which material was scraped and collected. The difference in DNA may be simply due to the MN systems having less area sampled, but the removable samples at the WI site also prevented material from being displaced from swabs by water in the system while surfaces were being sampled.

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Table 1. Median DNA concentrations of samples taken from the WI aquaponics systems. Medians with the same letter are not significantly different ($P > 0.05$).

Sample	Amount Sampled	Median DNA Concentration (ng/μl)
Fish Tank Plastic Wall	56.25cm ²	75.000 ^a
Mineralization Tank Water	25mls	4.570 ^b
Mineralization Tank Netting	56.25cm ²	19.040 ^a
Raft Tank Water	25mls	1.529 ^b
Raft Tank Plastic	56.25cm ²	34.100 ^a
Raft Tank Foam	56.25cm ²	18.510 ^{ac}
Raft Tank Detritus	variable [*]	20.400 ^a
Root Cuttings	variable [†]	4.010 ^{bc}

*A 25ml serological pipet tip was placed into detritus, and a pipet-aid was filled to 25mls with detritus and water.

†Approximately 5cm of the lower portion of roots were cut.

Table 2. Median DNA concentrations of samples taken from the MN aquaponics systems. Medians with the same letter are not significantly different ($P > 0.05$).

Sample	Amount Sampled	Median DNA Concentration (ng/μl)
Biofilter Gravel	25g	2.996 ^{ac}
Biofilter Water	25mls	0.000 ^b
Tank Water	25mls	0.000 ^b
Tank Wall	variable [*]	3.940 ^{ac}
Foam	variable [*]	0.709 ^{bc}
Root Cuttings	variable [†]	6.320 ^a
Pump Filter Medium	variable ^{††}	15.250 ^a

*Area swabbed was approximately 1-4cm²

†Approximately 15cm of the lower portion of roots were cut.

†† Approximately a 2.5cm × 2.5cm × 10cm piece of filter was cut.

Objective 2. —Purified DNA samples were submitted to the University of Wisconsin Biotechnology Center (<http://www.biotech.wisc.edu/>), which requested samples to be 20ng/μl or less. Samples that met that criterion were submitted undiluted, including those samples that had undetectable amounts of DNA. The UW Biotechnology Center performed PCR of the V3-V4 16S region, PCR cleanup, Illumina Mi-Seq next generation sequencing, and bioinformatic analyses using the Qiime pipeline (<http://qiime.org/>).

A total of 11,252,375 sequences were generated and analyzed. Of those, 10,998,062 (97.7%) were identified as prokaryotes (Bacteria or Archaea). There was a weak positive correlation between the concentration of DNA samples submitted and the number of reads generated from the sample (Fig. 2). Interestingly, samples with DNA concentrations too low to be detected (recorded as zero) frequently had greater than 40,000 DNA sequencing reads.

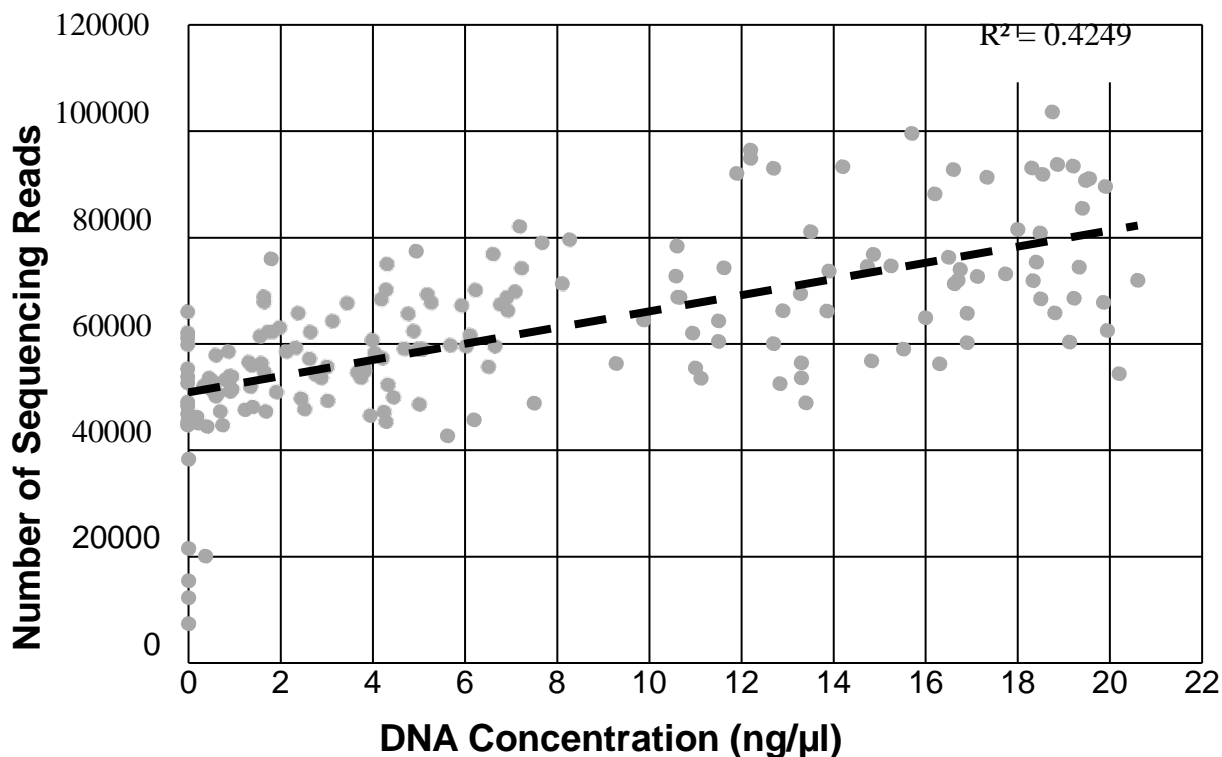


Figure 2. Scatter plot of the number of DNA sequences generated against the concentration of DNA sent for analysis. The data indicate a weak positive correlation between the concentration of DNA submitted for analyses and the number of sequences read during analysis.

The metagenomic analyses identified organisms from phyla to species, with 1,080 different operational taxonomic units (OTUs) at the species level. Only 10% of the species, however, were named (Table 3). As the taxonomic level approached species, the proportion of named groups decreased. Although these data indicate 1,080 species, it is possible there are more, because some sequences could not be resolved past taxonomic levels higher than genus and species. Because of the lack of species names for many of the organisms present, these analyses may be suboptimal for determining the abundance of pathogens, because pathogenic species share taxonomic groupings (genus, family, etc.) with non-pathogens.

It is unknown if different facilities will have different types of microbes in their systems. The two aquaponics facilities sampled differed in many ways, including design, fish species used, stocking densities, etc. Raup-Crick analyses were used to compare populations. Values 5 or less indicate significant dissimilarity, values 95 or greater indicate significantly similarity, and values from 6 to 94 indicate any population overlap is no different than random. At the phylum and class levels, the microbial populations are not significantly dissimilar (Table 4). From order to species, however, the Raup-Crick values indicated significant dissimilarity between communities of microbes MN and WI. Much of the endemic taxa at a facility, however, are rare taxa. Whether or not rare taxa should be included in the analyses is unknown, because we do not know if rare taxa perform significant functions in the system, e.g., nitrogen fixation, denitrification, iron reduction, sulfur reduction or oxidation, etc. In MN, 23% of the species identified are not present in WI, but those taxa make up only 0.3% of the microbial community sampled (Fig. 3A). Similarly, 16% of the species identified in WI systems were not present in MN systems, but that accounts for only 0.11% of the WI communities (Fig. 3B). Essentially, all species occurring at a frequency greater than 0.1% of the total population were present in both the MN and WI systems. This indicates that many rare taxa were sampled at each facility, but we do not know what role, if any, these taxa have in an aquaponics system. The analyses described below were performed with all taxa included, and many results show that the communities are significantly similar; removing rare taxa, however, was observed to result in conclusions that communities were different. Part of

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determining the significance of the rare taxa is determining the functional roles of the bacterial populations present in the system and determining what proportion of microbes are performing that function. As such, it may be more beneficial to dispense with analyzing these communities based on taxonomic identification and analyze based on their functional identity. We are continuing to analyze the data to determine the appropriate handling of rare taxa.

Table 3. The number of taxonomic groups identified and the number of taxonomic groups named from metagenomic analyses of aquaponics systems.

Taxonomic level	Total	Taxonomic Name Assigned	Percent Name Assigned
Phylum	50	48	96
Class	165	132	80
Order	326	210	64
Family	539	255	47
Genus	936	390	42
Species	1080	108	10

Table 4. Count of taxa identified in Wisconsin and Minnesota aquaponics facilities. The numbers represent the counts of taxa in the entire study (Total), in Wisconsin only (WI), in Minnesota only (MN), and in both WI and MN (Both). The MN/WI overlap is the percentage of the total count of taxa identified in both WI and MN. The Raup-Crick value indicates significant dissimilarity (0) to significant similarity (1).

Taxonomic level	Total	WI	MN	Both	MN/WI overlap	Raup-Crick
Phylum	50	46	49	45	90%	86
Class	165	142	155	132	81%	8
Order	326	275	295	244	75%	0
Family	539	449	483	393	73%	0
Genus	936	752	822	638	68%	0
Species	1080	864	938	722	67%	0

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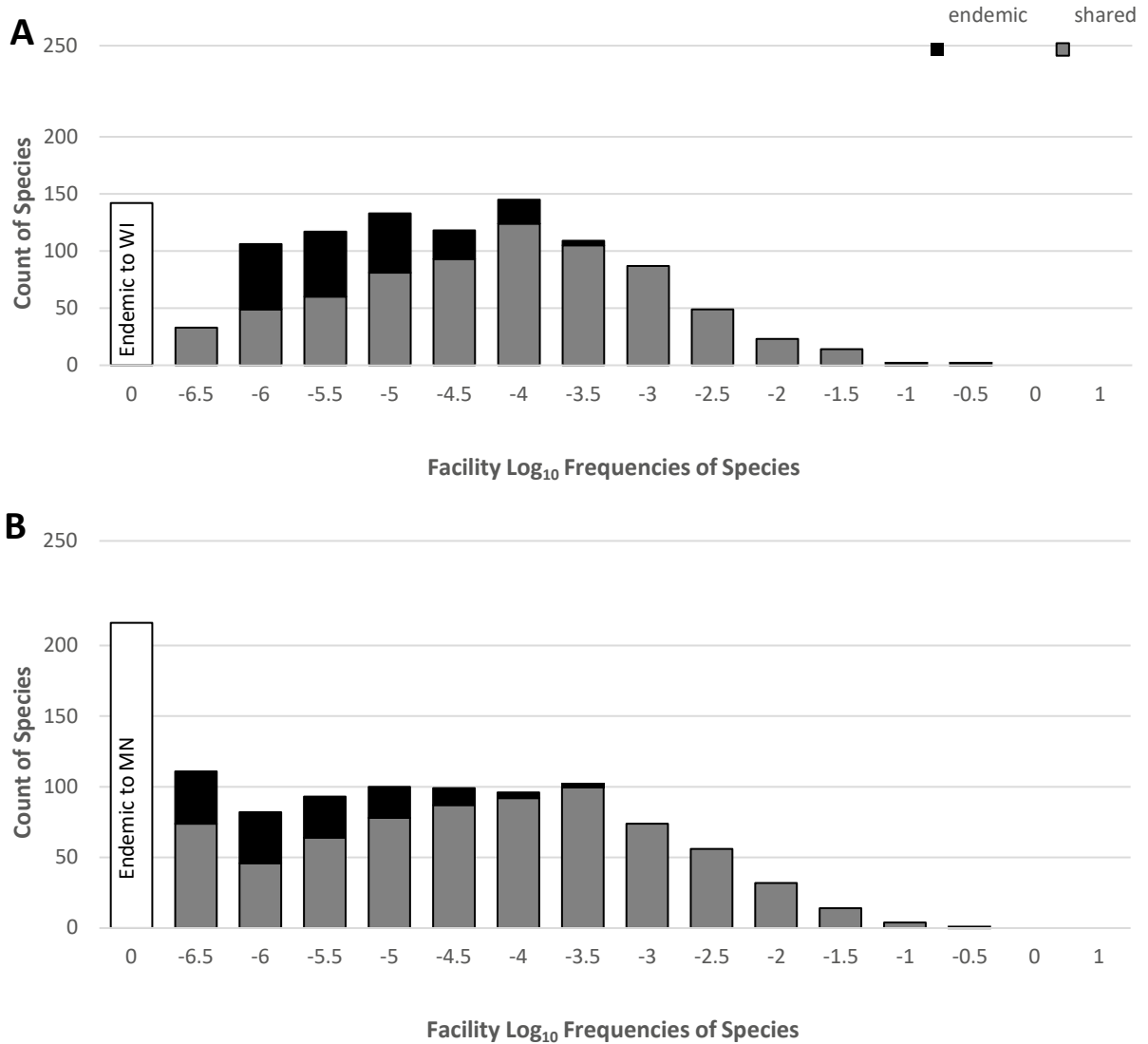


Figure 3. Frequency of species in MN (A) and WI (B) aquaponics systems. Bars represent the count of species occurring at a range of frequencies. Gray bars represent the taxa that are shared in the MN and WI systems, and black bars represent the taxa that are endemic to MN or WI. The white bar represents the number of taxa not present at that facility but present in the other.

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Treatment effects on microbial populations

The WI aquaponics systems were stocked with varying levels of hybrid walleye, but there were no significantly dissimilar populations resulting from the different stocking densities. The MN aquaponics systems had red wiggler worms (*Eisenia fetida*) stocked into the gravel biofilters of three systems and three systems without worms.

Sample site effects on populations

Comparisons of samples longitudinally through the WI aquaponics systems indicated significant similarities among all sample sites for all comparisons at the species level. The MN systems are also similar when evaluating longitudinally. At the species level, all sample populations are significantly similar. This indicates highly uniform communities of microbes throughout the MN and WI systems.

Microbes involved in nitrogen cycling

Conversion of ammonia waste products of fish to nitrite and nitrate is essential for an aquaponics system to maintain the health status of fish and provide plants with usable forms of nitrogen. Microbes involved in various aspects of the nitrogen cycle were identified using metagenomic analyses

Ammonia oxidizing bacteria (AOB) convert ammonia into nitrite. Members of the family Nitrosomonadaceae are common AOB. Members of Nitrosomonadaceae were present in both the WI and MN systems (Table 5), making up 0.42% and 0.011% of the populations, respectively. Unlike the WI systems, MN systems also contained the archaeal genus *Nitrosopumilus*, which are ammonia-oxidizing archaea (AOA). This group made up only 0.0005% of the total population, however. *Nitrosopumilus* is in Family Cenarchaeaceae, and unidentified members of this family were present in the system (0.00074%), suggesting the possibility that additional AOA are present. While *Nitrosopumilus* is present in MN systems, the higher occurrence of Nitrosomonadaceae suggests it has a greater role in ammonia oxidation than AOA. Wisconsin systems had a greater percentage of Nitrosomonadaceae ($P=0.004$, Mann-Whitney Test) than MN systems, but the percentage of *Nitrosopumilus* was not different between the sites ($P=0.397$, Mann-Whitney Test). The reason for the difference between MN and WI systems is not known, but could be related to system design, management, setup, or some other extrinsic factor.

Microbes that perform anaerobic ammonia oxidation (anammox) were also present in both MN and WI systems. This metabolic process is strictly anaerobic and reacts ammonia with nitrite to produce nitrogen gas and water, thus decreasing the levels of both toxic forms of nitrogen for fish. The nitrogen, however, becomes unavailable to plants. Microbes performing anammox are classified in Candidate Order Brocadiales, which comprised 0.0001% and 0.053% of the microbial population in MN and WI, respectively. Wisconsin systems had a significantly greater percentage of Brocadiales than MN systems ($P=0.002$). The majority of Brocadiales appears to be in the raft tank detritus, which is likely to encourage anoxic conditions and the growth of anaerobic bacteria. Comparable material was not available to be sampled from the MN systems, and it is possible that isolated anaerobic environments in the MN systems or elsewhere in WI systems may contain higher levels of anaerobic bacteria, including Candidate Order Brocadiales.

Nitrite oxidizing bacteria (NOB) convert nitrite to nitrate. Historically, this process was attributed to organisms in Genus *Nitrobacter*. This genus, however, was not identified in any of the samples. The Genus *Nitrobacter* is in the Bradyrhizobiaceae family, which was identified in the system. Not all members of this family were identified to the genus level, so it is possible that *Nitrobacter* is present, but not identified. Unknown members of Bradyrhizobiaceae comprised 0.0232% and 0.0092% of the microbial population in MN and WI, respectively. Although *Nitrobacter* is commonly associated with important NOB microbes, the Genus *Nitrospira* is readily found in environmental samples and oxidizes nitrite. *Nitrospira* was identified in MN and WI systems. In MN systems, *Nitrospira* made up 0.33% of the microbial population, whereas in WI, it made up 3.1% of the microbial population. The lack of other NOB identified suggests that *Nitrospira* is the primary NOB present in both WI and MN systems. Wisconsin systems had significantly greater percentages of *Nitrospira* ($P=0.014$, Mann-Whitney Test). Again, the reason for the difference between MN and WI systems is not known, but could be related to system design, management, setup, or some other extrinsic factor.

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Table 5. Percent of the microbial population comprised of microbes involved in nitrogen cycling.

Sampling Site	NH ₃ Oxidation		Anammox	NO ⁻ Oxidation
	Nitrosomonadaceae	<i>Nitrosopumilus</i>	Brocadiales	<i>Nitrospira</i>
MN				
Biofilter Gravel	0.0424	0.0032	0	1.2026
Biofilter Water	0.0016	0	0	0.0516
Tank Water	0.0015	0	0	0.0453
Foam Raft	0.0013	0	0	0.0307
Tank Wall	0.0011	0	0	0.0251
Plant Roots	0.0104	0.0003	0.0003	0.5050
Pump Filter	0.0171	0	0.0005	0.4488
System mean	0.0106	0.0005	0.0001	0.3299
WI				
Fish Tank Wall	1.0616	0	0.0009	4.7397
Mineralization Tank Water	0.0214	0	0.0039	0.4212
Mineralization Tank Netting	1.1663	0	0.0011	4.4472
Raft Tank Water	0.0047	0	0.0007	0.0897
Raft Tank Wall	0.3711	0	0.0001	6.6135
Foam Raft	0.3371	0	0.0003	6.0694
Plant Roots	0.2223	0	0.0008	1.0216
Raft Tank Detritus	0.1703	0	0.4121	1.6142
System mean	0.4194	0	0.0525	3.1271

Objective 3. — Progress is being made toward completing a fact sheet outlining the importance of microbial populations in aquaponics systems. Topics will include different types of microbes necessary for the system, where they may be found in the population, and recommendations on sampling populations. Data generated from this project will be included. Once the fact sheet is completed, the recorded presentation will be produced using similar content and published to the NCRAC website. This project produce a large amount of data, and the analysis of the data is not complete for oral presentations or preparation of a manuscript. Analysis of the data will continue, and a manuscript and presentation is expected.

Principal Accomplishments

This project demonstrates the use of sampling techniques to study the microbial populations in aquaponics systems. Analyses of the microbial populations indicate that the various surfaces of the system are colonized by a diverse population of microbes, whereas the water exhibits fewer types of microbes. The surfaces, although made of different materials or found in different areas of a system, have similar microbial populations. This information is useful for future studies, because it indicates that not every variety or location of a surface needs to be sample. Although the organisms present in the MN and WI aquaponics facilities are similar, the proportion of specific taxa may be different, as evidenced by the proportions of microbes involved in nitrogen cycling. Those organisms are present in MN systems, but their proportions are greater in WI systems, suggesting that design or management of aquaponics systems can result in greater populations of beneficial organism. Finally, we have developed the computer coding programs to take the data generated from the Qiime pipeline and deconstruct the Qiime outputs to analyze the dataset in ways not done by Qiime. This will provide more streamlined analyses of metagenomic data in future projects. Much of the time spent on data analysis was related to developing the code to appropriately analyze the data in a meaningful way for aquaponics.

Impacts

This is the first study that has used metagenomics to study multiple locations within replicate aquaponics systems at different aquaponics facilities. The information collected from this project will be useful to future researchers interested in studying microbial populations in aquaponics systems. The use of samplers that can be put into systems and allowed to colonize will allow for more uniform sampling among sites within systems, different systems, and different facilities to provide more accurate comparisons between samples. Because the communities of microbes on various surfaces, i.e., plastic tank walls, foam, and netting, were similar, the sampling of all different types of surfaces may not be required. The reduction in sample sites within the system will allow for more repeated sampling over time to evaluate the stability of microbial communities in an aquaponics system.

The identification of the microbes nitrogen-related microbes, i.e., *Nitrosomonadaceae*, *Brocadiales*, and *Nitrospira*, suggest they are the organisms primarily involved in nitrogen waste conversion allows for development of management strategies to support their specific growth for optimal fish and plant health. The general identification of different types of microbes and the locations they inhabit within a system may be beneficial in developing strategies to prevent or reduce the possibility of harmful microbes, e.g., plant, fish, and human pathogens, from establishing themselves in a system.

Recommended future activities

This project provides a snapshot of two facilities. Future projects are needed to determine if the communities in a system are stable over time or if they fluctuate seasonally or in relation to facility management practices. As such, we recommend future support of studies that include the evaluation of microbial communities to determine if the frequency of populations of beneficial microbes, e.g., nitrogen-cycling organisms, can be increased for better plant growth. Additionally, although the MN and WI facilities demonstrated notable similarities between the types of microbes that make up a majority of the community, we cannot determine if this indicates an “aquaponics microbial fingerprint” or if the populations simply represent any indoors aquatic ecosystem or recirculating system. We suggest the sampling of a non-aquaponics system, such as a recirculating aquaculture system, a hydroponics system, or some other artificial aquatic environment as controls for microbial community studies in aquaponics.

Publications, Manuscripts, Workshops, and Conferences

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

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SOME COMMONLY USED ABBREVIATIONS AND ACRONYMS

x	cross, by, or times
AIS	aquatic invasive species
anamnox	anaerobic ammonium oxidizing bacteria
AOA	ammonia oxidizing archaea
AOB	ammonia oxidizing bacteria
APHIS	Animal and Plant Health Inspection Service
AREF	Aquaculture Regional Extension Facilitator
AquaNIC	Aquaculture Network Information Center
BOD	Board of Directors
BW	body weight
°C	degrees Celsius
CES	Cooperative Extension Service
COD	chemical oxygen demand
CSFPH	Center for Food Security and Public Health
CVM	Center for Veterinary Medicine
EPC	epithelioma papulosum cyprini
°F	degrees Fahrenheit
FSR	final study report
ft, ft ² , ft ³	foot, square foot, cubic foot
FY	fiscal year
g	gram(s)
gal	gallon(s)
h	hour(s)
ha	hectare(s)
HACCP	Hazard Analysis and Critical Control Point
HCG	human chorionic gonadotropin
IAC	Industry Advisory Council
in	inch(es)
INAD	investigational new animal drug
ISU	Iowa State University
KAA	Kansas Aquaculture Association
kg	kilogram(s)
L	liter(s)
lb	pound(s)
LU	Lincoln University
m, m ² , m ³	meter(s), square meter, cubic meter
MAI	motile <i>Aeromonas</i> infection
MAS	motile <i>Aeromonas</i> septicemia
MDNRE	Michigan Department of Natural Resources and Environment
µg	microgram(s)
mg	milligram(s)
MC	Mill Creek
min	minute(s)
mL	milliliter(s)

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

Funding supplied through:



United States
Department of
Agriculture

National Institute
of Food and
Agriculture