North Central Regional Aquaculture Center

Annual Progress Report 2019-20

February 2021

30th Annual Progress Report

For the Period
September 1, 2019 to August 31, 2020

North Central Regional Aquaculture Center

339 Science Hall II
Iowa State University
Ames, IA 50011-3221
Telephone: (515) 294-5280 FAX: (515) 294-2995
Web site: http://www.ncrac.org

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Introduction

The U.S. aquaculture industry generated nearly $1.4 billion for over 3,000 producers in 2013 (USDA 2014). Though minor in a global context, accounting for 0.73% of total world value in 2015 (FAO 2017), the domestic impact of U.S. aquaculture is substantial, accounting for approximately almost 20% of the total U.S. seafood production (NOAA 2018). Yet, anticipated growth in the industry, both in magnitude and in species diversity, continues to fall short of expectations in many regions of the U.S.

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 continues to be met by imports. The U.S. imports a majority of its fish and shellfish and is currently the world's largest importer of edible seafood (valued at $21.5 billion in 2017; FAO 2017, NOAA 2018). Fish and shellfish imports are the second largest contributor to the U.S. trade deficit among agricultural products (USDA 2016). In 2017, the trade deficit was nearly $14.1 billion for edible fishery 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 currently funded through USDA National Institute of Food and Agriculture (NIFA). 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 12-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.

Organization 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.

Funds to operate NCRAC are granted by the USDA-NIFA USDA-National Institute of Food and Agriculture (NIFA) to ISU. ISU disperses funds and serves as legal and fiscal agent in the receipt and disbursement of funds. The Center at ISU also coordinates implementation and operation of individual projects as agreed upon by the Board of Directors as well as fiscal and technical reporting to the USDA-NIFA.

The staff of NCRAC at ISU included Joseph E. Morris, Director, Denise Birney, Administrative Specialist II, and Stephen Grausgruber, Graduate Extension Assistant for regional programming in 2018/19. In 2018 the NCRAC Director's NCRAC appointment decreased to 70% with the additional institution duties serving as Iowa State University Extension Specialist. Also, Denise Birney resigned in July 2019 and Quinn Zuercher was appointed as Administrative Specialist I in August 2019.

The Center Director has the following responsibilities (0.40 FTE [current grant], 30% of salary is from previous grants [FY16-FY18] for 70% NCRAC appointment):

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

The Administrative Specialist II (0.50 FTE [current grant] 50% NCRAC appointment) has the following responsibilities:

- Schedule meetings, make travel arrangements, attend meetings and take minutes;
- Maintain the administrative calendar;
- General office management, prepare correspondence;
- Answer or direct inquiries appropriately relating to aquaculture in general and the Center in particular;
- 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;
- Monitor Web site and keep Director and Program Specialist updated on changes/additions;
- Assist with grant application (pre-award);
- Maintain and monitor all budgetary matters for both the Center and sponsored projects including developing and monitoring sub-contracts with other parties for purposes of transferring funds for implementing all approved projects (post-award); and
- Manage procurement and travel for NCRAC.

The Graduate Extension student (0.5 FTE) has the following responsibilities:

- Interaction with associated information technology staff at Iowa State University regarding the NCRAC Web site;
- Regional Extension meetings;
- Regional presentations;
- Representation on NCRAC TC as Iowa's representative on extension;
- 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; 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* located on the NCRAC web site https://www.ncrac.org/ 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 32 grants from USDA for FY88 (Grant #88-38500-3885), FY89 (Grant #89-38500-4319), FY90 (Grant #90-38500-5008), FY91 (Grant #91-38500-5900), FY92 (Grant #92-38500- 6916), FY93 (Grant #93-38500-8392), FY94 (Grant #94-38500-0048), FY95 (Grant #95-38500-1410), FY96 (Grant #96-38500-2631), FY97 (#97-38500-3957), FY98(#98-38500-5863), FY99 (#99-38500-7376), FY00 (#00-38500- 8984), FY2001 (#2001-38500-10369), FY2002 (#2002-38500-11752), FY2003 (#2003-38500-12995), FY2004 (#2004-38500-14269), FY2005(#2005-38500- 15847), FY2006 (#2006-38500-16900), FY2007 (#2007-38500-18569), FY2008 (#2008-38500-19157), FY2009 (#2008- 38500-19157 extension) FY2010 (#2010-38500-20929), FY2011 (#2010-38500-20929 Amendment), FY2012 (2012-38500-19550), FY2013 (#2012- 38500-19550 Amendment), FY2014 (2014-38500-22138), FY2015 (2014-38500-19550 Amendment), FY2016 (2016-38500-25753), FY2017 (2016-38500-25753 Amendment), aFY18 (2018-38500-28887) and F20 (2020-38500-32560) with monies totaling $24,063,331. Currently, three 2-year grants are active (FY16, 18 and 20); the first 27 grants (FY88-14) have terminated and final reports provided to USDA-NIFA. 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 RFP for Pre-Proposal, Instructions for Submission of the full proposals, and Rapid Response Proposals for short-term projects.
- All pre-proposal outlines are initially reviewed by the Executive Committees of the IAC and TC/R, and TC/E (10 members). Reviews are provided to the NCRAC Board to select which proposals to accept for submission as full proposals. Full Proposals are then peer reviewed by individuals who are well qualified for a particular project because of their expertise and interests. Project outlines are mailed to three-four five reviewers within and outside the twelve state North Central Region. Final selection of projects to be submitted to USDA-NIFA for funding is done by the NCRAC Board with one final review done by the NCRAC community during the annual NCRAC meeting.
- The Out-of-Cycle Proposals are reviewed by the Executive Committees of the IAC and TC/R and TC/E (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 (if any).
- The Center then submits the revised project outlines as a Plan of Work (POW) to USDA for approval; process was changed in 2020 with only projects that do not meet federal Terns and Condition being submitted for approval.
- Once a POW is approved by USDA, the Center then prepares subcontracts for each participating institution. The Center receives all invoices for sub contractual 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, 2021, the Center has funded or is funding 137 projects through the first 28 grants received. Funding for these Center- supported projects is summarized in Table 1 below (pages 9-13). Information about funded projects is also available at the Center's Web site (http://www.ncrac.org). During this reporting period, the Publications Office at ISU produced and distributed a number of publications including fact sheets, technical bulletins, and videos. A complete list of all publications from this office is included in the on-line Appendix under Extension.

Other areas of support by the Administrative Office during this reporting period included: monitoring research and extension activities and developing progress reports; developing liaisons with appropriate institutions, agencies and clientele groups; soliciting, in coordination with the other RACs, written testimony for the U.S. House Appropriations Subcommittee on Agriculture, Rural Development, Food and Drug Administration, and Related Agencies and the U.S. Senate Appropriations Subcommittee on Agriculture, Rural Development, and Related Agencies; participating in the NCA; numerous oral and written presentations to both professional and lay audiences; working with other fisheries and aquaculture programs throughout the North Central Region; and maintaining the NCRAC Web site.

Project Reporting

As indicated in Table 1, NCRAC has funded a number of projects for many of the project areas it has selected for research and extension activities. For example, there have been 31 separately funded projects in regard to Extension/education and 12 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, 2018 to August 31, 2019. Projects, or Project components, that terminated prior to September 1, 2017 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 https://www.ncrac.org/.

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

Project Area	Project Number	Funding Level	Proposed Duration	Grant Number	
Aqua Drugs	1	$27,000	7/1/96-6/30/97	95-38500-1410	
	2	$950	12/1/96-11/30/97	95-38500-1410	
	3	$8,415	10/1/99-9/30/00	97-38500-3957	
	4	$223,677	6/1/04-11/30/05	2003-38500-12995	
	5	$60,000	7/15/04-7/14/05	2003-38500-12995	
	6	$50,000	11/1/04-10/31/06	2002-38500-11752	
	7	$129,936	1/1/06-12/31/06	2005-38500-15847	
	8	$150,000	9/1/08-8/31/10	2008-38500-19157	
	9	$27,880	9/1/09-8/31/10	2008-38500-19157	
	10	$100,000	9/1/11-8/31/31	2010-38500-20929	
	11	$240,000	9/1/12-8/31/14	2012-38500-19550	
Total		**$1,017,858**			7.40%
Aquaponics	1	$24,596	7/1/16-6/30/17	2014-38500-22138	
Total		**$24,596**			0.18%
Baitfish	1	$61,973	9/1/92-8/31/94	92-38500-6916	
	2	$111,997	9/1/06-8/31/08	2006-38500-16900	
	2	$88,003	9/1/06-8/31/08	2005-38500-18547	
Total		**$261,973**			1.90%
Conf./Wrkshp					
Env. Strategies Symp.	1	$5,000	9/1/00-5/31/01	96-38500-2631	
Nat. Aqua. Ext. Conf.	1	$3,005	10/1/91-9/30/92	89-38500-4319	
	2	$3,700	12/1/96-11/30/97	95-38500-1410	
	3	$4,500	11/1/02-10/31/03	00-38500-8984	
	4	$5,000	1/1/06-12/31/06	2005-38500-18547	
	5	$5,000	9/1/10-8/31/11	2008-38500-19157	
NCR Aqua. Conf.	1	$7,000	6/1/90-3/31/91	90-38500-5008	
	2	$3,000	12/9/98-6/30/99	96-38500-2631	
Percis III	1	$4,000	11/1/02-10/31/03	00-38500-8984	
Total		**$40,205**			0.29%
Crayfish	1	$49,677	9/1/92-8/31/94	92-38500-6916	
Total		**$49,677**			0.36%
Economics/Mkt	1	$127,338	5/1/89-12/31/91	88-38500-3885	
	1	$34,350	5/1/89-12/31/91	89-38500-4319	
	2	$53,300	9/1/91-8/31/92	91-38500-5900	
	3	$40,000	9/1/93-8/31/95	93-38500-8392	
	4	$47,916	9/1/99-8/31/01	97-38500-3957	
	5	$50,000	9/1/03-8/31/04	2002-38500-11752	

Project Area	Project Number	Funding Level	Proposed Duration	Grant Number	
	6	$23,565	9/1/10-8/31/11	2010-38500-20929	
	7	$75,276	9/1/12-8/31/14	2012-38500-19550	
	8	$198,608	7/1/2019-6/30/2021	2016-38500-25753	
Total		**$650,353**			4.73%
Base Ext	1	$39,221	5/1/89-4/30/91	88-38500-3885	
	1	$37,089	5/1/89-4/30/91	89-38500-4319	
	2	$31,300	3/17/90-8/31/91	89-38500-4319	
	3	$94,109	9/1/91-8/31/93	91-38500-5900	
	4	$110,129	9/1/93-8/31/95	91-38500-5900	
	5	$10,813	9/1/95-8/31/97	92-38500-6916	
	5	$20,391	9/1/95-8/31/97	95-38500-1410	
	6	$38,000	9/1/97-8/31/99	97-38500-3957	
	7	$94,000	9/1/99-8/31/01	99-38500-7376	
	8	$28,500	9/1/01-8/31/03	99-38500-7376	
	8	$18,154	9/1/01-8/31/03	2001-38500-10369	
	9	$28,000	9/1/03-8/31/05	2002-38500-11752	
	10	$211,545	9/1/05-8/31/07	2003-38500-12995	
	10	$7,735	9/1/05-8/31/07	2005-38500-15847	
	11	$21,850	9/1/07-8/31/09	2006-38500-16900	
	11	$92,469	9/1/07-8/31/09	2007-38500-18469	
	12	$37,966	9/1/08-8/31/10	2007-38500-18469	
	12	$22,539	9/1/08-8/31/10	2008-38500-19157	
	13	$29,000	9/1/09-8/31/11	2008-38500-19157	
	14	$35,700	9/1/11-8/31/13	2010-35800-20929	
	15	$45,000	9/1/13-8/31/15	2012-38500-19550	
	16	$23,175	9-1-15-8-31-16	2012-38500-19550	
	17	$50,000	9/1/16-8/31/18	2014-38500-22138	
Total		**$1,126,685**			8.19%
AREF	18	$100,000	9/1/03-8/31/05	2002-38500-11752	
Total		**$100,000**			0.73%
RAES	19	$199,624	9/1/05-5/31/09	2004-38500-14269	
	20	$150,000	9/1/09-8/31/11	2008-38500-19157	
	21	$196,612	9/1/11-8/31/13	2010-38500-20929	
	22	$101,820	9/1/13-8/31/14	2012-38500-19550	
	23	$103,347	9/1/14-8/31/16	2014-38500-22138	
	24	$124,993	9/1/16-8/31/18	2014-38500-22138	
Total		**$876,396**			6.37%
Other Ext.	25	$34,950	7/1/16-6/30/17	2014-38500-22138	
	26	$34,977	7/1/16-6/30/17	2014-38500-22138	
	27	$70,000	9/1/16-8/31/18	2014-38500-22138	
	28	$188,036	7/1/17-6/30/19	2016-38500-25753	

Project Area	Project Number	Funding Level	Proposed Duration	Grant Number	
	29	$151,739	7/1/17-6/30-19	2016-38500-25753	
	30	$150,000	10/1/2018-9/0/2020	2016-38500-2573	
	31	$132,368	10/1/2018-9/0/2020	2016-38500-2573	
Total		**$762,070**			5.54%
Total Ext.		**$2,865,151**			20.83%
Hybrid Striped Bass	1	$68,296	5/1/89-8/31/91	88-38500-3885	
	1	$68,114	5/1/89-8/31/91	89-38500-4319	
	2	$101,000	6/1/90-8/31/92	90-38500-5008	
	3	$96,550	9/1/91-8/31/93	91-38500-5900	
	4	$168,000	9/1/93-8/31/95	93-38500-8392	
	5	$150,000	9/1/95-8/31/97	95-38500-1410	
	6	$15,000	6/1/99-5/31/00	96-38500-2631	
	7	$98,043	9/1/01-5/31/04	98-38500-5863	
		$211,957	9/1/01-5/31/04	2001-38500-10369	
Total		**$976,960**			7.10%
Largemouth Bass	1	$170,000	9/1/05-5/31/07	2004-38500-14269	
	2	$155,000	9/1/14-8/31/16	2014-38500-22138	
Total		**$325,000**			2.36%
INADs/NADs	1	$55,241	9/1/93-5/14/00	89-38500-4319	
	2	$89,000	7/15/04-5/14/09	2003-38500-12995	
Total		**$144,241**			1.05%
Nutrition/Diets	1	$200,000	9/1/04-8/31/06	2002-38500-11752	
	2	$80,000	9/1/07-8/31/09	2006-38500-16900	
	3	$80,000	9/1/09-8/31/11	2008-38500-19157	
	4	$124,400	9/1/10-8/31/12	2008-38500-19157	
	5	$75,000	9/1/12-8/31/13	2010-28500-20929	
	6	$35,000	3/1/18-2/28/19	2016-38500-25753	
	7	$45,156	7/1/2019-6/30/2021	2016-38500-25753	
	7	$78,629	7/1/2019-6/30/2021	2018-38500-28887	
	8	$89,481	7/1/2019-6/30/2021	2016-38500-25753	
	8	$79,986	7/1/2019-6/30/2021	2018-38500-28887	
Total		**$887,652**			6.45%
Other					
Feed Training	1	$165,446	9/1/06-8/31/08	2005-38500-15847	
	1	$134,554	9/1/06-8/31/08	2006-38500-16900	

Project Area	Project Number	Funding Level	Proposed Duration	Grant Number	
Snail/Grub Mgmt	2	$225,000	9/1/07-8/31/09	2007-38500-18469	
RAS Microbial	3	$65,000	9/1/09-8/31/10	2008-38500-19157	
Winter Kill	4	$175,000	9/1/11-8/31/13	2008-38500-19157	
Field Assess.	5	$34,998	7/1/16-6/30/17	2014-38500-22138	
Total		**$799,998**			5.82%
Salmonids	1	$9,000	6/1/90-8/31/92	89-38500-4319	
	1	$120,799	6/1/90-8/31/92	90-38500-5008	
	2	$149,997	9/1/92-8/31/94	92-38500-6916	
	3	$199,290	9/1/94-8/31/96	94-38500-0048	
	4	$158,656	9/1/97-8/31/99	97-38500-3957	
Total		**$637,742**			4.64%
Sunfish	1	$130,758	6/1/90-8/31/92	90-38500-5008	
	2	$149,799	9/1/92-8/31/94	92-38500-6916	
	3	$173,562	9/1/94-8/31/96	94-38500-0048	
	4	$199,921	9/1/96-9/31/98	96-38500-2631	
	5	$199,748	9/1/99-8/31/01	99-38500-7376	
	6	$160,000	9/1/13-8/31/15	2012-38500-19550	
Total		**$1,013,788**			7.37%
Tilapia	1	$118,791	9/1/96-8/31/98	96-38500-2631	
	2	$150,000	9/1/98-8/31/00	98-38500-5863	
Total		**$268,791**			1.95%
VHS	1	$197,960	9/1/08-8/31/10	2008-38500-19157	
Total		**$197,960**			1.44%
Walleye	1	$177,517	5/1/89-8/31/91	89-38500-4319	
	2	$111,657	6/1/90-8/31/92	90-38500-5008	
	3	$109,223	9/1/91-8/31/92	91-38500-5900	
	4	$75,000	9/1/92-8/31/93	89-38500-4319	
	5	$150,000	9/1/93-8/31/95	93-38500-8392	
	6	$117,395	9/1/95-8/31/97	94-38500-0048	
	6	$59,835	9/1/95-8/31/97	95-38500-1410	
	7	$127,000	9/1/99-6/30/02	98-38500-5863	
	8	$97,775	7/1/2019-6/30/2020	216-38500-25753	
	8	$127,646	7/1/2019-6/30/2021	2018-38500-28887	
Total		**$1,153,048**			8.38%
Wastes/Eff.	1	$153,300	9/1/92-8/31/94	92-38500-6916	
	2	$100,000	9/1/96-8/31/98	96-38500-2631	
	3	$106,186	9/1/01-8/31/04	00-38500-8984	
	3	$88,814	9/1/01-8/31/04	2001-38500-10369	

Project Area	Project Number	Funding Level	Proposed Duration	Grant Number	
Total		**$448,300**			3.26%
White Papers	1	$4,999	7/1/98-12/31/98	96-38500-2631	
	2	$17,495	9/1/99-12/31/99	97-38500-3957	
Total		**$22,494**			0.16%
Yellow Perch	1	$76,957	5/1/89-8/31/91	88-38500-3885	
	1	$85,723	5/1/89-8/31/91	89-38500-4319	
	2	$92,108	6/1/90-8/31/92	90-38500-5008	
	3	$99,997	9/1/91-8/31/93	91-38500-5900	
	4	$150,000	9/1/93-8/31/95	93-38500-8392	
	5	$199,507	9/1/95-8/31/97	95-38500-1410	
	6	$185,458	9/1/97-8/31/99	97-38500-3957	
	7	$92,370	9/1/98-8/31/00	98-38500-5863	
	8	$326,730	9/1/01-5/31/04	00-38500-8984	
	8	$125,016	9/1/01-5/31/04	2001-38500-10369	
	9	$150,000	9/1/10-8/31/13	2010-38500-20929	
	10	$190,000	9/1/13-8/31/15	2012-38500-19550	
	11	$162,261	7/1/17-6/30/19	2014-38500-22138	
	12	$30,838	3/1/18-2/28/19	2016-38500-25753	
Total		**$1,966,965**			14.30%
137 Projects		**$13,752,752**			

Regular Project Reports

Project Title: Extension [Termination Report]
Total Funds Committed: $1,126,885
Initial Project Schedule: May 1, 1989 to August 31, 2019
Current Project Year: September 1, 2019 to August 31, 2020
Participants: D. E. Bauer, University of Nebraska-Lincoln; M. E. Clark, North Dakota State University; J. A. Held, University of Wisconsin-Stevens Point, Wisconsin; C. E. Hicks, Lincoln University; P. Hitchens, Southern IL University – Carbondale, Illinois; R. E. Kinnunen, Michigan State University; C. D. Lee, Kansas State University; J. E. Morris, Iowa State University; A. Pattillo, Iowa State University; A. Garcia, South Dakota State University, South Dakota; N. Phelps, University of Minnesota; K. K. Quagrainie, Purdue University, Illinois/Indiana Sea Grant; M. Smith, The Ohio State University; C. Weeks, Michigan State University.
Industry Liaison: Dan Vogler, Harrietta Hills Trout Farm, Michigan
Reason for Termination: Completion of project objectives.

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.

Technical Summary and Analysis

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 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 series in 2016 and 2017 in partnership with the National Aquaculture Association and U.S. Chapter of the World Aquaculture Society. 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. Videos are available at https://www.ncrac.org/video.

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.

Recent workshops include the 2017 Iowa Aquaculture Conference (videos of presentations located at https://www.ncrac.org/video) and the 2018 North Central Aquaculture Conference in Kansas City, Missouri (hosted by Missouri Aquaculture Association and NCRAC; presentations at https://www.ncrac.org/presentation/2018-north-central-aquaculture-conference).

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 Aquatic Invasive Species-Hazard Analysis Critical Control Point (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 coordinated a 2-day meeting of the NCRAC Publications Review Team in Des Moines, Iowa. This team of Extension, TC/E and TC/R members reviewed current NCRAC publications for content and whether or not they were still relevant to current aquaculture practices. Authors of past publications were contacted for identified updates in 2018. The departure of Pattillo in October 2017 resulted in Morris being appointed to ISU Extension in January 2018; Morris has since directed the new identified publications to be completed. ISU Extension staff have been doing the final edits and layout with the first publications have been distributed to the aquaculture community in 2019 with all publications to be completed in 2020.

Principal Accomplishments

Objective 1. — Participated in all NCRAC projects either as extension liaison or research, assisted the NCRAC community by presenting and networking to enhance the nation's knowledge of aquaculture production in the Midwest, and served as conduit between industry and research community.
Objective 2. — Networking among the extension community to enhance educational programs with minimal duplication in the region and the nation.
Objective 3. —Developed educational programs in the North Central Region include a large number of workshops, conferences, symposia, field-site visits that help to address educational needs of regional aquaculture community.

Impacts

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.

Recommended Follow-up Activities

NCRAC should summarize the Extension programs and their deliverables in manner that illustrates on the importance of regional aquaculture work.to North Central universities.

Publications, Manuscripts, Workshops, and Conferences

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

Project Title: A NCRAC-Sea Grant Partnership for Regional Aquaculture Extension Focused on Marketing and Consumer Demand **[** Progress Report]
Total Funds Committed: $150,000
Initial Project Schedule: November 1. 2018-October 30, 2020 [Extended to April 30, 2021]
Current Project Year: November 1, 2019-August 30, 2020
Participants: J. S. Carlton (Purdue University)
Extension Liaison: R. Kinnunen (Michigan State University), replaced by K. Quagrainie (Purdue University)
Industry Liaison: M. Emerson, Crystal Lake Fisheries, Missouri

Project Objectives

1. Hire a regional aquaculture extension specialist housed at Purdue University and jointly appointed in the North Central Region Sea Grant Programs and serving all 12 states of the North-Central Region.
2. Conduct a regional needs assessment to better understand what consumer- and marketing-oriented aquaculture programming is being done and how to best use extension to address needs and impediments.
3. Work with existing personnel throughout the North Central Region to develop and deliver extension programming to address consumer needs and impediments aimed at all of the states in the North Central Region.
4. Coordinate development of regional aquaculture extension networks by serving as a liaison among the Sea Grant programs, partnering universities, NCRAC stakeholders, and other stakeholders throughout the North Central Region.
5. Use quantitative and qualitative evaluation to assess the effectiveness of the specialist's program and to help plan subsequent years of the program.
6. Partner with stakeholders to develop funding extending beyond the initial two-year period.

Project Summary

Aquaculture is an important source of healthy protein for ever-expanding domestic and global populations. However, the US edible seafood trade deficit was over $14 billion in 2016. Aquaculture production in the North Central Region (NCR) could grow if producers have improved access to knowledge, skills, and technology and consumers demand this healthy, sustainable, locally produced food. This project is a partnership between the North-Central Regional Aquaculture Center (NCRAC) and Sea Grant, co-funded by NCRAC and Sea Grant, housed at Purdue University, and host a regional aquaculture specialist jointly appointed to the five NCR Sea Grant programs: Illinois-Indiana, Michigan, Minnesota, Ohio, and Wisconsin. The initial focus of the program will be aquaculture marketing and consumers. In the first two years of the project, the pro- gram will assess industry extension needs and impediments, deliver responsive, consumer-oriented programming and marketing, serve as a liaison among project partners and regional stakeholders, and seek future funding. The over- all goal of the project is increased consumer awareness of and demand for the locally grown, healthy farmed-fish protein, resulting in a more resilient aquaculture industry.

Anticipated Benefits

Short-term knowledge gains (timeframe: 1–2 years):

- Consumers will increase knowledge of the health, environmental, and eco- nomic benefits of locally produced seafood
- Consumer awareness of locally produced farmed seafood will increase
- Consumers will increase knowledge of how to clean and cook seafood
- Producers will have increased knowledge of consumer preferences and marketing techniques and understanding of relevant food supply chain regulations
- Program staff, NCRAC, USDA, and Sea Grant will increase their understanding of how to effectively partner on synergistic resource issues

Medium-term behavior changes (timeframe: 2–5 years)

- Consumers will increase their consumption of locally produced seafood
- Seafood producers, distributors, and sellers will adapt their practices based on consumer preferences
- The aquaculture industry will receive increased investment from existing and potential producers
- NCRAC, USDA, and Sea Grant will invest in continued partnerships on re- source issues.

Long-term condition changes (timeframe: 5+ years)

- Consumers will be aware of and demand locally produced aquaculture as a healthy, sustainable source of protein.
- The aquaculture industry in the NCR will be more resilient through in- creased sales, a better- understood market position, and increased consumer demand
- Enhanced quality of life for NCR residents thanks to increased production and consumption of locally grown seafood and a vibrant aquaculture industry
- A culture of collaboration and partnership between NCRAC, USDA, and Sea Grant

We will be creating aquaculture content for workshops to train educators and Extension personnel in STEM (Science, Technology, Engineering and Math) related fields and other industry-related concepts such as business operation, marketing, and financial management. Once development is completed, these workshop materials could be used in the NCR and throughout other RACs. With undergraduate enrollment declining at most universities, the addition of more applied courses/programs such as aquaculture could help reverse this trend. Few students are aware of the career opportunity available in aquaculture, so there is a need for identifying a clear career pathway that shows how education coupled with internship programs can lead to career opportunities in aquaculture. Ultimately, the aquaculture industry will benefit from an educated, skilled, young workforce that will help the U.S. aquaculture industry prosper and be ready to "carry the torch" for the industry as a generational change takes place. This can be best accomplished by the co-development of the aquaculture workforce.

Project Progress

Objective 1. Hire a regional aquaculture extension specialist housed at Purdue University and jointly appointed in the North Central Region Sea Grant Programs and serving all 12 states of the North-Central Region. — Ms. Amy Shambach was hired in summer 2019 to serve as our Regional Aquaculture Marketing Associate. She has been working across the states in the North-Central Region to achieve all of the objectives.

Objective 2. Conduct a regional needs assessment to better understand what consumer- and marketing-oriented aquaculture programming is being done and how to best use extension to address needs and impediments. — Our initial work was to assess what marketing- or consumer-facing aquaculture programming was occurring in the region in order to allow us to develop responsive programming. We invited 569 county extension staff in the region to participate in our needs assessment and approximately 29% of them completed the assessment. e delivered a summary of this assessment to NCRAC staff in early 2020 and have been developing programming and resources over the last 8 months based on our findings. We are in the process of supplementing this with a series of qualitative interviews with producers that we will use both in the latter part Phase 1 and the proposed Phase 2 of the project. We have contacted approximately 83 producers and conducted approximately 26 needs assessment interviews from a total of ten NCRAC states (MI, MN, MO, IA, IL, IN, NE, KS, OH, WI). Ten interviews are a substantial number for a needs assessment that gives us a good idea of what type of programming to develop and deliver, but we are trying to schedule an additional ten interviews to ensure that we are capturing the diversity of opinions in the region. In addition, we have worked are working with other researchers to supplement our needs assessments with a consumer-oriented needs assessment and hope to have those results inform our work in late Phase 1 and, if funded, early Phase 2.

Objective 3. Work with existing personnel throughout the North Central Region to develop and deliver extension programming to address consumer needs and impediments aimed at all of the states in the North Central Region. We provided an update on the extension programming to NCRAC staff earlier in the year. In brief, we have developed brochures, factsheets, a cookbook, and more products in addition starting a series of webinars on aquaculture marketing and facilitating stakeholder meetings. We are in the process of finalizing a website at EatMidwestFish.org that will be launched by mid-December. We will continue developing and delivering products and programs between now and the project end in Spring, 2021, with a pandemic-influenced focus on digital delivery.

Objective 4. Coordinate development of regional aquaculture extension networks by serving as a liaison among the Sea Grant programs, partnering universities, NCRAC stakeholders, and other stakeholders throughout the North Central Region. — Several project staff serve on the Sea Grant Great Lakes Aquaculture Collaborative (GLAC) and we have provided cross-project updates to NCRAC and to the GLAC to facilitate networking. In addition, we have ensured that NCRAC logos and information is included in products where applicable, helping NCRAC to share credit for some of the GLAC work that the project team has contributed to.

Objective 5. Use quantitative and qualitative evaluation to assess the effectiveness of the specialist's program and to help plan subsequent years of the program. — We have collected metrics evaluating our outputs, ranging from webinar attendees to number of social media followers. We will collect download numbers and other metrics as they become available.

Objective 6. Partner with stakeholders to develop funding extending beyond the initial two-year period. — We continue to seek long-term funding for this work.

Outreach Overview

All of our work is being distributed through our professional and personal networks, social media, the Eat Midwest Fish website, and more.

Target Audiences

Our outreach work is designed to reach two primary audiences: NCRAC-region producers and NCRAC-region consumers. The work to reach producers includes the marketing webinar series, our liaison work with the Great Lakes Aquaculture Collaborative, and some of the work on the Eat Midwest Fish website. The outreach related to finding and preparing farmed fish is directed at consumers.

Deliverables (Outputs)

Publications are listed in the NCRAC Appendix.

Outcomes/Impacts

The project is just ramping up, so we do not have any measurable impacts on stakeholders, but we have already significantly increased our knowledge of the potential aquaculture market to date by developing a database that consists names and contact information for many of the known food-fish producers and potential aquaculture distributors (e.g., seafood distributors, major restaurant groups, grocers, etc.) throughout the region.

Impacts Summary

Relevance. — The global demand for seafood is increasing. Catch fisheries are unable to provide all the seafood the market is demanding, leaving fish farmers to make up the difference. One might expect that as demand increases, the aquaculture industry in the U.S. would grow to meet it, but that is not the case. Since 2005, the overall trend is fewer aquaculture farmers in the U.S. We import over 90% of the seafood we eat

*Respons*e. — Illinois-Indiana Sea Grant (IISG) is has partnered with the North-Central Regional Aquaculture Center and other Great Lakes Sea Grant programs to begin a regional aquaculture extension program, providing science-based information to producers and consumers in the area. IISG has begun developing and delivering extension programming with both a consumer and producer focus and has worked with producers in the region.

Results. — By reaching out to 83 farmers across the NCRAC region for the programming needs assessment, we increased interactions between stakeholders and extension and improved visibility for NCRAC. More results will be forthcoming by the end of the project.

Recap. — Illinois-Indiana Sea Grant has formed a partnership with the North Central Regional Aquaculture Center and other Sea Grant programs and has begun providing critical science-based information to producers and consumers in the region.

Publications, Manuscripts, Workshops, and Conferences

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

Project Title: Supporting and Expanding Aquaculture in the Midwest Through Extension and Outreach **[**Progress report]

Total Funds Committed: $131,432

Initial Project Schedule: November 1, 2018-October 30, 2020

Current Project Year: November 1, 2018-August 30, 2019

Participants: M. Smith (Ohio State University)

Extension Liaison: A. Primus (University of Minnesota)

Industry Liaison: J. Blackburn, Fresh Harvest Farms, Ohio

Project Objectives

1. Build upon previously successful Extension and outreach programs to enhance the established North Central Region (NCR) industry by assisting farmers, educating educators, and assessing and prioritizing the needs of the NCR industry in ways that will not be probable at this time without NCRAC support.
2. Act in a liaison capacity on a variety of collegiate, state, regional, and national committees to ensure the NCR is well-represented when issues or opportunities that can or will affect the NCR aquaculture/aquaponic industry arises.
3. Develop and strengthen partnerships from within the NCR and outside the region among regulatory agencies, industry, academia, and other relevant entities to foster open, meaningful dialog on critical issues and build support for the NCR aquaculture/aquaponic industry.
4. Work closely with the liaisons of every NCRAC funded project to assist in developing and achieving strong deliverables to the industry.
5. Coordinate efforts for seeking non-NCRAC support for NCR aquaculture development; including consumer perception of aquaculture/aquaponics and technology transfer.

Project Summary

There are limited FTEs in Extension with aquaculture expertise in the Midwest. This project proposed and is accomplishing developing Extension programs that are definable and achievable to support the industry. This includes farm visits to learn of farmers problems and proposing potentials solutions or connecting the research(s) to the farmer, workshop development, liaison with other agencies/regions/support systems, and seek outside funding to enhance Midwest aquaculture.

Anticipated Benefits

One of the key components to this work is the development of a survey to assess how the industry feels about NCRAC and the work that is currently being developed. We believe that the research will help set the tone for an all-day discussion in early 2020 which will revolve around the researchers, extension, and the industry getting to learn more about each other so that the researchers can feel confident that the work they are seeking funding for is directly applicable to the researchers. Additionally, we are attempting to achieve some sort of consensus or agreement from the farmers about how to proceed with grant writing since there are more than 35 species cultured in the Midwest in at least six different production systems. Additional benefits to the industry include stated deliverables in the funded project.

Project Progress

Objective 1: — In this progress year, pre-COVID-19, the following tasks were completed:

- Visited farmers in four Midwest states (OH, WI, MO, IA).
- Created surveys for Midwest producers which built upon previous surveys conducted by former NCRAC RAES Dr. Weeks.
- Built upon previously successful round table discussions by developing a round table discussion at NCAC 2020 in Columbus, OH. Round table participants: 41 producers, 21 researchers, 12 Extension, and 2suppliers for a total of 76 participants.
- Started a regional "Fish Fact Sheet Friday" email to encourage producers to notice important educational materials that are there to support them and their business. ~Assisted NCRAC and the Ohio Aquaculture Association with the 2020 North Central Aquaculture Conference and NCRAC annual meeting.
- Due to the pandemic, it was also necessary to block off virtual office hours to encourage producers to join me and ask questions. Producers in the Midwest are also always informed they can contact me for support anytime in email. In 2020, most weeks emails were sent to the NCRAC listserv. ~
- When the pandemic first shut down the country, a comprehensive email was sent to the NCRAC listserv (send to Dr. Morris and shared) regarding numerous resources available to producers and suggestions for how to handle potential issues. Periodic emails were sent to NCRAC as more resources became available.

Objective 2 & 3: Tasks include:

- Participated in the first in-person meeting of the Great Lakes Aquaculture Collaborative in IL and participated in the Recirculating Aquaculture Salmon Network (RAS-N) at UWSP NADF.
- Applied for and became Student Director (Board member position) of the World Aquaculture Society.
- Applied for and became a Director (Board member position) with the United States Aquaculture Society. ~
- Wrote letters and participated in virtual meetings with the Ohio Division of Wildlife regarding their proposed tadpole and frog regulations. Regulations passed.
- During conversations with IAC during the 2020 NCRAC meeting in Columbus, OH, it became clear that the industry needed to be surveyed to understand if and how their business and markets were being affected by COVID-19. In collaboration with Virginia Tech and Engle-Stone Aquatic$, LLC, a quarterly survey was quickly developed and released with the support of the National Aquaculture Association. One journal editorial (JWAS) has been published and one article has been accepted for publishing in a COVID-19 special issue in AppliedEconomic Perspectives and Policy. Seventeen fact sheets have been created, with at least 12 more to be published in the upcoming months. Numerous presentations have been made on a local level (IN-IL Sea Grant webinar), regional level (Great Lakes Aquaculture Day), and national level (NAA-USAS webinar). ~Participated in the Great Lakes Aquaculture Day. Authored and presented on one topic and contributed to another presentation.
- Submitted, and had accepted, an abstract on the effects of the pandemic on our industry to enhance DNR's understanding of aquaculture. The conference is the2021 virtual Midwest Fish & Wildlife Conference, hosted by MN DNR. This is largely a DNR and academic conference that includes an aquaculture symposium.

Objective 4: Extension liaison on both out-of-cycle proposals submitted to NCRAC in 2020. One has been funded and one is awaiting decision. No funds were requested to conduct Extension work on one of the projects. ~Extension liaison and PI on a special RFP created by NCRAC. The RFP requested a team to investigate the effectiveness of NCRAC funded projects since its inception. This project is currently ongoing. No funds were requested to conduct Extension work on this project. ~Extension liaison, PI, or both on four of the fifteen full proposals submitted to NCRAC for the2020 RFP. Assisted at least three other teams who submitted full proposals with making connections to producers who would be interested in collaborating on their project.

Objective 5: ~Wrote language which led to the creation of an Extension Educator with Ohio Sea Grant. This person's job is to partially assist with COVID-19 survey data, as well as aquaculture/aquaponic work in Ohio. ~Participating in a Purdue led proposal which, if funded would increase and extend Extension FTE in the NCR with non-NCRAC funding.

Outreach Overview

Personal farm visits with hands-on water quality workshop in north Central Indiana (conveniently located for several surrounding states), phone calls/emails with producers and the groundwork being laid for future communication.

Target Audiences

The target audience of the extension work will be aquaculture producers.

Deliverables (Outputs)

Hands-on water quality workshops, social media posts as applicable, assist the OAA and NCRAC Director with development of the 2020 North Central Aquaculture Conference, and sought aquaculture funding for Midwest support

Outcomes/Impacts

Producers in the Midwest and the rest of the United States utilized the results of our COVID-19 survey data to obtain local, state, or federal financial support for their business. ~Producers, researchers, and Extension in the Midwest found the roundtable discussions useful and meaningful. NCRAC Board members state the proposals that were received in 2020 were relevant and applied. High quality proposals are likely to lead to projects that have meaningful impact on the industry. ~Open office hours have allowed producer, interested producers, and researchers the opportunity to discuss ideas or questions they have.

Impacts Summary

Relevance. — Although there continues to be interest in all facets of aquaculture in the NCR, there are limited FTEs in Extension with aquaculture expertise.

Response. — Surveys were created, farms were visited, facts sheets and webinars were created, available Extension information was relayed to the industry, office hours were created for any producer to participate, and grant money was sought and received to increase Extension FTEs in the region.

Results. — Farmers were able to obtain funding to support their business during the pandemic due to the available fact sheets and the JWAS editorial. Fact sheets were referenced by U.S. Senators and Members of Congress in letters to the Secretary of Agriculture as they sought inclusion of additional aquaculture products in relief funding. ~Producers, Extension, and researchers worked cohesively during the roundtable discussions. Knowledge was gained and information was exchanged. ~Producers, interested producers, and researchers participated in open office hours.

Recap. — While the pandemic drastically changed what this year's progress was supposed to look like, this team adjusted and created meaningful information for the industry.

Project Title: Genetically Improved All-Female Walleye for Intensive Aquaculture Production in the Great Lakes Region [Progress Report]
Total Funds Committed: $225,421
Initial Project Schedule: July 1, 2019-June 30, 2021 [Extended to April 30, 2021]
Current Project Year: November 1, 2019-August 30, 2020
Participants: K. Dabrowski (Ohio State University), Osvaldo Jhonatan Sepulveda-Villet (University of Wisconsin-Milwaukee)
Extension Liaison: Alex Primus (University of Minnesota)
Industry Liaison: Adam Hater, Jones Fish, Cincinnati, Ohio

Project Objectives

The overall goal of the proposed project is to use genetic methods to produce triploid walleye *Sander vitreus* that will result in superior growth of the female genotype (30% growth advantage over males) (Malison et al. 1990) and avoidance of nutrients expenditure associated with sexual maturation by polyploidy (sterilization). Specific objectives are to: 1. Produce meiotic gynogenetic (XX) walleye and to compare two methods (immersion and feeding) to sex reverse gynogenetic fish into neomales (sperm producing XX fish) using 17α-methyltestosterone(MT). 2. Optimize the use of pressure shocks to produce triploid walleyes. 3. Compare growth, survival, and gonad development of the following four experimental progeny groups: (a) diploid walleyes (sex genotypes: XX & XY), (b) triploid walleyes (XXX & XXY), (c) all-female diploid walleyes (XX), and (d) all-female triploid walleyes (XXX). These experiments will be conducted in raceway tanks (OSU, UW-Madison, and Reef Systems Coral FarmInc, New Albany, OH) and micro-ponds (Northey Farms LLC, Deerfield, WI). 4. Refine walleye sperm cryopreservation methods and develop a pilot cryobank for walleye neomale sperm to allow for immediate availability to research laboratories and fish farms in the North Centralregion 5. Record short videos over the span of 2 years of research and extension (work on the farms) that will include all the phases of life cycle of walleye and the methods conducted in the laboratory, including production of gynogens and triploids (pressure shock), sperm cryopreservation and use in practical field conditions, and results of the project.

Project Summary

This project aims to optimize and combine feminization and triploidy to produce walleye *Sander vitreus* with superior production traits. We are working toproduce gynogenetic masculinized walleye (XX-genotype) and cross these individuals with walleye females. Resulting progenies will be pressure shocked to produce triploid (sterile) all-female offspring. Our proposed methods eliminate possibility of escaped domesticated fish interbreeding with wild stocks, thus addressing major public concerns about impact of aquaculture on conservation of aquatic resources. We will compare growth, survival, and gonad development of: triploids of female walleye x male walleye cross, control diploids of female walleye x male walleye cross, all-female triploids offemale walleye x walleye neomale cross, and control diploids of female walleye x walleye neomale cross in tanks. These techniques are likely to accelerate growth, enhance production efficiency, and improve flesh quality. The technologies developed will be immediately delivered to industry. Neomale sperm will be cryopreserved and stored in a pilot cryo-bank and will be made available to research laboratories and fish farms.

Anticipated Benefits

The technologies and resources gained from this research will directly benefit the aquaculture industry by increasing production efficiency and providing means for production of improved triploid all-female stocks for grow-out. The economic analysis included in this proposal will substantiate our predictionson the improvements gained by production of triploid all-female walleye stocks. This project will expand the production of walleye in the North Central Region by increasing the profitability of walleye aquaculture through these improved strains. This technology has proven successful in other commercial species, such as the production of all-female triploid rainbow trout, produced and sold by Troutlodge, Washington, U.S. By providing year-round availability of walleye neomale sperm, there will be a reduction in the size of broodstock operations needed by reducing the number of breeding males required to conduct out-of-season spawning in commercial facilities. There is also an opportunity for the future development of a commodity market forhigh-quality, validated walleye gametes for commercial hatchery use (again, the triploid rainbow trout currently produced by Troutlodge is a convincing example). There is also a major economic incentive for the production and sale of triploid all-female walleye eggs to states, agencies, and programs that don't have their own broodstocks, similar to what is currently done with rainbow trout. Troutlodge Inc, the largest trout egg producer in the word, charges$34/1000 triploid all-female rainbow trout eggs, compared to $15/1000 diploid mixed-sex eggs.

Project Progress

Produce meiotic gynogenetic (XX) walleye and compare two methods (immersion and feeding) to sex reverse gynogenetic fish into neomales (spermproducing XX fish) using 17α-methyltestosterone (MT). - Gynogenetic progenies were not produced in 2020 as a result of the COVID state-wideshutdowns in Ohio and Wisconsin. The shutdown limited OSU research capabilities and eliminated sampling efforts of Ohio Department of NaturalResources, from which we receive walleye gametes. Thus, wild sourced gametes were unavailable. In addition, age-2 walleye females in OSU lab hadnot reached sexual maturation by the 2020 spawning season. UWM attempted to secure genetically defined strains of walleye from colleagues at UW-Stevens Point, but the enforced shutdowns due to COVID-19 emergency impeded this activity. However, OSU did produce meiotic gynogenetic walleyein 2018 and these fish were subjected to sex reversal (half of those progenies) using MT via feeding method. In an effort to continue work throughout thepandemic, we carried out grow-out of 2018 gynogenetic fish, and monitored growth and survival in comparison to non-sex reversed control gynogens.Further, to prepare for the Spring 2021 spawning season completion of Obj 1, we conducted gynogenesis trials with Yellow perch eggs using bothWalleye (our 2-year old stock) and Yellow perch sperm. We evaluated UV-irradiation of sperm and tested physical shock methods that we will use toproduce walleye gynogens in 2021. We determined walleye sperm is successfully irradiated when diluted 1:10 in Lahnsteiner extender and exposed to9,000 J/m2 UV. Yellow perch sperm was successfully irradiated when diluted 1:10 in Lahnsteiner extender and exposed to 9,500 J/m2 UV. We determined a heat shock of 28-29°C, applied at 5 minutes post fertilization (mpf), for a 10-minute duration was successful in doubling the chromosome ofhaploid Yellow perch meiotic gynogens produced via UV-irradiated Walleye or Yellow perch sperm, and could be used to produce walleye gynogens. 2.Optimize the use of pressure shocks to produce triploid walleyes.- Triploid walleye were produced in April 2019. We evaluated effectiveness of 3 differentpressure shocks applied 4mpf: 7,000psi, 40 minute duration; 8,000psi, 30 minute; and 9,000psi,12 minute. Triploidy induction rate and embryonicsurvival were highest in the 9,000psi group (95% and 17.5%). Control, diploid walleye were 100% diploid, with eyed-stage embryonic survival of 11.4%.The 9,000psi shocked group and control, diploid fish were reared in aquaria for first feeding with live Artemia nauplii.Survival of control, diploid groups(44.9±8.8%) was higher than that of the triploid groups (30.0±2.7%) at the end of feeding in aquaria (35 days post fertilization, dpf). However, there wasno significant difference in mean weight between control, diploid (53.4±12.7mg) and triploid walleye (50.0±11.4mg). Fish were transferred to 60L tanks ina flow-through system for grow-out and transitioned solely to dry diet (Otohime B2) after 40 days of exogenous feeding (58dpf) on Artemia. No significantdifferences in weight between control, diploid and triploid groups were observed during this phase of rearing. At 250dpf (December 2020), fish from eachexperimental group were PIT tagged and then combined and transferred to 400L tanks for common garden grow-out. Table 1 reports the growth ofexperimental groups over the course of grow-out. We have not observed a growth advantage of triploid walleye compared to diploids from hatchingthrough15 months of age. However, it is likely that a growth advantage will not be seen until control, diploid fish begin to sexually mature and grow thegonads, while it is likely that triploid individuals will not mature sexually, instead, continuing to invest energy in body growth. 3. Compare growth, survival,and gonad development of: (a) diploid walleyes (sex genotypes: XX & XY), (b) triploid walleyes (XXX & XXY), (c) all-female diploid walleyes (XX), and (d)all-female triploid walleyes (XXX). These experiments will be conducted in raceway tanks (OSU, UW-Madison, and Reef Systems Coral Farm Inc, NewAlbany, OH) and micro-ponds (Northey Farms LLC, Deerfield, WI). - There has been no progress toward this objective due to state-wide shutdowns andtravel restrictions resulting from the pandemic. We aim to complete this objective in 2021. UWM will use an internally developed genome for walleye toseek gene candidates for sex-determination to assist in evaluating these objectives following the modified timeline. 4. Refine walleye spermcryopreservation methods and develop a pilot cryobank for walleye neomale sperm. - UWM attempted to secure genetically defined strains of walleyefrom colleagues at UW- Stevens Point to test cryopreservation methods, but the enforced COVID shutdowns impeded this activity. We continued torefine cryopreservation of percid semen using a controlled rate freezer, and yellow perch semen as a proxy to walleye.

Outreach Overview

Results of triploidy induction (objective 2), gynogenesis (objective 1), and hormonal sex reversal (objective 1) experiments conducted at OSU wer epresented at the 2020 Aquaculture America conference in Honolulu, Hawaii in February 2020. We anticipate publication of these “Year 1” results in peer-reviewed scientific journals in the year 2021. Results of the gynogenesis experiments were also published in the World Aquaculture Magazine within an article entitled: Sterility in Aquaculture – Advances, Performance, Impacts.

Target Audiences
Fish farmers in the North Central Region, fish farmers across the U.S., aquaculture industry professionals, fisheries managers, scientists and researchers, graduate and undergraduate students.

Deliverables (Outputs)
The research conducted during 2019 and 2020 directly contributed to the education of undergraduate students enrolled in the OSU Aquaculture course during both spring semesters, as students were trained in fish reproduction, embryology, and larviculture through hands-on learning. In addition, this project provided 4 undergraduate interns an opportunity to gain experience in hatchery methods, fish husbandry, and research throughout 2019 and2020. The first year of this project also directly contributed to the training of 3 graduate students, one of which completed her doctorate degree December 2020. We have also determined an optimal pressure shock conditions for walleye meiotic gynogenesis. UWM's share of this effort resulted in two graduating MS thesis students (Haley Lucas, and Sonya Ponzi). Additionally, resources developed through this and a previous NCRAC-funded project allowed the enrichment of four courses offered at our school (Principles of Aquaculture systems, Sustainable Finfish Aquaculture and Nutrition Principles, Fish Health, and Wisconsin Aquaponics: Hemp and Hops). Two MS theses were produced, and two journal manuscripts are in development for publication.

Outcomes/Impacts
Short term outcomes: • Increased knowledge of optimized methods to obtain triploid walleye through pressure shocks • Increased knowledge of performance (growth, survival) of mixed sex triploid walleyes in comparison to mixed sex diploid walleyes in indoor culture • Increased knowledge of methods to obtain gynogenetic walleye through use of irradiated yellow perch or walleye sperm • Increased knowledge and optimization of the production and performance from early life stage through adulthood of sex reversed gynogenetic walleye Medium term outcomes: • Delivery of technology developed thus far to the scientific community and industry professionals through oral presentations at conferences and a magazine article •Undergraduate students gaining knowledge and understanding of this technology through participation in OSU and UWM courses and internships

Impacts Summary
Relevance. — There is a high potential for walleye to become a major contributing species to private aquaculture in the North Central Region and beyond. However, thegap in knowledge on their production potential and value have delayed the development of this species for aquaculture. Therefore, advances in research that provide solutions to the challenges associated with intensive culture, high density, formulated feeds, of walleye could result in a more profitable aquaculture industry.
*Respons*e. — The proposed project will specifically address the questions of sex ratio and superior growth of triploid sterile all-female walleye. During the first year ofthe project, we completed objective 2, optimization of pressure shocks to produce triploid walleye, and began work on objective 1, production of meioticgyno genetic walleye and subsequent hormonal sex reversal. For the first time, we are collecting data on the growth and survival of 100% female,gynogenetic, sex reversed and triploid walleye stocks and comparing them to traditional diploid mixed sex stocks, in order to quantify the value of culturing female monosex triploid walleye.
Results. — The proposed project directly addresses a major constraint to the aquaculture industry in the North Central Region and has begun providing critical knowledge, essential to the development of this new alternative fish species for U.S. aquaculture, walleye, to the professional and scientific communities. We anticipate that as this project progresses, we will gather additional knowledge, which will lead to changes in industry priorities as walleye aquaculture expands in the NCR and beyond. We have also provided graduate and undergraduate students with valuable, hands-on training in these technologies, which will aid in the project's long-term goals as these individuals enter the workforce.
Recap. — We have developed technology to produce walleye triploids through pressure shock, as well as all-female walleye gynogens and hormonally sex-reversed gynogens. These technologies are being further developed and refined, and will be disseminated to industry after completion of the project.

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

Table 1: Weight data of triploid and control, diploid walleye from 28 through 470 days post fertilization at OSU.

Treatment Group	Days post fertilization (dpf) 28	35	125	250	470
Control, diploid	21.5±5.0mg	53.4±12.7mg	5.1 ±1.2g	23.8±7.2g	51.7±9.4g
Triploid	16.3±4.4mg	50.0±11.4mg	4.7 ±1.2g	19.6±10.2g	48.6±14.1g

Project Title: Choice of Seafood: An Analysis of the North Central Region Market for Farm-Raised Seafood [Progress report]

Total Funds Committed: $198,608

Initial Project Schedule: July 1, 2019-June 30, 2021

Current Project Year: July 1, 2019-August 30, 2020

Participants: S. Valle de Souza (Michigan State University), B. Knudson (Michigan State University), K. Quagrainie (Purdue University)

Extension Liaison(s): R, Kinnunen (Michigan State University) and P. Hitchens (Southern Illinois University-Carbondale)

Industry Liaison(s): D. Vogler and E. Birchmeier

Project Objectives

1. To design survey questions to identify, through seafood marketing channels' choice:
    a. consumer's preferred species,
    b. consumer's perception and willingness to pay for alternative forms of seafood: fresh, refrigerated, frozen, processed (fillet, smoked and canned),
    c. consumer's quality expectations,
    d. factors influencing consumer purchase of aquaculture products,
    e. other possible benefits and attributes NCR aquaculture products can offer to consumers, and
    f. niche market location and potential for specific/unique aquaculture species.
2. To compare consumer perception and preference for locally originated versus out-of-the-NCR-region and out-of-country;
3. To identify consumer willingness to pay a premium price for a local/regional brand;
4. To identify preferred forms of seafood: fresh, refrigerated, frozen, processed (fillet, smoked and canned);
5. To identify consumer quality expectation;
6. To identify factors influencing consumer purchase of farm-raised seafood versus wild catch;
7. To identify other possible benefits and attributes NCR aquaculture products can offer to consumers;
8. To identify niche market location and potential for specific/unique aquaculture species;
9. To disseminate research results in a multi-regional format using tangible technique-centered bulletins for conversion of farm structure or production methods, if our research identifies production systems, species or best practice certification labels required by market players.

Deliverables:

1. Reporting of consumers' preferences identified by statistical analysis of survey data, using restaurants, retail, processors/packing sheds and wholesalers purchase choices.
2. Reporting of restaurants, retail, processors/packing sheds and wholesale market preferences and willingness to pay defined by species and alternative forms of seafood: fresh, refrigerated, frozen, processed (fillet, smoked and canned).
3. Identification of other factors influencing restaurants, retail, processors/packing sheds and wholesale market purchase of aquaculture products, which may suggest best marketing strategies and other benefits or attributes NCR aquaculture products can offer to consumers.
4. Reporting of niche market location and respective demographics, including current players in the supply chain, for specific aquaculture species, and suggesting marketing strategies and value-adding product modifications.
5. Results will be disseminated through extension and outreach programs including a range of educational fact sheets and data bulletins to be published in the NCRAC Fact Sheet Series. Researchers will also be available for presentations in interested states' aquaculture associations, and will publish refereed journal articles.

Project Summary

This project proposed a survey within the North Central Region (NCR) to identify marketing channels' choices of seafood according to their constraints and demand perception. Assuming that marketing channels define the pool of options from which consumers choose seafood species and product form, this survey were to be designed to identify their preferences and willingness to pay for farm-raised seafood, by species, quantity required, quality perception and preferred form of product.

Anticipated Benefits

Research results will provide vital information for the NCR aquaculture industry defining species with high market potential and species-specific niche markets, and identifying new benefits and attributes sought by these marketing channels and their final consumers, which NCR farms can offer. This research addresses a significant information gap in the NCR. To our knowledge, the last seafood marketing channel-specific survey was published in 1999. Since then, NCR demographics changed significantly directly affecting current demand for seafood. A comparison between the 2000 and 2010 census datasets shows acute changes in population cultural background, significant increase in income and changes in household characteristics, all drivers of consumer choices. Further information will be drawn from this project pertaining retail market size and distribution.

Project Progress

Objective 1. — Literature review and secondary data collection have taken place that address a-f questions. Secondary data of research developments were collected through the work of the entire team from peer reviewed journal articles, as well as from government-provided statistical data (i.e. USDA Aquaculture Census, USDA Agriculture Census) and updates from other research developments presented in industry webinars (i.e. Virginia Tech News, Sea Grant Seminars and workshops). Further secondary data on markets were obtained by extension liaison personnel daily monitoring seafood market updates (i.e. Seafood Source, Mintel), and through conversations with wholesale buyers on fish sales to the live markets, conversations with producers concerning COVID-19 impact on sales, and benefits from the Coronavirus Stimulus Program to aquaculture farmers. Three survey instruments were designed to address abovementioned questions (a-e) to (1-v). Distribution of the first survey, targeting retailers started on December 19th, 2019. Data collected from this first survey was however insufficient to be consider a good representation of the NCR market for seafood, due to unforeseen shortcomings of the distribution lists from MarketMaker database, initially proposed for this project. Another two survey instruments were then designed to complement information, directly targeting restaurants and consumers. Distribution was also expanded to country-wide to provide the extended benefit of a benchmarking comparison of results. These surveys were released by the end of this reporting period, on August 31st, 2020.

Objectives 2-8.— Primary data collected from these surveys are now being parsed and analyzed for reporting in year 2 of the project addressing these objectives.

Objective 9.— Objective was initially addressed through oral presentations at three NCR conferences, however disrupted by COVID-19 travel and lockdown restrictions. Dissemination of research results will retake its course after COVID-19 restrictions are lifted and will include results from survey data analysis.

Outreach Overview

Outreach activities related to Objective 9 will take place in year 2 of the project. Results will be disseminated through extension and outreach programs, including educational fact sheets and data bulletins to be published in the NCRAC Fact Sheet Series, and through publication of extension and journal articles.

Target Audiences

The North Central Region Aquaculture Industry, as well as restaurants and retailers purchasing seafood from this industry.

Deliverables (Outputs)

Workshops will be delivered in year 2 of the project, upon conclusion of data analysis.

Outcomes/Impacts

Initial literature review and secondary data analysis was presented at the Michigan Aquaculture Association Annual Meeting, the Ohio Aquaculture Association Conference and at the NCRAC Annual Meeting, informing the NCR aquaculture community and industry about consumer preferences changes following underlying changes in demographics, and about structural changes in the USA aquaculture industry.

Impacts Summary

Relevance. — Impact summary will be presented in year 2 of the project, upon conclusion of data analysis and result dissemination.

Response. — Impact summary will be presented in year 2 of the project, upon conclusion of data analysis and result dissemination.

Results. — Impact summary will be presented in year 2 of the project, upon conclusion of data analysis and result dissemination.

Recap. — Impact summary will be presented in year 2 of the project, upon conclusion of data analysis and result dissemination.

Project Title: Nutritional Programming of Yellow Perch Larvae Using Live Food as a Vehicle [Progress Report]
Total Funds Committed: $123,785
Initial Project Schedule: July 1, 2019-June 30, 2021
Current Project Year: November 1, 2019-August 30, 20
Participants: K. Kwasek and B. Rader (Southern Illinois University-Carbondale), V. McCracken (Southern Illinois University-Edwardsville (Ohio State University
Extension Liaison: M. Smith (Ohio State University)
Industry Liaison: J. Bowzer, ADM Animal Nutrition, Decatur, Illinois

Project Objectives

1. To determine if Nutritional Programming of yellow perch larvae via live food improves dietary plant protein utilization in yellow perch during later life stages.
2. To determine the mechanism underlying the Nutritional Programming responsible for improved dietary plant protein utilization:
    a. To assess if Nutritional Programming changes gut microbial communities responsible for improved digestion of dietary plant protein.
    b. To determine if Nutritional Programming mitigates any inflammatory or morphological changes in the gut responsible for improved digestion of dietary plant protein.
3. To communicate the Nutritional Programming concept via live food, Nutritional Programming feeding strategy protocol, and live food enrichment formulation that could be used by fish farmers and feed manufacturing industry, to improve plant protein-based diets utilization during yellow perch grow-out phase.

Project Summary

Replacement of fishmeal (FM) in aquaculture diets with plant protein (PP) has been an ongoing challenge. High-quality PP concentrates are widely used since their digestibility can be comparable to FM. However, their price can exceed the cost of marine raw materials. Progress with utilization of lower-quality PP has been made but a number of concerns must be overcome to maintain acceptable growth rates and feed efficiency values at high FM substitution levels. Nutritional Programming (NP) is a promising approach to offset the negative effects of dietary PP by modifying specific physiological responses during early development leading to fish with long-lasting ability to assimilate a previously undesirable PP. We propose an unconventional NP strategy with dietary PP for yellow perch (YP) *Perca flavescens* using live food as a vehicle. We believe this innovative feeding approach will become a practical way for enhancing utilization of diets based on high levels of cost-effective plant raw materials. Consequently, this study will contribute to expansion of YP production and development of competitive aquafeed market within the North Central Region (NCR) by providing feed manufacturers and farmers with possibility of using bigger raw material basket allowing for more flexibility in formulations of diets deprived of FM.

Anticipated Benefits

We believe that NP induced at first feed is a much more effective way of improving YP acceptance and utilization capacity of dietary PP compared to the "traditional" NP method, which is normally induced with dry feed during later fish stages. The combination of live food and PP will provide all the nutrients required for proper growth and development and at the same time expose the fish to alternative raw materials and/or anti-nutritional factors delivered in low enough concentrations to induce long-lasting adaptation of the fish towards the same dietary components later in their life without impairing the larval well-being. If proven, this feeding strategy will become a feasible and practical way for enhancing YP utilization of diets based on almost any raw material. The outcome of this study will provide the fish farmers and feed industry within the NCR with the possibility of using bigger and more cost-effective raw material basket and hence, allow for more flexibility in formulations of diets deprived of FM. This will consequently lead to the development of competitive aquaculture feed market that will contribute to the intensification of more sustainable production of YP and other important fish species in the NCR.

Project Progress

Objective 1.— Due to covid-19 and travel bans imposed by SIU-C yellow perch eggs could not be obtained from out-of-state as initially planned or from Illinois state hatcheries which were closed in Spring 2020. The result if these Covid-19 closers has been that the YP feeding trial has been postponed to Spring 2021).

Objective 2. —Preliminary data were gathered from an alternative NP trial on largemouth bass as a model species. The objectives of this model study were to 1) determine the optimal window of time post-hatch that will result in the best effects of NP (reflected by the highest growth performance on a plant-based diet during pre-adult stage); and 2) determine the effect of timing of NP on molecular and histological responses of the intestinal tract.

Largemouth bass larvae were distributed into tanks with three replicates per treatment at an initial stocking density of 200 larvae/L. Three groups were programmed at separate times with one positive control group for four groups in total (Figure 1. 1) The first group (NPL) was nutritionally programmed with live *Artemia* nauplii enriched with soybean meal at first feed (6 dph) for ten days. After 10 days all fish were switched to fishmeal-based diet. 2) The second group was programmed starting at 16 dph with a dry plant-based diet for ten days (NPD1) following the *Artemia* nauplii feeding. After 10 days all fish were switched to fishmeal-based diet. 3) The third group (NPD2) was programmed at 26 dph with a dry plant-based diet for ten days following the *Artemia* nauplii feeding. After 10 days all fish were switched to fishmeal-based diet. 4) The fourth group was positive control group (PC), which received a fishmeal-based diet for the entire duration of the trial following the *Artemia* nauplii feeding.

After the programming all groups were raised using the fishmeal diet feeding regimen until 100 dph when the transition to plant-based diets started. During the *Artemia* and fishmeal diet feeding all groups were provided with their food up to apparent satiation. Just prior to plant-based diet transition, densities in the tanks were adjusted to 30 fish per tank to ensure all groups were able to reach their full growth potential during the plant-based diet feeding. This was the start of the PP challenge phase. During the PP challenge all groups were fed at restricted feeding rate (% biomass). Fish were weighed every two weeks and feed volume was adjusted accordingly. The challenge phase was finished when 200% growth had been reached in all the tanks.

At the end of the trial (172 dph) the following parameters were determined: final average growth, weight gain, and survival rate. A total of 3 fish from each tank were taken for whole body proximate analysis. In addition, the digestive tracts of three fish per tank were dissected and preserved in liquid nitrogen to analyze gene expression. Finally, additional three digested tracts from each tank were sampled for histological analysis.

Results

The survival during the PP challenge phase was the lowest in the NPD1 group with an average survival of 84 ± 0.05 %. NPL, PC, and NPD2 groups showed survival of 95 ± 0.01 %, 93 ± 0.02 %, and 96 ± 0.04% respectively (± standard deviation).

At the start of the PP challenge the NPD1 group was the smallest, with each fish weighing 11.69 ± 0.95g on average. The second smallest was the NPD2 group, weighing 15.69 ± 0.47g, then NPL and PC were the largest weighing 18.74 ± 0.79g and 18.46 ± 0.26g respectively. This trend of NPL and PC weighing the most (and being roughly similar), followed by NPD2 and then NPD1 continued for the duration of the PP challenge phase. The final average weights at 172 dph were: 59.04 ± 2.03g, 45.62 ± 5.59g, 51.91 ± 1.58g, and 62.64 ± 2.75g for NPL, NPD1, NPD2 and PC groups respectively. However, the weight gained every two weeks as a percent of initial body mass showed different results. Consistently during the experiment NPD1 showed the highest amount of weight gain (%; Figure 2). This was followed by NPD2 and control, and NPL showing the lowest weight gain percent. Statistically significant differences in weight gain (%) between groups were present throughout the plant-protein challenge phase as well as in the numerical weight gain (g).

Work is currently underway to examine the expression of inflammatory genes in the intestine, as well as the extent of inflammation and the health status of the digestive tract via histological analysis.

Preliminary Conclusions

The present study found that the NPL and PC groups presented the best growth performances at the beginning and at the end of the PP challenge in terms of the average fish weight. However, NPD1 group presented the highest dietary soybean meal utilization capacity reflected by the highest weight gain compared to the remaining groups. This could possibly suggest that although the fish growth during the NP phase and shortly after was compromised by the early exposure to soybean meal in a form of dry feed, this early disadvantage led to improved coping mechanism in the NPD1 group with the same NP trigger (soybean meal) during the grow-out phase. However, it is also important to note that NPD1 presented the lowest survival and the lowest starting and final average weights which might have contributed to higher percentage of the weight gain throughout the PP challenge phase.

The current study suggests that the timing of NP and the NP method (SBM delivery) seems critical to improve dietary PP utilization. The early dry feed exposure seems to result in improved growth performance of the fish in their later stages, however, the NPL approach results in uncompromised performance of the fish during the overall production cycle since this group followed the performance of the positive control throughout the trial. It is also possible that although NPD1 was better at dietary soybean meal utilization for growth, the intestinal tract presented lower resistance to soybean meal-induced inflammation leading to lower survival of the fish during the PP challenge. This, however, requires further confirmation using the remaining molecular and histological analyses results.

This study will help determine the most optimal and beneficial timing of programming induction to achieve the highest performance of carnivorous fish fed plant-based diets during the grow-out phase. We are also confident that the gene expression and histological results will help elucidate the mechanism behind NP and help strengthen our methodology for assessing the same parameters in the YP trial in 2021.

Objective 3. — initially scheduled for May 2021, moved to May 2022 due to covid-19. Since a graduate student had already been hired in August 2019 an alternative project was developed for Spring 2020 on a species that was available. The project focused on NP and we believe it greatly contributes to the funded NCRAC project. Specifically, we are confident that the additional study performed strengthens our knowledge on NP in carnivorous fish species such as YP, its mechanism of action, and its most beneficial approach method.

Outreach Overview

A workshop at Millcreek Perch Farm in Marysville, Ohio, will be organized for Midwest fish farmers to facilitate the transfer of knowledge regarding alternative feeding strategies and live food enrichments to improve growth and feeding efficiencies of important local aquaculture species, including YP and largemouth bass. We will introduce the farmers to the concept of NP and how to use this feeding strategy in the most effective way to enhance utilization of commercial feeds based on PP sources. We will also discuss conventional live food feeding techniques currently used by the industry to facilitate an open dialogue between farmers.

We understand that changing a farmer's typical protocols for feed-training YP and largemouth bass on the farm will not be easy or immediately adopted by the entire industry. Our desire is for a hands-on workshop to discuss the necessary protocols and how easy this could be adopted into a farmer's typical practices. The co-owner of Millcreek Perch Farm is heavily involved in NCRAC, and we believe that successful demonstrations during the workshop will help OSU Extension in delivering the results from this proposed work. Smith also has camera equipment that can be utilized for recording the workshop, explaining in detail the protocol the farmer would ideally follow, and the benefits of the research results. This work would then be displayed on NCRAC's Vimeo website and several Midwest aquaculture websites. Smith will also be responsible for transferring the results to the rest of the NCRAC Extension/outreach community and beyond. The results will also be presented at the next North Central Aquaculture Conference.

The workshop is scheduled for May 2022 (after project extension requested due to covid-19).

Target Audiences
Aquaculture industry, specifically, feed and raw materials producers, as well as fish farmers who wish to increase survival and growth performance of their fish at a decreased cost.

Deliverables (Outputs)
None developed at this time

Outcomes/Impacts
The preliminary results from the largemouth bass study suggests that the timing of NP and the NP method (SBM delivery) seems critical to improve dietary PP utilization. Although programming during the first feeding using live food-enriched soybean meal is a practical way of inducing programming and leads overall to the highest survival and fish size as opposed to dry feed programming, the latter results in a better utilization of dietary soybean meal during the grow-out. However, further investigation is still needed to provide a recommendation on the most beneficial NP approach and will be included in the final report.

Although largemouth bass was used a model species in the study, we believe that this approach is applicable to other carnivorous fish including YP. We also believe that the gene expression and histological results from this study will help elucidate the mechanism behind NP and help strengthen our methodology for assessing the same parameters in YP trial in 2021.

Impacts Summary
Relevance. — The inability of carnivorous fish to grow satisfactorily on lower cost feeds with higher inclusion of soybean meal and similar quality PP sources.

*Respons*e. — The NP concept was tested during different time windows of larval/juvenile development to assess which window leads to the best fish performance on soybean-meal based diet during the grow-out phase
Results. — The NP overall provides an alternative option to improve dietary soybean-meal utilization in fish without changing dietary formulation. In addition, the largemouth bass study strengthens our knowledge on NP, its mechanism of action, and its most beneficial approach method.

Recap. — The growth performance of fish fed soybean-meal diets during the grow-out depends on the timing of early exposure of the fish to soybean meal.

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

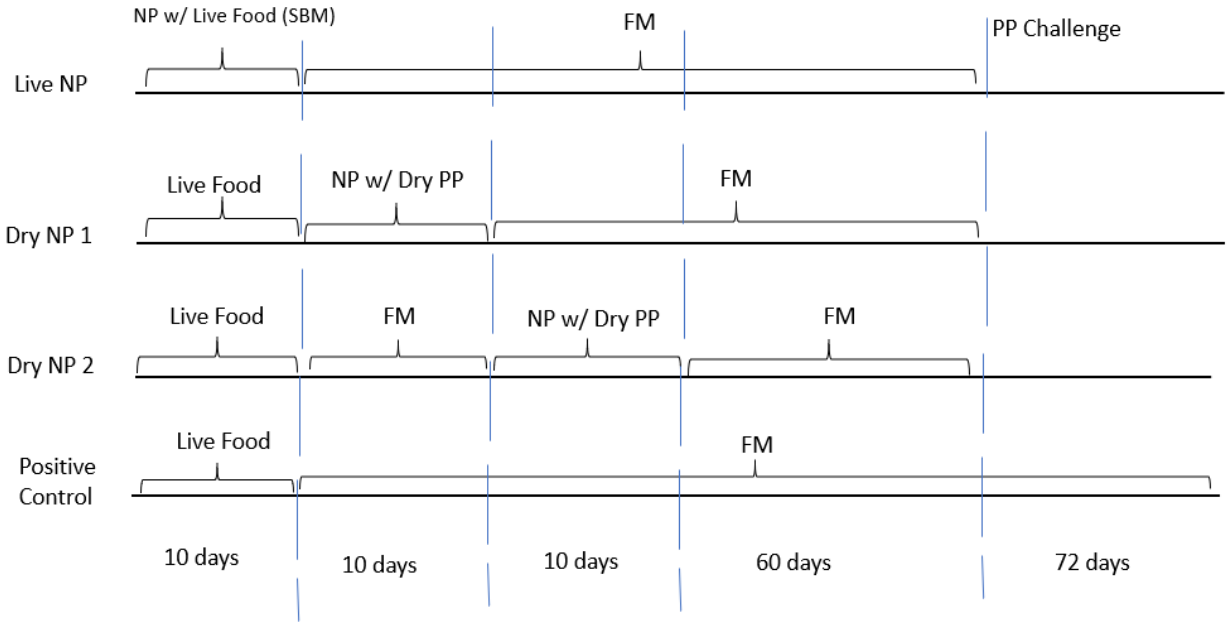

Figure 1. Schedule of nutritional programming. Groups are presented with the time they were nutritionally programed (NP) using either live food or dry plant-protein diets (PP), or fed fishmeal diets (FM).

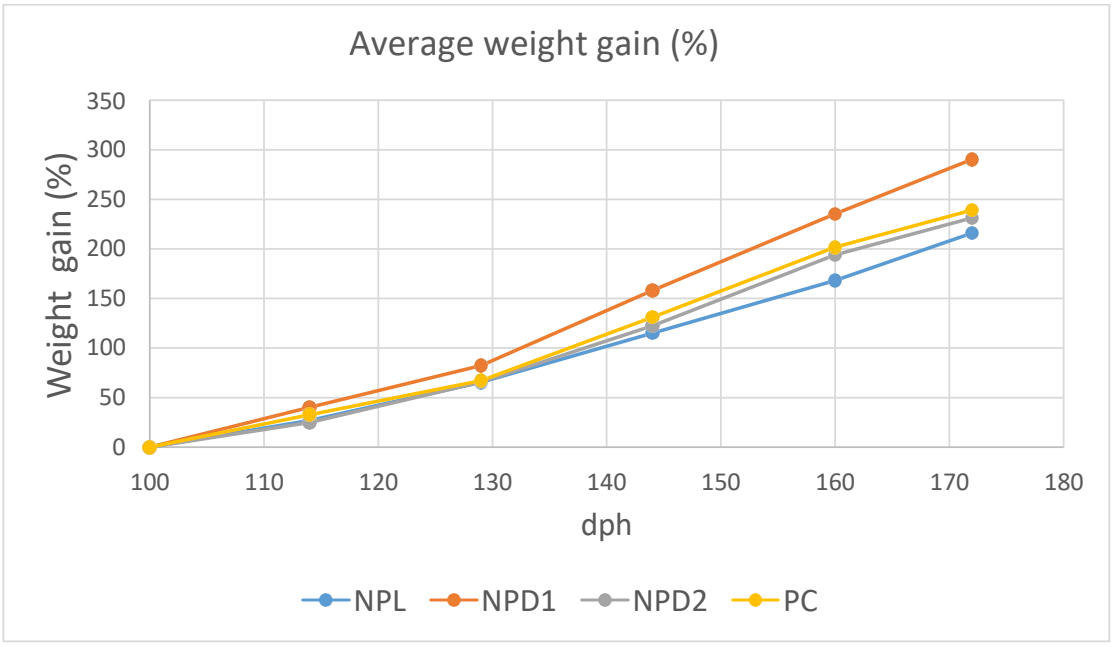

Figure 2. Average weight gain as a percentage of the initial starting weight at 100 dph. This timeframe represents the weight gained during the plant-protein challenge phase where all groups were fed the same plant-based diet apart from the positive control (PC).

Project Title: Optimization of Practical Feed Formulation to Improve Fish Health and Production of Yellow Perch (*Perca flavescens*) [Progress Report]
Total Funds Committed: $225,421
Initial Project Schedule: July 1, 2019-June 30, 2021
Current Project Year: November 1, 2019-August 30, 2020
Participants: Dong-Fang Deng and R. Newton (University of Wisconsin-Milwaukee), K.
Extension Liaison: J. Poletto (University of Nebraska
Industry Liaison: Rich Lackaff

Project Objectives

The ultimate goal of this proposal is to increase the profitability of yellow perch aquaculture by developing nutritionally balanced and cost-effective feed. To achieve this goal, the objectives of our two-year project are to:

1) Optimize practical feed formulation by determining the optimal dietary carbohydrate in feed for yellow perch based on growth performance and nutrient utilization;
2) Evaluate effects of different diets on gut microbial ecology and stress tolerance of yellow perch;
3) Determine production efficiency of the new feed at laboratory and commercial farms; and
4) Transfer technology and disseminate findings to industries to enhance the applications of findings.

Project Summary

There is no practical feed available for feeding yellow perch (*Perca flavescens*). Current commercial feeds do not provide optimal nutrients for this fish and fatty liver, extra viscera lipid, and suboptimal growth are commonly observed. Our previous research shows that different starch sources have different effects on yellow perch with wheat starch added at a level of 20% in the feed inducing significant fatty liver and viscera lipid accumulation in perch. Thus, in this project we will optimize dietary carbohydrate used in fish feed to enhance fish health and growth performance as well as reduce feed cost for yellow perch. Specifically, we will investigate how different carbohydrates influence 1) growth performance and nutrient utilization in laboratory and farm conditions; 2) fish tolerance in response to environmental stressors such as temperature shocks or hypoxia challenge; and 3) bacterial community composition and active community fraction in the host gut across diet regimes. This work will integrate lab studies and farm testing to evaluate production efficiency of the new practical feed compared with a commercial feed in selected farms. This proposal combines expertise in nutrition, feed processing, microbial ecology and extension research, to generate a comprehensive evaluation of feed quality. We will use the outcome to train students and skilled workforces to support the aquaculture industry.

Anticipated Benefits

The feed industry can make use of our findings to produce specific feed for yellow perch farming. Yellow perch farmers will have feed specific for perch grow-up. By using the new feed, we expect that yellow perch farms will increase their production profit because feed is one of the major costs in yellow perch production. This project will provide trainings to students and industrial collaborators on feed nutrition and fed management. We will help to train the next generation workforce by involving undergraduate students and graduate students in research extension activities.

Project Progress

Objective 1.— We proposed to determine an optimal practical feed formulation in a lab-based test but due to the restriction of working capacity during Covid-19, we could not spawn fish in March and could not manufacture test diets using a commercial pilot mill due to facility lockdown and limited access to the research facility. Thus, the research plan was postponed. To prepare for the upcoming trial, we successfully spawned some fish in August, 2020 using out-life cycle broodstocks and now have fingerlings available at University of Wisconsin-Milwaukee. A feeding trial will start as soon as we can get feed manufactured by our Co-PI. We also had difficulties in getting ingredients for feed preparation but expect that the test feed will be made during the last week of December, 2020 or the first week of January, 2021. A feeding trial will start in January, 2021 as soon as the feed are ready.

In the interim, a preliminary feeding trial was conducted to test six diets that contained similar levels of carbohydrates (15, 20 and 25%) provided from two sources of carbohydrates (wheat flour and corn flour). The feed formulations contained the sample levels of carbohydrate proposed in the proposal and has similar lipid levels. Fish were fed based on estimated feeding rates (2-5% body weight daily) and four times daily (9:00, 12:00, 15:00; 18:00). The feeding trial lasted for 10 weeks. At the end of the 10-week feeding trial, all fish were fasted for 24 h before they were batch-weighed and counted to obtain final survival and total weight of each tank. Four individuals were then euthanatized with an overdose of MS222 (500 mg·L-1) and kept at -80°C until used for proximate composition (moisture, crude protein, lipid, ash) analyses. Another four fish from each tank were euthanatized for measurement of individual body weight and full

length to calculate the condition factor (CF). Subsequently, these four fish were dissected to obtain weight of the liver, viscera, and carcass for calculations of hepatosomatic index (HSI), viscera somatic index (VSI), and carcass index (CSII), respectively (See Table 2).

Preliminary results indicated no mortality was observed during the feeding trial time period. The percent of weight gain (%WG), and specific growth rate (SGR, % BW.day were significantly higher for fish fed the wheat flour-based diets than those of fish fed the corn flour diets ($P \leq 0.05$). Feed conversion ratio (FCR) was lower for Fish fed the wheat flour-based diets were shown to have a lower FCR than those fed the corn flour- based diets (Table2). The flour sources did not lead to different effects on the values of condition factor (CF), carcass index (CSI), and viscera somatic index (VSI) of yellow perch ($P>0.05$). The hepatosomatic index (HSI) was significantly higher in fish fed the wheat flour-based diets than those fed the corn flour-based diets. Yellow perch fed 20% carbohydrate had lower growth and condition factor than those fed 15% or 25% carbohydrate. FCR was significantly increased in yellow perch fed the diets containing 20% carbohydrate when compared to those fed diets containing 15% or 23% carbohydrate. Increase in dietary carbohydrate level significantly increased the HSI of yellow perch but did not influence the values of CF, CSI, and VSI. No significant interaction influence on growth performance and morphology parameters was observed between carbohydrate sauces and their inclusion levels.

Fish fed the wheat flour-based diet were observed to have significantly higher lipid content in their whole fish and liver tissue than the fish fed the corn flour-based diet (Table 2). The moisture content was significantly lower in the perch fed the corn flour-based diets than those observed in fish fed the wheat flour-based diets. The protein or ash level was not statistically different between fish fed wheat or corn flour diets. Yellow perch fed 25% flour-based diets contained a higher level of lipid and lower moisture content than those fed 15% or 20% flour-based diets. In contrast, the level of liver protein was lower in fish fed 25% flour than those fed 15% or 20% flour based diets. No interaction effect was observed between carbohydrate source and the added levels.

Initial finds from this study indicate that under the current testing conditions, wheat flour promoted a better growth in yellow perch juvenile than corn flour but it also tended to induce lipid accumulation in liver tissue or whole fish. The diets containing 25% wheat or corn flour did not depress growth when compared the diet containing 15% flour. It was unexpected that the fish fed 20% flour had the lowest growth rate. For this study, we still need to finish other analysis related to fish health evaluation. Therefore, no solid conclusion can be drawn at this stage. In addition, the cold extruding method could not generate good degree of gelatinization in starch. This could impact the digestibility and physical quality of feed pellets. We expected that test diet made by using a pilot feed mill will overcome the above issues. This will be tested in the upcoming feeding trial. After this preliminary study, we have established reliable lab protocols and students were well trained on the process of lab study on feed testing.

Objective 2. — As a part of this objective, Newton will compare the microbial community composition in fish intestines among fish in proposed diet trials. As the diet trials have been delayed due to the SARS-CoV-2 pandemic, to date no fish intestines have been collected. This will occur as soon as the diet trials are able to start again. In preparation for the upcoming trials, lab supplies have been purchased for collection of fish intestines and subsequent DNA extraction. One undergraduate student researcher also has been hired into the Newton lab and trained to carry out DNA extractions.

Objective 3.— This objective is proposed to be conducted in Year-2 of the project. We have identified two fish farms that we will collaborate with for farm testing of the feed formulation generated from objective 1.

Objective 4. — we proposed to "Transfer technology and disseminate findings to enhance the applications generated from this project." Part of this Objective included establishing a training program whereby undergraduate students would be trained in the Poletto Fish Physiology laboratory at the University of Nebraska Lincoln (UNL) and subsequently placed in internships or jobs at local aquaculture facilities as already-skilled workers with knowledge of aquaculture practices. To this end, a local aquaponics and aquaculture facility was identified and visited by students from the 2020 Ichthyology course at UNL, after which two undergraduate students were recruited to the Poletto Lab. One undergraduate was fully trained to proficiency in the laboratory, and another is currently being trained. Due to a delay in the establishment of funds at UNL, all students worked as volunteers, and any domestic travel to aquaculture facilities was prohibited by UNL due to the SARS-CoV-2 pandemic. Finally, a training manual was developed to standardize the training process, and a list of potential aquaculture facilities with which UNL will establish internship programs was assembled

Outreach Overview

Due to the delayed research planned in year-1, outreach plan was postponed. Currently, the major outreach activities have been focused in training students and engage them in the project planning. Two undergraduate students were recruited to the Poletto Lab at UNL. One undergraduate was fully trained to proficiency in the laboratory, and another is currently being trained. Three undergraduate students were identified at UWM to be tainted with lab protocols

including fish culture system management, maintenance of broodstock, feed processing and analysis in nutrition and microbial. The PI presented the project ideas and preliminary study at the Wisconsin Aquaculture Association annual meeting. We have connected with two fish farms at Wisconsin for farm testing when we identify a feed formulation to be used. We will conduct outreach activities in the spring 2021 to train farmers on protocols that needed for a farm testing when we obtained results from the lab and farm test trials. Outreach activities will also focus on engaging local communities through technology –these may be social media platforms, local TV and radio programming, and opportunities for students and adults through university classes and local events. Ultimately, we seek to not only disseminate information about diet development, and secure a partner in the feed industry, but also to help local farmers enhance their practices in a way that promotes more, better, and more pervasive use of aquaculture at the local and regional level.

Target Audiences

Yellow perch producers will benefit with an optimal feed for growing perch at a cost-effective approach. Feed industry will be able to adopt the new findings to make feed targeted on yellow perch; Students and researchers will gain new knowledge on fish feed nutrition and develop collaborations with feed industry and fish farmers. Collaboration farmers will receive trainings on feed management.

Deliverables (Outputs)

No data is available up to this report point due to the postponed plan of year 1 research

Outcomes/Impacts

No data is available up to this report point due to the postponed plan of year 1 research

Impacts Summary

Relevance. Yellow perch is a high demand seafood in the Great Lake regions. Feed is one of the major components accounting for yellow perch production cost. Aquaculture production and profitability of yellow perch are challenged by suboptimal feed, which is produced for Salmonid species of fish. Current commercial feed used to feed yellow perch causes adverse impacts on yellow perch health and production efficiency.
*Respons*e. — This project is to develop practical feed targeted on yellow perch production by investing feed formulations optimizing with proper levels and sources of carbohydrate, and train industrial partners on feed management. An optimal feed formulation will be developed by lab studies and tested at fish farms. Theproject was delayed due to Covid-19 pandemic.
Results. — No impact measurement up to this point due to the delayed plan of this project._
Recap. — The outcome of this project will provide yellow perch producer with cost effective feed and help to train fish famers and the next generation workforce for the aquaculture industry.

Publications, Manuscripts, Workshops, and Conferences
See the Appendix for a cumulative output for all NCRAC-Funded Yellow Perch activities.

Table 1. Growth performance and morphology measurements of yellow perch fed with test diets containing different levels of wheat or corn flour for 10 weeks.

CHO source	CHO level (%)	Weight gain (%)	SGR (%.BW.d)	FCR	CF	CSI(%)	VSI(%)	HSI(%)
Wheat	15	233.3	1.72	1.36	1.21	88.3	7.38	1.03
Wheat	20	216.3	1.64	1.48	1.18	87.7	7.71	1.12
Wheat	25	235.1	1.73	1.37	1.22	87.6	8.13	1.44
Corn	15	206.0	1.60	1.50	1.19	88.3	7.43	0.84
Corn	20	171.8	1.43	1.74	1.16	88.1	7.67	0.96
Corn	25	218.6	1.66	1.38	1.22	87.1	8.25	1.23
Pooled SE		11.64	0.05	0.07	0.01	0.6	0.42	0.04
Source		**Means of main effect**						
Wheat		228.2[a]	1.69[a]	1.40[b]	1.21	87.8	7.74	1.20[a]
Corn		198.8[b]	1.56[b]	1.54[a]	1.19	87.8	7.79	1.01[b]
	Level (%)							
	15	219.7[a]	1.66[a]	1.43[b]	1.20[a]	88.3	7.41	0.93[c]
	20	194.1[b]	1.53[b]	1.61[a]	1.17[b]	87.9	7.69	1.04[b]
	25	226.8[a]	1.69[a]	1.38[b]	1.22[a]	87.3	8.19	1.33[a]
	ANOVA: P-values							
Source		0.009	0.008	0.033	0.001	0.968	0.898	0.000
Level		0.037	0.026	0.017	0.009	0.264	0.205	0.000
Source X Level		0.500	0.392	0.256	0.001	0.705	0.980	0.844

Initial body weight of fish was 14.8 ±0.13 (mean body weight ±SE, n=21).
Treatment means represent the average values of three tanks per treatment. Main effect means indicated by different letters are significantly different at $p \leq 0.05$ by Fisher LSD test.
WG (percentage of weight gain, %) = (FBW – IBW)/IBW × 100
SGR (specific growth rate, % body weight per day) = 100*Ln (final body weight/initial body weight)/feeding duration (days)
FCR（Feed conversion ratio） = (feed intake per tank, g) /(total final fish weight g –total initial fish weight g + dead fish g).
CF (condition factor, g/cm^3) = 100* (body weight, g)/ $(\text{body length, cm})^3$
CSI (carcass index,%) = 100* degutted fish weight (g)/fish body weight, g
VSI (viscerosomatic index, %) = 100× (viscera weight, g)/ (body weight, g)
HSI (hepatosomatic index, %) =100× (liver weight, g)/ (body weight, g)

Table 2. Proximate composition of whole body and liver tissue of yellow perch fed with test diets containing different levels of wheat or corn flour for 10 weeks (% wet weight).

		Whole body				Liver		
CHO source	**CHO level (%)**	Moisture	Ash	Protein	Lipid	Moisture	Protein	Lipid
Wheat	15	66.00	4.72	18.05	11.80	59.45	12.35	16.73
Wheat	20	65.64	4.72	18.92	11.73	56.33	12.39	18.41
Wheat	25	65.14	4.63	18.02	12.59	58.38	10.79	18.46
Corn	15	66.37	4.75	18.21	11.02	62.15	13.03	14.56
Corn	20	66.48	4.87	18.15	10.50	60.60	13.06	15.38
Corn	25	65.43	4.70	17.89	11.96	61.17	11.60	15.87
Pooled SE		0.04	0.09	0.48	0.36	1.08	0.44	1.05
Source		**Means of main effect**						
Wheat		65.60	4.69	18.33	12.04[a]	58.05[b]	11.84	17.87[a]
Corn		66.09	4.77	18.08	11.16[b]	61.31[a]	12.56	15.27[b]
	Level (%)							
	15	66.19[a]	4.74	18.13	11.41[b]	60.80	12.69[a]	15.64
	20	66.06[ab]	4.79	18.53	11.11[b]	58.46	12.73[a]	16.90
	25	65.29[b]	4.66	17.95	12.28[a]	59.77	11.19[b]	17.17
	ANOVA: P-values							
Source		0.152	0.276	0.539	0.011	0.003	0.068	0.011
Level		0.089	0.383	0.488	0.019	0.139	0.006	0.336
Source X Level		0.500	0.761	0.813	0.623	0.687	0.723	0.985

Data is presented as mean ±SE. Treatment means represent the average values of three tank per treatment. Main effect means indicated by different letters are significantly different at $p \leq 0.05$ by Fisher LSD test.

Out-of-Cycle Project Reports

Project Title: Formulation and Assessment of a New Generation of Starter Diets for Largemouth Bass (*Micropterus salmoides*) and Yellow Perch (*Perca flavescens*) larvae [Termination Report]

Project Period: March 1, 2017-February 28, 2019; extension to August 30, 2020.

NCRAC Funding Level: $35,000

Participants: K. Dabrowski, The Ohio State University, Ohio; T. Barry, University of Wisconsin- Madison.

Extension Liaison: Alex Primus, University of Minnesota

Industry Liaison: Adam Hater, Jones Fish Farm, Ohio

Reason for Termination: Completion of project objectives.

Project Objectives:

1. To raise larval LMB and YP on live rotifer/brine shrimp nauplii diets as a control and transition to commercial formulated feed (Otohime) or laboratory prepared microparticulate diets.
2. To prepare a *Pichia pastoris* (yeast) culture in order to obtain sufficient biomass of the product proven to be successful in diets for marine and freshwater fish larvae. In addition, we will express salmon trypsinogen in *Pichia* to increase the protein digestion capacity of larval fish fed with this ingredient.
3. To compare growth rate, survival, and swim bladder inflation of LMB and YP in side-by-side laboratory (OSU and UW-Madison) and practical, on-farm (Coral Reef, New Albany, OH) experiments during the larval-juvenile transition period.

Project Summary

Previous experiments provided evidence that rearing largemouth bass and yellow perch larvae on formulated diets from beginning of food intake to replace live feeds results in high mortality despite food acceptance. Therefore, we analyzed formulated diets that included a yeast species successfully used in initial feeding of other fish species, and modified this yeast to express proteolytic enzyme and facilitate digestion process. Enrichment of live food with Pichia had a positive effect on performance of juvenile Zebrafish, compared to algae enrichment alone. We found Zebrafish fed formulated dry diet at first-feeding had inferior performance to those fed live feed. Transformation of fish trypsinogen linear DNA into Pichia was expected to increase larval fish protein digestion capacity. Salmon trypsinogen gene was cloned and transformed into Pichia, but trypsinogen protein was not expressed. In 2020, Pichia was transformed to express cod trypsinogen with fusion protein GFP, but only GFP expressed alone provided fluorescent signal. Results of this work linked failure of expression of trypsinogen to the specific Salmon transgene, and concluded lack of expression is not an issue in the yeasts themselves or the expression system used.

Technical Summary and Analysis

Objective 1.— Experiments carried out in OSU lab in 2019 concentrated on zebrafish as the surrogate, model Experiments carried out in OSU lab in 2019 concentrated on zebrafish as the surrogate, model species only due to the delay of the award in the first year of the project. In 2019 spawning season of both yellow perch (May) and largemouth bass (June) were already completed before the first culture batch of Pichia was produced. In 2020, because of the COVID-19 pandemic, travel and laboratory work was not allowed from March to August. The first feeding experiment addressed the effect of live food (*Artemia* naupli) enrichment with Pichia yeast along other commercial enrichments on zebrafish performance during juvenile stage and follow-up to the phase of maturation. Zebrafish larvae wer estocked at the density of 110-114 larvae in 6 cylindrical containers (6L) and conditions followed the procedure described earlier, 3 ppt saline, constant light and 28–29 ºC (Dabrowski and Miller (2018). During the first 7 days (5-11 days post fertilization; dpf), larvae in all containers were fed marine rotifers *Brachionus plicatilis*. At 12 dpf, larvae were divided into 3 groups (2 replicates) and transitioned to feeding with enriched Artemia, with one of 3 prepared diets; Nannochloropsis algae (NA) (Nanno 3600 Instant Algae®), baker's yeast (*Saccharomyces cerivisiae*) (SC), or live, concentrated Pichia yeast (PY). This phase lasted 3 weeks. At 34 dpf, fish from each group was divided into 3 replicates and were transferred to a recirculating Zebrafish rack system (2 L containers) and fish were transitioned to feeding dry food (Otohime B2® diet). Fish were fed 4-5 times/day based on fish biomass at 26.5-27 °C and photoperiod at 12:12 (L:D). The mean body weight for fish was 60.7, 79.7 and78.7, and survival 87, 82.7 and 85.9%, for NA, SC and PY, respectively. Differences between treatments were not significant at this point. Following transition to dry feed differences in performance widened, and zebrafish at 91 dpf showed differences in the mean weight, 296.6,316.3 and 307.7 mg. We concluded that enrichment of live food with Pichia during larval-juvenile transition have positive effect on fish performance in comparison to algae enrichment alone. The second experiment in 2019 addressed the estimation of live feed (enriched rotifers and/or Artemia nauplii) (labelled with Fluorescent GFP-Pichia; Green Fluorescent Protein expressed) in comparison to dry formulated feed acceptance, intestinal transition time and performance (growth and survival in the similar rearing unite set-up). Larvae used in this experiment (Casper

(transparent) strain, 7 dpf and wild type pigmented fish, 5 dpf, were divided randomly into 6 groups with 2 replicates (40 larvae/replicate). Larvae were subjected to six feeding treatments (T) as follows: 1. Treatment 1(Rotifer): larvae were fed on rotifers during the 2phases of experiment (Rotifers were fasted for 16 h). 2. Treatment 2 (Rotifer-P): larvae were fed on Pichia enriched rotifers during the 2 phases of experiment (Rotifers were enriched with Pichia for 16 h). 3. Treatment 3 (Dry diet-P): larvae were fed on Pichia containing dry diet (size 106-212 μm) during the 2 phases of experiment. 4. Treatment 4 (Rotifer-Artemia): larvae were fed on rotifers during the first phase, 7 days (rotifers were fasted for 16 h) then shifted to Artemia during the second phase, 7 days (artemia were starved for 17-18 h). 5. Treatment 5 (Rotifer-Artemia-P): larvae were fed on rotifers during the first phase (rotifers were starved for 16 h) then shifted to Pichia enriched Artemia during the second phase (Artemia were enriched for 17-18 h). 6. Treatment 6 (Rotifer-Dry diet-P): larvae were fed on rotifers (rotifers were starved for 16h) during the first phase then shifted to Pichia containing dry diet (size 106-212 μm). Fish were fed manually 4-5 times /day. Fish for observations under the fluorescent microscope (Nikon 80i Epifluorescent microscope) collected after 1 h of feeding (Figure 1). Green light images demonstrate intake of fluorescent marker in rotifers (A and B) and Artemia nauplii (Fig. 1C and D). The anesthetized fish from the first 3groups were taken at 8dpf and at 13th dpf (1st day of phase 1 and 2, respectively). In the second phase, samples were taken from the other 3groups (4-6) at 15 dpf (1st day of phase 2) and at 20 dpf (5 days after feeding of the designed food in this phase). After 21 dpf, larvae were counted in all the tanks for survival and samples (n = 12) were taken for measuring weight (g) and length (mm) of juveniles (Table 1). These samples were kept in -80oC to be used for the gene expression (trypsin enzyme) analysis. At the completion of this experiment significant differences were recorded in mean weight of zebrafish that indicate highly inferior performance of fish fed formulated dry feed (Table1).

Dr.Rappley's laboratory carried out studies aimed at transforming linear DNA sequence of Atlantic salmon and cod (in 2020) trypsinogens into Pichia cells. To address this objective, previously characterized trypsin-II (ST-II) mRNA sequence of salmon trypsin (CAA49678.1 trypsin II) was used to extract the full sequence of salmon trypsinogen. >XM_014155449.1, predicated Salmo salar trypsinogen II, mRNA sequence,was then used. A synthetic gene was made that lacked the secretion signal so that the trypsin would be maintained within the yeast cells rather than secreted into the medium. This gene was then inserted into a cloning vector (pCR600), and transformed into E.coli cells following the direction of (Invitrogen kit manufacturer). After that, the plasmid DNA of the recombinant clones was purified, digested SalI to produce linear DNA, and transformed into Pichia GS115. Four transformants of each construct were tested for salmon trypsinogen expression by immunoblotting using the FLAG or 6xHIS epitope tags. For constitutive expression vectors (i.e. glyceraldehyde-3-phosphate dehydrogenase(GAPDH) promoter, and translation elongation factor (TEF1), the cells were grown in 2 ml of minimum glucose medium overnight at 30oC. For constructs that use the methanol-regulated AOX1 promoter, cells were first gown up overnight in 2 ml of minimum glycerol (1%) medium at30oC. After that, cell were harvested by centrifugation and cultured in minimum Pichia base with added methanol to final concentration of 0.5, 1and 2 % and incubated at 30oC with shaking. Cloning of Salmon trypsinogen II (ST-II) gene and its transformation into Pichia pastoris were successfully achieved and confirmed by sequencing. For constructs using the TEF1 promoter, none of the transformants showed expression of the trypsinogen protein. However, expression of a GFP transgene under control of the same promoter was successfully detected. The same results were obtained when trypsinogen genes were expressed from the methanol-induced promoter AOX1. These results directly linked the failure in expression of trypsinogen to the Salmon trypsinogen transgene and not a problem in the yeasts themselves or the expression system employed. Consequently, future work will attempt to use alternate fish trypsinogens such as cod trypsinogen and cunner fish (*Tautogolabrus adsperus*) (Macouzet et al.2005). We have requested synthesis of primers. Targeted Audiences Yellow Perch operations in the North Central Region (NCR) have all experienced difficulties in out of season reproduction and raising perch larvae in captivity, in recirculated systems. The industry has long recognized that expanding Yellow Perch culture from ponds to indoor systems would require replacement of live feeds with nutrient complete diets from larval stage to broodstock. Cost-effective starter diets or minimizing duration of live feed (rotifer and brine shrimp) use is a prerequisite of the economic viability of perch culture within NCR.

Principal Accomplishments

Through the first experiment, we concluded that enrichment of live food with Pichia during larval-juvenile transition have positive effect on fish performance in comparison to algae enrichment alone. We developed a protocol for enriching live food with Pichia yeast, and utilized an optimal rearing system. In experiment two, we developed a protocol for producing Pichia containing dry diet for first feeding and compared that to live food. At the completion of this experiment, we recorded significant differences in mean weight of zebrafish, indicating highly inferior performance of fish fed formulated dry feed (Table1). These results during the feeding trials are directly linked to the failure in the yeast's expression of trypsinogen to the Salmon trypsinogen transgene and not a problem in the yeasts themselves or the expression system employed. We anticipate that use of an alternate fish trypsinogen will result in expression of this protein, increasing performance of fish fed Pichia containing dry diet. We have thus developed a protocol for preparing this yeast with a trypsinogen transgene, though alternate sources need to be investigated, and subsequently preparing diets containing this transgenic yeast for first feeding. In 2020, we extended studies on Zebrafish and included effect of nutritional history of juveniles on the reproductive capacity of Zebrafish. Namely, Artemia were enriched with 3different products: Nannochloropsis algae, Saccharomyces cerevisiae and Pichia pastoris yeast for 24 h enrichment period. Growth performance was not significantly different among the different groups at the end of both phases (enriched Artemia phase and dry feed phase) ($P<0.050.05$). Female weight and length as well as male length were not affected by the Artemia enrichment ($P<0.05$). Larvae fed Saccharomyces cerevisiae enriched artemia showed the highest male weight compared with the other 2 groups. Pichia enriched Artemia fed as juveniles showed improved average fecundity ($P < 0.05$), fertilization rate and average spawning efficiency ($P<0.05$).

Impacts

This work has made a significant contribution to the development of formulated diets for first feeding in commercial species in order to replace live feeds. The results produced in this project provide valuable and necessary preliminary knowledge and methods for formulating dry diets containing transgenic yeast to enhance fish performance. Availability of these diets in the future, after further research and development, will make culture of Largemouth bass and yellow perch feasible year-round. Industry would be able to avoid the limitations of unpredictable weather effects on pond zooplankton and the short growing season in the NCR.

Recommended follow-up activities

We anticipate publishing these studies in peer-reviewed scientific journals in 2021. These experiments have developed a protocol that can be applied to commercial species in the future. Additionally, future work will attempt to use fish trypsinogens other than Salmon, such as cod and cunner fish, in order to achieve expression of the trypsinogen proteins in Pichia. We anticipate that successful expression of these proteins will result in greater performance of fish fed these Pichia containing diets as first food.

Table 1. Growth and survival of Zebrafish larvae at 21 dpf (initial mean weight of zebrafish larva is 0.22 mg).

Treatments	Body weight (mg)	Body length (mm)	Survival (%)
Rotifer	61.2± 5.0 ^{a}	19.7 ± 0.5 ^{a}	80.6 ± 9.1
Rotifer-P	69.1± 2.1^{a}	20.4 ± 0.2 ^{a}	78.4 ± 3.1
Dry diet- P	8.1 ±2.6 ^{b}	6.8 ± 1.0 ^{d}	62.4 ± 2.2
Rotifer-Artemia	60.5 ±3.7^{a}	17.3 ± 0.1 ^{b}	85.9 ± 6.6
Rotifer- Artemia-P	61.0 ±3.7 ^{a}	17.2 ±0.5 ^{b}	82.8 ± 6.6
Rotifer-Dry diet-P	16.3 ±1.7 ^{b}	11.6 ± 0.2^{c}	89.0 ± 2.2
P-value	< 0.0001	< 0.0001	0.0334

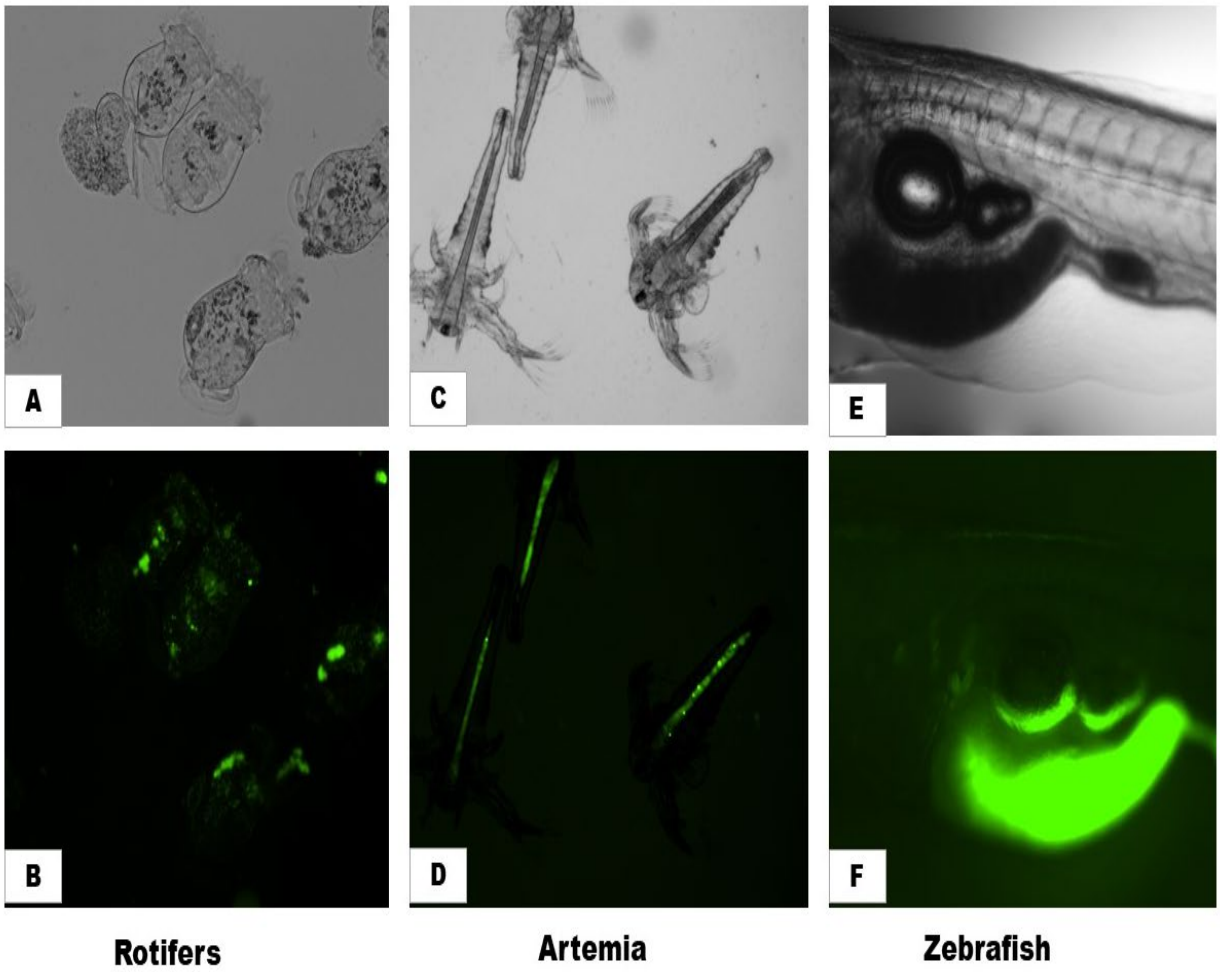

Figure 1. Enriched rotifers (A and B) which were enriched with Pichia for 16 h; Enriched *Artemia* (C and D) which were enriched with *Pichia* for 17-18 h; zebrafish larvae (E and F) as larvae was fed on rotifers during the first phase then shifted to Pichia containing dry diet during the second phase. First row of pictures is under natural light while the second row is using fluorescence. All pictures were captured using the Nikon 80i fluorescent microscope, 4x.

Publications, Manuscripts, Workshops, and Conferences

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

Some Commonly Used Abbreviations and Acronyms

AIS	aquatic invasive species
APHIS	Animal and Plant Health Inspection Service
ARS	Agriculture Research 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
FSR ft, ft^2, ft^3	final study report 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
INAD	investigational new animal drug
ISU	Iowa State University
KAA	Kansas Aquaculture Association
LU	Lincoln University
m, m^2, m^3	meter(s), square meter, cubic meter
MAI	motile *Aeromonas* infection
MAS	motile *Aeromonas* septicemia
MDNRE	Michigan Department of Natural Resources and Environment
µg	microgram(s)
mg	milligram(s)
MC	Mill Creek
min	minute(s)
mL	milliliter(s)
mm	millimeter(s)

MSU	Michigan State University
MT	methyltestosterone
N	number
NAA	National Aquaculture Association
NADA	new animal drug application
NASAC	National Association of State
NCC	National Coordinating Council
NCR	North Central Region
NCRAC	North Central Regional Aquaculture
NIFA	National Institute of Food and Agriculture
NOB	nitrite oxidizing bacterial
OCARD	Ohio Center for Aquaculture Research and Development
OSU	The 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 thousand
Purdue	Purdue University
RAC(s)	Regional Aquaculture Center(s)
RAES	Regional Aquaculture Extension
RAET	Regional Aquaculture Extension Team
RAS	recirculating aquaculture system
RS	Rimler-Stotts
SPAH	Schering-Plough Animal Health
TC	Technical Committee (TC/E = Technical
™	trademark
TSA	Tryptic Soy Agar
UMESC	Upper Midwest Environmental Sciences
USDA	U.S. Department of Agriculture
USFWS	U.S. Fish and Wildlife Service
UW-Madison	University of Wisconsin-Madison
UW-Milwaukee	University of Wisconsin-Milwaukee
VHS	viral hemorrhagic septicemia
VHSv	viral hemorrhagic septicemia virus
WATER	Wisconsin Aquatic Technology and Environmental Research

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