

**NORTH CENTRAL
REGIONAL AQUACULTURE CENTER**



ANNUAL PROGRESS REPORT 2010-11

FEBRUARY 2012

ANNUAL PROGRESS REPORT

For the Period
September 1, 2010 to August 31, 2011

February 2012

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

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INTRODUCTION

The U.S. aquaculture industry is an important sector of U.S. agriculture generating over \$1.16 billion in 2009 for producers. Yet, anticipated growth in the industry, both in magnitude and in species diversity, continues to fall short of expectations.

Much of what is known about aquaculture science is a result of institutional attention given to our traditional capture of wild fisheries with the goal of releasing cultured fishes into public waters for enhancement of declining public stocks. Despite extensive efforts to manage wild populations for a sustained yield, as a nation we consume substantially greater amounts than we produce. Much of the United States' demand for seafood has been met by imports. The value of imported fisheries products has substantially increased over the last two decades. In 2010, the U.S. imported \$27.4 billion of fisheries products and the trade deficit was \$5 billion for all fisheries products, most of which was for edible fish and shellfish.

Landings for most commercial capture fisheries species and recreational fisheries of the United States have been relatively stable during the last decade, with many fish stocks being over exploited. In this situation, aquaculture provides an opportunity to reduce the trade deficit and meet the rising U.S. demand for fish products. A strong domestic aquaculture industry is needed to increase U.S. production of fish and shellfish. This can be achieved by a partnership among the Federal Government, State and local public institutions, and the private sector with expertise in aquaculture development.

Congress recognized the opportunity for making significant progress in aquaculture

development in 1980 by passage of the National Aquaculture Act (P.L. 96-362). Congress amended the National Agricultural Research, Extension, and Teaching Policy Act of 1977 (P.L. 95-113) in Title XIV of the Agriculture and Food Act of 1981 (P.L. 97-98) by granting authority to establish aquaculture research, development, and demonstration centers in the United States in association with colleges and universities, State Departments of Agriculture, Federal facilities, and non-profit private research institutions. Five such centers have been established: one in each of the northeastern, north central, southern, western, and tropical/subtropical Pacific regions of the country. The Food, Conservation, and Energy Act of 2008 (P.L. 110-246), otherwise known as the Farm Bill, has reauthorized the Regional Aquaculture Center program at \$7.5 million per annum. As used here, a center refers to an administrative center. Centers do not provide monies for brick-and-mortar development. Centers encourage cooperative and collaborative aquaculture research and extension educational programs that have regional or national application. Center programs complement and strengthen other existing research and extension educational programs provided by the U.S. Department of Agriculture (USDA) and other public institutions. As a matter of policy, centers implement their programs by using institutional mechanisms and linkages that are in place in the public and private sector.

The mission of the Regional Aquaculture Centers (RACs) is to support aquaculture research, development, demonstration, and extension education to enhance viable and profitable U.S. aquaculture production which will benefit consumers, producers, service industries, and the American economy.

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The North Central Regional Aquaculture Center (NCRAC) was established in February 1988. It serves as a focal point to assess needs, establish priorities, and implement research and extension educational programs in the twelve state agricultural heartland of the United States which includes Illinois, Indiana, Iowa, Kansas, Michigan, Missouri, Minnesota, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin. NCRAC also provides coordination of interregional and national programs through the National Coordinating Council for Aquaculture (NCC). The council is composed of the RAC directors and USDA aquaculture personnel.

ORGANIZATIONAL STRUCTURE

Michigan State University (MSU) and Iowa State University (ISU) work together to develop and administer programs of NCRAC through a memorandum of understanding. MSU is the prime contractor for the Center and has administrative responsibilities for its operation. The Director of NCRAC is located at MSU. ISU shares in leadership of the Center through an office of the Associate Director who is responsible for all aspects of the Center's publications, technology transfer, and outreach activities. In 2012 NCRAC became solely administered by Iowa State University where the Office of the Director is located.

During the time period of this report the staff of NCRAC at MSU included Ted R. Batterson, Director, and Liz Bartels, Executive Secretary. The Center Director has the following responsibilities:

- ▶ Developing and submitting proposals to the USDA National Institute of Food and Agriculture (NIFA; formerly the Cooperative State Research, Education and Extension Service) which, upon approval, becomes a grant to the Center;
 - ▶ Developing appropriate agreements (sub-contracts) with other parties, including ISU for the Associate Director's office, for purposes of transferring funds for implementation of all projects approved under the grants;
 - ▶ Serving as executive secretary to the Board of Directors, responsible for preparing agenda and minutes of Board meetings;
 - ▶ Serving as an ex-officio (non-voting) member of the Technical Committee and Industry Advisory Council;
 - ▶ Coordinating the development of research and extension plans, budgets, and proposals;
 - ▶ Coordinating and facilitating interactions among the Administrative Center, Board of Directors, Industry Advisory Council, and Technical Committee;
 - ▶ Monitoring research and extension activities;
 - ▶ Arranging for review of proposals for technical and scientific merit, feasibility, and applicability to priority problems and preparing summary budgets and reports as required;
 - ▶ Recruiting other Administrative Center staff as authorized by the Board of Directors;
 - ▶ Maintaining liaison with other RACs; and
 - ▶ Serving on the NCC.
- NCRAC's Office for Publications and Extension Programs at ISU is under the direction of Joseph E. Morris, Associate Director. The Associate Director has the following responsibilities:
- ▶ Coordinating, facilitating, and executing regional aquaculture extension program activities;
 - ▶ Serving as head of Publications for NCRAC, including editor of the fact

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sheet, technical bulletin, culture manual, and video series as well as of the NCRAC Newsletter;

- ▶ Serving as the NCRAC liaison with national aquaculture extension programs, including in particular, extension programs of the other four USDA Regional Aquaculture Centers; and
- ▶ Serving as a member of NCRAC's Extension Executive Committee.

The Board of Directors (BOD) is the primary policy-making body of the NCRAC. The BOD has established an Industry Advisory Council (IAC) and Technical Committee (TC). Membership of the BOD consists of four persons from the IAC, a representative from the region's State Agricultural Experiment Stations and Cooperative Extension Services, a member from a non-land grant university, representatives from the two universities responsible for the center: Michigan State and Iowa State, and chairs of the two subcommittees of the Center's Technical Committee. The IAC is composed of representatives from each state's aquaculture association and six at-large members appointed by the BOD who represent various sectors of the aquaculture industry and the region as a whole. The TC is composed of a sub-committee for Extension (TC/E) and a sub-committee for Research (TC/R). Directors of the Cooperative Extension Service within the North Central Region appoint representatives to the TC/E. The TC/R has broad regional make-up and is composed of scientists from universities and state agencies with varied aquacultural expertise who are appointed by the BOD. Each sub-committee of the TC has a chairperson who serves as a member of the BOD.

NCRAC functions in accordance with its *Operations Manual* which is periodically

amended and updated with BOD approval. It is an evolving document that has changed as the Center's history lengthens. It is used for the development of the cooperative regional aquaculture and extension projects that NCRAC funds.

ADMINISTRATIVE OPERATIONS

Since the inception of NCRAC on February 1, 1988, the role of the Administrative Center has been to provide all necessary support services to the BOD, IAC, TC, and project work groups for the North Central Region as well as representing the region on the NCC. As the scope of the NCRAC programs expand, this has entailed a greater work load and continued need for effective communication among all components of the Center and the aquaculture community.

The Center functions in the following manner.

- ▶ After BOD approval of Administrative Center costs, the Center submits a grant to USDA/NIFA/Grants Management Branch for approval. To date the Center has received 22 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), FY2008/09 (#2008-38500-19157), and FY2010/11 (#2010-38500-20929) with

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monies totaling \$17,686,202. Currently, three grants are active (FY07-11); the first 19 grants (FY88-06) have terminated.

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

Through August 31, 2011, the Center has funded or is funding 89 projects through 514 subcontracts from the first 22 grants received. Funding for these Center-supported projects is summarized in Table 1 below (pages 6–8). Information about

funded projects is also available at the Center's Web site (<http://www.ncrac.org>). During this reporting period, the Publications Office at ISU produced and distributed a number of publications including fact sheets, technical bulletins, and videos. A complete list of all publications from this office is included in the Appendix under Extension.

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

PROJECT REPORTING

As indicated in Table 1, NCRAC has funded a number of projects for many of the project areas it has selected for research and extension activities. For example, there have been thirteen separately funded projects in regard to Extension and nine 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

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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, 2010 to August 31, 2011. Projects, or Project components, that terminated prior to September 1, 2010 have

been reported on in earlier documents (e.g., 1989-1996 Compendium Report and other Annual Progress Reports).

A cumulative list of all publications, manuscripts, papers presented, or other outputs for all funded NCRAC project areas is contained in the Appendix.

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Table 1. North Central Regional Aquaculture Center funded projects.

| Project Area | Project Number | Proposed Duration Period | Funding Level | Grant Number | |
|--------------------------------|---|--------------------------|------------------------------|----------------------------|------------------|
| Aquaculture Drugs | 1 | 7/1/96-6/30/97 | \$27,000 | 95-38500-1410 | |
| | 2 | 12/1/96-11/30/97 | \$950 | 95-38500-1410 | |
| | 3 | 10/1/99-9/30/00 | \$8,415 | 97-38500-3957 | |
| | 4 | 6/1/04-11/30/05 | \$223,677 | 2003-38500-12995 | |
| | 5 | 7/15/04-7/14/05 | \$60,000 | 2003-38500-12995 | |
| | 6 | 11/1/04-10/31/06 | \$50,000 | 2002-38500-11752 | |
| | 7 | 1/1/06-12/31/06 | \$129,936 | 2005-38500-15847 | |
| | 8 | 9/1/08-8/31/10 | \$150,000 | 2008-38500-19157 | |
| | 9 | 9/1/09-8/31/10 | <u>\$27,880</u> \$677,858 | 2008-38500-19157 | |
| Baitfish | 1 | 9/1/92-8/31/94 | \$61,973 | 92-38500-6916 | |
| | 2 | 9/1/06-8/31/08 | \$111,997 | 2006-38500-16900 | |
| | | | <u>\$88,003</u> \$261,973 | 2005-38500-18547 | |
| Conferences/Workshops/Symposia | Environmental Strategies Symposium | 1 | 9/1/00-5/31/01 | \$5,000 | 96-38500-2631 |
| | | | | | |
| | Nat'l. Aquaculture Exten. Workshop/Conference | 1 | 10/1/91-9/30/92 | \$3,005 | 89-38500-4319 |
| | | 2 | 12/1/96-11/30/97 | \$3,700 | 95-38500-1410 |
| | | 3 | 11/1/02-10/31/03 | \$4,500 | 00-38500-8984 |
| | | 4 | 1/1/06-12/31/06 | \$5,000 | 2005-38500-18547 |
| | | 5 | 9/1/10-8/31/11 | <u>\$5,000</u> \$21,205 | 2008-38500-19157 |
| | NCR Aquaculture Conference | 1 | 6/1/90-3/31/91 | \$7,000 | 90-38500-5008 |
| | | 2 | 12/9/98-6/30/99 | <u>\$3,000</u> \$10,000 | 96-38500-2631 |
| | Percis III | 1 | 11/1/02-10/31/03 | \$4,000 | 00-38500-8984 |
| Crayfish | 1 | 9/1/92-8/31/94 | \$49,677 | 92-38500-6916 | |
| Economics/Marketing | 1 | 5/1/89-12/31/91 | \$127,338 | 88-38500-3885 | |
| | | | \$34,350 | 89-38500-4319 | |
| | 2 | 9/1/91-8/31/92 | \$53,300 | 91-38500-5900 | |
| | 3 | 9/1/93-8/31/95 | \$40,000 | 93-38500-8392 | |
| | 4 | 9/1/99-8/31/01 | \$47,916 | 97-38500-3957 | |
| | 5 | 9/1/03-8/31/04 | \$50,000 | 2002-38500-11752 | |
| | 6 | 9/1/10-8/31/11 | <u>\$23,565</u> \$376,469 | 2010-38500-20929 | |

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|--|-----------------|--------------------|------------------|------------------|
| Extension ("Base" Extension—Project Nos. 1-13; Aquaculture Regional Extension Facilitator [AREF]—Project No. 14; and Regional Aquaculture Extension Specialist [RAES]—Project Nos. 15 and 16) | 1 | 5/1/89-4/30/91 | \$39,221 | 88-38500-3885 |
| | 2 | 3/17/90-8/31/91 | \$37,089 | 89-38500-4319 |
| | 3 | 9/1/91-8/31/93 | \$31,300 | 89-38500-4319 |
| | 4 | 9/1/93-8/31/95 | \$94,109 | 91-38500-5900 |
| | 5 | 9/1/95-8/31/97 | \$110,129 | 91-38500-5900 |
| | 6 | 9/1/97-8/31/99 | \$10,813 | 92-38500-6916 |
| | 7 | 9/1/99-8/31/01 | \$20,391 | 95-38500-1410 |
| | 8 | 9/1/01-8/31/03 | \$38,000 | 97-38500-3957 |
| | 9 | 9/1/03-8/31/05 | \$94,000 | 99-38500-7376 |
| | 10 | 9/1/05-8/31/07 | \$28,500 | 99-38500-7376 |
| | 11 | 9/1/07-8/31/09 | \$18,154 | 2001-38500-10369 |
| | 12 | 9/1/08-8/31/10 | \$28,000 | 2002-38500-11752 |
| | 13 | 9/1/09-8/31/11 | \$211,545 | 2003-38500-12995 |
| | 14 | 9/1/03-8/31/05 | \$7,735 | 2005-38500-15847 |
| | 15 | 9/1/06-8/31/09 | \$21,850 | 2006-38500-16900 |
| | 16 | 9/1/09-8/31/11 | \$92,469 | 2007-38500-18469 |
| | | \$37,966 | 2007-38500-18469 | |
| | | \$22,539 | 2008-38500-19157 | |
| | | \$29,000 | 2008-38500-19157 | |
| | | \$100,000 | 2002-38500-11752 | |
| | | \$199,624 | 2004-38500-14269 | |
| | | \$150,000 | 2008-38500-19157 | |
| | | <u>\$1,422,434</u> | | |
| Hybrid Striped Bass | 1 | 5/1/89-8/31/91 | \$68,296 | 88-38500-3885 |
| | 2 | 6/1/90-8/31/92 | \$68,114 | 89-38500-4319 |
| | 3 | 9/1/91-8/31/93 | \$101,000 | 90-38500-5008 |
| | 4 | 9/1/93-8/31/95 | \$96,550 | 91-38500-5900 |
| | 5 | 9/1/95-8/31/97 | \$168,000 | 93-38500-8392 |
| | 6 | 6/1/99-5/31/00 | \$150,000 | 95-38500-1410 |
| | 7 | 9/1/01-5/31/04 | \$15,000 | 96-38500-2631 |
| | | \$98,043 | 98-38500-5863 | |
| | | <u>\$211,957</u> | 2001-38500-10369 | |
| | | \$976,960 | | |
| Largemouth Bass | 1 | 9/1/05-8/31/07 | \$170,000 | 2004-38500-14269 |
| National Coordinator for Aquaculture INADs/NADAs | 1 | 9/1/93-8/31/94 | \$2,000 | 89-38500-4319 |
| | | 5/15/95-5/14/96 | \$5,000 | 94-38500-0048 |
| | | 5/15/96-5/14/97 | \$6,669 | 92-38500-6916 |
| | | | \$3,331 | 95-38500-1410 |
| | | 5/15/97-5/14/98 | \$15,000 | 96-38500-2631 |
| | | 5/15/98-5/14/99 | \$13,241 | 94-38500-0048 |
| | | 5/15/99-5/14/00 | \$10,000 | 95-38500-1410 |
| | 2 | 7/15/04-7/14/05 | \$9,000 | 2003-38500-12995 |
| | | 9/15/05-8/31/06 | \$15,000 | 2004-38500-14269 |
| | | 9/1/06-8/31/08 | \$40,000 | 2006-38500-16900 |
| | 5/15/08-5/14/09 | <u>\$25,000</u> | 2007-28500-18469 | |
| | | \$144,241 | | |

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|--|---|-----------------|------------------|------------------|
| Nutrition/Diets | 1 | 9/1/04-8/31/06 | \$200,000 | 2002-38500-11752 |
| | 2 | 9/1/07-8/31/09 | \$80,000 | 2006-38500-16900 |
| | 3 | 9/1/09-8/31/11 | \$80,000 | 2008-38500-19157 |
| | 4 | 9/1/10-8/31/12 | \$43,363 | 2008-38500-19157 |
| | | | <u>\$81,037</u> | 2010-28500-20929 |
| | | | \$484,400 | |
| Other Feed Training Carnivorous Fish | 1 | 9/1/06-8/31/08 | \$165,446 | 2005-38500-15847 |
| | | | <u>\$134,554</u> | 2006-38500-16900 |
| | | | \$300,000 | |
| Snail Management/Grub Control | 1 | 9/1/07-8/31/09 | \$212,495 | 2007-38500-18469 |
| RAS Microbial Communities | 1 | 9/1/09-8/31/10 | \$65,000 | 2008-38500-19157 |
| Salmonids | 1 | 6/1/90-8/31/92 | \$9,000 | 89-38500-4319 |
| | | | \$120,799 | 90-38500-5008 |
| | 2 | 9/1/92-8/31/94 | \$149,997 | 92-38500-6916 |
| | 3 | 9/1/94-8/31/96 | \$199,290 | 94-38500-0048 |
| | 4 | 9/1/97-8/31/99 | <u>\$158,656</u> | 97-38500-3957 |
| | | \$637,742 | | |
| Sunfish | 1 | 6/1/90-8/31/92 | \$130,758 | 90-38500-5008 |
| | 2 | 9/1/92-8/31/94 | \$149,799 | 92-38500-6916 |
| | 3 | 9/1/94-8/31/96 | \$173,562 | 94-38500-0048 |
| | 4 | 9/1/96-9/31/98 | \$199,921 | 96-38500-2631 |
| | 5 | 9/1/99-8/31/01 | <u>\$199,748</u> | 99-38500-7376 |
| | | \$853,788 | | |
| Tilapia | 1 | 9/1/96-8/31/98 | \$118,791 | 96-38500-2631 |
| | 2 | 9/1/98-8/31/00 | <u>\$150,000</u> | 98-38500-5863 |
| | | \$268,791 | | |
| Viral Hemorrhagic Septicemia (VHS) | 1 | 9/1/08-8/31/10 | \$197,960 | 2008-38500-19157 |
| Walleye | 1 | 5/1/89-8/31/91 | \$177,517 | 89-38500-4319 |
| | 2 | 6/1/90-8/31/92 | \$111,657 | 90-38500-5008 |
| | 3 | 9/1/91-8/31/92 | \$109,223 | 91-38500-5900 |
| | 4 | 9/1/92-8/31/93 | \$75,000 | 89-38500-4319 |
| | 5 | 9/1/93-8/31/95 | \$150,000 | 93-38500-8392 |
| | 6 | 9/1/95-8/31/97 | \$117,395 | 94-38500-0048 |
| | | | \$59,835 | 95-38500-1410 |
| | 7 | 9/1/99-6/30/02 | <u>\$127,000</u> | 98-38500-5863 |
| | | \$927,627 | | |
| Wastes/Effluents | 1 | 9/1/92-8/31/94 | \$153,300 | 92-38500-6916 |
| | 2 | 9/1/96-8/31/98 | \$100,000 | 96-38500-2631 |
| | 3 | 9/1/01-8/31/04 | \$106,186 | 00-38500-8984 |
| | | <u>\$88,814</u> | 2001-38500-10369 | |
| | | \$448,300 | | |
| White Papers | 1 | 7/1/98-12/31/98 | \$4,999 | 96-38500-2631 |
| | 2 | 9/1/99-12/31/99 | <u>\$17,495</u> | 97-38500-3957 |
| | | \$22,494 | | |

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|--------------|---|----------------|------------------|------------------|
| Yellow Perch | 1 | 5/1/89-8/31/91 | \$76,957 | 88-38500-3885 |
| | | | \$85,723 | 89-38500-4319 |
| | 2 | 6/1/90-8/31/92 | \$92,108 | 90-38500-5008 |
| | 3 | 9/1/91-8/31/93 | \$99,997 | 91-38500-5900 |
| | 4 | 9/1/93-8/31/95 | \$150,000 | 93-38500-8392 |
| | 5 | 9/1/95-8/31/97 | \$199,507 | 95-38500-1410 |
| | 6 | 9/1/97-8/31/99 | \$185,458 | 97-38500-3957 |
| | 7 | 9/1/98-8/31/00 | \$92,370 | 98-38500-5863 |
| | 8 | 9/1/01-5/31/04 | \$326,730 | 00-38500-8984 |
| | | \$125,016 | 2001-38500-10369 | |
| | 9 | 9/1/10-8/31/13 | <u>\$150,000</u> | 2010-38500-20929 |
| | | | \$1,583,866 | |

NORTH CENTRAL REGIONAL AQUACULTURE CENTER

PROJECT REPORTS

NORTH CENTRAL REGIONAL AQUACULTURE CENTER

AQUACULTURE DRUGS: EFFECTIVENESS RESEARCH LEADING TO APPROVALS FOR CONTROLLING MORTALITY IN COOLWATER AND WARMWATER FINFISH DUE TO AEROMONAD INFECTIONS WITH TERRAMYCIN 200 FOR FISH® (OXYTETRACYCLINE DIHYDRATE) AND AQUIFLOR® (FLORFENICOL)¹

Project *Progress Report* for the Period
September 1, 2008 to August 31, 2011

NCRAC FUNDING: \$95,000 (September 1, 2008 to August 31, 2011)

PARTICIPANT:

Mark P. Gaikowski Upper Midwest Environmental Sciences Center Wisconsin

Industry Advisory Council Liaison:

Mark Willows North American Fish Farmers Cooperative North Dakota

PROJECT OBJECTIVES

- | | |
|--|---|
| <p>(1) Identify the etiologic agent (<i>Aeromonas</i> spp.) from isolates collected from disease outbreaks in the NCR and characterize the disease syndrome before conducting any effectiveness studies.</p> <p>(2) Have active, established Investigational New Animal Drug (INAD) exemptions or work with the sponsors of publicly disclosable INADs for Terramycin 200 for Fish® and Aquaflor®.</p> | <p>(3) Develop draft pivotal effectiveness study protocols with the concurrence of the two drug sponsors (Phibro Animal Health=PAH for Terramycin 200 for Fish® and Schering-Plough Animal Health=SPAH for Aquaflor®).</p> <p>(4) Submit the draft pivotal effectiveness study protocols through established INADs for Terramycin 200 for Fish® and Aquaflor® for protocol concurrence from CVM before beginning the effectiveness studies.</p> |
|--|---|

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

NORTH CENTRAL REGIONAL AQUACULTURE CENTER

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

ANTICIPATED BENEFITS

Disease constitutes the largest single cause of economic losses in aquaculture as represented by some investigators. There are few treatments available for current and emerging aquaculture diseases. The control of mesophilic or motile *Aeromonas* infections (MAI) is extremely relevant to the aquaculture industry in the North Central

Region (NCR) as it has experienced a loss of income in commercially important food fish species and baitfish. These economic losses result directly from fish mortality due to MAI and from opportunistic secondary infections, and indirectly because of unappealing visual appearance of food fish with gross external lesions.

Both Terramycin 200 For Fish® (oxytetracycline dihydrate) and Aquaflor® (florfenicol) have been shown to be effective against a wide variety of Gram-negative bacterial pathogens of fish including certain *Aeromonas* spp. (e.g. *A. salmonicida*). It is likely that one or both of these antibacterials will effectively reduce mortality associated with motile *Aeromonas* septicemia (MAS) in coolwater and warmwater fish. This research will provide valuable information to commercial and public fish culturists and enable them to effectively reduce production loss in cool- and warmwater fish caused by *Aeromonas* species.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

Etiologic Agent

Observations of clinical signs and gross necropsy were performed on muskellunge and tilapia which exhibited mortality and clinical signs of MAS at two separate NCR fish culture facilities. Clinical signs noted included hemorrhages, ulcerative skin lesions and excess ascetic fluid. At both facilities, the kidney was observed to have discoloration or changes in the overall texture. All observations made were consistent with those previously reported from outbreaks of various motile *Aeromonas* species.

Microbiological samples were obtained from diseased (moribund) fish exhibiting clinical signs of MAS and inoculated onto Tryptic

AQUACULTURE DRUGS

Soy Agar (TSA) plates and incubated at 30°C (86°F) for 24 h. Creamy to tan, round, shiny colonies were inoculated onto a separate TSA plate to obtain pure cultures. Rimler-Shotts (RS) plates were inoculated with a single colony of a pure culture and incubated at 30°C (86°F) for 24 h. If yellow colony growth occurred, then cultures were inoculated onto a BBL Crystal™ for identification. Identifications made from isolates obtained from NCR facilities are listed in Table 1.

A third isolate was obtained from walleye exhibiting mortality potentially characteristic of MAS at another NCR fish culture facility. Clinical signs were not obtained as the isolate was submitted directly to the U.S. Fish and Wildlife Service La Crosse Fish Health Center for diagnosis. Identification was accomplished as previously described and is provided in Table 1.

Three additional isolates were obtained from channel catfish exhibiting clinical signs and mortality indicative of MAS. Though outside of the NCR, these isolates were collected from what appears to be a highly virulent strain of *A. hydrophila* which could readily cause mortality in the NCR. Clinical observations provided by the collecting pathologist were consistent with those

previously reported for MAS. Identification was accomplished as previously described and is provided in Table 1.

Characterize the Disease Syndrome

Challenge trials began in October 2009 to characterize the disease syndrome. Five isolates were used during each challenge trial, with two species each of cool and warmwater fish. Mortality, morbidity, and clinical signs were observed for 14 days after challenge initiation. Samples were collected from mortalities to confirm infection. Isolates were also tested to determine sensitivity to oxytetracycline dihydrate and florfenicol.

Progress in Year 1 was delayed because there were few public or private aquaculture facilities that had outbreaks of MAI.

OBJECTIVE 2

The Upper Midwest Environmental Sciences Center (UMESC) currently has an established INAD for both Aquaflor® and Terramycin 200 for Fish®.

OBJECTIVE 3

Because there were few outbreaks of MAI in Year 1, development of the effectiveness protocol was delayed. A protocol titled “Field effectiveness of Aquaflor® (florfenicol) and Terramycin 200 For Fish®

Table 1. Species of origin, year, and details of aeromonad isolates obtained for study.

| Isolate | Source species | Year | Details | Source |
|---------|-----------------|------|--------------------------------|------------------------------------|
| 1 | Channel catfish | 2007 | <i>A. hydrophila</i> | University of Arkansas, Pine Bluff |
| 2 | Tilapia | 2009 | <i>A. veronii</i> | Private fish farm (NCR) |
| 3 | Muskellunge | 2009 | <i>A. sobria/A. veronii</i> | Spirit Lake Fish Hatchery (NCR) |
| 4 | Walleye | 2009 | <i>A. hydrophila</i> | Rathbun State Fish Hatchery (NCR) |
| 5 | Goldfish | 2005 | <i>A. hydrophila/A. caviae</i> | Wisconsin Vet Diagnostic Lab |
| 6 | Channel catfish | 2009 | <i>A. hydrophila</i> | Auburn University |
| 7 | Channel catfish | 2009 | <i>A. hydrophila</i> | Auburn University |
| 8 | Channel catfish | 2009 | <i>A. hydrophila</i> | Auburn University |
| 9 | Channel catfish | 2007 | <i>Aeromonas</i> spp. | Mississippi State University |

NORTH CENTRAL REGIONAL AQUACULTURE CENTER

(oxytetracycline dihydrate) to control mortality in coolwater and warmwater finfish due to Motile Aeromonad infections” was submitted to the U.S. Food and Drug Administration’s Center for Veterinary Medicine (CVM) in October 2009.

OBJECTIVE 4

In Year 2, a protocol was submitted through established INADs for Terramycin 200 for Fish® and Aquaflor® titled “Field effectiveness of Aquaflor® (florfenicol) and Terramycin 200 For Fish® (oxytetracycline dihydrate) to control mortality in coolwater and warmwater finfish due to Motile Aeromonad infections” on February 4, 2010 to CVM for concurrence. A stop review request was submitted by UMESC to allow CVM to review an H-submission (prepared by UMESC and submitted on July 6, 2010). The H-submission summarized literature and data to support the current protocol and requested CVM to consider infection by any motile aeromonad species to be a potential cause of MAI. The H submission is complete and the protocol was revised and re-submitted for CVM concurrence in Year 3.

In Year 3, the protocol titled “Field effectiveness of Aquaflor® (florfenicol) and Terramycin 200 For Fish® (oxytetracycline dihydrate) to control mortality in coolwater and warmwater finfish due to Motile Aeromonad infections” was submitted to CVM after review of the H submission described above. Protocol concurrence was received on June 2, 2011. CVM provided protocol concurrence with a protocol titled “Identification of motile aeromonads isolated from Study Number AEH-09-MAS-02” on June 17, 2011. The “Identification of motile aeromonads isolated from Study Number AEH-09-MAS-02” protocol will be used to identify to species the motile

aeromonad species isolated in the pivotal effectiveness field trials.

OBJECTIVE 5

A pivotal effectiveness field study was conducted at the Spirit Lake Fish Hatchery, Spirit Lake, IA with juvenile muskellunge which exhibited signs of a motile aeromonad infection. The pivotal field trial is now complete and data generated during the trial are being summarized for inclusion in a final study report.

WORK PLANNED

OBJECTIVE 5

Conduct two additional pivotal effectiveness studies on Terramycin 200 for Fish® and Aquaflor® according to Good Clinical Practice regulations and the CVM-concurred protocols.

OBJECTIVE 6

Effectiveness data will be analyzed and draft final study reports will be prepared for Terramycin 200 for Fish® and Aquaflor® no more than four months after the studies are completed.

OBJECTIVE 7

The respective draft study reports will be submitted to PAH and SPAH for their review.

OBJECTIVE 8

Submit the final study reports through established INADs for Terramycin 200 for Fish® and Aquaflor® to CVM for acceptance no more than two months after PAH and SPAH have completed their reviews of the draft study reports.

OBJECTIVE 9

Ensure that all questions and concerns about the final study reports are answered no more than one month after receiving comments from CVM.

AQUACULTURE DRUGS

OBJECTIVE 10

If CVM accepts the data as proving effectiveness for the aeromonad infections encountered in the NCR, provide the acceptance letter and effectiveness studies to PAH and SPAH so that they can pursue supplemental new animal drug application (NADA) approvals for their respective drug products.

approvals by CVM for either, or both, Terramycin 200 for Fish® (oxytetracycline dehydrate) and Aquiflor® (florfenicol), which, if approved, would allow aquaculturists the use of these antibacterials to reduce mortality associated with MAS in coolwater and warmwater fish.

IMPACTS

The effectiveness studies of this project should lead to supplemental NADA

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

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

SUPPORT

| YEAR | NCRAC- USDA FUNDING | OTHER SUPPORT | | | | | TOTAL SUPPORT |
|--------------|---------------------------|-----------------|----------|------------------|-------|---------|------------------|
| | | UNIVER- SITY | INDUSTRY | OTHER FEDERAL | OTHER | TOTAL | |
| 2008-09 | \$37,000 | | | \$2,600 | | \$2,600 | \$39,600 |
| 2009-10 | \$28,000 | | | | | | \$28,000 |
| 2010-11 | \$30,000 | | \$3500 | \$20,000 | \$200 | | \$53,700 |
| TOTAL | \$95,000 | | | \$22,600 | \$200 | \$2,600 | \$121,300 |

NORTH CENTRAL REGIONAL AQUACULTURE CENTER

AQUACULTURE DRUGS: DRUG APPROVAL RESEARCH ON 17 α -METHYLTESTOSTERONE (OFFICIAL TRANSFER OF 17 α - METHYLTESTOSTERONE [MT] ANALYTICAL METHOD FOR FEED)²

Project *Progress Report* for the Period
September 1, 2009 to August 31, 2011

NCRAC FUNDING: \$27,880 (September 1, 2009 to August 31, 2010)

PARTICIPANTS:

| | | |
|---|---|--------------|
| Mark Gaikowski | Upper Midwest Environmental Sciences Center | Wisconsin |
| Nilmini Wijewickreme | Maxxam Analytics [formerly CANTEST Ltd.] | B.C., Canada |
| <i>Industry Advisory Council Liaison:</i> | | |
| Mark Willows | Binford Eagle Fisheries | North Dakota |
| <i>Extension Liaison:</i> | | |
| Kevin Fitzsimmons | University of Arizona | Arizona |

PROJECT OBJECTIVES

- | | |
|---|---|
| (1) Develop study protocols to conduct the MT feed method transfer of the MT analytical feed method. | (5) Assay control and medicated feed samples according to the study protocols concurred with by CVM. |
| (2) Submit method transfer study protocols to the Center for Veterinary Medicine (CVM) for concurrence. | (6) Complete report of analysis and submit along with raw data to the Upper Midwest Environmental Sciences Center (UMESC). |
| (3) Provide final study protocols to participating laboratories. | (7) Compare and discuss the results of both the CANTEST Ltd. (CANTEST) reference (expert) and transferred (naïve) analyses of the MT transfer study samples based on the MT analytical feed method developed by the University of Wisconsin-Madison (UW-Madison). |
| (4) Prepare and ship medicated feed to participating laboratories. | |

² NCRAC has funded nine Aquaculture Drugs projects. This Progress Report is for the ninth Aquaculture Drugs project. It is a 1-year funded project that began January 1, 2009. A Termination Report for the first project is contained in the 1997-98 Annual Progress Report; a Termination Report for the second project is contained in the 1996-97 Annual Progress Report, a Termination Report for the third project is contained in the 2001-02 Annual Progress Report, a Termination Report for the fourth project is contained in the 2006-07 Annual Progress Report, and Termination Reports for the sixth and seventh projects are contained in the 2007-08 Annual Progress Report. A fifth project, which provided \$60,000 for a portion of the funds required to purchase sufficient radiolabeled AQUIS[®] for use in a total residue depletion study in rainbow trout, is reported on under the Termination Report for the National Coordinator for Aquaculture New Animal Drug Applications (NADAs) in the 2008-09 Annual Progress Report. A Progress Report for the eighth project is contained elsewhere in this report.

NORTH CENTRAL REGIONAL AQUACULTURE CENTER

- (8) Determine whether any changes are needed to the MT analytical feed method developed by UW-Madison based on the results of the MT feed transfer study.
- (9) Validate that the naïve analyst at CANTEST can analyze the MT feed samples according to the analytical feed method developed by UW-Madison.
- (10) Compile Final Study Report (FSR), archive raw data, and submit FSR to CVM through the UMESC MT investigational new animal drug (INAD) exemption.
- (11) Respond to CVM comments.
- (12) Gain acceptance from CVM for the MT feed method transfer study.

ANTICIPATED BENEFITS

The results from this project will directly affect the potential for approval of MT by the U.S. Food and Drug Administration's Center for Veterinary Medicine (CVM). The data from this study, if accepted by CVM, support the potential approval of MT-medicated feed for use in tilapia. MT-medicated feed is used to produce greater than 80% phenotypic male populations, a significant benefit to U.S. producers because male tilapia generate more biomass with less effort in less time making them more cost efficient to raise.

PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

A study protocol was developed to conduct the work for a method transfer trial for the analytical method to determine MT concentrations in fish feed.

OBJECTIVE 2

The study protocol was submitted to CVM for review. The protocol was returned with

review comments which were used to revise the protocol to a final draft.

OBJECTIVE 3

The final protocol was provided to Maxxam Analytics (formerly CANTEST; Burnaby, British Columbia, Canada), the company providing the reference and participating laboratories for the work.

OBJECTIVE 4

Control (non-medicated) tilapia feed was prepared at Rangen Inc. The control feed was used to prepare MT-medicated feed at Rangen Inc. Control and MT-medicated feed were shipped from Rangen Inc. to UMESC.

A feed production report was prepared and submitted by UMESC to Rangen Inc. Rangen Inc. submitted the feed production report to Rangen's confidential INAD authorization file.

Control feed samples were shipped from UMESC to Maxxam Analytics to initiate the method familiarization phase of the study.

OBJECTIVE 5

Participating laboratory analysts (analysts with no previous experience performing the method) were involved in a method familiarization analysis session to ensure the participating laboratory analysts could successfully perform the method to determine MT concentrations in feed before analyzing feed samples for the method transfer phase of the study. Control feed samples shipped by UMESC to Maxxam Analytics were stored at $-20 \pm 10^{\circ}\text{C}$. Eight 5 ± 0.1 g of control un-medicated feed samples were weighed. Two samples were not fortified (control samples). The remaining samples were fortified with an appropriate volume of $1,000 \mu\text{g/g}$ MT stock standard to obtain matrix equivalent MT

AQUACULTURE DRUGS

concentrations of 30, 60, and 90 µg/g. Duplicates were analyzed for each fortification level.

Participating laboratory analysts demonstrated that there was no MT interference from matrix constituents in control feed. Analysts met the acceptance criteria of <15 µg/g of MT equivalent interference in the control feed extract.

Participating laboratory analysts obtained mean percent recoveries of 87.6, 93.8, and 101.6% from samples fortified with MT at 30, 60, and 90 µg/g, respectively. These data were within the method acceptable percent recovery range (>80% and <110%). Based on the results obtained during the method familiarization phase, participating laboratory analysts were successful performing the method for determining MT concentrations in feed.

Thereafter, UMESC shipped to Maxxam Analytics control and MT-medicated feed to be used in the method transfer phase of the study. During the method transfer phase, the participating laboratory analysts were blinded from the identity of the samples they processed and the results from the phase determined if the method could be accurately and precisely used by naïve analysts to determine MT concentrations in fish feed.

Control and medicated feed samples shipped by UMESC to Maxxam Analytics were stored at $-20 \pm 10^{\circ}\text{C}$. Forty control feed samples were weighed. Ten control samples were not fortified (control samples). The remaining control feed samples were fortified with an appropriate volume of 1,000 µg/mL MT stock standard to obtain matrix equivalent MT concentrations of 30, 60, and 90 µg/g (10 samples/concentration). Ten samples of MT-medicated feed were

weighed from each of two MT-medicated feed batches (expected MT concentration of 60 µg/g). The reference laboratory analysts (analysts with previous experience performing the method) processed five control samples, five samples from each fortification level, and five samples from each batch of MT-medicated feed. The participating laboratory analysts processed the same list of samples.

OBJECTIVE 6

Reference and participating laboratory results from the method transfer phase were submitted by Maxxam Analytics to UMESC for review.

OBJECTIVE 7

The method transfer phase results from the reference and participating laboratories were compared. Participating laboratory analysts demonstrated the matrix equivalent MT concentrations in the control feed met the acceptance criteria of <15 µg/g of MT equivalent interference in the control feed extract. In comparison, reference laboratory analysts also demonstrated the matrix equivalent MT concentrations in the control feed met the acceptance criteria of <15 µg/g of MT equivalent interference in the control feed extract.

Participating laboratory analysts obtained mean percent recoveries of 97.6, 99.5, and 109.7% from samples fortified to obtain matrix equivalent MT concentrations of 30, 60, and 90 µg/g, respectively. These data were within the method acceptable percent recovery range (>80% and <110%). In comparison, reference laboratory analysts obtained mean percent recoveries of 81.3, 77.2, and 73.4% from samples fortified to obtain matrix equivalent MT concentrations of 30, 60, and 90 µg/g, respectively. Two of the three mean recoveries were not within

NORTH CENTRAL REGIONAL AQUACULTURE CENTER

the method acceptable percent recovery criteria.

Participating laboratory analysts obtained a mean matrix equivalent MT concentration in medicated feed Batch 1 of 58.9 µg/g with a method precision of 9.6% (% relative standard deviation) and a mean matrix equivalent MT concentration in Batch 2 of 60.9 µg/g with a method precision of 6.5%. In comparison, reference laboratory analysts obtained a mean matrix equivalent MT concentration in medicated feed Batch 1 of 43.0 µg/g with a method precision of 8.5% and a mean matrix equivalent MT concentration in Batch 2 of 42.8 µg/g with a method precision of 11%.

OBJECTIVE 8

An investigation was undertaken to determine the cause of failure of the reference laboratory analysts to obtain mean method recoveries from fortified samples in the acceptable range. As a result of the investigation, it was determined that the participating laboratory analysts made slight modifications to the method. Because the method percent recovery data obtained by the participating laboratory analysts were within the method's acceptable range, the modifications were used to revise the existing method.

OBJECTIVE 9

The revised method was used by reference and participating laboratory analysts to process the sample sets described in *OBJECTIVE 5* for the method transfer phase of the study.

Using the revised method, participating and reference laboratory analysts demonstrated the matrix equivalent MT concentrations in the control feed met the acceptance criteria of <15 µg/g of MT equivalent interference in the control feed extract.

Using the revised method, participating laboratory analysts obtained mean percent recoveries of 96.9, 104.0, and 105.0% from samples fortified to obtain matrix equivalent MT concentrations of 30, 60, and 90 µg/g, respectively. These data were within the method acceptable percent recovery range. In comparison, reference laboratory analysts obtained mean percent recoveries of 86.9, 86.5, and 84.9% from samples fortified to obtain matrix equivalent MT concentrations of 30, 60, and 90 µg/g, respectively. These data were also within the method acceptable percent recovery range.

Using the revised method, participating laboratory analysts obtained a mean matrix equivalent MT concentration in medicated feed Batch 1 of 54.3 µg/g with a method precision of 1.7% and a mean matrix equivalent MT concentration in Batch 2 of 58.6 µg/g with a method precision of 8.4%. In comparison, reference laboratory analysts obtained a mean matrix equivalent MT concentration in medicated feed Batch 1 of 47.1 µg/g with a method precision of 2.3% and a mean matrix equivalent MT concentration in Batch 2 of 49.1 µg/g with a method precision of 1.9%.

OBJECTIVE 10

The reference laboratory and the participating laboratory submitted to UMESC final reports describing the results from the processing of sample sets described in *OBJECTIVE 5* for the method transfer phase of the study. UMESC included those reports in a comprehensive final report. UMESC archived all the raw data, submitted to INAD I-011395 the comprehensive final report, and requested CVM to review the report.

OBJECTIVE 11

The following statement was in a letter UMESC received from CVM on June 21,

AQUACULTURE DRUGS

2011: “We incomplete your phased investigational new animal drug submission for the proposed MASCULINIZING FEED FOR TILAPIA (17 α -methyltestosterone) Type C Medicated dated December 17, 2010.” UMESC efforts to address the review responses are ongoing.

WORK PLANNED

OBJECTIVE 11

UMESC will review with CVM their final report review comments.

OBJECTIVE 12

UMESC will work with CVM to develop a corrective course of action. If necessary, the final report will be revised. The revised report will be submitted to CVM and UMESC will request that the method transfer study data described in the final study report be accepted.

IMPACTS

Legal use of MT in the U.S. is dependent on CVM approval. Approval is contingent on providing data that will fulfill their data requirements. One of the outstanding data requirements is a method transfer trial where a laboratory naïve to the method for determining MT concentrations in feed must

adequately perform the method. The results from this work should fulfill the outstanding data requirement and have a direct effect on the potential for MT approval.

Tilapia is the fifth most consumed seafood in the United States. The approval of MT-medicated feed for use in tilapia to produce greater than 80% phenotypic male populations would be of significant benefit to the U.S. producers. Male tilapia generate more biomass with less effort in less time making them more cost efficient to raise. Approval of MT will provide advantages for those producers who currently do not have the space, time, or money to produce genetically male tilapia populations. The production of male tilapia populations is critical to the U.S. tilapia industry if producers are to remain competitive with foreign tilapia producers.

SUPPORT

NCRAC has provided \$27,880 which is the entire amount allocated for this 1-year project.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

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

NORTH CENTRAL REGIONAL AQUACULTURE CENTER

5th NATIONAL AQUACULTURE EXTENSION WORKSHOP/CONFERENCE³

Project *Termination Report* for the Period
September 1, 2010 to August 31, 2011

NCRAC FUNDING LEVEL: \$5,000 (September 1, 2010 to August 31, 2011)

PARTICIPANTS:

| | | |
|-------------------------------------|---|------------------|
| Jimmy Avery | Mississippi State University | Mississippi |
| <i>National Steering Committee:</i> | | |
| Ron Blair | University of Tennessee Extension | Tennessee |
| Gef Flimlin | Rutgers Cooperative Extension | New Jersey |
| Gary Fornshell | University of Idaho Extension | Idaho |
| David Heikes | University of Arkansas Pine Bluff | Arkansas |
| Gary Jensen | USDA National Institute of Food and Agriculture (NIFA) | Washington, D.C. |
| Gene Kim | NOAA National Sea Grant | Maryland |
| Ron Kinnunen | Michigan State University | Michigan |
| Max Mayeaux | USDA National Institute of Food and Agriculture (NIFA) | Washington, D.C. |
| Paul Olin | University of California-San Diego | California |
| Robert Romaine | Louisiana State University | Louisiana |
| Tetsuzan Benny Ron | University of Hawaii | Hawaii |
| Jeff Silverstein | USDA Agricultural Research Service | Washington, D.C. |
| Nathan Stone | University of Arkansas Pine Bluff | Arkansas |
| Laura Tiu | Ohio State University | Ohio |
| Craig Tucker | Mississippi State University | Mississippi |
| Marc Turano | North Carolina State University | North Carolina |
| Forrest Wynne | Kentucky State University | Kentucky |

REASON FOR TERMINATION

The project objectives were completed.

(2) Demonstrate and conduct hands-on experience with state-of-the-art computer applications for improving delivery of extension programs.

PROJECT OBJECTIVES

(1) Learn successful approaches to problem-solving through case studies that can be replicated in other states, i.e., lessons learned.

(3) Identify national extension priorities and critical issues with development of corresponding action plans for implementation.

³NCRAC has provided funding along with the four other Regional Aquaculture Centers for five national aquaculture extension meetings; the first was called a National Aquaculture Extension Workshop whereas the second through fifth were called National Aquaculture Extension Conferences. This termination report is for the fifth meeting, which was held June 5-7, 2011 in Memphis, Tennessee.

NORTH CENTRAL REGIONAL AQUACULTURE CENTER

- (4) Identify potential interregional extension projects, such as curriculum development or national decision support databases.
- (5) Share educational materials and programs in addition to expertise.
- (6) Strengthen regional and national communication networks to improve services to clientele.
- (7) Examine successful extension components and outcomes to research projects and develop approaches to improve integration nationwide.
- (8) Develop a collective strategy to define extension's role in measuring impacts of Regional Aquaculture Center (RAC) projects and collaboration with others in academia and private sector.
- (9) Improve business management skills related to aquaculture and enhance knowledge concerning marketing of aquatic products.

PRINCIPAL ACCOMPLISHMENTS

The 5th National Aquaculture Extension Conference was held June 5-7, 2011 in Memphis, Tennessee and built upon the previous conferences held in March 1992 in Little Rock, Arkansas; April 1997 in Annapolis, Maryland; April 2003 in Tucson, Arizona; and April 2007 in Cincinnati, Ohio. This conference is the only opportunity for extension aquaculture professionals nationwide to plan, organize, implement, and evaluate a professional growth and enhancement program that aims to improve personal performance and effectiveness. As programs undergo scrutiny and downsizing, and seeing as how extension capabilities in aquaculture vary across the nation,

investment in human capital is of enormous benefit. As more university faculty are asked to address multiple responsibilities of extension, teaching, and research functions, effectiveness becomes increasingly important.

The conference provided participants new contacts, knowledge about new topics, information about new tools to enhance productivity, appreciation of experience and perspectives on issues, a chance to replicate model programs, gain in expanding professional networks, opportunities to contribute to the development of others, insights into participating in regional and national initiatives, and growth in skills development relevant to responsibilities. The conference provided a forum for professionals from the Sea Grant Marine Advisory Service, the Cooperative Extension Service, and other outreach programs to seek collaboration for mutual benefit to the aquaculture industry and the public.

Despite the tough economic times at universities, the conference had an excellent turnout. There were 108 participants representing 38 states, the District of Columbia, and Micronesia. Participants represented both Land Grant universities as well as NOAA Sea Grant programs from across the United States.

Given the shortened agenda compared to previous conferences, this was a very efficient conference in terms of shared information. There were 49 oral presentations by speakers and panel members as well as 34 poster presentations.

Roger Barlow, President of The Catfish Institute and Executive V.P. of Catfish Farmers of America, gave a presentation on the recent challenges to the U.S. farm-raised

NATIONAL AQUACULTURE EXTENSION CONFERENCE

catfish industry. Robert Rheault, East Coast Shellfish Growers Association, made a presentation on the value of growers associations and how Extension can assist in their formation.

Meryl Broussard, Deputy Director, Agriculture and Natural Resources, USDA NIFA, and Gary Jensen, National Program Leader for Aquaculture, USDA NIFA, spoke about opportunities, trends, and the future outlook for Extension. Michael Rice, NOAA Aquaculture Science Coordinator, and Gene Kim, NOAA Aquaculture National Program Leader, discussed coordination and Sea Grant Extension programming.

Additional plenary themes on Day 1 included Extension partnerships with federal research laboratories, trends and outlook for Extension participation in the Regional Aquaculture Centers, the increasing role of Extension in federal programs targeting aquaculture, and Extension's role in combating misconceptions about aquaculture.

The freshwater concurrent session on Day 2 focused on aquatic plants, economic tools for struggling producers, emerging technologies for finfish production, and an introduction to eXtension and Aquaculture Communities of Practice. The marine concurrent session included discussions on case studies in marine Extension, Extension's role in expanding markets for aquaculture products, legal issues and informational resources, input into Sea Grant aquaculture research, and a review of new aquaculture Extension projects.

Professional development took center stage in Day 2's plenary sessions with discussions on promotion and tenure issues and the use of communication and information

technology tools. The conference wrapped up with a panel of Extension specialists discussing case studies of economic development.

Resource Sharing

Conference planners were vitally interested in each participant having the opportunity to share information concerning successful programs and projects. Due to the limited number of oral presentation slots, conference participants were encouraged to submit a poster presentation. Each speaker and poster presenter was required to provide an extended abstract for the conference proceedings. All participants were asked to bring resource materials that were not available over the internet. Tables were provided in the meeting area for participants to display these materials. The extended abstracts, attendees contact information, and resource materials (newsletters, production manuals, promotional pieces, and program descriptions) were provided on electronic media devices.

Evaluation

A post-conference questionnaire was used to collect input from participants to identify strengths and weaknesses and to solicit ideas to improve planning for subsequent conferences. Respondents were asked to rate the usefulness of sessions on a scale of 1 to 5 with 1 being low and 5 being highly useful. Additional question required only "Yes" or "No" responses.

Value

In order to reduce travel costs, this year's conference was shortened from 4 days to 2 days. When asked to rate the benefits of the program considering time/travel cost, 80% of respondents ranked it as moderately high (4) to high (5). When asked to rate the overall value, 75% ranked it as moderately high (4) to high (5). Concerning future

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conferences, 89% indicated that they would attend future conferences and 96% indicated that they would recommend other Extension personnel to attend.

Benefit to Participants

Due to shrinking state and federal budgets, there has been increasing emphasis by funding agencies for conference participants to show increased knowledge concerning funding opportunities or direct benefits to clientele resulting from conferences of this type. However, participants indicated that establishing contacts was still a very important benefit of this conference. When asked what they gained from the program, the number one response (97%) was “Names of other people to contact.” The number two response (65%) dealt with “Resource materials to use.” Additional stated benefits included:

- Link to National Law Library
- New communication strategies
- Information on aquatic herbicides
- Collaboration opportunities
- Insight into funding problems at USDA

Benefit to Clientele

When asked about the benefits to clientele directly related to attendance of this (and past) conference(s), the top two responses were (1) “able to use new contacts to help solve local problems” and (2) “gained awareness of information resources available from others.” Additional stated benefits included:

- Being able to help farmers access new tools and information

- New approaches to problem solving
- Knowledge of the economic tools provided
- Increased knowledge of funding opportunities that can benefit producers

IMPACTS

- There were 108 participants representing 38 states, the District of Columbia, and Micronesia. Participants represented both Land Grant universities as well as NOAA Sea Grant programs from across the United States.
- The top three responses to what was gained from the conference were (1) contacts, (2) resource materials, and (3) a better understanding of Regional Aquaculture Center and Sea Grant programs.
- Concerning future conferences, 89% indicated that they would attend future conferences and 96% indicated that they would recommend other Extension personnel to attend.

RECOMMENDED FOLLOW-UP ACTIVITIES

Attendees will be sent a post-conference. The location for the next conference has not yet been determined.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

See the Appendix for a cumulative output for all NCRAC-funded National Aquaculture Extension Workshops/Conferences.

NATIONAL AQUACULTURE EXTENSION CONFERENCE

SUPPORT

| YEARS | NCRAC- USDA FUNDING | OTHER SUPPORT | | | | | TOTAL SUPPORT |
|--------------|------------------------------------|-------------------------|-----------------|--------------------------------------|--------------|--------------|--------------------------|
| | | UNIVER- SITY | INDUSTRY | OTHER FEDERAL^a | OTHER | TOTAL | |
| 2010-11 | \$5,000 | | | \$6,500 | | \$11,500 | \$11,500 |
| TOTAL | \$5,000 | | | \$6,500 | | \$11,500 | \$11,500 |

Southern Regional Aquaculture Center contributed \$5,000 and Center for Tropical and Subtropical Aquaculture contributed \$1,500. Individual travel grants were supported by several RACs and the NOAA National Sea Grant College Program.

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NCRAC AND MARKETMAKER™⁴

Project *Progress Report* for the Period
September 1, 2010 to August 31, 2011

NCRAC FUNDING: \$23,565 (September 1, 2010 to August 31, 2011)

PARTICIPANTS:

| | | |
|--|-----------------------|------|
| Joseph E. Morris | Iowa State University | Iowa |
| <i>Extension Liaison:</i> Richard Clayton | Iowa State University | Iowa |

PROJECT OBJECTIVES

- (1) Conduct a survey of all North Central Region (NCR) aquaculture producers for data that will be assimilated into the MarketMaker™ system.
- (2) Undertake outreach activities to educate and register NCR producers into the MarketMaker™ system.
- (3) Develop a “how to” tutorial case study tool that will instruct NCR producers on how to conduct market research using the MarketMaker™ system.

ANTICIPATED BENEFITS

This project will result in producer to consumer value-chain visibility that will immediately begin to address all of the five stated goals of the NCRAC program:

- Develop transferable (marketing/outreach)* technology to enable producers to be profitable;
- Disseminate relevant educational materials to achieve profitable margins of operation (through increased market

exposure, visibility, outreach and delivery efficiency);

- Engage in research (cooperative market-research and outreach initiative that incorporates detailed industry value-chain data) partnerships between industry, universities, and public agencies;
- Deliver demonstrations and regular aquaculture extension programs (with respect to Market-Maker training and communication skills); and
- Foster open dialogue and networking throughout the North Central aquaculture community.

* Information in parenthesis qualifies the goal with respect to this proposed project.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

Activities in this objective have been delayed until Spring 2012 due to limited number of staff with Clayton’s departure.

⁴ NCRAC has funded six Economics/Marketing projects. Termination Reports for the first two projects are contained in the 1989-1996 Compendium Report; a Termination Report for the third project is contained in the 1996-97 Annual Progress Report; a Termination Report for the fourth project is contained in the 2002-03 Annual Progress Report; and a Termination Report for the fifth project is contained in the 2003-04 Annual Progress Report. This Progress Report is for the sixth project. It is a 1-year project that began September 1, 2010.

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OBJECTIVE 2

An effort to host a ½-day meeting on the use of MarketMaker™ with a walleye workshop was developed for summer 2011 in conjunction with Chris Weeks in January 2011. However, due to limited number of projected anticipants, the decision was made to postpone this meeting until a later time.

OBJECTIVE 3

A 51-page MarketMaker™ tutorial has been developed by Iowa State University Value Added Agriculture Program.

WORK PLANNED

OBJECTIVE 1

In 2012, Allen Pattillo will work with Morris to contact all producers served by NCRAC by electronic or paper survey and follow-up all non-response or ambiguous survey responses with telephone inquiries. This will be tasked to NCRAC student workers. As the data set is assembled, it will be entered into the Market-Maker system via ISU's Value-added Agriculture

Project student workers under the supervision of a Market-Maker staff specialist.

OBJECTIVE 2

Upon completion of the survey in Objective 2, Pattillo and Morris will develop an online educational module that will assist in the registration of individual operations in the region.

OBJECTIVE 3

The final draft of the MarketMaker™ tutorial will be provided to regional and national audiences.

SUPPORT

NCRAC has provided \$23,565 which is the entire amount allocated for this 1-year project.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

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

EXTENSION⁵

Project *Progress Report* for the Period
May 1, 1989 to August 31, 2011

NCRAC FUNDING LEVEL: \$912,125 (May 1, 1989 to August 31, 2011)

PARTICIPANTS:

| | | |
|-----------------------|---|--------------|
| Dennis E. Bauer | University of Nebraska-Lincoln | Nebraska |
| Fred P. Binkowski | University of Wisconsin-Milwaukee | Wisconsin |
| Mark E. Clark | North Dakota State University | North Dakota |
| Richard D. Clayton | Iowa State University | Iowa |
| James M. Ebeling | Ohio State University | Ohio |
| Mark E. Einstein | Purdue University | Indiana |
| Robert D. Espeseth | University of Illinois | Illinois |
| Donald L. Garling | Michigan State University | Michigan |
| Jeffrey L. Gunderson | University of Minnesota-Duluth | Minnesota |
| F. Robert Henderson | Kansas State University | Kansas |
| Charles E. Hicks | Lincoln University | Missouri |
| Chester L. Hill | North Dakota State University | North Dakota |
| John N. Hochheimer | Ohio State University | Ohio |
| Paul B. Jarvis | North Dakota State University | North Dakota |
| Anne R. Kapuscinski | University of Minnesota | Minnesota |
| Terrence B. Kayes | University of Nebraska-Lincoln | Nebraska |
| David L. Klinkebiel | North Dakota State University | North Dakota |
| Ronald E. Kinnunen | Michigan State University | Michigan |
| Christopher C. Kohler | Southern Illinois University-Carbondale | Illinois |
| David J. Landkamer | University of Minnesota | Minnesota |
| Charles D. Lee | Kansas State University | Kansas |
| Frank R. Lichtkoppler | Ohio State University | Ohio |
| Terry A. Messmer | North Dakota State University | North Dakota |
| Brian K. Miller | Purdue University | Indiana |

⁵NCRAC has funded a number of Extension activities, both as stand-alone projects or as components of species-or topical-specific projects, including 13 stand-alone projects deemed “Base” Extension. This Progress Report is for components of the first 13 “Base” Extension projects; a Progress Report for the 12th “Base” Extension project (an Addendum to the 11th “Base” Extension project) is contained elsewhere in this report. The first three “Base” projects were chaired by Donald L. Garling, the fourth was chaired by Fred P. Binkowski, and projects 5-13 were chaired by Joseph E. Morris. A Project Component Termination Report for one of the objectives of the fifth “Base” Extension project is contained in the 1997-98 Annual Progress Report; a Project Component Termination Report for one objective of “Base” Extension projects 1-8 is contained in the 2003-04 Annual Progress Report. The 13th “Base” project is a 2-year funded project that began September 1, 2009. Fred P. Binkowski chaired the 14th stand-alone Extension project (the Aquaculture Regional Extension Facilitator [AREF]); a Termination Report for which was contained in the 2004-05 Annual Progress Report. Laura G. Tiu chaired the 15th stand-alone Extension project (Regional Aquaculture Extension Specialist [RAES]); a Termination Report for that project was contained in the 2008-09 Annual Progress Report. Christopher Weeks chairs the 16th stand-alone Extension project (Regional Aquaculture Extension Specialist [RAES]); a Progress Report for that project is contained elsewhere in this report.

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PARTICIPANTS (continued):

| | | |
|----------------------|---|------------------|
| Jerry B. Mills | South Dakota State University | South Dakota |
| Jeff Mittlemark | University of Minnesota | Minnesota |
| Joseph E. Morris | Iowa State University | Iowa |
| Kenneth E. Neils | Kansas State University | Kansas |
| Burton F. Pflueger | South Dakota State University | South Dakota |
| Robert A. Pierce II | University of Missouri | Missouri |
| Michael D. Plumer | University of Illinois | Illinois |
| Kwamena K. Quagraine | Purdue University | Illinois/Indiana |
| Shawn H. Sanders | North Dakota State University | North Dakota |
| Daniel A. Selock | Southern Illinois University-Carbondale | Illinois |
| John P. Slusher | University of Missouri | Missouri |
| Fred L. Snyder | Ohio State University | Ohio |
| Brian R. Stange | North Dakota State University | North Dakota |
| LaDon Swann | Purdue University | Indiana/Illinois |
| Laura G. Tiu | Ohio State University | Ohio |
| Geoffrey Wallat | Ohio State University | Ohio |

PROJECT OBJECTIVES

- (1) Strengthen linkages between North Central Regional Aquaculture Center (NCRAC) Research and Extension Work Groups.
- (2) Enhance the NCRAC extension network for aquaculture information transfer.
- (3) Develop and implement aquaculture educational programs for the North Central Region (NCR).

ANTICIPATED BENEFITS

Members of the NCRAC Extension Work Group have promoted and advanced commercial aquaculture in a responsible fashion through an organized education/training outreach program. 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 to enhance current and future production methodologies for selected species, e.g., freshwater shrimp, hybrid striped bass, yellow perch, and walleye, through hands-on workshops and field demonstration projects;
- ▶ Improved lines of communication between interstate aquaculture extension specialists and associated industry contacts;
- ▶ Access to aquaculture information by the industry at any time via the Internet, including such things as photographs, publications, and traditional as well as educational streaming videos (which are under development);
- ▶ An enhanced legal and socioeconomic atmosphere for aquaculture in the NCR; and
- ▶ Continued development of state producer organizations that are engaged in identifying and providing solutions to industry issues.

EXTENSION

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Examples follow for each of the objectives from the thirteen projects funded to date going back to 1989; however, greater emphasis is placed on more recent activities.

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 non-funded collaborator on a variety of projects including the NCRAC 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.

Since the beginning of NCRAC in 1989 numerous publications have been developed that address regional aquaculture issues. However, there continues to be a need to review these past publications. For instance, in 2009 the updating process for a series of publications titled “Managing Iowa Fisheries” was completed by Rich Clayton. These publications cover topics from aquatic vegetation and pond management to

aquaculture. All are topics that are relevant to fish production in Iowa as well as the NCR. The text in these publications was updated with new pictures included for on-line media delivery. The complete series is now available on-line as well as in print. Since this initial review, an on-going review of NCRAC extension publications has continued.

On February 26-27, 2009 the members of the NCRAC Regional Aquaculture Extension Team (RAET) and the North Central Region Strategic Planning Group held a joint meeting in Kansas City, Missouri during the NCRAC Annual Program Planning Meeting. The purpose of the meeting was to ascertain whether there are strategies that the team could employ to help the aquaculture industry develop within the region. One conclusion was that there is a need to develop a better communication system to streamline what can be done to help the industry grow. There are many extension and research programs in place, yet a common complaint from people within the aquaculture industry is that the information is either hard to find or not available. A revised NCRAC Web site was developed in 2010 and 2011 to address the need to better present the information to the public.

There was a consensus that the following points need to be met in order for the aquaculture industry to grow within the region: demand for a desirable product, a targeted species, opportunity, and an available market. Primary points of discussion included a state-by-state review of the current status of the aquaculture industry in terms of production, availability, and demand of targeted species.

Recommendations to NCRAC were suggested in areas of public education,

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extension and outreach education, marketing, work with regulatory agencies, and research. An action plan, with the goal of improving growth in the aquaculture industry, was then developed.

OBJECTIVE 2

The demand for aquaculture extension education programs cannot be met by the few aquaculture-designated specialists in the NCR. A NCRAC white paper on extension presents several strategies to address this concern.

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. Many of these requests have been met by providing fact sheets, technical bulletins, and detailed responses to both generalized and specialized questions. This extension network is critical to being able to match specific aquaculture questions with the best source of information, e.g., crawfish and leech information with Gunderson; yellow perch information with Binkowski, Tiu, and Wallat; and sunfish and walleye information with Morris.

The Aquaculture Network Information Center (AquaNIC [<http://aquanic.org/>]) was established at Purdue University in 1994 through funds from the U.S. Department of Agriculture's Cooperative State Research, Education, and Extension Service and the Illinois-Indiana Sea Grant College Program. In subsequent years, NCRAC has provided continued financial support for AquaNIC. The hardware for this Web site is housed in the Department of Animal Sciences at

Purdue University and is coordinated by the Mississippi-Alabama Sea Grant Consortium, the Alabama Cooperative Extension System, and the Illinois-Indiana Sea Grant College Program.

AquaNIC was the first U.S. aquaculture Web site and has globally been one of the most widely accessed and cited aquaculture Web sites. Approximately 1,200 individual, educational, commercial, and governmental Web sites link to AquaNIC as a source of on-line aquaculture information. AquaNIC was visited by more than 1.0 million people who viewed almost 750,000 pages for the time period from September 2010 through March 2011. This translates into more than 3,000 visitors/day. AquaNIC was taken offline April 1, 2011 due to lack of funding.

As with any long-term organization, there have been changes in NCRAC extension personnel since the inception of the project. For instance, Landkamer was the primary aquaculture extension contact for Minnesota. In the intervening years, he was replaced by Kapuscinski who was, in turn, replaced by Gunderson. Two other individuals were replaced in 1994. In Kansas, Neils replaced Henderson and in Illinois, Kohler replaced Selock. Lee replaced Neils in Kansas in 1996. Hochheimer, who replaced Ebeling in Ohio, left Ohio State University; Tiu was appointed as the aquaculture extension specialist for Ohio in 1998. Sanders, appointed as the extension contact for North Dakota in 1998, resigned; Paul Jarvis was appointed in 1999 and he has since been replaced by Mark Clark. In 2005 Pflueger replaced Mills as the appointed NCRAC Extension contact for South Dakota. In 2005 Bauer was designated to replace Kayes in Nebraska. In 2000, Swann resigned from Purdue/Illinois Sea Grant; Felkner served Indiana in the interim and in 2006

EXTENSION

Quagraine was appointed as state extension specialist at Purdue University. Plumer served Illinois until 2010 when, upon his retirement, Dave Shiley was appointed. In 2007, two long term extension contacts, Tiu and Morris, were replaced as NCRAC extension contacts by Wallat and Clayton, respectively. In 2010 Tiu was again appointed as extension contact for Ohio State University. In 2011 there were a number of changes in extension contacts in the NCR with Clayton (Iowa), Pierce (Missouri) and Shiley (Illinois) replaced by Pattillo, Hicks, and Quagraine, respectively.

Lee developed and published the 2008-2009 Kansas Aquaculture Association (KAA) Directory as well as maintained the KAA Web site and updated material provided by the KAA while also providing assistance to KSU Extension and general public. He also provided assistance to private pond owners on fish culture, management and aquatic vegetation control.

Pierce served as the Extension liaison for the Lincoln University Aquaculture Program by co-coordinating aquaculture Extension and outreach educational activities on the culture and production of sunfish for food markets; developing and reviewing Extension publications; and reviewing aquaculture research proposal submissions developed to enhance the capacity of Lincoln University's aquaculture research and outreach program.

Hicks followed up Pierce's activities by developing a new publication on freshwater prawn (shrimp) culture, provided assistance in establishing a freshwater prawn and cage culture business at Gaylord Farm, Butler, Missouri as well as participated in a regional sunfish culture workshop at Columbus, Ohio.

In North Dakota, Clark developed an updated list of state producers for submission to the NCRAC Publications Office as well as worked with state public agency personnel concerning state/federal regulations for North Dakota producers

Continued progress toward enhancing the NCRAC extension network for aquaculture information transfer has been accomplished through the North Central Aquaculture Regional Extension Facilitator Web site (www.ncaref.org) which continues to receive thousands of visits from a wide variety of clients.

On August 22, 2008, Binkowski and the Great Lakes WATER (Wisconsin Aquatic Technology and Environmental Research) Institute staff hosted the National Aquaculture Association Board members and guests for a tour of the WATER Institute's aquaculture facilities followed by a traditional Milwaukee Friday night yellow perch fish fry. In September 2008, the U.S. Trout Farmer's Association held the Midwest Aquaculture Conference in Milwaukee, Wisconsin. In 2010 Kinnunen and Morris attended a NCRAC Regional Aquaculture Extension Team Investment Workshop in Milwaukee, Wisconsin, chaired by Fred Binkowski (University of Wisconsin-Milwaukee [UW-Milwaukee]). At this workshop four different types of aquaculture with financial institution representatives were represented.

Discussion included possible roadblocks to larger investments in aquaculture in the region. In 2010 and 2011 Kinnunen has continued to be involved in several facets of fish processing and HACCP (hazard analysis and critical control point) training in both aquatic invasive species and food safety.

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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). There have been workshops on general aquaculture, fish diseases, early life stage culture, recirculation systems, cage culture, aquaculture business planning, pond management (fish and vegetation), water quality, and taxa-specific topics, e.g., baitfish, channel catfish, crayfish, hybrid striped bass, leach, rainbow trout, sunfish, walleye, and yellow perch culture, as well as in-service training for high school vocational-agricultural teachers. Depending on the workshop, the number in attendance often exceeded 100. Through these workshops, critical issues in the private aquaculture industry have been identified, e.g., market availability, economic returns, and regulatory concerns.

NCRAC Extension contacts have served as editors for regional aquaculture newsletters as well as in-state aquaculture association newsletters; served on state aquaculture advisory councils and state aquaculture task forces; and assisted in the planning and implementation of state aquaculture association meetings.

In addition to the previously mentioned areas, NCRAC Extension contacts have been instrumental in fostering the continued growth of the aquaculture industry in the region through a variety of activities and many have worked with industry and governmental representatives to produce state aquaculture plans and improved governmental regulations.

All fish processors, including those who handle aquaculture products, are now

required by law to process their fish following HACCP guidelines. Kinnunen and Gunderson have conducted numerous HACCP training workshops throughout the NCR. These workshops served to train fish processors on the principles of HACCP and to give them knowledge on how to develop and implement a HACCP plan for their specific facility. Attendees, who come from throughout the NCR, represent both public and private audiences as well as Native American groups.

NCRAC Extension contacts have also been responsive to arising issues for the NCR aquaculture industry. For instance, the aquaculture industry is accused of being an important vector for the further spread of exotic species such as zebra mussels, Eurasian watermilfoil, and round gobies. To better identify the risks of spreading exotic species and to reduce those risks, an AIS (aquatic invasive species)-HACCP approach has been developed by Kinnunen and Gunderson and taught to private fish farmers, wild bait harvesters, state and federal agency natural resource personnel, and Native Americans. An AIS-HACCP plan has also been developed to address the growing concern of biosecurity, particularly in regard to diseases such as viral hemorrhagic septicemia (VHS). Kinnunen and Gunderson have also taught other members of the NCR aquaculture extension community about their AIS-HACCP program, in essence, they've "trained the trainers" and all AIS-HACCP materials are available at www.seagrant.umn.edu/ais/haccp.

In-service training of secondary teachers has taken place in a number of states. For instance, teachers in Iowa, Ohio, and Wisconsin have received instruction in aquaculture.

EXTENSION

Several states have on-site facilities that are used for extension programming, e.g., the Piketon facilities operated by Ohio State University are used to inform the public about aquaculture as well as foster grass root support for this agriculture enterprise. The facilities at Iowa State University and the University of Wisconsin-Milwaukee have also been used in a similar fashion.

The Ohio Center for Aquaculture Research and Development hosts three electronic list serves, the most popular of which is the Aqua-Ohio list serve. Over 150 clients subscribe to this list serve which allows for timely dissemination of aquaculture related news and resources. This information is further disseminated by the list subscribers to additional interested parties.

In early fall 2007 a question was raised by regional producers as to the possibility of bringing aquatic stakeholders together from various backgrounds to discuss the regulatory and administrative discrepancies among states when it comes to aquatic livestock, biosecurity, and commerce. The concept of a meeting/forum evolved into an action plan to try and accomplish this task. A forum was designed to explore federal and state regulations that are impacting the profitable and efficient interstate movement of aquatic livestock for both private and public purposes in hopes of finding consistent uniform methods for the NCR and other states currently under the federal order for VHS. The concept of this Forum was to discuss improvement and revision of state regulations and policies whereby aquatic livestock for both public and private purposes can be enhanced while also maintaining animal health. The five delegate groups represented: private producers, public producers (such as hatchery personal), animal health representative (veterinarians), state natural

resources, and agriculture state agencies; representatives were invited from fourteen states. The states in the NCR (Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin) and two others affected by the federal order on VHS (New York and Pennsylvania) were chosen. Issues that the 37 forum participants were in consensus on in rank order were:

- ▶ no uniformity in state regulations;
- ▶ limited availability of fish health officials; and
- ▶ no uniformity of testing standards among states.

The complete report for this meeting can be found at: www.aquaticlivestock.org/.

This forum impacted the NCR by bringing some of these key players (delegates) to a neutral table to discuss these common issues which had never been done before with aquatic livestock producers. Many of the delegate groups had never sat down to discuss their issues with the other stakeholder groups. Some delegates didn't realize that other delegates have the same issues, e.g., private producers and public producers both have to deal with changing transportation regulations.

Kinnunen coordinated a 3-day Seafood HACCP Training course that was held at Bay Mills, Michigan, December 9-11, 2008. Formal evaluations from attendees rated the course as excellent. The 33 attendees included state and tribal fishermen/processors, fish farmers, state regulators, along with representatives from major firms from around the U.S. dealing with fishery products.

Kinnunen has been effective in providing outreach/extension materials to many

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culturists. For instance, he provided preventative information and AIS-HACCP materials to the Colorado Division of Wildlife regarding the control of quagga mussel veligers on Kokanee salmon eggs. Kinnunen's role in this area is also exemplified by his attendance at the Trade Workshop II that was sponsored by the Great Lakes Commission. Those in attendance learned about the success that NCRAC has had with AIS-HACCP and how it has been widely adopted by the baitfish and aquaculture industries and may provide a model for other sectors to follow.

Additional activities by Kinnunen included coordination of a 3-day Seafood HACCP Training course that was held at Keweenaw Bay Indian Community. The 31 attendees included state and tribal fishermen/processors, fish farmers, Indian Health Service, along with a representative whose company sells the fish to McDonalds® for their fish sandwiches.

A NCR baitfish workshop was hosted by Chris Weeks (Michigan State University [MSU]) and Jeff Gunderson (University of Minnesota-St. Paul) in La Crosse, Wisconsin. Speakers included Kinnunen (MSU), Morris (Iowa State University), Jeff Nuese (UW-Milwaukee), Gunderson, Fischer (University of Wisconsin-Stevens Point), Weeks, and Gaikowski (Upper Midwest Environmental Sciences Center) as well as industry representatives such as Barry Thoele. Nathan Stone (University of Arkansas-Pine Bluff) also presented an overview of the baitfish industry. The approximately 30 participants heard presentations regarding new information on baitfish culture as well as associated disease issues.

WORK PLANNED

Efforts will continue in regard to strengthening linkages between research and extension work groups as well as enhancing the network for aquaculture information transfer. Participants will also continue to provide in-service training for CES, Sea Grant, and other land owner assistance personnel.

Educational programs and materials will be developed and implemented including AIS-HACCP workshops that will be planned as needed in the NCR as well as workshops on aquatic plant management for aquaculture facilities, prawn production, and larval fish culture. Any other workshops developed and hosted by state aquaculture extension contacts will be advertised in surrounding states to take advantage of the NCRAC extension network and the individual expertise of the Extension Work Group participants. There are also plans to enhance Web-based communications through the use of streaming videos and electronic fact sheets. Streaming videos will include the following topics:

- ▶ yellow perch culture,
- ▶ freshwater shrimp culture,
- ▶ culture pond construction,
- ▶ water quality assessment,
- ▶ fry-pond fertilization regimes, and
- ▶ aquatic vegetation management.

In addition, a Web site for predator management and fish grub control (using information from the recently completed NCRAC snail management/grub control project) will be finalized and linked to NCRAC's Web site (<http://www.ncrac.org>).

Current NCRAC extension materials will continue to be reviewed and updated by regional extension contacts in partnerships with the NCRAC administrative office.

EXTENSION

IMPACTS

Examples include:

- ▶ Development of aquaculture education programs for the NCR has provided “hands-on” opportunities for prospective and experienced producers. More than 10,000 individuals have attended workshops, conferences, or symposia organized and delivered by members of the NCRAC Extension Work Group.
- ▶ Fact sheets, technical bulletins, videos, and CDs have served to inform a variety of clients about numerous aquaculture practices for the NCR. For instance, “Making Plans for Commercial Aquaculture in the North Central Region” is often used to provide clients with initial information about aquaculture, while species-specific publications have been used in numerous regional meetings. The Center’s Web site provides immediate availability to many of the products that have been developed by the Extension Work Group (e.g., fact sheets as PDF files) and with the further development of streaming videos, not only will clients have the benefit of being able to read about aquaculture for free on a 24-h basis, they will also be able to see it in action. This ability to enhance technology transfer should result in a more economically-successful aquaculture industry in the NCR.
- ▶ Fish processors who have attended NCRAC-sponsored HACCP Training Workshops have learned the principles of HACCP with regards to its importance in insuring the production of a safe fishery product. HACCP plans have been implemented by workshop attendees who are now keeping records of their daily processing and Sanitation Standard Operating Procedures. Hundreds of fish processors and/or aquaculturists have attended HACCP Training Workshops.
- ▶ AIS-HACCP workshops have been attended by commercial culturists, state and federal natural resource personnel as well as Native Americans, many of whom have implemented the principles of AIS-HACCP into their operations
- ▶ Quagraine has developed workshops that were mainly hands-on, which enabled participants to acquire skills in building cages, handling fish, using financial spreadsheets, etc. Some farmers are now building their own cages and using financial spreadsheets for their aquaculture operations.
- ▶ Hicks assisted in the establishment of the Gaylord Farms Cage Culture and Prawn business and securing for them a Sustainable Agriculture Research and Education (SARE) grant for expansion of their operations.
- ▶ Regional aquaculture information (i.e., workshop announcements, fact sheets, and product marketing) is quickly and efficiently distributed to aquaculture clients in Ohio and neighboring states. This results in Ohio fish farmers being well informed about activities and information that can enhance the success of their businesses.
- ▶ Nearly 100 fish farmers attended the Ohio Bluegill Workshop in 2011. Farmers were able to network and are now working together, buying feed and fingerlings in bulk and cooperatively marketing together in order to reduce individual costs. The PowerPoint presentations and video/audio from the event are available free and located at http://southcenters.osu.edu/aqua/extension/osu_bluegill_aquaculture_workshop.htm
- ▶ The expansion of the Aqua-Ohio List serve as served to improve the transfer of regional aquaculture information (i.e.

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workshop announcements, fact sheets, and product marketing) more quickly and efficiently distributed to aquaculture clients in Ohio and neighboring states.

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

SUPPORT

| YEARS | NCRAC- USDA FUNDING | OTHER SUPPORT | | | | | TOTAL SUPPORT |
|---------------|---------------------------|-----------------|----------|------------------|----------|-------------|------------------|
| | | UNIVER- SITY | INDUSTRY | OTHER FEDERAL | OTHER | TOTAL | |
| 1989-91 | \$107,610 | \$237,107 | | | | \$237,107 | \$344,717 |
| 1991-93 | \$94,109 | \$152,952 | | | | \$152,952 | \$247,061 |
| 1993-95 | \$110,129 | \$198,099 | | \$250,000 | \$55,000 | \$503,099 | \$613,228 |
| 1995-97 | \$31,204 | \$149,325 | \$5,000 | \$84,000 | | \$238,325 | \$269,529 |
| 1997-99 | \$38,000 | \$110,559 | | | | \$110,559 | \$148,559 |
| 1999-01 | \$94,000 | \$108,124 | | | | \$108,124 | \$202,124 |
| 2001-03 | \$46,654 | \$99,702 | | | | \$99,702 | \$146,356 |
| 2003-05 | \$28,000 | | | | | | \$28,000 |
| 2005-07 | \$219,280 | | | | | | \$219,280 |
| 2007-09 | \$114,139 | | | | | | \$114,319 |
| 2009-11 | \$29,000 | | | | | | \$29,000 |
| TOTALS | \$912,125 | \$1,055,868 | \$5,000 | \$334,000 | \$55,000 | \$1,449,868 | \$2,361,993 |

EXTENSION ADDENDUM⁶

Project *Progress Report* for the Period
September 1, 2008 to August 31, 2011

NCRAC FUNDING: \$60,505 (September 1, 2008 to August 31, 2011)

PARTICIPANTS:

| | | |
|--|--|-----------|
| Glenda D. Dvorak | Iowa State University | Iowa |
| Christopher F. Hartleb | University of Wisconsin-Stevens Point | Wisconsin |
| Myron J. Kebus | Wisconsin Department of Agriculture, Trade, and Consumer Protection | Wisconsin |
| Ronald E. Kinnunen | Michigan State University | Michigan |
| Jeannette McDonald | University of Wisconsin-Madison | Wisconsin |
| Joseph E. Morris | Iowa State University | Iowa |
| <i>Industry Advisory Council Liaison:</i> | | |
| William West | Blue Iris Fish Farm, Black Creek | Wisconsin |

PROJECT OBJECTIVES

- (1) To develop an online Fish Health Certificate Program for producers, providing them with relevant risk assessment and management principles and practices to reduce losses due to fish diseases and set up mechanisms to collect data on the impact of the training on the individual fish operations and the industry in general.
- (2) Development and presentation on workshops focused on AIS-HACCP training.

ANTICIPATED BENEFITS

Aquatic animal health and fish disease management are extremely relevant to the aquaculture industry in the North Central Region (NCR) because the industry has experienced both long- and short-term disease issues. These issues have resulted in

both significant changes to the regulation of the industry and economic losses associated with fish mortalities. Thus, greater requirements for disease detection and assessment on the farm are needed. The aquaculture industry has requested more information on fish health and mechanisms by which fish farmers can be better trained to prepare, identify, and manage disease outbreaks on the farm. Though previous attempts at educating and assisting fish farmers with aquatic disease issues have addressed the subject with printed information and workshops, few have had region-wide impact and none have attempted to prepare the aquaculture industry for whole farm disease management.

For this proposed extension project, a series of online fish health learning modules developed for the aquaculture industry will

⁶ NCRAC has funded a number of Extension activities, both as stand-alone projects or as components of species- or topical-specific projects. This Progress Report is for one of the 13 stand-alone "Base" Extension projects and is an Addendum to the 11th "Base" Extension project which is chaired by Joseph E. Morris. This is a project that had two years of funding and began September 1, 2008.

EXTENSION ADDENDUM

be created and implemented to better educate the fish farmer about aquatic diseases and on-farm fish health management. An Internet-based set of educational modules will present best management practices that will assist the fish farmer in developing biosecurity plans as well as educating about and bringing to the forefront risk factors in farm management and disease control. Fish farmers will not only be shown techniques for evaluating disease introduction, transmission, and basic pathological signs, they will also be shown how to minimize disease occurrence and prepare for infections and proper disease risk management along with explanations and examples of veterinary inspection, health assessment, and disease treatments. This proposed work will also aid the fish farmer in understanding the veterinary health assessment report and, upon completion of the online learning modules, the fish farmer can obtain a certificate of completion that can help veterinarians recognize the fish farmer and his/her aquaculture facility as one educated on methods of disease prevention and one prepared to cooperate with the veterinarian in implementing proper treatment procedures.

The Aquatic Invasive Species-Hazard Analysis and Critical Control Point (AIS-HACCP) approach has many advantages. It can effectively deal with a diverse industry and diverse risk factors associated with a variety of plant, invertebrate, vertebrate, and pathogen AIS. If it develops as it has in the seafood industry, this approach should prove to be a good partnership between industry and government regulators. It can help avoid overly restrictive regulations, and, if properly applied, can be effective at reducing the risk of spreading AIS via baitfish harvest and aquaculture practices. The HACCP approach concentrates on the

points in the process that are critical to the environmental safety of the product, minimizes risks, and stresses communication between regulators and the industry. With proper cooperation among industry representatives, resource management agencies, and other AIS experts, the AIS-HACCP approach will reduce the risk that AIS will be established in new locations while maintaining the economic viability of the baitfish and aquaculture industries. It can provide a mechanism for AIS-free certification, and it can instill confidence in the public that state and federal fish stocking programs are conducting their activities in an environmentally responsible manner.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

Development of an online Fish Health Certificate Program for producers that will provide them with relevant risk assessment and management principles and practices to reduce losses due to fish diseases is now complete. Part one of the Fish Health Certificate Program included the development of a six module Web-based learning program. Modules 1-6 of the asynchronous learning program have undergone peer review, revisions based on those reviews were made, and the modules have been published, available at http://ce.vetmed.wisc.edu/Fish_Producer_Courses. The modules contain information about:

- (1) Introductory principles and practices such as regional fish production, farm types in the NCR, principle culture systems, and the myriad of regulatory agencies involved in U.S. aquaculture.
- (2) Risk management and biosecurity methods that can assist producers in reducing the risk of introduction of diseases at aquaculture facilities. This

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module reviews topics such as Best Management Practices, loss events, continuing education, veterinary services, record keeping, and links to state and federal guidelines and policies.

- (3) Water quality management and monitoring, and disease prevention including reviews of water characteristics, physical and chemical water components, and effluent discharge at aquaculture facilities.
- (4) Fish health inspections, with particular emphasis on what producers should expect at an inspection, how producers can prepare for inspections, regulatory consequences, supplies and equipment required at an inspection, and how samples are collected, shipped, and what type of voucher specimens may be collected.
- (5) Veterinary health assessments and reports are presented showing typical results of a fish health inspection. Information included shows a producer how they can use the information to improve fish health management at their facility. This includes a review of treatments and medications and the role of follow-up assessments.
- (6) Case studies describing diseases based on water quality problems, environmental diseases, bacterial infections, and ectoparasites have been developed. Case studies specific to Koi herpes virus, largemouth bass virus, infectious salmon anemia, spring viraemia of carp, and viral hemorrhagic septicemia have been developed based on actual “real-world” examples.

Evaluation and outcome assessment tools have been developed. Mechanisms are in place to collect data on the finished products.

The online program will have free access for the first year for those who complete a pre-program survey. To access the program, producers can follow these instructions:

- (1) Go to VetMedCE.org.
- (2) Click “Sign up now” to create an account (this is free).
- (3) Click on “Take a course.”
- (4) Navigate to the courses by clicking on “Fish Health Courses for Producers and Veterinarians” > “Fish Producer Courses.”
- (5) Click on the “Complete Fish Health for Producers Program” link at the bottom of the list.
- (6) To take the program for free, click on the “survey link” on the course homepage.
- (7) Copy the access code from the bottom of the survey.
- (8) Click the “Register” button on the original course Web page.
- (9) Paste the access code into the field on the RIGHT side of the screen.

OBJECTIVE 2

A publication entitled “Biosecurity for Aquaculture Facilities in the North Central Region” was developed and is now available through the North Central Regional Aquaculture Center. Kinnunen coordinated a 3-day AIS HACCP Training course that was held at Bay Mills, Michigan in 2008. Formal evaluations from attendees rated the course as excellent. The 33 attendees included state and tribal fishermen/processors, fish farmers, and state regulators along with representatives from major firms from around the U.S. dealing with fishery products.

Kinnunen has provided preventative information and AIS-HACCP materials to the Colorado Division of Wildlife regarding the control of quagga mussel veligers on Kokanee salmon eggs. Kinnunen’s role in

EXTENSION ADDENDUM

this area is also exemplified by his attendance at the Trade Workshop II that was sponsored by the Great Lakes Commission. Those in attendance learned about the success of AIS-HACCP and how it has been widely adopted by the baitfish and aquaculture industries and may provide a model for other sectors to follow.

In 2010 Kinnunen conducted 1-day AIS-HACCP Training Workshops in Ashland, Nebraska and Spirit Lake, Iowa. Those in attendance included state fish hatchery and fish management personnel, private sector aquaculture personnel, and an aquatic veterinarian. Attendees indicated in a written evaluation that they would use the material learned and implement plans at their own facilities within the next several months.

Kinnunen also coordinated a second 3-day Seafood HACCP Training course at Bay Mills, Michigan along with Mike Erdman (Menominee County Extension Director) and Jim Thannum (Great Lakes Indian Fish and Wildlife Commission). Formal evaluations from attendees rated the course as excellent. The 40 attendees included state and tribal fishermen/processors, fish farmers, state regulators, along with representatives from major firms from around the U.S. dealing with fishery products.

In addition to the noted workshops, Kinnunen met with the Board of Directors of the Michigan Aquaculture Association to continue the process of developing a strategic plan for aquaculture development in Michigan. Additional contacts made by Kinnunen in support of this objective include a veterinarian who is compiling information for the Michigan Department of Agriculture on the subjects of aquaculture biosecurity and AIS-HACCP as well as

several agency staff and private environmental consultants.

The Michigan Aquaculture Association Conference was held in Mt. Pleasant where Kinnunen gave an update on AIS-HACCP and Aquaculture Biosecurity. In addition, the Michigan Aquaculture Strategic Plan was discussed in conjunction with Ed Mahoney (Michigan State University).

In 2011 Kinnunen worked with Rick Weidenhammer (Michigan Wholesale Bait Association) regarding the proposed plans by the Michigan Department of Natural Resources and Environment (MDNRE) to have baitfish trucks hauling bait tested for Asian Carp via DNA testing. The plan also calls for prevention using the AIS-HACCP program.

Kinnunen also attended a meeting hosted by the Wisconsin Department of Natural Resources in Manitowish Lakes, Wisconsin to discuss the invasion of spiny water fleas into lakes in northern Wisconsin and Michigan's western Upper Peninsula. He shared with the group the AIS-HACCP program and how natural resource management agencies could use this program to prevent the spread of aquatic invasive species by way of their assessment operations. Similar efforts included: (1) Wild Rivers Invasive Species Coalition Annual Meeting; (2) a Central Upper Peninsula all agency meeting that included officials from the U.S. Fish and Wildlife Service, U.S. Forest Service, National Park Service, and Michigan MDNRE where the program efforts were highlighted; and (3) began evaluating the use of AIS-HACCP at Cabala's Master Walleye Circuit fish tournaments and attended events in Escanaba and Sault Ste. Marie, Michigan. Kinnunen developed a display on aquatic invasive species and surveyed tournament

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anglers on their current practices to prevent the spread of AIS. At these two tournaments he evaluated procedures that could be critical control points to prevent the spread of AIS. Tournaments attract participants from many states and have the potential to spread AIS.

WORK PLANNED

Six months after completing the Fish Health Certificate Program, each participant will be sent a follow-up survey. The follow-up survey will help determine if any actions, changes in practices, policies, or procedures have been implemented at the participant's facility following the completion of the course. The survey will also test the retention of knowledge from the course (i.e., intermediate outcomes).

IMPACTS

The complete Fish Health Certificate Program was peer reviewed this past summer and has recently been published. Tools have been designed and developed to evaluate the Web-based program and the level of knowledge gained by the participants through outcome indicators.

These indicators include assessments using short evaluation surveys of usefulness, accessibility, and user-friendliness, along with individual module and full-program knowledge indicators based on short-term survey of outcomes. An evaluation survey and assessment is an integral part of the Fish Health Certificate Program. Each participant must complete a survey and an assessment before they receive their certificate. Data is automatically collected and collated.

AIS-HACCP workshops have been attended by commercial culturists, state and federal natural resource personnel, as well as Native Americans, many of whom have implemented the principles of AIS-HACCP into their operations.

SUPPORT

NCRAC has provided \$60,505 which is the entire amount allocated for this 2-year project.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

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

EXTENSION ADDENDUM

REGIONAL AQUACULTURE EXTENSION SPECIALIST (RAES)⁷

Project *Termination Report* for the Period
September 1, 2009 to August 31, 2011

NCRAC FUNDING: \$150,000 (September 1, 2009 to August 31, 2011)

PARTICIPANTS:

| | | |
|--|---------------------------------------|-----------|
| Ronald E. Kinnunen | Michigan State University | Michigan |
| Joseph E. Morris | Iowa State University | Iowa |
| Larry G. Olsen | Michigan State University | Michigan |
| Christopher Weeks | Michigan State University | Michigan |
| <i>Industry Advisory Council Liaison:</i> | | |
| John Reynolds | Midwest Fish and Crayfish, Merrifield | Wisconsin |
| <i>Extension Liaison:</i> | | |
| Geoffrey Wallat | Ohio State University | Ohio |

REASON FOR TERMINATION

The objectives were completed and the funds terminated. However, an additional RAES project is underway for the period September 1, 2011 to August 31, 2013 under new objectives. This termination report is for the 2009-2011 RAES project established under the following objectives.

PROJECT OBJECTIVES

- (1) In conjunction with the NCRAC Industry Advisory Council and state aquaculture extension contacts, assess and prioritize North Central Region (NCR) industry needs, focusing on issues with regional significance.
- (2) Develop and implement strategies to address pertinent needs - interact with pertinent NCRAC and non-NCRAC aquaculture initiatives to accomplish identified strategies.

- (3) Develop and facilitate “linkages” among agencies, industry, academia, and other relevant entities to foster open, meaningful dialog on critical NCR issues.
- (4) Coordinate efforts for seeking non-NCRAC support to facilitate information and technology transfer to the industry.

PRINCIPLE ACCOMPLISHMENTS *OBJECTIVE 1*

In anticipation of this objective, the RAES (project PI), along with the Industry Advisory Council (IAC) chair, William Lynch, formulated and conducted a NCR Aquaculture Industry Survey in 2009 focusing on critical needs and priorities as perceived by the NCR aquaculture community. In all, 232 surveys were mailed to a random sample of a target of 20 individuals in each state within the NCR.

⁷ NCRAC has funded a number of Extension activities, both as stand-alone projects or as components of species- or topical-specific projects. This Termination Report is for one of the 16 stand-alone Extension projects, the second Regional Aquaculture Extension Specialist (RAES) project, which was chaired by Christopher Weeks. It was a 2-year project that began September 1, 2009.

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Participants were representatives from industry, State and Federal environmental agencies, and academia. The survey received 62 responses for a response rate of 27%. Participants were asked to rate impediments facing the industry and priorities they felt were necessary to help improve aquaculture industry development in the region. Results from the survey have been incorporated into a NCR Aquaculture Critical Needs Assessment Report which is currently under review by the RAES Project Oversight Committee and expected to be distributed to NCRAC members before the 2012 NCRAC Annual Program Planning Meeting. Survey results have also been made available online (http://www.ncrac.org/roadmap/webinfo/publications/09_survey_results.pdf).

OBJECTIVE 2

Over the course of the project, the RAES has given several presentations on subjects specific to regional aquaculture industry development in Indiana, Michigan, Missouri, and Wisconsin, and has met and talked with producers from all 12 NCR states. Through both survey response and discussion, the majority of industry members identified restrictive and confusing regulations as the greatest impediment to industry growth. To that end the RAES committed substantial effort to help clarify regulations imposed on NCR aquaculture producers, including the following tasks:

- Monitored and updated the NCRAC Web site *State Importation and Transportation Requirements for Cultured Aquatic Animals*. The site was originally developed by the current RAES in 2003, and improved in 2009 with help from the NCRAC Associate Director's office as part of the RAES project. Views to this Web site have increased from approximately 500 hits per month in 2009-2010, to an average

of 800–1,000 views per month in 2011. The Web site's pages have also been linked to aquaculture regulation searches from the USDA Animal and Plant Health Inspection Service (APHIS) aquaculture Web site.

- Summarized import requirements for NCR states on the NCR Aquaculture Roadmap Web site in table format. The summary is unique, concise, and has received good feedback: http://www.ncrac.org/roadmap/webinfo/pdf_downloads/NCRAC%20import%20ref.pdf.
- Sought out information from aquaculture related list serves, news, and personal contact information from across the nation for dissemination to the industry.
- Worked with regulators and industry personnel across the region to try to minimize disruption of commerce due to viral hemorrhagic septicemia (VHS).
- Encouraged active participation by the NCR aquaculture community in regulatory rule making processes. Examples include posting of, and industry solicitation for Federal Register comments on APHIS reactions to VHS and the publication of the National Aquatic Animal Health Plan.

Information transfer was another major focus area for the RAES. In 2009, the RAES initiated a project to enhance the NCR Aquaculture Roadmap from its original short PDF file, into a user friendly aquaculture information outreach tool. The Roadmap Web site provides easy access to all Regional Aquaculture Center publications, other non-copyrighted articles, regulations, NCR state associations, extension Web sites, extension, research, and state agency contacts across the region, upcoming events, and other materials: <http://www.ncrac.org/roadmap/index.htm>.

In 2010, the RAES with the NCRAC IAC Chair, William Lynch, submitted written recommendations to the NCRAC Board for a revision to the project selection protocol followed at NCRAC Annual Program Planning Meetings. The recommended changes were designed to more narrowly define key targeted priorities, allow for increased Technical Committee involvement, and improve the effectiveness of the NCRAC project selection. The Board approved the recommended protocol revisions, which were initiated at the 2011 Annual Program Planning Meeting.

Current news items and potentially useful topical information were actively sought from several national and international sources for distribution to the aquaculture community through the NCR Fish Culture List Serve. The list serve has grown from under 90 subscribers at the start of the RAES project to about 200 at present. The NCR Fish Culture List Serve is maintained out of Iowa State University and supported by NCRAC's Associate Director's office. Subscription is available through the NCR Aquaculture Roadmap Web site.

OBJECTIVE 3

In 2009-2010 the RAES gave presentations at the Michigan Aquaculture Association Meeting, Michigan Department of Agriculture Meeting for Aquatic Animal Health, the Wisconsin Workshop for Veterinarians on Fish Regulatory Medicine, and the Indiana Aquaculture Workshop. The RAES also facilitated and presented at the NCRAC Baitfish Workshop held at the La Crosse Fish Health Center, Wisconsin, and participated in the National Aquaculture Association's (NAA) 4P Workshop, as well as a Hazard Analysis Critical Control Point Workshop on VHS and biosecurity, both of which were held in Ohio. A goal of the RAES throughout the course of these

meetings has been to network with industry, academia, and regulatory agency personnel for the purpose of promoting NCR aquaculture industry interests and development.

The RAES project team has identified three organizations where increased membership base among NCRAC members would likely help promote NCR industry interests. These include NAA, Farm Bureau, and the National Association of State Aquaculture Coordinators (NASAC). The RAES is now a member of Michigan Farm Bureau and is under the nomination process for becoming a member of their Aquaculture Advisory Committee. The RAES also attended the National Aquaculture Extension Conference in June 2011 hosted by USDA's National Institute of Food and Agriculture (NIFA) and NASAC and gave a poster presentation on the RAES project. The RAES is now a member of the USDA-sponsored eXtension Freshwater Aquaculture Group and serves in an "ask the expert" role on regional issues. The RAES also has plans to get more involved with NAA (as part of the 2011-2013 RAES project).

OBJECTIVE 4

The RAES has recently submitted a final version of an amended proposal to Michigan Sea Grant entitled "Integrated Assessment - Expansion of Michigan's Existing Commercial Aquaculture Activities into a Major Sustainable Seafood Production Industry." The proposal is expected to be recommended for funding by Michigan Sea Grant and begin in spring 2012. This project will provide for a full time position through Michigan State University for two years and approximately \$268,000 for use in development and implementation of a commercial seafood sector strategic plan in Michigan. The RAES will act in a supervisory role only, and will pursue other

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funding opportunities for aquaculture research and extension for other NCR states in similar fashion (for the 2011-2013 project). The RAES has also served on three State and Federal grant review panels for U.S. aquaculture program development (National Oceanic and Atmospheric Administration, Southern Regional Aquaculture Center, and Ohio Sea Grant).

In 2011, the RAES completed a review entitled "Incorporating Deliverables into the NCRAC Project Development Process (Review)." The report will be made available to NCRAC members prior to the 2012 NCRAC Annual Program Planning Meeting. Other information transfer activities developed, facilitated, and maintained by the RAES have been described above.

IMPACTS

- Increased knowledge, awareness, and accessibility of information regarding aquaculture, baitfish, interstate transport, and health certification requirements to the industry and aquaculture community in the NCR.
- Increased knowledge (and potential interests) in the area of aquatic animal health to qualified and/or certified fish health inspectors.
- Increased non NCRAC funding support for NCR aquaculture industry development.

RECOMMENDED FOLLOW-UP ACTIVITIES

Two assessment/reviews have been completed and will be distributed to NCRAC members prior to the 2012 NCRAC Annual Program Planning Meeting:

- NCR Aquaculture Critical Needs Assessment Report

- Incorporating Deliverables into the NCRAC Project Development Process (Review)

A new RAES project has begun (2011-2013) under new objectives described below:

1. Continue RAES support to the NCR aquaculture community through ongoing activities in areas of liaison services, leadership, assessing and addressing industry needs, and information transfer.
2. Develop and implement strategies to address and promote aquaculture sustainability in the NCR.
3. Develop and strengthen partnerships from within the NCR and outside the region among regulatory agencies, industry, academia, and other relevant entities to foster open, meaningful dialog on critical issues and build support for the NCR aquaculture industry.
4. Coordinate efforts for seeking non-NCRAC support for NCR aquaculture development.
5. Examine regional aquaculture development and assess NCRAC research and extension activities in terms of impacts on the NCR aquaculture industry. Make recommendations for improving NCRAC projects in terms of incorporating measures of program success.

SUPPORT

NCRAC has provided \$150,000 which is the entire amount allocated for this 2-year project.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

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

NUTRITION/DIETS—RAPID DETERMINATION OF AMINO ACID REQUIREMENTS OF YELLOW PERCH AND TILAPIA⁸

Project *Progress Report* for the Period
September 1, 2009 to August 31, 2011

NCRAC FUNDING: \$40,000 (September 1, 2009 to August 31, 2011)

PARTICIPANTS:

| | | |
|---|----------------------------------|--------------|
| Robert S. Hayward | University of Missouri-Columbia | Missouri |
| <i>Industry Advisory Council Liaison:</i> | | |
| Mark Willows | Binford Eagle Fisheries, Binford | North Dakota |
| <i>Extension Liaison:</i> | | |
| Joseph E. Morris | Iowa State University | Iowa |

PROJECT OBJECTIVES

- (1) Conduct a full literature search on amino acid composition, amino acid requirements, and feed formulations for yellow perch and Nile tilapia.
- (2) Evaluate body amino acid composition of yellow perch and Nile tilapia.
- (3) Evaluate limiting amino acid requirements of yellow perch and Nile tilapia.
- (4) Evaluate amino acid availability of dietary ingredients for yellow perch and Nile tilapia.
- (5) Develop a least-cost formulation model available to the NCR aquaculture industry within a two-year period for yellow perch and Nile tilapia.
- (6) Coordinate findings from this study with the Technical Committee Extension Subcommittee of NCRAC.

ANTICIPATED BENEFITS

Although strong demand exists for both yellow perch and Nile tilapia, current high production costs need to be reduced to enhance profit margins. As with other aquaculture species, feed costs represent a significant percentage of producers' variable costs. Current trout diets contain 4–50% protein, with the majority of the feed costs owing to the use of fish meal based protein. Replacement of fish meal with other animal protein and plant protein sources can result in significant savings in fish feed production costs.

This study, while optimizing nutrient requirements, will remove excess protein from the current diets for both species. This project further seeks to reduce the feed cost by using highly digestible as well as economically available local feedstuffs that benefits both fish producers as well as local grain producers. It is anticipated that this project will reduce current feed costs by at least 40% for yellow perch and by 30% for

⁸NCRAC has funded three Nutrition/Diets projects. The Termination Report for the first project is contained in the 1997-98 Annual Progress Report. The Termination Report for the second Nutrition/Diets project is contained in the 2008-09 Annual Progress Report. This Progress Report is for the third project. It is a 2-year project that began September 1, 2009.

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Nile tilapia while the growth rate of fish will be increased or maintained at current production levels.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

Literature searches have been conducted on feedstuffs' amino acid composition, amino acid requirements, and feed formulations for grow out-stage yellow perch and Nile tilapia.

OBJECTIVE 2

A no-cost extension will be requested to extend this project whereby this objective will be addressed.

OBJECTIVE 3

The experiment is currently being done to determine lysine requirement of Nile tilapia. The experiment will run for eight weeks, and will ultimately determine the exact lysine requirement of grow out-stage Nile tilapia.

OBJECTIVE 4

The digestibility study for grow out-stage Nile tilapia has been completed. Nine common feedstuffs including fish meal, poultry byproduct meal, meat and bone meal, blood meal, soybean meal, peanut meal, corn gluten meal, as well as wheat and corn, have been tested in this experiment. Digestible energy, protein and amino acids have been evaluated. These data will be important mainly for the ideal protein diet formulation and least-cost diet formulation.

OBJECTIVES 5-6

A no-cost extension will be requested to extend this project.

WORK PLANNED

OBJECTIVE 1

The literature will continue to be searched for additional information on development of least-cost diet formulation for grow out-stage Nile tilapia.

OBJECTIVE 2

Whole body amino acid compositions of yellow perch and Nile tilapia will be conducted. The body amino acid profiles will provide a reference for formulating a protein and amino acid balanced diet for the two focal species.

OBJECTIVE 3

Lysine requirement for Nile tilapia will be determined using information from the first year's experiment.

OBJECTIVE 4

The digestibility study for yellow perch will be conducted once an adequate number of yellow perch has been collected. After numerous efforts and failures to secure perch of the appropriate size, and bring them into the lab at the University of Missouri-Columbia fully healthy, the decision has been made to conduct this research at the Piketon Center operation by Ohio State University. This gets around the major problem of transporting these fish, as they are available on site.

OBJECTIVES 5-6

Upon the completion of the previous objectives, a low-cost feed formulation for both yellow perch and Nile tilapia will be developed and provided to the extension contacts for distribution.

IMPACTS

Diets for North Central Region species that are effective, economical, and can be developed within reasonable time periods are needed for the advancement of the

NUTRITION/DIETS

aquaculture industry in this region. The development of appropriate and effective diets in this project has the potential of greatly increasing the profitability of the aquaculture industry in this region.

SUPPORT

NCRAC funds provided to date total \$40,000; a total of \$80,000 has been allocated for this 2-year project.

PUBLICATIONS, MANUSCRIPTS, WORKSHOPS, AND CONFERENCES

See the Appendix for a cumulative output for all NCRAC-funded Nutrition/Diets activities.

NORTH CENTRAL REGIONAL AQUACULTURE CENTER

EVALUATION OF THE NEWLY-DEVELOPED, LEAST-COST EXPERIMENTAL DIET FOR BLUEGILL AT COMMERCIAL DENSITIES IN PONDS AT TWO OR MORE FACILITIES IN THE NORTH CENTRAL REGION⁹

Project *Progress Report* for the Period
September 1, 2010 to August 31, 2011

NCRAC FUNDING LEVEL: \$43,363 (September 1, 2010 to August 31, 2011)

PARTICIPANTS:

| | | |
|------------------|-----------------------|----------|
| Paul B. Brown | Purdue University | Indiana |
| Charles E. Hicks | Lincoln University | Missouri |
| Joseph E. Morris | Iowa State University | Iowa |
| Robert A. Rode | Purdue University | Indiana |
| James E. Wetzel | Lincoln University | Missouri |

PROJECT OBJECTIVES

- (1) Using as consistent protocols as possible across locations, evaluate/determine performance of recently-developed NCRAC least-cost juvenile (3" minimum total length) bluegill diet versus an "industry standard" diet at two distinct latitude locations at standard pond stocking densities for one growing season. Stocking densities to be determined by the investigator(s) and producer(s).
- (2) Coordinate dissemination of project results with the NCRAC Technical Committee/Extension Subcommittee.

driven by, broader U.S. and worldwide transitions in the seafood industry. Aquaculture-related business in the NCR continues to be an "emerging" industry in that selection of appropriate species and associated culture practices including feed selection is on-going.

As with any animal industry, feed costs can be a considerable component. It is a known fact that feeds often account for $\geq 50\%$ of the variable costs in aquaculture budgets. To reduce these variable costs there have been numerous research efforts in the NCR as well as the nation addressing the possible uses of lower-cost foodstuffs, e.g., vegetable or animal by-product protein.

ANTICIPATED BENEFITS

Growth in the North Central Region's (NCR) aquaculture industry mirrors, and is

Clearly, a substantial need exists to reduce these costs and develop more nutritionally

⁹NCRAC has funded four Nutrition/Diets projects. The Termination Report for the first project is contained in the 1997-98 Annual Progress Report; the Termination Report for the second Nutrition/Diets project is contained in the 2008-09 Annual Progress Report; and the Progress Report for the third project is contained elsewhere in this Annual Report. This Progress Report is for the fourth project. It is a 2-year project that began September 1, 2010.

NORTH CENTRAL REGIONAL AQUACULTURE CENTER

adequate diets for established as well as emerging aquaculture species in the NCR. Although significant insights have come from these efforts, no diets yielding advantages beyond those offered by existing practical diets have resulted, in terms of growth performance, cost, or improved fish health, for any NCR culture species. The aim of the proposed study is to evaluate a diet for juvenile northern bluegill (*Lepomis macrochirus macrochirus*) that is significantly less costly than currently available diets for sunfish, while yielding a growth rate that is at least equal to an industry standard sunfish diet. Such a diet formulation is now available to the NCR as the result of a recently funded North Central Regional Aquaculture Center (NCRAC) project (see <http://www.ncrac.org/FundedProjects/Nutrition2.htm>) developed by Robert Hayward (University of Missouri-Columbia). The formulation now needs to be evaluated by comparing its performance against an “industry standard” diet in a commercial production pond setting.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

Prior to the initiation of the funded project, Lincoln University (LU) staff conditioned monosex groups of adult northern bluegill and select brood fish ($N = 216$; 72 males and 144 females). In late May 2010 half of the brood fish (36 males and 72 females) were sent to Iowa State University (ISU) and half were retained by LU. The fish were placed into hatchery ponds at both sites. All ponds were managed for optimal fish production following pond fertilization practices and management for sunfish fingerling production. In October 2010, fingerlings were harvested from ponds located at both locations with ISU fish being held in the

campus facility until the following spring. LU staff over-wintered fish in ponds.

In April-May 2011 ponds located at ISU, LU, and Purdue University (Purdue) were stocked at 19,772 fish/ha (8,000 fish/acre) with ISU ponds stocked with a combination of ISU and LU fish, and Purdue ponds stocked with LU fish. All stocked age-1 fish originated from the same parental stock source. Average size of fish ranged from 8.5–13.1 g (0.3–0.5 oz) at Purdue, 10.4–15.1 g (0.4–0.5 oz) at LU, and 5.9–12.8 g (0.2–0.4 oz) at ISU.

Starting the first week of May fish at all locations were fed either the standard diet (SilverCup Extruded Trout; 40% protein and 12% fat) or the bluegill open formula diet to satiation using feeding rings to limit waste. Initial analysis of the fish sampled in late June indicated no significant differences between the two diets at LU and ISU but at Purdue fish fed the standard diet were significantly larger.

WORK PLANNED

Pond culture will continue until October 2011 for a culture period of approximately 180 days with fish at all sites being harvested during the same timeline. Fish will be harvested by seining followed by hand picking of stranded fish during draining. At the end of the culture season, a representative randomly selected sample ($N = 100+$) from each pond will be dressed for fillets with weights taken for whole body, gilled, and gutted carcass, fillet, viscera, and liver. A subsample of randomly selected fillets ($N = 20$) will be homogenized prior to proximate composition analysis. Proximate composition will be made of four replicates from each subsample.

NUTRITION/DIETS

IMPACTS

Results garnered from this research will provide the aquaculture industry with relevant field-tested information related to the culture of bluegills using least cost experimental diets.

PUBLICATIONS, MANUSCRIPTS, WORKSHOPS, AND CONFERENCES

See the Appendix for a cumulative output for all NCRAC-funded Nutrition/Diets activities.

NORTH CENTRAL REGIONAL AQUACULTURE CENTER

COMPARISON, IDENTIFICATION, AND ROLE OF MICROBIAL COMMUNITIES IN RECIRCULATING SYSTEMS IN THE NORTH CENTRAL REGION¹⁰

Project *Termination Report* for the Period
September 1, 2009 to August 31, 2011

NCRAC FUNDING: \$65,000 (September 1, 2009 to August 31, 2011)

PARTICIPANTS:

| | | |
|--------------------|-----------------------------------|-----------|
| Lutgarde M. Raskin | University of Michigan | Michigan |
| James S. Diana | University of Michigan | Michigan |
| Russell L. Cuhel | University of Wisconsin-Milwaukee | Wisconsin |
| Carmen Aguilar | University of Wisconsin-Milwaukee | Wisconsin |

Industry Advisory Council Liaison:

| | | |
|------------|-------------------------------|----------|
| Russ Allen | Seafood Systems, Inc., Okemos | Michigan |
|------------|-------------------------------|----------|

Extension Liaison:

| | | |
|---------------|-------------------------------|--------------|
| Mark E. Clark | North Dakota State University | North Dakota |
|---------------|-------------------------------|--------------|

REASON FOR TERMINATION

Project completed.

PROJECT OBJECTIVES

- (1) Characterize the microbial communities in established production scale marine and freshwater recirculating aquaculture systems (RAS). These systems have been operational and producing aquatic organisms for more than one year.
- (2) Once these microbial communities have been identified, the role(s) of these microbial communities within the nitrogen cycle will be quantified with the goal of increasing the efficiency of the RAS (increased survival, growth, and density, etc. of aquatic organisms).
- (3) Coordinate the results of this project with the Technical Committee Extension Subcommittee of NCRAC.

PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

Freshwater Recirculating Aquaculture Systems (RAS)

One of the recirculating systems is located at the Great Lakes Water Institute at the University of Wisconsin-Milwaukee (UW-Milwaukee). This indoor, freshwater RAS, housing approximately 10,000 yellow perch, has been in operation for approximately 10 years. The system is comprised of a production tank housing the yellow perch, followed by an ozonation step, pH correction using sodium bicarbonate, a floating plastic bead filter designed for solids removal, a fluidized bed sand filter, and a whiffle ball aerator. The plastic bead filter and the sand filter were analyzed for biological activity.

DNA was extracted from biomass samples obtained from both the plastic bead filter

¹⁰This 1-year funded project was chaired by Lutgarde M. Raskin and began September 1, 2009. In other areas of this report, this project is referred to as RAS Microbial Communities.

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and the sand filter of the freshwater RAS. Clone libraries were generated targeting the 16S rRNA gene of *Bacteria*, *Archaea*, and *Planctomycetes*.

The bacterial clone libraries for the plastic bead filter and the sand filter resulted in 231 and 239 sequences, respectively. The plastic bead filter contained a diverse bacterial community. It was primarily comprised of *Alphaproteobacteria*, *Acidobacteria*, and *Planctomycetes*. However, it contained members of the *Actinobacteria*, *Verrucomicrobia*, *Bacteroidetes*, *Oputitae*, *Nitrospira*, *Deinnococcus*, *Fusobacteria*, *Flavobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, and *Deltaproteobacteria*. The bacterial community in the sand filter was less diverse. The *Alphaproteobacteria* made up over 50% of the sequences in this library. This community also contained members of the *Acidobacteria*, *Planctomycetes*, *Sphingobacteria*, *Actinobacteria*, *Verrucomicrobia*, *Bacteroidetes*, *Nitrospira*, *Deinnococcus*, *Flavobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, and *Deltaproteobacteria*. Surprisingly, no ammonia oxidizing bacteria (AOB) were detected in either of the clone libraries, whereas nitrite oxidizing bacteria (NOB) were contained with the phylum *Nitrospira*.

The archaeal clone libraries for the plastic bead and sand filters contained 47 and 60 sequences, respectively. The archaeal diversity in both of the filters was low. The detected *Archaea* belonged almost exclusively to the *Thermoprotei*. No ammonia oxidizing archaea (AOA) were detected in either filter.

Planctomycetes clone libraries were constructed specifically to detect the presence of anaerobic ammonia oxidizing (anammox) bacteria. The clone libraries for

the plastic bead and sand filters contained 78 and 37 sequences, respectively. The plastic beads contained members of the genera *Planctomyces*, *Zavarzinella*, *Pirellula*, *Rhodopirellula*, and *Blastopirellula*. The sand filter community contained members of the *Planctomyces* and *Blastopirellula*. None of these genera contain any known species that perform the anammox metabolism.

In order to supplement the clone library data, 454-pyrosequencing was performed, targeting the bacterial and archaeal 16S rRNA gene. Pyrosequencing resulted in 460 and 248 bacterial sequences for the plastic bead and sand filter samples, respectively. The plastic bead filter was comprised primarily of *Alphaproteobacteria*, which made up more than 50% of the sequences. Other groups that were present were *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, *Actinobacteria*, *Acidobacteria*, *Planctomycetes*, *Nitrospira*, *Fusobacteria*, *Bacteroidetes*, *Chloroflexi*, *Firmicutes*, and *Deinnococcus-Thermus*. The sand filter contained *Alphaproteobacteria*, *Bacteroidetes*, *Acidobacteria*, and *Nitrospira* at high abundances. The remainder of the community was comprised of *Betaproteobacteria*, *Deltaproteobacteria*, *Gammaproteobacteria*, *Verrucomicrobia*, *Gammatimonadetes*, *Planctomycetes*, *Deinnococcus-Thermus*, *Actinobacteria*, and *OP10*. No AOB were detected in either sample. In both samples, *Thermoprotei* was the only archaeal group that was detected and classified.

Marine RAS

The marine RAS located at Seafood Systems, Inc. in Okemos, Michigan consists of nine culture tanks in series followed by a trickling filter. Because the facility is located away from the coast, artificial seawater was prepared from a commercial

RAS MICROBIAL COMMUNITIES

salt solution. The marine RAS relies on biofiltration in a multi-stage, nitrifying trickling filter that contains two types of biofilm attachment media: plastic bioballs and crushed oyster shells. Microbial biomass from five biofilters and water samples from the effluent of the culture tanks and biofilter compartments were collected on August 10, 2010. The first five compartments contained bioballs, while the last compartment contained oyster shells.

At the time of sampling, the pH in the system ranged from 8.52–8.67. The alkalinity in the system ranged from 308–470 mg/L as CaCO₃, and the total chemical oxygen demand (COD) ranged from 44–63 mg/L. The ammonia concentrations were very low (in the µg-N/L range) and showed a generally decreasing trend across the tanks and the biofilter compartments. Both the ammonia and nitrite concentrations were below the toxicity level.

The composition of the microbial community in the biofilter compartments was determined by analysis of the bacterial 16S rRNA gene sequences obtained from 454-pyrosequencing. Analysis of the sequences revealed that the most abundant groups at the phylum level were the *Bacteroidetes*, *Nitrospira*, *Planctomycetes*, and *Proteobacteria*. Populations of interest within the microbial community included AOB, anaerobic ammonia oxidizing (anammox) bacteria, NOB, and *Vibrio* species. AOB of the genera *Nitrosococcus* and *Nitrosospira* were present. The abundance of AOB in all compartments was less than 1% of sequences analyzed. NOB from the phyla *Proteobacteria* and *Nitrospira*, genera *Nitrobacter*, and *Nitrospira*, respectively, were detected. The most abundant NOB in all compartments were the nitrite-oxidizing *Nitrospira*. All

sequences in the *Nitrospira* phylum were nitrite-oxidizing NOB. *Nitrospira* were the dominant NOB at the time of sampling, which was likely due to the low nitrite concentrations in the biofilter. Also, *Vibrio* species were not detected in any of the samples. The genus *Vibrio* is of importance because it contains shrimp pathogens, specifically *Vibrio harveyi*.

OBJECTIVE 2

The freshwater RAS was monitored for various water quality parameters, including ammonia, nitrite, nitrate, COD, alkalinity, total suspended solids (TSS), and volatile suspended solids (VSS). Samples were taken at five points throughout the system: the production tank, the pH adjustment tank, the plastic bead filter effluent, the sand filter effluent, and the whiffle ball aerator effluent. The total ammonia nitrogen (TAN) and nitrite concentrations were consistently removed to levels of 0.05 mg NH₄⁺-N/L and 0.2 mg NO₂⁻-N/L, respectively.

To determine the nitrification capacity and the ability of the system to respond to a high ammonia concentration, the system was spiked with ammonia and the concentrations of ammonia and nitrite were monitored over the course of several hours. The ammonia concentration decreased from 6.3 mg/L NH₄⁺-N (450 µM) to less than 0.35 mg/L NH₄⁺-N (25 µM) in just under two hours and a significant reduction in ammonia concentration was observed within the first half hour. The nitrite concentrations were initially 0.003 mg/L NO₂⁻-N (0.2 µM) but increased to just over 0.014 mg/L NO₂⁻-N (1 µM) within the first hour. The concentration stabilized at this level and decreased to pre-spike levels within 2.5 h. These data indicate that the nitrification capacity of this system is sufficient to allow the system to respond to high ammonia concentrations. This suggests that the

system would be able to respond to an ammonia shock or could accommodate denser fish stocking or a higher feed rate in the production tank.

Batch experiments were also conducted on the media from both the plastic bead filter and the sand filter to determine the ammonia and nitrite oxidizing activity of the biologically active media. In these experiments, media (either plastic beads or sand) was sampled from the biological filters, placed in bottles with water sampled from the system and spiked with either ammonia or nitrite. For the bottles spiked with ammonia, ammonia and nitrite concentrations were measured to determine how quickly the ammonia was removed and whether there was nitrite buildup in the water. In the assay with biofilter sand, the ammonia level dropped from an initial concentration of 13.3 mg/L $\text{NH}_4^+\text{-N}$ (950 μM) to below 2.8 mg/L $\text{NH}_4^+\text{-N}$ (200 μM) in 40 h and began to level off. After 130 h, the ammonia level was down to 1.8 mg/L $\text{NH}_4^+\text{-N}$ (130 μM). The nitrite concentration in this assay remained less than 0.02 mg N/L for the duration of the experiment. Ammonia and nitrite oxidation rates of the biofilter sand were proportional to the amount of sand added to the batch experiment, with the rates increasing linearly with increased sand concentration.

Similar experiments were performed using plastic beads from the floating bead filter. For the beads, the ammonia was removed almost as quickly as in the assay with the sand. However, the nitrite was not removed quickly enough to counteract the nitrite production from ammonia oxidation, resulting in a buildup of nitrite in the system. Based on these experiments, one can conclude that both the sand and the plastic beads have some ammonia-oxidizing capacity. However, the sand has

significantly greater capacity for ammonia oxidation than the beads. This is consistent with the results from the monitoring of the RAS. Also, given that there was no nitrite buildup in the sand experiments and that the plastic beads had little nitrite oxidation capacity, one can conclude that the sand filter is the primary location of nitrite oxidation. The clone library analysis of the two filters showed similar abundances of *Nitrospira* in both the plastic bead and sand filters (3.1 and 3.5%, respectively). However, pyrosequencing revealed a much greater abundance of *Nitrospira* in the sand filter (12.9%) versus the plastic bead filter (3.5%). This supports the finding in the batch experiment that the sand is primarily responsible for nitrite oxidation.

Unfortunately, the marine RAS system was not available during the time period for which experiments were planned for nitrification capacity experiments.

OBJECTIVE 3

Information garnered from this project will be reviewed by members of the Extension Workgroup in developing possible new materials for the industry.

IMPACTS

- Improve knowledge of nitrogen cycle in RAS and generate means for stable management of toxic nitrogen compounds.
- Understand how operational changes (e.g., backwashing, change in ammonia load) affect biofilter operation.
- Characterize the microbial communities in the two biological filters.
- Improve economic viability of RAS by finding ways to improve process efficiency (i.e., underutilized nitrification potential suggests that a greater stocking density or a larger

RAS MICROBIAL COMMUNITIES

production tank is possible for the current water treatment system).

- In collaboration with a Mathematics-Biology Initiative at UW-Milwaukee, an undergraduate student has made progress on a model using physical characteristics of the RAS, flow rates, bench-derived process rates, and time series chemical analysis.

RECOMMENDED FOLLOW-UP ACTIVITIES

To better understand the microbial community dynamics in a RAS, it would be necessary to perform a detailed assessment of the abundance and activity of ammonia and nitrite oxidizers in biofilters over an extended time period. To optimize the efficiency of this type of RAS, it would be important to understand the stability of the

system under higher ammonia loadings. This could provide information to investigate the possibility of increased stocking densities and thus greater economic efficiency. It would also be useful to understand the system's response to spikes in ammonia or nitrite loading to evaluate its ability to respond to sudden changes in water quality and protect the fish from acute toxicity.

SUPPORT

NCRAC has provided \$65,000 which is the total amount allocated for this project.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

See the Appendix for a cumulative output for all NCRAC-funded RAS Microbial Communities activities.

NORTH CENTRAL REGIONAL AQUACULTURE CENTER

SNAIL MANAGEMENT/GRUB CONTROL¹¹

Project *Progress Report* for the Period
September 1, 2007 to August 31, 2011

NCRAC FUNDING: \$20,500 (September 1, 2007 to August 31, 2011)

PARTICIPANTS:

| | | |
|---|---|-----------|
| Gregory W. Whitledge | Southern Illinois University-Carbondale | Illinois |
| Christopher F. Hartleb | University of Wisconsin-Stevens Point | Wisconsin |
| Todd Huspeni | University of Wisconsin-Stevens Point | Wisconsin |
| Joseph E. Morris | Iowa State University | Iowa |
| Richard D. Clayton | Iowa State University | Iowa |
| Industry Advisory Council Liaison: | | |
| Rex Ostrum | Ostrum Acres Fish Farm, McCook | Nebraska |
| Extension Liaison: | | |
| Joseph E. Morris | Iowa State University | Iowa |

PROJECT OBJECTIVE

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

ANTICIPATED BENEFITS

Grub infections in fish culture ponds are extremely relevant to the aquaculture industry in the North Central Region (NCR) as the industry has experienced a loss of income in both commercially important food fish species and baitfish. These economic losses result both directly from fish mortality due to trematode infection, and indirectly because of unappealing visual presentation of food fish fillets containing grubs. Outcomes of this project should help

culturists in dealing effectively and economically with these infestations.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

A search has been initiated by Iowa State University staff to review literature to date concerning the three main control methods for snails: biological, chemical, and mechanical. This information will then be combined with information garnered from this research project to develop an interactive Web page for fish producers to access and obtain information potentially relevant to their snail problems. Among the various options, information regarding effectiveness, application costs, legal implications, and potential for impact on pond general ecology, e.g., zooplankton dynamics in fish fingerling ponds, will be listed. This Web page will be hosted on the revised North Central Regional Aquaculture Center (NCRAC) Web site.

¹¹ This Progress Report is for the second objective of this project. A Project Component Termination Report for the first objective is contained in the 2009-10 Annual Progress Report. This is a project that had two years of funding and is chaired by Gregory W. Whitledge. It began September 1, 2007.

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WORK PLANNED

Iowa State University

In 2012 the completed database on snail control will be shared with all project investigators to insure that the information is complete. Additional information garnered from the recently completed research will be included. Following project review of this database, a Web page will then be developed and placed on the NCRAC Web site.

IMPACTS

Project results will provide valuable information regarding the effectiveness and efficiency of several potentially useful approaches for controlling snail populations and associated grub infestations in aquaculture ponds in the NCR. Previously untested treatments for snail control in ponds (the crayfish *Orconectes virilis*,

freshwater prawn, hybrid sunfishes, biocontrol with natural dominant trematodes, and integrated chemical and biological controls) are being evaluated. Results will also provide insight into the degree of snail population control required to limit grub prevalence in cultured fishes in ponds where food fish are raised.

SUPPORT

To date, NCRAC has provided \$20,500 which is the total amount allocated for this objective.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

See the Appendix for a cumulative output for all NCRAC-funded Snail Management/Grub Control activities.

VIRAL HEMORRHAGIC SEPTICEMIA (VHS)¹²

Project *Progress Report* for the Period
September 1, 2008 to August 31, 2011

NCRAC FUNDING: \$197,960 (September 1, 2008 to August 31, 2011)

PARTICIPANTS:

| | | |
|---|---|-----------|
| Jeffrey J. Rach | Upper Midwest Environmental Sciences Center | Wisconsin |
| Glenda D. Dvorak | Iowa State University | Iowa |
| Ronald E. Kinnunen | Michigan State University | Michigan |
| Jeffrey A. Malison | University of Wisconsin-Stevens Point | Wisconsin |
| Industry Advisory Council Liaison: | | |
| Christopher Weeks | Michigan State University | Michigan |

PROJECT OBJECTIVES

- (1) Determine the safety and efficacy of iodine disinfection on walleye and northern pike eggs infected with VHS.
- (2) Prepare and electronically disseminate a VHS “response” packet that specifically targets fish farm producers. The packet would address aspects of the disease (clinical signs, routes of transmission) and prevention practices to minimize introduction and spread. The packet will also contain Web sites and information sources where fish farmers can obtain the most current, up-to-date status of the disease.
- (3) Conduct a series of six biosecurity workshops held at different fish farms across the region, targeting different production systems (flow-through, pond, and recirculation systems).
- (4) Utilize the existing Aquatic Invasive Species (AIS) Hazard Analysis Critical Control Point (HACCP) Training Curriculum to develop specific fish

disease HACCP plans for each of the six facilities involved in the workshops.

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

ANTICIPATED BENEFITS

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

¹²This 2-year funded project is chaired by Jeffrey A. Malison and it began September 1, 2008.

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

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

The proposed use of the AIS-HACCP approach has many advantages. It can effectively deal with a diverse industry and diverse risk factors associated with a variety of plant, invertebrate, vertebrate, and pathogen AIS. If it develops as it has in the seafood industry, it should prove to be a good partnership between industry and government regulators. It can help avoid overly restrictive regulations, and, if properly applied, can be effective at reducing the risk of spreading AIS via baitfish and aquaculture practices. The HACCP approach concentrates on the points in the process that are critical to the environmental safety of the product, minimizes risks, and stresses

communication between regulators and the industry. With proper cooperation between industry representatives, resource management agencies, and other AIS experts, the AIS-HACCP approach will reduce the risk that AIS will be established in new locations while maintaining the economic viability of the baitfish and aquaculture industries. It can provide a mechanism for AIS-free certification, and it can instill confidence in the public that state and federal fish stocking programs are conducting their activities in an environmentally responsible manner.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

Adult walleye and northern pike were collected from the Mississippi River (Pool 9) and spawned at the Upper Midwest Environmental Sciences Center (UMESC) by personnel from the U.S. Fish and Wildlife Service (USFWS) Genoa National Fish Hatchery. Immediately after sperm activation, fertilized eggs were taken to a controlled access laboratory with effluent disinfection where egg challenge, disinfection, and incubation activities occurred. Immediately on entry into the laboratory, eggs were challenged at either 10^5 or 10^8 plaque-forming units/mL (PFU/mL) for 30 min. The virus used for this study was isolated by the USFWS La Crosse Fish Health Center from emerald shiners (*Notropis atherinoides*) collected from Lake Erie in 2006. Eggs challenged at 10^5 PFU/mL were progeny of different male/female pairings than those challenged at 10^8 PFU/mL. Walleye egg adhesion was reduced by immersing the eggs in a bentonite solution for ~2 min during VHSV challenge. Immediately after challenge, eggs were assigned to one of the four treatment groups (Table 1 in the Appendix to this report).

Eggs were held in well water for at least 90 min post-fertilization before being distributed to miniature egg jars. Eggs were maintained in egg jars until hatch with no other chemical treatments applied. Egg and fry samples were collected and the presence or absence of VHSv determined using epithelioma papulosum cyprini (EPC) cells. Assays used for determining the presence of VHSv were conducted according to the USFWS Standard Procedures for Aquatic Animal Health Inspections/American Fisheries Society Fish Health Section Blue Book (2007) procedures.

VHSv was not isolated from any iodophor-disinfected treatment groups (Appendix Table 1, treatment groups 2-4). However, VHSv was isolated from control eggs (Appendix Table 2) immediately after challenge and for up to four days after challenge in northern pike eggs challenged at 10^8 PFU/mL. The virus was not detected in positive control eggs one day post-challenge for either northern pike or walleye eggs challenged at 10^5 PFU/mL nor was it detected in fry of either control or iodophor-disinfected treatment groups.

Though some iodophor treatments reduced hatch, eggs and fry appeared to develop normally. Iodophor disinfection did not substantially reduce northern pike egg hatch but walleye egg hatch was reduced when eggs were held for 30 or 60 min in the iodophor disinfection solution (Appendix Table 3).

Egg iodophor disinfection appears to effectively eliminate VHSv (strain IVb) from the surface of walleye and northern pike eggs. Although iodophor egg disinfection reduced walleye egg hatch in this study, previous UMESC toxicity studies indicated that when applied shortly after fertilization (~5 min), similar iodophor

disinfection treatment regimens did not alter egg hatch. Incorporation of iodophor disinfection at 100 ppm during gamete collection from non-salmonid fishes immediately post-fertilization (<5 min) for 30 min or at 90 min after fertilization for 10 min may reduce VHSv (strain IVb) transmission without affecting egg hatch.

In Year 2, adult walleye were collected from North Dakota and spawned at UMESC. Immediately after sperm activation, fertilized eggs were taken to a controlled access laboratory where egg disinfection and incubation activities occurred. The study objective was to determine the safety of iodophor surface disinfection at target doses of 0, 1, or 2× the recommended dose rate (100 mg/L) for multiple exposure durations at various times post fertilization and for 1 or 2 disinfection events (Appendix Table 4). The second disinfection event was administered at the approximate midpoint between fertilization and the first cell division.

The study was conducted in replicate egg jars supplied with well water in a continuous flow system. Egg jars were connected to one of four individually plumbed headbox systems. Each headbox supplied water to 12 egg jars. There were six egg jars per side (block) with one treatment randomly assigned to each block of six egg jars. Eggs (25 ± 5 mL) were assigned to each jar according to a completely randomized distribution scheme.

Very poor fertilization rates were realized. Although adult walleye appeared healthy at spawning and females had free flowing eggs, most male walleye provided very little milt. When notochord development was checked it was apparent that fertilization had not occurred. This trial was terminated

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before embryo hatch due to the low fertilization rate.

Also in Year 2 adult hybrid striped bass were collected from Oklahoma by the Oklahoma Department of Fish and Game and spawned at UMESC. Adult female striped bass were injected with human chorionic gonadotropin (HCG) to stimulate oocyte maturation. After staging, ripe female striped bass eggs were fertilized with male white bass milt. The study objective was to determine the safety of iodophor surface disinfection at target doses of 0, 0.25, 0.50, or 1× the recommended dose rate (100 mg/L) for multiple exposure durations (Appendix Table 5). The study was conducted according to the methods described above for walleye.

The hybrid striped bass eggs were very sensitive to iodophor disinfection. Hatch rate in the 25 mg iodophor/L disinfection group was similar to that of the untreated controls; hatch was very limited in the 50 mg iodophor/L disinfection group and nonexistent in the 100 mg iodophor/L disinfection group.

Iodophor concentrations safe to disinfect hybrid striped bass eggs are substantially less than those used to disinfect the surfaces of eggs of other fish species. Presently it is not clear whether iodophor disinfection is suitable for surface disinfection of hybrid striped bass eggs.

Although walleye safety data were not collected during this spawning year at UMESC, other laboratories did collect data which, when combined with previous UMESC data and data available from the literature, should describe the safe treatment regimens for walleye. UMESC did collaborate with the Missouri Department of Conservation on the effect of iodophor

disinfection on walleye egg hatch and fingerling survival. These data are being summarized and will be submitted to UMESC.

In Year 3, UMESC will evaluate vertical transmission of VHSv from adult spotfin shiners (*Notropis spilopterus*) to eggs. Adult spotfin shiners will be obtained from Woodside Farms (Bellevue, Ohio) and will be exposed to VHSv by injection prior to spawning. Adults and eggs will be tested for the presence/absence of VHSv. A portion of the eggs will be allowed to hatch and the fry tested for the presence or absence of VHSv.

OBJECTIVE 2

The VHS “response” packet was developed by Iowa State University in April 2009. The packet is an 18-page PDF document containing information for aquaculture producers on the signs, susceptible species, and prevention of VHS. A “Biosecurity for Aquaculture Facilities” PowerPoint® presentation (36 slides with speaker notes) was also developed in April 2009. All of the materials have been forwarded to other Project Leaders (Malison and Kinnunen) to be incorporated into the biosecurity workshop objective of this project (Objective 3). Additionally, these materials have been posted for download on the Center for Food Security and Public Health (CFSPH) Web site (<http://www.cfsph.iastate.edu/DiseaseInfo/MoreInfo/VHS.htm>) and the Focus on Fish Health Web site (www.focusonfishhealth.org).

OBJECTIVE 3

In 2009/2010, eight planned VHS-biosecurity workshops were conducted at aquaculture facilities in the NCR. Michigan State University and University of Wisconsin Extension Aquaculture

Specialists partnered with local and regional animal health professionals to present information on fish disease transmission, VHS and HACCP planning specific to developing a biosecurity plan for aquaculture facilities. Details are as follows:

- ▶ May 14, 2009, Indiana – Bodin State Fish Hatchery (recirculating aquaculture system), 27 total in attendance.
- ▶ June 25, 2009, Missouri – Crystal Lakes Fisheries (flow through), 29 total in attendance.
- ▶ August 20, 2009, Michigan – Michigan Bait and Fish Farm (flow through), 24 total in attendance.
- ▶ September 17, 2009, Wisconsin – Gollon Bait and Fish Farm (pond), 23 in attendance.
- ▶ April 6, 2010, Wisconsin – U.S. Geological Survey UMESC (research), 50 in attendance.
- ▶ April 21, 2010, Michigan – Keweenaw Bay Indian Fish Hatchery (raceway/pond), 10 in attendance.
- ▶ May 6, 2010, Ohio – Calala’s Water Haven (pond), 12 in attendance.
- ▶ June 17, 2010, South Dakota – Porter’s Bait Farm (pond and flow through), 20 in attendance.

Evaluations of the workshops indicated that the participants found the information helpful (average score of 4.56 on a scale of 5), intended to use the information (average score 4.58), and the information was presented in an easy to understand format (average score 4.57). HACCP plans were developed for each of the hosting facilities with special emphasis on system type (pond, recirculating, or flow-through) and business activities (wild stocking, egg and fingerling production, or grow out for food). It was interesting to note that the initial skepticism of the participants was overcome by program emphasis on the economic

consequences of disease introduction and the critical control point analysis that is the basis of a HACCP plan. This analysis provides the framework to make biosecurity decisions that are effective and economical.

OBJECTIVE 4

- ▶ Bodin State Fish Hatchery already had a HACCP biosecurity plan in place. Comments were made to improve a few critical control points (visitor access and logs).
- ▶ Crystal Lakes Fisheries had their own biosecurity plan which was used as a basis for drawing up a HACCP biosecurity plan.
- ▶ Michigan Bait and Fish Farms already had a HACCP biosecurity plan in place from previous work with Michigan State University Sea Grant Extension.
- ▶ Gollon Bait and Fish Farm had their own biosecurity plan which was used as a basis for drawing up a HACCP biosecurity plan.
- ▶ U.S. Geological Survey UMESC had a biosecurity plan developed which was reviewed and recommendations for improvement were made.
- ▶ Keweenaw Bay Indian Fish Hatchery is working on developing biosecurity measures and recommendations were made on critical control points.
- ▶ Calala’s Water Haven produces and sells softshell crayfish and an AIS-HACCP plan was developed for this part of their bait operation.
- ▶ Porter’s Bait Farm produces and sells fathead minnows and an AIS-HACCP plan was developed for this part of their bait operation.

WORK PLANNED

OBJECTIVE 5

To be completed as described in the original proposal.

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OBJECTIVE 6

Production of the HACCP biosecurity video is nearing completion. The DVD script has undergone review and the appropriate edits have been incorporated, the audio portion of the DVD has been recorded and the video components have been selected. The final editing and production is currently being undertaken by UW-Extension media services and a final version of the DVD is expected by December. Also, following the completion of the biosecurity workshop videos and model HACCP plans, these materials will be posted by ISU for free access on the CFSPH and Focus on Fish Health Web sites.

IMPACTS

- ▶ The project demonstrated that coolwater fish eggs retain VHSV for up to 4 days following immersion challenge but that eggs may not retain VHSV through egg hatch (all fry, including controls, were VHSV negative).
- ▶ The project demonstrated that iodophor disinfection may safely and effectively reduce the risk associated with VHSV exposure during spawning/egg take operations from wild brood fish.
- ▶ The project demonstrated that hybrid striped bass are sensitive to iodophor disinfection.
- ▶ A U.S. Geological Survey Fact Sheet was published in FY 2010 (see <http://pubs.usgs.gov/fs/2009/3107/>).
- ▶ To date, there have been no reports of VHS having been found in any NCR fish farm or hatchery, nor is there any evidence suggesting that VHS has been spread via fish movements into or out of

any fish farms. VHS has changed how fish farmers do business in the NCR whether farmers are located in a state directly impacted by the Federal Order or a state that has farmers doing business in the Great Lakes states. Through workshops and educational materials on biosecurity, farmers have become aware of the risks and potential hazards diseases from outside sources bring. Biosecurity was not a word of common vocabulary before 2007 and now is incorporated as part of their business plan. State agencies have responded with their own set of rules requiring additional testing and fish certifications. Farmers have been able to utilize biosecurity strategies to minimize the impacts these rules have or they have been able to continue business by complying with requirements in new rules when biosecurity plans are mandatory.

- ▶ The majority of the attendees at the workshops indicated that they would implement biosecurity/AIS-HACCP plans at their facilities based on the information learned at the workshops.
- ▶ The VHS-HACCP instructional DVD will further increase the ability of aquaculture producers to develop effective and economical HACCP-based biosecurity plans to control the spread of VHSV as well as address potential AIS and disease concerns in the future.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

See the Appendix.

VHS

SUPPORT

| YEAR | NCRAC- USDA FUNDING | OTHER SUPPORT | | | | | TOTAL SUPPORT |
|--------------|---------------------------|-----------------|----------|------------------|---------|----------|------------------|
| | | UNIVER- SITY | INDUSTRY | OTHER FEDERAL | OTHER | TOTAL | |
| 2008-09 | \$116,870 | | | \$23,422 | \$3,900 | \$27,322 | \$144,192 |
| 2009-10 | \$81,090 | | | \$50,000 | | \$50,000 | \$131,090 |
| TOTAL | \$197,960 | | | \$73,422 | \$3,900 | \$77,322 | \$275,282 |

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APPENDIX

Table 1. Iodophor disinfection treatment groups. Disinfection was initiated immediately after viral challenge except that disinfection of Treatment group 4 was initiated 60 minutes after challenge.

| Treatment group | Iodophor disinfection | Time initiated post-fertilization (min) | Disinfection duration (min) | Iodophor concentration (ppm) |
|-----------------|-----------------------|---|-----------------------------|------------------------------|
| 1 | No | NA | NA | 0 |
| 2 | Yes | ~30 | 30 | 100 |
| 3 | Yes | ~30 | 60 | 100 |
| 4 | Yes | ~90 | 10 | 100 |

Table 2. Detection of VHSV (# positive samples/# samples tested) in northern pike and walleye positive control eggs and fry.

| Day post challenge | Northern pike | | Walleye | |
|--------------------|------------------------|------------------------|------------------------|------------------------|
| | 10 ⁵ PFU/mL | 10 ⁸ PFU/mL | 10 ⁵ PFU/mL | 10 ⁸ PFU/mL |
| Day 0a | 3/3 | 3/3 | 2/3 | 3/3 |
| Day 0b | 2/3 | 3/3 | 2/3 | 3/3 |
| Day 1 | 0/6 | 6/6 | 0/6 | 2/6 |
| Day 2 | 0/6 | 6/6 | NT | NT |
| Day 3 | 0/6 | 6/6 | NT | NT |
| Day 4 | 0/6 | 4/6 | NT | NT |
| Day 5 | 0/6 | 0/6 | NT | NT |
| Fry | 0/6 | 0/6 | 0/6 | 0/6 |

a-eggs tested during challenge

b-eggs tested after water hardening

NT-Not tested

Table 3. Percent hatch of northern pike and walleye eggs.

| Treatment Group | Percent Hatch | | | |
|-----------------|------------------------|------------------------|------------------------|------------------------|
| | Northern pike | | Walleye | |
| | 10 ⁵ PFU/mL | 10 ⁸ PFU/mL | 10 ⁵ PFU/mL | 10 ⁸ PFU/mL |
| 1 | 61 | 65 | 44 | 38 |
| 2 | 53 | 67 | 56 | 5 |
| 3 | 49 | 67 | 19 | 0 |
| 4 | 61 | 69 | 54 | 43 |

VHS

Table 4. Iodophor disinfection treatment groups for walleye eggs.

| Treatment Group | Concentration (mg iodophor/L) | Duration (min) | Time initiated post fertilization (min) | Second iodophor disinfection duration (min) |
|-----------------|-------------------------------|----------------|---|---|
| 1 | NA | NA | NA | NA |
| 2 | 100 | 10 | 90 | NA |
| 3 | 100 | 30 | immediately | NA |
| 4 | 200 | 10 | 90 | NA |
| 5 | 200 | 30 | immediately | NA |
| 6 | NA | NA | NA | NA |
| 7 | 100 | 10 | 90 | 10 |
| 8 | 100 | 30 | immediately | 10 |

Table 5. Iodophor disinfection treatment groups for striped bass eggs.

| Treatment Group | Concentration (mg iodophor/L) | Duration (min) | Time initiated post fertilization (min) |
|-----------------|-------------------------------|----------------|---|
| 1 | NA | NA | NA |
| 2 | 25 | 10 | 90 |
| 3 | 25 | 30 | immediately |
| 4 | 50 | 10 | 90 |
| 5 | 50 | 30 | immediately |
| 6 | NA | NA | NA |
| 7 | 100 | 10 | 90 |
| 8 | 100 | 30 | immediately |

NORTH CENTRAL REGIONAL AQUACULTURE CENTER

DETERMINATION OF PRODUCTION PARAMETERS OF SELECTED YELLOW PERCH LINES AT COMMERCIAL DENSITIES IN PONDS AT TWO OR MORE FACILITIES IN THE NORTH CENTRAL REGION¹³

Project *Progress Report* for the Period
September 1, 2010 to August 31, 2011

NCRAC FUNDING: \$67,926 (September 1, 2010 to August 31, 2011)

PARTICIPANTS:

| | | |
|---|---------------------------------------|-----------|
| Hanping Wang | Ohio State University | Ohio |
| Christopher F. Hartleb | University of Wisconsin-Stevens Point | Wisconsin |
| William E. Lynch, Jr. | Mill Creek Perch Farms, LLC | Ohio |
| Jeffrey A. Malison | Coolwater Farms, LLC | Wisconsin |
| Industry Advisory Council Liaison: | | |
| Charles E. Hicks | Lincoln University | Missouri |
| Extension Liaison: | | |
| Laura G. Tiu | Ohio State University | Ohio |

PROJECT OBJECTIVES

- (1) Using consistent protocols, assess survival and growth rate of two replications of first-year fingerlings of improved lines of yellow perch as compared to fingerlings from local brood stock (feed-trained fingerlings to be stocked at 60,000/acre (150,000 fish/ha).
- (2) Using consistent protocols assess 2nd year survival, growth rate, and market parameters (production, fillet yields, percent market size) of both replications of improved lines of yellow perch as compared to local fish.

- (3) Disseminate results to industry and to end-user customers via fact sheets, scientific publications, and an on-farm field day.

ANTICIPATED BENEFITS

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

¹³ NCRAC has funded nine Yellow Perch projects. This Progress Report is for the ninth Yellow Perch project. It is a 3-year funded project that is chaired by Hanping Wang and began September 1, 2010. Termination Reports for the first three projects are contained in the 1989-96 Compendium Report; a Termination Report for the fourth and fifth projects is contained in the 1997-98 Annual Progress Report; a Termination Report for the sixth project is contained in the 1999-00 Annual Progress Report; a Termination Report for the seventh project is contained in the 2000-01 Annual Progress Report; and a Termination Report for the eighth project is contained in the 2004-05 Annual Progress Report.

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completion of this project, multiplication stations will be established to produce enough fry/fingerlings from improved strains for fish farmers in the NCR. Success in this project should be similar to that achieved for striped bass, rainbow trout, and catfish. Improved strains should show increased growth by 20–25% per generation and have a tremendous positive impact on the NCR yellow perch aquaculture industry.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

Ohio

Using the previously developed 2nd generation of selected brood fish, the 3rd generation of selected lines was created via marker-assisted cohort selection (MACS). When a majority of the 2nd generation improved lines reached harvest size, approximately 500 of the best fish were chosen for selected lines based on their body weight and breeding value, PIT tagged, and genotyped at the Ohio State University (OSU) Center for Aquaculture Research and Development (OCARD) using microsatellite markers. Molecular genetic pedigrees were determined and genetic relatedness charts were constructed for mating. Among the selected fish, about 300 pairs of the least-related fish having the highest breeding value were selected for factorial, mass, and pair mating; 215 survival families and a total of 1.2–1.5 million fry were produced. In addition, a control line of local brood fish from Ohio was also produced.

All the brood fish candidates were genotyped using eight microsatellite loci developed and optimized at Ohio State University's Aquaculture Genetics and Breeding Laboratory in Piketon. Polymerase chain reaction (PCR) was performed using a BioRad PTC-200 DNA engine thermal cycler. Genotyping was

performed using an ABI 3130 DNA Sequencing and Genotyping System and genotypes were automatically scored using Genemapper. Parentage assignment of the improved line and “control” fish was performed using microsatellite profiles for the eight loci and the exclusion-based approach implemented in the program CERVUS 3.0.

Factorial and single-pair matings based on the genetic pedigree and relatedness chart were conducted in 50.0 cm-diameter (19.7 in-diameter) flow-through tanks in March 2011 when fish had reached sexual maturity. One or two injections of human chorionic gonadotropin (HCG) at the dosage of 200–600 IU/kg (91–273 IU/lb) body weight based on females' need and maturity were used to synchronize spawning. The fertilized egg ribbon from each mating was collected daily from spawning tanks starting 2 days post-injection.

One hundred egg ribbons were produced from the improved line at OCARD in Piketon. Twenty of them were delivered to Mill Creek (MC) Perch Farms in Ohio on March 16, 2011. No ribbons were shipped to Wisconsin sites because transport permits were not ready by the March spawning time. At both the Piketon Station and MC Perch Farm, egg ribbons were incubated in tanks with flow-through well water. Two days post hatch, fry were siphoned and counted for stocking in the nursery ponds. At the same time, 30 and 18 families from the Ohio control line were produced by mass spawning at MC Perch Farm and OCARD, respectively.

At OCARD, 320,000 improved fry were stocked at 80,000 fry each to four 0.08 ha (0.2 acre) ponds for nursery (1,040,000/ha; 421,000/acre), 50,000 local fry (650,000/ha; 263,000/acre) were stocked into an

YELLOW PERCH ASSESSMENT

additional pond. At MC Perch Farm, approximately 200,000 improved fry were stocked to two 0.08 ha (0.2 acre) ponds, and 200,000 local fry were stocked in two other similar ponds. All ponds were fertilized twice before stocking and once every week during the nursery period. Fry at the two locations were pond-reared until they reached 25.0–35.0 mm (1.0–1.4 in) (~6 weeks), at which time they were harvested and moved into indoor tanks for feed-training.

Feed-training was conducted in 1.8–3.0 m (6.0–10.0 ft) round tanks with a stocking density of 4.0–5.0 kg (8.9–11.0 lb) fingerlings/m³ at a temperature of 20–24°C (68–75°F) for 3–4 weeks. Fish were fed AquaMax Fry Powder and high protein Starter 100 with high protein using automatic feeders. Feeding rates were about 5% of body weight (BW) for the first 2 days, and then increased to 7–8% BW.

For Year 1 rearing, the two test sites conducted replicated tests of the improved fish versus the local-strain using two types of rearing tests: (1) at OCARD, the selected line of yellow perch and a local-strain (control) were reared in separate ponds, each having two replicates, with a density of 69,300 fish/ha (28,000/acre); (2) at MC Perch Farm, the selected line and a local-strain were raised communally in two 0.08 ha (0.2-acre) ponds at a density of 232,900 fish/ha (94,300/acre). AquaMax Starter 100 to AquaMax Grower 400 feed was used for all experimental ponds with a feeding rate of 3% BW (OCARD) and satiation feeding (MC Perch Farms). Feeding amount and rates were adjusted monthly based on an assumed survival of 75% and calculated biomass using mean weight at the Piketon site.

All of the ponds were harvested at the end of October (MC Perch Farm) and in early November 2011 (OCARD). One hundred and fifty fish from each of the separate ponds at Piketon, and 500 fish from each of the communal ponds at MC Perch Farm were sampled, individually weighed and finclipped. Finclip samples were preserved individually with 95-100% alcohol in small vials. Eight molecular markers were used to assign selected and local-strain yellow perch to their family of origin for communal rearing.

At MC Perch Farm, improved yellow perch grew significantly larger than local yellow perch native to the farm in two communal ponds, where both improved and unimproved fish grew in the same environment. The improved line outweighed the local strain by 32.00% on average at the end of the Year 1 test (October). Fingerling survival in the MC Perch Farm's communal ponds with improved fish was as high as they have ever experienced.

In the OCARD ponds, improved fish exhibited a 27.16% higher survival rate and a 22.01% higher production than the local Ohio strain by the end of October of Year 1. Although the 27.16% higher survival rate of the improved fish resulted in a significantly higher density and lower feed rations (rations were calculated based on the same assumed survival rate for all the ponds), the improved line still had a higher mean body weight (37.82 g; 1.33 oz) than the local Ohio strain (37.62 g; 1.33 oz). A significantly greater reduction of CV_{wt} was observed for the improved line than the unimproved fish, indicating the size variation of the improved fish was smaller, and their percentage of marketable size fish would be higher by the end of Year 2.

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Wisconsin

The co-investigators and collaborators in Wisconsin could not conduct their portion of the studies for Objective 1 as originally planned because of issues regarding the interstate transport of fish and fish eggs that arose from recently changed state and federal viral hemorrhagic septicemia restrictions and testing. Because of these issues, Objectives 1 and 2 were delayed by one year and instead will commence in 2012 and 2013, respectively.

WORK PLANNED

OBJECTIVE 1

Both on-farm and on-station tests of selected lines with local control lines will be conducted at two locations in the state of Wisconsin using both separate rearing and communal rearing methods as performed in Ohio. The two sites are the University of Wisconsin-Stevens Point Northern Aquaculture Demonstration Facility and Coolwater Farms, LLC. Consistent rearing protocols will be adhered to the two selected sites.

OBJECTIVE 2

In Year 2, the selected and local lines of large yellow perch fingerlings will be reared in ponds at two Ohio sites. In the autumn of Year 2, all of the ponds will be harvested and the key production parameters (e.g., survival, growth, feed conversion for separate rearing, fillet yield) will be evaluated. Differences between females and males will be carefully measured.

OBJECTIVE 3

A yellow perch workshop is planned for spring of 2012. At this workshop, research results will be presented to all attendees. Information continues to be shared over the yellow perch list serve hosted by OCARD.

IMPACTS

The impact of this project will be primarily through the delivery of superior yellow perch strains to farmers for use in a wide range of culture and exposure conditions across the NCR. The greatest return on investment for this project is the ultimate reduction in production costs due to increased growth rate and reduced feed costs by using genetically improved strains. Success in this project should be similar to that achieved for striped bass, rainbow trout, and catfish. Improved strains should show increased growth by 20–25% per generation and have a tremendous positive impact on the NCR yellow perch aquaculture industry.

SUPPORT

NCRAC funds provided to date total \$67,926; a total of \$150,000 has been allocated for this 3-year project.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

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

APPENDIX

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APPENDIX

AQUACULTURE DRUGS

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Salmonid Culture, East Lansing, Michigan, March 23-24, 1990. (Donald L. Garling)

Midwest Regional Cage Fish Culture Workshop, Jasper, Indiana, August 24-25, 1990. (LaDon Swann)

Aquaculture Leader Training for Great Lakes Sea Grant Extension Agents, Manitowoc, Wisconsin, October 23, 1990. (David J. Landkamer and LaDon Swann)

Regional Workshop of Commercial Fish Culture Using Water Reuse Systems, Normal, Illinois, November 2-3, 1990. (LaDon Swann)

1st North Central Regional Aquaculture Conference, Kalamazoo, Michigan, March 18-21, 1991. (Donald L. Garling, Lead; David J. Landkamer, Joseph E. Morris and Ronald Kinnunen, Steering Committee)

Crayfish Symposium, Carbondale, Illinois, March 23-24, 1991. (Daniel A. Selock and Christopher C. Kohler)

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- Fish Transportation Workshops, Marion, Illinois, April 6, 1991 and West Lafayette, Indiana, April 20, 1991. (LaDon Swann and Daniel A. Selock)
- Regional Workshop on Commercial Fish Culture Using Water Recirculating Systems, Normal, Illinois, November 15-16, 1991. (LaDon Swann)
- 1st National Aquaculture Extension Workshop, Ferndale, Arkansas, March 3-7, 1992. (Joseph E. Morris, Steering Committee)
- Regional Workshop on Commercial Fish Culture Using Water Recirculating Systems, Normal, Illinois, November 19-20, 1992. (LaDon Swann)
- In-Service Training for CES and Sea Grant Personnel, Gretna, Nebraska, February 9, 1993. (Terrence B. Kayes and Joseph E. Morris)
- Aquaculture Leader Training, Alexandria, Minnesota, March 6, 1993. (Jeffrey L. Gunderson and Joseph E. Morris)
- Investing in Freshwater Aquaculture, Satellite Videoconference, Purdue University, April 10, 1993. (LaDon Swann)
- National Extension Wildlife and Fisheries Workshop, Kansas City, Missouri, April 29-May 2, 1993. (Joseph E. Morris)
- Commercial Aquaculture Recirculation Systems, Piketon, Ohio, July 10, 1993. (James E. Ebeling)
- Yellow Perch and Hybrid Striped Bass Aquaculture Workshop, Piketon, Ohio, July 9, 1994. (James E. Ebeling and Christopher C. Kohler)
- Workshop on Getting Started in Commercial Aquaculture Raising Crayfish and Yellow Perch, Jasper, Indiana, October 14-15, 1994. (LaDon Swann)
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- Aquaculture in the Age of the Information Highway. Multimedia session, 18 month meeting of the Sea Grant Great Lakes Network, Niagara Falls, Ontario, May 6, 1995. (LaDon Swann)
- AquaNIC. Annual Meeting of the Aquaculture Association of Canada, Nanaimo, British Columbia, June 5, 1995. (LaDon Swann)
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- Rainbow Trout Production: Indoors/Outdoors, Piketon, Ohio, July 8, 1995. (James E. Ebeling)
- North Central Regional Aquaculture Center Hybrid Striped Bass Workshop, Champaign, Illinois, November 2-4, 1995. (Christopher C. Kohler, LaDon Swann, and Joseph E. Morris)
- 3rd North Central Regional Aquaculture Conference, Indianapolis, Indiana, February 6-7, 1997. (LaDon Swann)
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- Description of the Aquaculture and Bait Fish Industries: Threat Evaluation and Identification of Critical Control Points, International Joint Commission Workshop on Exotic Policy, Milwaukee, Wisconsin, September 22-26, 1999. (Jeffrey L. Gunderson)
- Fisheries Management in the North Central Region, 9th National Extension Wildlife, Fisheries, and Aquaculture Conference, Portland, Maine, September 29-October 2, 1999. (Joseph E. Morris, and S.K. Whitcomb)
- Internet Resources for Aquaculture Education and Communications: Present and Future, 9th National Extension Wildlife, Fisheries, and Aquaculture Conference, Portland, Maine, September 29-October 2, 1999. (LaDon Swann)

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- Yellow Perch Producers' Forum, Hudson, Wisconsin, January 21-22, 2000. (Joseph E. Morris and Jeffrey L. Gunderson)
- Organic Aquaculture Standards Workshop, Minneapolis, Minnesota, June 23-24, 2000. (Anne R. Kapuscinski)
- "I've got this hog barn..." Videoconference Workshop, Lima, Ohio, November 16, 2002. (Laura G. Tiu)
- Applications of HACCP in Aquaculture, Aquaculture America 2003, Louisville, Kentucky, February 18-21, 2003. (Ronald E. Kinnunen)
- Food Safety Issues Related to Aquaculture, Aquaculture America 2003, Louisville, Kentucky, February 18-21, 2003. (Ronald E. Kinnunen)
- The ANS-HACCP Approach: Reducing the Risk of Spreading Aquatic Nuisance Species, Aquaculture America 2003, Louisville, Kentucky, February 18-21, 2003. (Ronald E. Kinnunen)
- Use of Natural Ponds for Fish and Baitfish Production, Aquaculture America 2003, Louisville, Kentucky, February 18-21, 2003. (Ronald E. Kinnunen)
- Overviews on Production, Nutrition, Economics, and Fish Health Management for Yellow Perch, *Perca flavescens*, Aquaculture America 2003, Louisville, Kentucky, February 18-21, 2003. (Fred P. Binkowski, Ronald E. Kinnunen, and Geoffrey Wallat)
- Hybrid Walleye Workshop, Jackson, Missouri, March 5, 2003. (Ronald E. Kinnunen and Robert A. Pierce II)
- Extension Program Assessment: An Extension Specialist's View, 3rd National Aquaculture Extension Conference, Tucson, Arizona, April 7-11, 2003. (Joseph E. Morris)
- Great Lakes Native American Involvement in Fisheries Extension Programs, 3rd National Aquaculture Extension Conference, Tucson, Arizona, April 7-11, 2003. (Ronald E. Kinnunen and Charles Pistis)
- On Farm Demonstration of Freshwater Shrimp Culture in Southern Ohio, 3rd National Aquaculture Extension Conference, Tucson, Arizona, April 7-11, 2003. (Laura G. Tiu)
- Potential Recovery and Beneficial Use of Aquaculture Effluents and Waste By-Products, Aquaculture 2004, Honolulu, Hawaii, March 1-4, 2004. (Joseph E. Morris and Fred P. Binkowski)
- Introduction to Recirculating Aquaculture Workshop, Bellevue, Ohio, March 20, 2004. (Laura G. Tiu)
- Great Lakes Native American Involvement in Fisheries Extension Programs, American Fisheries Society Annual Meeting, Madison, Wisconsin, August 25, 2004. (Ronald E. Kinnunen)
- Channel Catfish Culture in Midwestern Plastic-Lined Ponds, American Fisheries Society Annual Meeting, Madison, Wisconsin, August 25, 2004. (Joseph E. Morris)
- Aquaculture Field Day, Lincoln University Carver Farm, Missouri, October 2004. (Robert A. Pierce)
- Yellow Perch Aquaculture Workshop, Bad River Tribal Hatchery Program, Milwaukee, Wisconsin, December 2004. (Fred P. Binkowski)
- Yellow Perch and Lake Sturgeon Workshop, Lac du Flambeau Tribal Hatchery, Milwaukee, Wisconsin, February 2005. (Fred P. Binkowski)
- Yellow Perch Aquaculture Workshop, Kearney, Nebraska, February 26, 2005. (Fred B. Binkowski)
- Hazard Analysis Critical Control Point (HACCP) Training for Commercial Fish Processors (poster), International Association of Great Lakes Research Conference, Ann Arbor, Michigan, May 24, 2005. (Ronald E. Kinnunen)
- Great Lakes Native American Involvement in Fisheries Extension Programs, International Association of Great Lakes Research Conference, Ann Arbor, Michigan, May 24, 2005. (Ronald E. Kinnunen and Charles Pistis)
- Why AIS-HACCP? Overview and Rationale, International Association of Great Lakes Research Conference, Ann Arbor, Michigan, May 24, 2005. (Ronald E. Kinnunen and Jeffery L. Gunderson)

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- Aquaculture Overview, National Farm and Ranch Business Management Education Association Annual Conference, Wooster, Ohio, June 13, 2005. (Laura G. Tiu)
- AIS-HACCP Training Workshop, American Fisheries Society Annual Conference, Lake Placid, New York, September 10, 2006. (Ronald E. Kinnunen)
- Yellow Perch Spawning Workshop, Milwaukee, Wisconsin, November 2, 2006. (Fred B. Binkowski).
- AIS-HACCP Train-the-Trainer Workshop, Columbus, Ohio, February 9, 2007. (Ronald E. Kinnunen and Jeff Gunderson)
- Conversion of Livestock Barns into Fish Production Facilities IP Videoconference, Purdue University, West Lafayette, Indiana, March 8, 2007. (Kwamena K. Quagraine)
- Tri-State Aquaculture Conference/Workshop. Ashland, Nebraska, March 17, 2007. (Fred B. Binkowski and Joseph E. Morris)
- Freshwater Prawn Production Workshop, Sellersburg, Indiana, April 14, 2007. (Kwamena K. Quagraine)
- Using Sensory Analysis to Better Position a Fish Product in the Market Place, 4th National Aquaculture Extension Conference, Cincinnati, Ohio, May 1-3, 2007. (Ronald E. Kinnunen)
- The HACCP Approach to Prevent the Spread Of Aquatic Invasive Species by Aquaculture and Baitfish Operations, 4th National Aquaculture Extension Conference, Cincinnati, Ohio, May 1-3, 2007. (Ronald E. Kinnunen)
- The VHS Virus in the Great Lakes Region, 92nd Annual Meeting and Professional Improvement Conference, National Association of County Agricultural Agents, Grand Rapids, Michigan, July 17, 2007. (Ronald E. Kinnunen)
- The HACCP Approach to Prevent the Spread of Aquatic Invasive Species by Aquaculture and Baitfish Operations, 92nd Annual Meeting and Professional Improvement Conference, Association of County Agricultural Agents, Grand Rapids, Michigan, July 17, 2007. (Ronald E. Kinnunen)
- AIS-HACCP Training Workshop, Clare, Michigan, July 30, 2007. (Ronald E. Kinnunen)
- AIS-HACCP Training Workshop, Rogers, Minnesota, September 6, 2007. (Ronald E. Kinnunen and Jeff Gunderson)
- Michigan Aquaculture and Salmonid Aquaculture in the North Central Region, Great Lakes Sea Grant Network Conference, Chicago, Illinois, September 18, 2007. (Ronald E. Kinnunen)
- AIS-HACCP Training Workshop, Stevens Point, Wisconsin, October 26, 2007. (Ronald E. Kinnunen and Phil Moy)
- AIS-HACCP Training Workshop, Pierre, South Dakota, January 4, 2008. (Ronald E. Kinnunen and Jeff Gunderson)
- MarketMaker, Michigan Aquaculture Association Annual Conference, Clare, Michigan, February 12, 2008. (Ronald E. Kinnunen)
- North Central Regional Aquaculture Center VHS Project, Michigan Aquaculture Association Annual Conference, Clare, Michigan, February 12, 2008. (Ronald E. Kinnunen)
- VHS: a Regional Industry Perspective, Illinois VHS Conference and Workshop, Rend Lake, Indiana, April 26, 2008 (Christopher T. Weeks)
- AIS-HACCP Use in the Baitfish and Aquaculture Industries, Organisms in Trade Workshop, Great Lakes Commission, Romulus, Michigan, June 10, 2008. (Ronald E. Kinnunen)
- AIS-HACCP Training Workshop, Indianapolis, Indiana, July 23, 2008. (Ronald E. Kinnunen and Kristin TePas)
- Seafood HACCP Training Workshop, Bay Mills, Michigan, December 9-11, 2008. (Ronald E. Kinnunen)
- North Central Regional Aquaculture Center Seeks Input from the Missouri Aquaculture Industry, Missouri Aquaculture Association Meeting and Biosecurity Workshop, Jefferson City, Missouri, January 23, 2009. (Christopher T. Weeks)
- AIS-HACCP Training Workshop, Ashland, Nebraska, October 15, 2009. (Ronald E. Kinnunen and Richard Clayton)

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- Aquaculture in Michigan – A Brief Overview of Status, Regulatory Structures and Impacting Factor, Lansing, Michigan, January 22, 2010. (Christopher T. Weeks)
- Michigan Aquaculture Association, Aquaculture Biosecurity/AIS-HACCP Update, Mt. Pleasant, Michigan, February 25, 2010. (Ronald E. Kinnunen)
- Seafood HACCP Training Workshop, Bay Mills, Michigan, March 9-11, 2010. (Ronald E. Kinnunen)
- Certified Pesticide Applicators Workshop, Aquatic Plant Identification and Control, Wetmore, Michigan, March 19, 2010. (Ronald E. Kinnunen and Jim Islieb)
- Interstate Movement of Live Fish Regulations in the North Central U.S., Workshop for Veterinarians, Madison, Wisconsin, April 26-27, 2010. (Christopher T. Weeks)
- AIS-HACCP Training Workshop, Spirit Lake, Iowa, June 16, 2010. (Ronald E. Kinnunen and Joseph Morris)
- Baitfish Regulations and State Industry Contacts, NCRAC Baitfish Workshop, La Crosse, Wisconsin, September 21, 2010. (Christopher T. Weeks)
- Biosecurity and VHS Virus Update, NCRAC Baitfish Workshop, La Crosse, Wisconsin, September 21, 2010. (Ronald E. Kinnunen)
- Early Spawning and Associated Laboratory Practices of Golden Shiners, NCRAC Baitfish Workshop, La Crosse, Wisconsin, September 21, 2010. (Joseph E. Morris)
- Pond Fertilization Strategies for Northern Climates, NCRAC Baitfish Workshop, La Crosse, Wisconsin, September 21, 2010. (Joseph E. Morris)
- Interstate Movement of Live Fish Regulations in the North Central U.S., Workshop for Veterinarians, Frankfort, Indiana, September 30, 2010. (Christopher T. Weeks)
- Online fish health program for fish farmers. The 35th Annual Eastern Fish Health Workshop, Shepherdstown, Shepherdstown, West Virginia, May 24-28, 2010. (Myron J. Kebus)
- NCRAC Baitfish Workshop, La Crosse, Wisconsin, September 21, 2010. (Jeff Gunderson, Joseph E. Morris and Ronald E. Kinnunen)
- Missouri Aquaculture – Status, Progress, Priorities, Information Access and Concerns. Missouri Aquaculture Association Annual Meeting, Lincoln University Carver Farm, January 10, 2011. (Christopher T. Weeks)
- Ohio State University Bluegill Aquaculture Workshop, Columbus, Ohio, February 11, 2011. (Charles Hicks, Laura Tiu)
- Seafood HACCP Training Workshop, Baraga, Michigan, February 15-18, 2011. (Ronald E. Kinnunen)
- Growing Power Conference, Fish Processing, Milwaukee, Wisconsin, September 11, 2010. (Ronald E. Kinnunen)

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FEED TRAINING CARNIVOROUS FISH

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Sims, D.W. 2007. Effects of feed training methods and light intensity on survival and feed training success of largemouth bass *Micropterus salmoides* and effectiveness of new bird repellent devices in a commercial aquaculture setting. Master's thesis. Southern Illinois University-Carbondale.

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Kelly, A.M., and C.C. Kohler. 1996. Sunshine bass performance in ponds, cages, and indoor tanks. Progressive Fish-Culturist 58:55-58.

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- Schnick, R.A. 2007. Dialog with Upper Midwest Environmental Sciences Center on Fisheries Management Chemicals and Drugs Program. Upper Midwest Environmental Sciences Center Partner Meetings, La Crosse, Wisconsin, May 10-11, 2007.
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APPENDIX

SOME COMMONLY USED ABBREVIATIONS AND ACRONYMS

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

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