

**North Central Regional Aquaculture Center Compendium Report  
for the Period May 1, 1989 to August 31, 1996**

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## Introduction

The U.S. aquaculture industry continues to be one of the fastest growing sectors within U.S. agriculture, although at a lesser rate than what occurred during the 1980s. Production in 1994 reached 666 million pounds and generated approximately \$751 million for producers. The impact of U.S. aquaculture is substantial accounting for approximately 181,000 jobs and generating an estimated \$5.6 billion annually. Yet, anticipated growth in the industry, both in magnitude and in species diversity, continues to fall short of expectations.

Much of what is known about aquaculture science is a result of institutional attention given to our traditional capture of wild fisheries with the goal of releasing cultured fishes into public waters for enhancement of declining public stocks. Despite extensive efforts to manage wild populations for a sustained yield, as a nation we consume substantially greater amounts than we produce. Much of the United States' demand for seafood has been met by imports. The U.S. imports over 40% of its fish and shellfish and, after Japan, is the world's second largest importer of seafood. Fisheries imports are the largest contributor to the U.S. trade deficit among agricultural products, and the second largest after petroleum, among all natural resources products. The value of imported fisheries products more than doubled during the 1980s and has continued to increase in the 1990s. In fact, the \$12.5 billion value for 1995 was a record. In 1995, the trade deficit was \$4.2 billion for all fisheries products, \$3.5 billion 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 overexploited. 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/sub-tropical Pacific regions of the country. The 1996 Federal Agriculture Improvement and Reform Act (FAIR) (P. L. 104-127) 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.

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 12 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.

At the present time the staff of NCRAC at MSU includes Ted R. Batterson, Director and Liz Bartels, Executive Secretary. The Center Director has the following responsibilities:

- 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;
- with assistance of the Economics and Marketing Work Group, Technical Committee, or others preparing a summary of regional aquaculture, including production statistics and sales, and identifying

technical, financial, and institutional constraints to expanding production. The summary shall include sections addressing established industries, development industries, and opportunities for new product development, and recommended research needs;

- maintaining liaison with other RACs; and
- serving on the NCC.

At the present time the staff of NCRAC's Office for Publications and Extension Administration at ISU includes Joseph E. Morris, Associate Director and Glenda Dike, Secretary. The Associate Director has the following responsibilities:

- serving as head of Publications for NCRAC, including editor of the Center's newsletter;
- serving as the NCRAC liaison with national aquaculture extension programs, including in particular, extension programs of the other four USDA RACs; 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 two persons from the IAC (the chair and an at-large member), a representative from the region's State Agricultural Experiment Stations and Cooperative Extension Services, a member from a non-land grant university and representatives from the two universities responsible for the center: Michigan State and Iowa State. 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 subcommittee 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 an ex-officio 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 inception of NCRAC 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/CSREES/Grants Management Branch for approval. To date the Center has received nine 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), and FY96 (Grant #96-38500-2631) with monies totaling \$6,440,981. Currently, five grants are active (FY92-96); the first four grants (FY88-91) have terminated.
- The Center annually coordinates a program planning meeting which sets priorities for the next funding cycle and calls for regional workshops to develop project outlines to address priority problem areas.
- Work Groups, which are formed at the workshops, submit project outlines to the Center. The projects are peer reviewed by experts from both within and outside the region.
- The BOD, using 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 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

objective(s) are then included in a workshop announcement that is broadly distributed throughout the North Central Region. The workshops are 1-day events to establish a work group that will develop a project outline over the summer months. Work group members will be those who have demonstrated that they have the expertise and facilities for undertaking the proposed work in regard to a particular objective or objectives. The proposed work cannot deviate from the objective or objectives included in the workshop announcement. The work group elects a chair and secretary. The chair is responsible for submitting the project outline to the NCRAC Director; the secretary is responsible for preparing minutes from the workshop that are distributed to all attendees. All project outlines are peer reviewed. The reviewers' comments are used by the BOD in making the final selection of projects and level of funding at the following year's annual Program Planning meeting. All work group members are apprised of the BOD decisions. Revisions of projects approved by the BOD are submitted by the work group chair to the NCRAC Director. The revised project outlines are then included in a POW that is submitted to USDA. Upon approval by USDA, the Center issues subcontracts to the funded work group members.

### *Time frame*

- Program Planning meeting: early winter.
- Workshops: late-spring, early summer.
- Project outlines developed over the summer by work group members who participated in the workshops. These project outlines are then submitted to the Center in the fall and peer reviewed.
- The Board of Directors at the following year's Program Planning Meeting selects the projects to be funded.
- Project outline revised and submitted to the Center by May.
- Revised projects are then submitted in June as a POW (or an amendment to a POW) to USDA for approval. Once approved by USDA subcontracts are let by the Center with a start date of September 1.

By following this procedure, it takes approximately 18 months from the time of identifying a priority area until inception of a project to address the issue in question.

### *Workshops*

The purpose of the workshops is to bring together those who are best qualified to work on project objectives by virtue of a demonstrated record of expertise and access to facilities required in the project. These people form a work group for the purpose of writing a project outline to address the problem in question. The following criteria typically apply to those projects that are funded by NCRAC.

- Involves participation by two or more states in the North Central Region;
- requires more scientific manpower, equipment, and facilities than generally available at one location;
- approach is adaptable and particularly suitable for inter-institutional cooperation resulting in better use of limited resources and a saving of funds;
- will complement and enhance ongoing extension and research activities by participants, as well as offer potential for expanding these programs;
- is likely to attract additional support for the work which is not likely to occur through other programs and mechanisms;
- is sufficiently specific to promise significant accomplishments in a reasonable period of time (usually up to 2 years);
- can provide the solution to a problem of fundamental importance or fill an information gap;
- can be organized and conducted on a regional level, assuring coordinated and complementary contributions by all participants.

The NCRAC program pays no overhead to participating institutions nor tuition remission, has no brick-and-mortar money, and relies on in-place salaried personnel, equipment, and facilities to carry out the projects. Due to the collaborative and cooperative nature of these regional projects, no one individual or institution receives a significant portion of the total project funds.

### **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 five separately funded projects in regard to Extension and six for walleye. 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 Plans of Work (POWs) or amendments to POWs for the grants as indicated in Table 1. Many times, the projects within a particular area are merely continuations of previously funded activities; while at other times they are addressing new objectives. Presented below are Progress or Termination Reports for all projects that were underway or completed during the period May 1, 1989, to August 31, 1996. May 1, 1989, marked the beginning of the first projects funded by NCRAC.

All publications, manuscripts, or papers presented for all funded NCRAC project areas are listed in the Appendix.

# North Central Regional Aquaculture Center (NCRAC)

**Table 1. North Central Regional Aquaculture Center funded projects.**

Project area	Project number	Proposed duration period	Funding level	Grant number
<b>Extension</b>	1	5/1/89-4/30/91	\$39,221	88-38500-3885
			\$37,089	89-38500-4319
	2	3/17/90-8/31/91	\$31,300	89-38500-4319
	3	9/1/91-8/31/93	\$94,109	91-38500-5900
	4	9/1/93-8/31/95	\$110,129	91-38500-5900
	5	9/1/95-8/31/97	\$10,875	92-38500-6916
			\$25,725	95-38500-1410
			\$348,448	
<b>Economics and marketing</b>	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
			\$254,988	
<b>Yellow Perch</b>	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	\$200,000	95-38500-1410
			\$704,785	
<b>Hybrid Striped Bass</b>	1	5/1/89-8/31/91	\$68,296	88-38500-3885
			\$68,114	89-38500-4319
	2	6/1/90-8/31/92	\$101,000	90-38500-5008
	3	9/1/91-8/31/93	\$96,550	91-38500-5900
	4	9/1/93-8/31/95	\$168,000	93-38500-8392
	5	9/1/95-8/31/97	\$160,000	95-38500-1410
			\$661,960	
<b>Walleye</b>	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	\$117,897	94-38500-0048
			\$57,103	95-38500-1410
			\$798,397	
<b>Sunfish</b>	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	\$174,999	94-38500-0048
			\$455,556	
<b>Salmonids</b>	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	\$200,000	94-38500-0048
			\$479,796	
<b>NCR Aquaculture Conference</b>	1	6/1/90-3/31/91	\$7,000	90-38500-5008
<b>National Aqua. Extension Workshop</b>		10/1/91-9/30/92	\$3,005	89-38500-4319
<b>Crayfish</b>	1	9/1/92-8/31/94	\$49,677	92-38500-6916
<b>Baitfish</b>	1	9/1/92-8/31/94	\$61,973	92-38500-6916
<b>Wastes/Effluents</b>	1	9/1/92-8/31/94	\$153,300	92-38500-6916
<b>National Aquaculture INAD/NADA Coordinator</b>	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	
			\$17,000	

# Project Termination or Progress Reports

## Extension

*Progress Report for the Period May 1, 1989 to August 31, 1996*

### **NCRAC funding level**

\$328,923 (May 1, 1989 to August 31, 1996)

### **Participants**

Fred P. Binkowski, University of Wisconsin-  
Milwaukee, Wisconsin

James E. Ebeling, Ohio State University, Ohio

Donald L. Garling, Michigan State University,  
Michigan

Jeffrey L. Gunderson, University of Minnesota,  
Minnesota

F. Robert Henderson, Kansas State University, Kansas

John Hochheimer, Ohio State University, Ohio

Anne R. Kapuscinski, University of Minnesota,  
Minnesota

Terrence B. Kayes, University of Nebraska-Lincoln,  
Nebraska

Ronald E. Kinnunen, Michigan State University,  
Michigan

Christopher C. Kohler, Southern Illinois University-  
Carbondale, Illinois

David J. Landkamer, University of Minnesota,  
Minnesota

Charles Lee, Kansas State University, Kansas

Joseph E. Morris, Iowa State University, Iowa

Kenneth E. Neils, Kansas State University, Kansas

Robert A. Pierce II, University of Missouri, Missouri

Daniel A. Selock, Southern Illinois University-  
Carbondale, Illinois

LaDon Swann, Purdue University Indiana/Illinois

#### **Administrative Advisor:**

David C. Petritz, Purdue University, Indiana

### **Project objectives**

1. Strengthen linkages between North Central Regional Aquaculture Center (NCRAC) research and extension work groups.
2. Enhance the North Central Region (NCR) extension network for aquaculture information transfer.
3. Provide in-service training for Cooperative Extension Service, Sea Grant Advisory Service, and other landowner assistance personnel.
4. Develop and implement aquaculture educational programs for the NCR.
5. Develop aquaculture materials for the NCR including extension fact sheets, bulletins, manuals/guides, and instructional video tapes.

### **Anticipated benefits**

The NCRAC Extension Work Group will promote and advance commercial aquaculture in a responsible fashion through an organized education/training outreach program. The primary benefits will be:

- 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., walleye, hybrid striped bass, yellow perch, salmonids, and sunfish, through hands-on workshops and field demonstration projects;
- improved lines of communication between interstate aquaculture extension specialists and associated industry contacts; and
- an enhanced legal and socioeconomic atmosphere for aquaculture in the NCR.

## Progress and principal accomplishments

### Objective 1

Due to the efforts of aquaculture extension personnel in the NCR, NCRAC's Board of Directors formally adopted guidelines for extension's involvement in all NCRAC-funded projects. These guidelines integrate research and extension activities so that extension service personnel can better serve their clientele groups. In addition, aquaculture Extension Work Group members have:

- served as extension liaisons, if not active researchers, for every funded NCRAC project;
- assisted in writing and developing the NCRAC Walleye Culture Manual that was edited by Bob Summerfelt of Iowa State University;
- assisted with the planning, promotion, and implementation of the hybrid striped bass, walleye and yellow perch workshops held throughout the region;
- helped conduct a survey of crayfish producers in the NCR and completed a report on *Orconectes immunis* for inclusion in the Crayfish Work Group report;
- provided the NCRAC Economics and Marketing Work Group with information relevant to that group's efforts to develop cost of production budgets and expected revenues for the commercial production of food-sized hybrid striped bass, walleye, and yellow perch in the NCR;
- participated as Steering Committee members for a regional public forum regarding the National Aquaculture Development Plan of 1996;
- assisted NCRAC in obtaining information on the 1995 status of aquaculture in the NCR. The information will be used to develop NCRAC's regional aquaculture situation and outlook (S&O) report. Extension specialists often coordinated the effort to develop a cover letter, prepare a mailing list and send the survey out, and to follow up to assure a high response rate;
- conducted educational programs for the Wisconsin Aquaculture Association on non-indigenous aquatic nuisance species and implications for aquaculture as well as participating in the annual meeting of the Great Lakes Fish Health Committee providing input as it relates to aquaculture.

### Objective 2

The demand for aquaculture extension education programs cannot be met by the few specialists in the

NCR (4.0 FTE). Networking of specialists and Cooperative Extension Service (CES) designated contacts has maximized efficiency of education programs and minimized duplication. The NCRAC Extension Project is designed to assess and meet the information needs of the various clientele groups through cooperative and coordinated regional educational programming. In fact, individual state extension contacts often respond to 10-15 calls per month from outside their respective state as well as interacting with colleagues with mutual concerns related to developing aquaculture activities.

Prior to mid-1994, little coordination of international aquaculture information sharing existed. National and international agencies producing information could only be obtained by contacting the respective sources of this information. Also, individual CES personnel relied heavily on information produced by individual states or through regional cooperative projects. As Internet access extended beyond educational institutions and governmental agencies, a clear need developed to utilize the Internet to reach a much broader audience. In the age of an "information overload" the need for a centralized gateway to the ever increasing number of aquaculture resources in electronic format was apparent.

The development of the Aquaculture Information Network Center (AquaNIC) has been instrumental in reaching the public with valuable and timely information. It has been funded, in part, by NCRAC and has currently over 4,000 contacts per month from more than 50 countries to this web site. AquaNIC receives direction from a national steering committee from public and private sector aquaculture. AquaNIC began on a Gopher Server in July 1994 and moved to a World Wide Web server in January 1996. AquaNIC ([ag.ansc.purdue.edu/aquanic/](http://ag.ansc.purdue.edu/aquanic/)) houses more than 1,650 extension publications, governmental documents, image files, comprehensive e-mail lists, newsletters, calendars, job announcements, and résumés. In addition, AquaNIC has 190 pointers to other aquaculture and fisheries related web sites. Ongoing promotional campaigns through mouse pads and access information cards has increased the level of awareness of this new resource available to the world aquaculture industry. It is the gateway to the world's electronic resources in aquaculture including the Regional Aquaculture Centers.



AquaNIC also serves as the home of NCRAC's web site ([ag.ansc.purdue.edu/aquanic/ncrac](http://ag.ansc.purdue.edu/aquanic/ncrac)) which was developed in conjunction with NCRAC administrative staff and the Illinois-Indiana Sea Grant Program. The web site provides electronic versions of NCRAC extension publications, directories, operations manuals, and newsletters.

Aquaculture handbooks have been developed and distributed to each NCRAC designated aquaculture extension specialist and selected CES and Sea Grant field staff member.

As with any organization, there have been changes in NCRAC extension personnel since the inception of the project. Landkamer was the primary aquaculture extension contact for Minnesota. However, he left the university and Kapuscinski became the primary contact person until 1992 when Gunderson assumed that responsibility. In 1994 there were two changes: in Kansas, Neils replaced Henderson and in Illinois, Kohler replaced Selock. There continues to be changes in NCRAC extension personnel since the inception of the project; Hochheimer has replaced Ebeling in Ohio while Lee replaced Neils in Kansas in 1996.

### **Objective 3**

In-service training for CES and Sea Grant personnel and other landowner assistance personnel have been held in most of the states in the region. Training has been in the areas of basic aquaculture and safe seafood handling including HACCP (Hazard Analysis Critical Control Point).

### **Objective 4**

A number of workshops, conferences, videos, field-site visits, hands-on training sessions, and other educational programs have been developed and implemented.

There have been workshops on general aquaculture, fish diseases, commercial recirculation systems, aquaculture business planning, crayfish culture, pond management, yellow perch and hybrid striped bass culture, rainbow trout production, in-service training for high school vocational-agricultural teachers, and polyploid induction in sunfish held in the region.

Two North Central Region Aquaculture Conferences have been held. The first in Kalamazoo, Michigan, was held in March 1991. The second was held in February

1995 in Minneapolis, Minnesota. These regional meetings were attended by hundreds of individuals including persons from Canada. The next conference is scheduled for February 1997 in Indianapolis, Indiana.

On April 10, 1993, over 700 viewers from 35 states and Canada watched the first national interactive teleconference on aquaculture, "Investing in Freshwater Aquaculture," that was broadcast from Purdue University. It was a televised satellite broadcast for potential fish farmers. The program consisted of 10, 5- to 7-minute video tape segments which addressed production aspects of channel catfish, crayfish, rainbow trout, hybrid striped bass, tilapia, yellow perch, baitfish, and sportfish. A set of course materials was available prior to the program. Three times during the program, a question and answer period was available to the audience through a toll free telephone number. Questions not answered during the program were answered by mail afterwards. The entire teleconference is available as a videotape from NCRAC's Publications Office, as well as two other videotapes by the University of Nebraska-Lincoln that are reprises of the broadcast.

### **Objective 5**

Numerous fact sheets, technical bulletins, and videos have been written or produced by various participants of the Extension Work Group. These are listed in the Appendix.

### **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 landowner assistance personnel. Educational programs and materials will be developed and implemented. This includes development of a sunfish culture guide, yellow perch culture guide and videos, hybrid striped bass culture guide, a publication on fee-fishing (sunfish), tilapia culture information packet, and a publication on yellow perch culture in flowing water systems.

Additional workshops developed and hosted by state extension contacts will be advertised in surrounding states to take advantage of the NCRAC Extension Network and the individual expertise of Extension Work Group participants.

Several additional NCRAC fact sheets, technical bulletins, and videos will be developed by various Work Group members.

## Impacts

- In-service training for CES and Sea Grant personnel has enabled those professionals to respond to initial, routine aquaculture questions from the general public.
- Development of aquaculture education programs for the NCR has provided “hands-on” opportunities for prospective and experienced producers. Approximately 5,000 individuals have attended workshops or conferences organized and delivered by the NCRAC Extension Work Group. Clientele attending regional workshops learned of aquaculture development strategies in other areas of the country and acquired information which was of direct use to their own enterprises. Education programs also created situations where problems encountered by producers were expressed to extension personnel who later relayed them to researchers at NCRAC work group meetings for possible solutions through the research effort.
- Fact sheets, technical bulletins, and videos 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 on walleye, trout, and catfish have been used in numerous regional meetings and have been requested by clients from throughout the United States. Publications on organizational structure for aquaculture businesses, transportation of fish in bags, and others are

beneficial to both new and established aquaculturists. In a 1994 survey, NCRAC extension contacts estimated that NCRAC publications were used to address approximately 15,000 client questions annually.

- NCRAC extension outreach activities have helped to foster a better understanding and awareness for the future development of aquaculture in the region.
- In the brief time since AquaNIC began more than 25,000 people from 49 countries have chosen to use AquaNIC as an alternative to or in conjunction with traditional means of obtaining information. Primary users by countries are: U.S. (40%), Canada (5%), Australia (3%), and the United Kingdom (2%). As a gateway to electronic resources in aquaculture, AquaNIC has increased the timeliness and variety of information available to outreach educators, governmental agencies, and individual users while more effectively utilizing existing personnel resources. AquaNIC can be accessed anytime and, therefore, alleviates the challenges associated with office hours, time zones or weekends. Several groups have recognized the benefits AquaNIC provides to the world aquaculture industry and have established long-term partnerships with AquaNIC to assist them in distribution of their resources. Key groups using AquaNIC to house their web sites include: the World Aquaculture Society, NCRAC, Indiana Aquaculture Association, and the Illinois Aquaculture Industry Association.

## Publications, manuscripts, workshops, or conferences

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

## Support

Years	NCRAC USDA funding	Other support				Total Support	
		University	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
1995-96	\$17,075	\$70,968				\$70,968	\$88,043
<b>Total</b>	<b>\$328,923</b>	<b>\$659,126</b>		<b>\$250,000</b>	<b>\$55,000</b>	<b>\$964,126</b>	<b>\$1,293,049</b>

## Economics And Marketing

*Project Termination Report for the Period May 1, 1989 to August 31, 1993*

### **NCRAC funding level**

\$214,988 (May 1, 1989 to August 31, 1993)

### **Participants**

Susan B. Kohler, Southern Illinois University-Carbondale, Illinois

Marshall A. Martin, Purdue University, Indiana

Patrick D. O'Rourke, Illinois State University, Illinois

Jean R. Riepe, Purdue University, Indiana

#### ***Extension liaisons:***

Donald L. Garling, Michigan State University, Michigan

Terrence B. Kayes, University of Nebraska-Lincoln, Nebraska

LaDon Swann, Purdue University, Indiana

### **Reason for termination**

The objectives for this work were completed.

### **Project objectives**

1. Identify existing and needed economic data; develop statistical reporting methods; design an information management system and prototype annual situation/outlook report on the North Central Region (NCR) aquaculture industry; begin collecting and compiling a regional database; and prepare a situation/outlook report.
2. Develop and implement an extension program designed to educate current and potential aquaculture producers on the need to provide accurate economic information on their operations.
3. Investigate economic production and marketing feasibility for selected species currently produced in the NCR and other species which offer commercial potential.
4. Identify existing policy impediments and incentives for expanded aquaculture development in each participating state within the NCR.

### **Principal accomplishments**

The first Situation and Outlook (S&O) Report for the 12-state NCR was published in August 1993. The

report was developed to compile preliminary statistics and information about aquaculture in the NCR. Data and information contained in the report was obtained from a variety of sources including an extensive mail survey conducted in 1991 to gain information about the aquaculture industry in the NCR during 1990. Accurate information from respondents to that and other surveys was the result of the extension educational program of the project. Numerous contacts were established with state agencies interested in commercial aquaculture (e.g., natural resources, environmental licensing/permitting, and agriculture agencies), state aquaculture associations, state extension services, Sea Grant programs, and economic development groups in each of the states within the NCR seeking input and support for information gathering components of the project. Key individuals respected by the aquaculture industry were chosen from those various groups to facilitate legitimation of the data and to explain the value of accurate economic data reporting.

Two surveys of retail, wholesale, and other firms that comprise the traditional marketing channels for fish and seafood products within the NCR were completed in 1990 and 1991. The results of these surveys determined that channel catfish, trout, salmon (salt- and freshwater), freshwater shrimp, and tilapia were the five cultured freshwater species that were most frequently sold in the NCR. The species that were judged to have the most marketing potential were walleye, yellow perch, bluegill (sunfish), largemouth bass, and frogs. The surveys also indicated that the general perception of farm-raised products was positive within seafood distribution channels. Compared to wild-caught species, farm-raised aquaculture products were perceived as being fresher, of higher quality, and had greater price stability.

Trout and catfish cost of production budgets were developed. Based on the 1990 survey of producers in the NCR, these two species are the largest revenue generators for this region's growers.

There were 65 trout producers who grew trout and sold in excess of \$1,000 during 1990. Twenty-nine of these producers were chosen for the cost of production study to reflect differences in sizes of operations. Nineteen of the facilities were visited in person; the remaining 10 were sent a questionnaire and then interviewed by telephone. The initial 19 were also contacted by telephone, as needed, to clarify initial responses when questions arose. Of the 10 participants who were not visited, five did not respond to the telephone survey. Three questionnaires were not used because the data provided was too incomplete to develop budgets. The remaining 21 producer surveys provided the data base for the trout study. The level of cooperation received from trout growers in completing a very lengthy and difficult questionnaire was a very pleasant surprise.

Data were collected for the calendar year 1991. Producers were asked to provide information about variable and fixed operating costs of raising trout, the prices of variable and fixed inputs for which producers would have price data, type(s) of fish stocked, stocking size, market size, food conversion rate, and physical relationships such as water temperature and flow rates.

The 21 producers were divided into three groups. The small group contained nine producers with gross sales of \$1,250 to \$45,000, with average sales of \$20,039. These producers sold an average of 8,845 kg (19,500 lb) live weight. The medium group contained seven producers with sales ranging from \$92,178 to \$130,000, and averaging \$108,220. Output averaged 278,671 kg (61,435 lb) live weight. The large group contained five producers who averaged \$324,184 in sales and 72,746 kg (160,375 lb) live weight in output. The smallest of this group sold \$225,000 during 1991.

Variable plus fixed operating costs were \$21,140 for the average small producer resulting in negative operating revenues of \$1,101; so there was a negative balance before any allocation to the operator's labor, management and investment. The medium and large groups had variable plus fixed costs of \$95,927 and \$239,510, respectively, leaving returns of \$12,293 and \$84,674, respectively, available for operator's labor, management, and investment.

The cost and revenue data were of questionable validity at best. Most of the trout producers interviewed have very weak cost accounting skills and, therefore, have

limited ability to evaluate whether their trout operations are profitable or not. In addition, trout operations as a group are very complex when compared to other agricultural production enterprises because of the number of growing ranges for the fish (hatching eggs, selling or buying fingerlings, selling or buying stockers, and selling food size fish). Also, there are a large variety of production facilities, i.e., ponds, raceways, cages, etc. and all possible combinations of these facilities, plus variations in the costs of obtaining water. Experience suggests that very basic educational programs in management, cost accounting, and budgeting would be highly beneficial to these producers in NCR.

It was determined that states within the NCR are in various stages of aquaculture policy development. Minnesota, Illinois, and Indiana have enacted legislation promoting the development of the aquaculture industry. At the other end of the spectrum, North and South Dakota have so few producers that private fish culture is not likely to reach the public agenda for quite some time. Most of the states in the region fall somewhere in between, with aquaculture interests working toward the development of a state aquaculture development plan or the implementation of legislation supporting the industry.

Five major resource policy issues for aquaculture in the region were identified: (1) regulatory jurisdiction, (2) predator control, (3) water quality, (4) regulation of game and non-native species, and (5) environmental contamination. While producers have generally favored the classification of aquaculture as agriculture, in hope of avoiding environmental regulation, the research indicated that there is little reason to believe that such a reclassification will resolve the underlying substantive issues.

Most policies affecting the growth and marketing of fish in the NCR are found in state natural resources agencies. They are found in these agencies because they were developed to regulate open access fisheries, and now are the only policies that apply to cultured settings. However, for aquaculture to become more feasible, reconsideration of this regulatory policy framework is necessary. A cautious and studied approach is suggested for any attempt to revise the regulatory structure that affects fish farmers. Producer issues in many cases have similarities to those for other

livestock enterprises, but often in increased intensity because of application in water instead of on land.

The investigators found that one of the barriers to the growth and development of the aquaculture industry in the NCR was a lack of comprehensive information on the state laws and regulations that affect the industry. One of the outputs of the project was the publication of a digest that includes the laws related to the marketing and production of aquacultural products for the 12-state NCR. The publication entitled “Aquaculture Law in the North Central States: A Digest of State Statutes Pertaining to the Production and Marketing of Aquacultural Products” was published in May 1992 as the first publication in NCRAC’s Technical Bulletin Series.

## Impacts

The S&O Report provided a widely distributed report of the state of aquaculture in the NCR. It indicated that farm-raised aquaculture products have become more important in fulfilling seafood markets in the United States.

The marketing study showed that the primary species being cultured in the NCR are highly marketable and are well-accepted in commercial seafood marketing channels.

The benefits of the cost of production budgets are two. First, for the first time, budgets using North Central trout and catfish producer data are available for use by regional producers and NCRAC Extension agents in

assisting producers and others to assess the profitability of existing and proposed fish enterprises. These budgets are also useful in helping producers assess the feasibility of growing other species in the region. Second, these budgets provide an educational tool in the hands of Extension agents to teach fish growers how to improve cost accounting and budgeting procedures on their operations. Improved cost accounting will make producers better managers and assist regional researchers in assessing the feasibility of growing particular species under varying conditions.

Incorporation of cost of production budget parameters into budget software will give current or potential fish producers, financial institutions, and policy makers regional results about the feasibility of producing trout and catfish in various locations of the NCR. In addition, the data on some costs such as water and facilities will be transferable to other species of interest.

## Recommended follow-up activities

A new Economics and Marketing Work Group will begin to develop cost of production budgets and expected revenues for the raising of food-sized walleye, yellow perch, and hybrid striped bass on farms in the NCR.

## Publications, manuscripts, or papers presented

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

## Support

Years	NCRAC USDA funding	Other support				Total support	
		University	Industry	Other federal	Other		Total
1989-91	\$161,688	\$59,683				\$59,683	\$221,371
1991-92	\$53,300	\$66,457				\$66,457	\$119,757
<b>Total</b>	<b>\$214,988</b>	<b>\$126,140</b>				<b>\$126,140</b>	<b>\$341,128</b>

## Economics And Marketing

*Progress Report for the Period September 1, 1993 to August 31, 1996*

### **NCRAC funding level**

\$40,000 (September 1, 1993 to August 31, 1996)

### **Participants**

Susan B. Kohler, Southern Illinois University-  
Carbondale, Illinois

Marshall A. Martin, Purdue University, Indiana

Patrick D. O'Rourke, Illinois State University, Illinois

Jean R. Riepe, Purdue University, Indiana

#### ***Extension liaisons:***

Donald L. Garling, Michigan State University,  
Michigan

Terrence B. Kayes, University of Nebraska-Lincoln,  
Nebraska

LaDon Swann, Purdue University, Indiana

### **Project objective**

Develop cost of production budgets and expected revenues for the raising of yellow perch, walleye, and hybrid striped bass on farms in the North Central Region (NCR).

### **Anticipated benefits**

The overall goal of this collaborative project is to enhance walleye, yellow perch, and hybrid striped bass production by developing enterprise budgets for various production systems for these species in the NCR. This supports the mission of the North Central Regional Aquaculture Center (NCRAC), especially by conducting research "for the enhancement of viable and profitable commercial aquacultural production in the United States for the benefit of producers, consumers, and the American economy."

The cost of production and budgeting components of this project offer the potential to help in identifying production systems for walleye, yellow perch, and hybrid striped bass most likely to be commercially viable in the NCR. Information on production costs is quite limited for these species, especially walleye and yellow perch. Enterprise budgets for real and prototype systems will enable producers or potential producers to

assess the expected costs for their own operation, for a new operation, or for increased production in their present operation in an objective and comprehensive manner.

This project will benefit the aquaculture industry in the NCR in several ways, even though there are some limitations in using these budgets given the "emerging" status of the industry and the small number of commercial producers in these three species.

- First, objectively developed cost information is typically more accurate than subjectively developed cost information or no information on costs at all. These budgets will give producers an idea of how enterprise budgets should be organized, what types of data need to be collected, and why good record keeping is essential. The production values and relationships upon which the cost structure are based, while not standardized in the industry, should serve as a rough rule-of-thumb by which aquacultural producers can gauge their management skills.
- Second, enterprise budgets are an excellent management tool and are the cornerstone for financial analysis of aquaculture operations for producers and investors. These budgets may stimulate potential and current aquacultural producers to put together budgets and analysis for their own unique enterprises.
- Third, enterprise budgets are also the cornerstone for sensitivity analysis (yet another management tool). Undertaking sensitivity analysis will enable economists, producers, and potential producers to better understand the relative importance of cost and production items in the budget and the impact on profitability.
- Finally, realizing that the budgets produced under the auspices of this project will not be the final, definitive budgets for production of these species in the NCR, they will serve as a solid starting base from which to better understand the potential profitability of alternative species, production systems, life stages, etc.

In a more indirect way, the enterprise budgets will accomplish two other important things.

- One, the budgets may help guide research and extension decisions concerning hybrid striped bass, walleye, and yellow perch by NCRAC work group participants, the Industry Advisory Council (IAC), the Board of Directors, and the supporting committees.
- Second, the budgets will provide an opportunity for the economists and other personnel developing the budgets to interact with aquaculture producers, researchers, and extension personnel in the NCR. This type of interdisciplinary interaction is vital for the improved understanding and communication of all vital aspects of aquaculture in the NCR.

Economic feasibility analysis will help producers evaluate technical advances in fish production. This contribution is critical as a guide to future research funding in the various species and production systems suitable for commercial production. The distribution of research results from this project will provide a structured and objective framework for profitability and financial analysis of hybrid striped bass, walleye, and yellow perch aquaculture systems for producers, financial institutions, and others.

### Progress and principal accomplishments

#### *Hybrid striped bass*

Kohler has compiled a review of the literature on hybrid striped bass production and production costs. The literature reviewed is summarized in an annotated bibliography. This bibliography will be available to anyone needing the information.

Kohler has developed hybrid striped bass cost of production estimates based on six recent published reports on hybrid striped bass production. These estimated production costs were presented at the NCRAC Hybrid Striped Bass Workshop in November 1995.

#### *Walleye*

O'Rourke and Illinois State University graduate students continued an extensive walleye production and culture literature review. The primary focus of the literature review was to evaluate research findings that might be useful in ascertaining the cost of production for walleye fingerlings and food-sized fish under

intensive and extensive culture regimes. Very little economic research was found and even less was found that was documented well enough to be useful.

Work has advanced on identifying and analyzing the cost of production for food-sized walleye in intensive culture systems. The second M.S. thesis on walleye to come from this project was officially finished in December 1995. It reported on an economic feasibility analysis of a tank based intensive food-sized walleye system.

#### *Yellow perch*

Riepe's analysis of yellow perch production in ponds and cages is reported in NCRAC Extension Fact Sheet #111 and NCRAC Extension Technical Bulletin #111, both ready for release. While developing cost estimates for yellow perch aquaculture, Riepe investigated feed and fingerling prices and procurement with various suppliers. A fact sheet on managing feed costs was developed and is in final review by Riepe as a NCRAC Extension Fact Sheet.

### Work planned

The distribution of research results from this project is proceeding, primarily for the walleye species. The research on cost of production in tank culture systems for fingerlings and food sized walleye will be organized in fact sheet or technical bulletin format for release to producers, financial institutions, and others.

Riepe will complete the review of the fact sheet on managing feed costs. This is expected to be published as a NCRAC Extension Fact Sheet.

### Impacts

Kohler and O'Rourke presented the review of hybrid striped bass production costs as well as profitability and volume-cost business analysis tools at the NCRAC Hybrid Striped Bass Workshop in November 1995. The information developed and presented is anticipated to be directly useful to the attendees (producers and potential producers) as they consider their own operations and intentions in light of the cost data and analytical tools presented.

This project has already benefited the aquaculture industry in the NCR through the workshop presentations. As a result of this NCR project, economists have been able to develop and deliver

presentations on economic issues in aquaculture production to current and potential aquacultural producers. These presentations and the publications which follow may reduce the impacts of uninformed investment decisions by current and potential aquaculture entrepreneurs.

**Publications, manuscripts, and papers presented**

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

**Support**

Years	NCRAC USDA funding	Other support				Total Support
		University	Industry	Other Federal	Other	
1993-95	\$40,000	\$59,683				\$59,683
<b>Total</b>	<b>\$40,000</b>	<b>\$59,683</b>				<b>\$59,683</b>

**Yellow Perch**

*Project Termination Report for the Period May 1, 1989 to August 31, 1993*

**Total funding level**

\$354,785 (May 1, 1989 to August 31, 1993)

**Participants:**

- Fred P. Binkowski, University of Wisconsin-Milwaukee, Wisconsin
- Paul B. Brown, Purdue University, Illinois
- David A. Culver, Ohio State University, Ohio
- Konrad Dabrowski, Ohio State University, Ohio
- Donald L. Garling, Michigan State University, Michigan
- Terrence B. Kayes, University of Nebraska-Lincoln, Nebraska
- Jeffrey A. Malison, University of Wisconsin-Madison, Wisconsin
- Robert J. Sheehan, Southern Illinois University-Carbondale, Illinois

**Extension liaison:**

Donald L. Garling, Michigan State University, Michigan

**Non-funded collaborators:**

- Forrest Williams, Bay Port Aquaculture, Inc., West Olive, Michigan
- David Northey, Coolwater Farms, Dousman, Wisconsin

**Reason for termination**

The objectives for the first three NCRAC-funded projects on Yellow Perch were completed.

**Project objectives**

1. Compare the survival, growth, feed conversion, and proximate composition of offspring from selected Northern, Southern, and Great Plains stocks of yellow perch, at different life history stages and at different temperatures.
2. Evaluate the survival, growth, and feed conversion of yellow perch raised at various loadings or rearing densities in selected flow-through and pond culture systems.
3. Evaluate and improve the efficiency of various methods of inducing triploidy in yellow perch, and compare the survival and growth to market size of the triploids produced with that of normal diploids.
4. Compare pond and intensive culture methods for the production of yellow perch fingerlings.

**Principal accomplishments**

The principal goal of this work was to develop practical strategies for commercial yellow perch aquaculture under the diverse environmental conditions that exist in



the NCR. Much of this goal was realized. During its 4-year history, the two projects examined: (1) the suitability of selected wild perch brood stocks obtained from different geographic locales as candidates for potential brood stock development; (2) the applicability of selected conventional production technologies to perch aquaculture; (3) the potential of using chromosomal triploidy induction to enhance growth; and (4) the relative merits of pond versus intensive culture methods for the production of perch fingerlings, and the nutrient composition of live-food organisms versus perch fry raised to different sizes (stages of development) under different culture conditions (different pond sites and laboratories, pond versus intensive culture).

With respect to objective 1, studies at the University of Wisconsin-Milwaukee (UW-Milwaukee) found variations in percentage of survival and swim bladder inflation between perch fry from different stocks, and research at Purdue University (Purdue) identified significant differences in the growth of perch fingerlings from these same stocks at various rearing temperatures. In overview, these variations and differences appeared to be primarily reflective of the geographic locales from which the brood fish and fertilized eggs of the different stocks were selected, which is a factor that should be considered when selecting brood stock for the production of perch. Thus, producers in the northern and southern parts of the NCR should probably use brood stock from their own respective parts of the region, and not expend undue time and resources seeking “super” perch from stocks with presumed superior performance traits that have not been documented by properly controlled experimental procedures.

As part of objective 2, Michigan State University (MSU) and University of Wisconsin-Madison (UW-Madison) researchers unequivocally demonstrated that perch can be raised to market size using conventional aquaculture production technologies in a time frame similar to that of such important commercially cultured species as channel catfish. The demonstration of this fact is perhaps one of the project’s most important practical benefits, because it underscores the importance of matching species selection for aquaculture development with climatic conditions and available resources. This research effort was also important because it helps refute the notion that, except

for salmon and trout, finfish aquaculture in the North, owing to the shorter growing season, cannot be competitive with aquaculture production at southern or tropical latitudes. The temperature requirements of perch for successful reproduction and optimum growth make the commercial culture of this species in the principal catfish producing states of the South highly unlikely.

Regarding objective 2, MSU investigators, working with Bay Port Aquaculture Inc. of West Olive, Michigan, have demonstrated that perch can be raised on a commercial scale at high densities in flow-through tanks, using research-based procedures for estimating carrying capacity based on the dissolved oxygen requirements and ammonia tolerance limits of perch. Such an approach to perch aquaculture, employing intensive procedures similar to those used in commercial trout production, should be particularly applicable to situations where an inexpensive, abundant source of high-quality temperate (i.e., 18-24°C; 64.4-75.2°F) water is or can be made available for “grow out” - from natural springs, wells, and/or the utilization of dependable waste heat or clean cooling water from such providers as electric power generating stations.

Using a different approach, UW-Madison researchers, working with Coolwater Farms of Dousman, Wisconsin, have demonstrated that the commercial-scale culture of perch in ponds can be feasible, if sufficient quantities of inexpensive groundwater (which ranges between about 8 and 14°C across the NCR; 46.4 and 57.2°F) is available to moderate pond water temperature highs during the summer and ice formation during the winter. Such groundwater addition also helps maintain elevated dissolved oxygen concentrations, and facilitates ice control during the winter to provide access for management and feeding and to prevent equipment damage. The benefit of this approach is that it provides a ready means of producing commercial quantities of perch in those parts of the region where pond construction is feasible and groundwater is abundant and available at a reasonable cost.

Studies by researchers at the UW-Madison and Southern Illinois University-Carbondale (SIUC) on objective 3, have shown that while direct triploidy induction in fertilized eggs produces perch that exhibit retarded gonadal development and somewhat higher fillet yields than is observed in normal diploid fish,

direct triploidy induction does not significantly enhance growth. Investigators at the UW-Madison have developed effective procedures to induce triploidy in perch either by heat or hydrostatic pressure shocks, but have shown that such shocks exert a negative influence on growth independent of ploidy change. Accordingly, unless perch can be marketed on the basis of fillet yield or lack of reproductive competence, instead of total body weight, it is difficult to envision how direct triploidy induction can benefit commercial perch aquaculture. Researchers at the UW-Madison have also developed procedures for producing tetraploid brood perch, which presumably can be backcrossed with diploid fish to produce triploid eggs via natural fertilization, rather than by using physical or chemical shock treatments. Triploid perch produced by crossing tetraploid and diploids may grow faster than diploids, but this potential benefit has not yet been tested either experimentally or in practice.

Researchers at the UW-Madison and UW-Milwaukee, working collaboratively on objective 4, have clearly demonstrated that with recently developed “best available” techniques, fry and early-fingerlings perch raised in ponds exhibit better survival and growth and far fewer problems with swim bladder inflation and spinal deformities than perch reared intensively in tanks since hatching. Furthermore, after habituation to formulated feed and intensive culture conditions, pond-reared perch often continue to out-grow fish reared entirely by intensive methods. Over the years, UW-Madison investigators have continued to develop improved procedures for incubating and hatching perch eggs, rearing and harvesting ever-increasing numbers of perch fingerlings from ponds (up to 1,000,000 per surface ha; 2,470,097/acre), and habituating early-fingerlings (16-18 mm total length; 0.63-0.70 in) to formulated feed and intensive culture conditions using internal tank lighting. The development of these procedures represents another one of the project’s most important practical benefits, because they provide fish farmers with a ready means of producing large numbers of perch fingerlings that are habituated to formulated feed and ready for “grow out” to market size.

After 3 years of research on objective 4, UW-Madison and UW-Milwaukee investigators have found that despite significant improvements in procedures and fry survival, problems with swim bladder inflation and cannibalism continue to be serious impediments to the

large-scale intensive production of perch fingerlings. UW-Milwaukee researchers demonstrated that problems with early development and habituation of fry to intensive culture conditions were not as serious with perch originating from the Prquimans River in North Carolina, as with perch fry originating from other locales. Ohio State University (OSU) investigators discovered no significant differences in the amino acid compositions of young perch from Wisconsin and Ohio, suggesting similar nutritional needs across perch stocks. Researchers from OSU also found suggestive evidence, but no causal or clear-cut functional linkages, that dietary ascorbic acid deficiencies may be responsible for the high incidence of spinal deformities often observed in perch larvae reared under intensive culture conditions, and that certain long-chain fatty acids may be important in the diets of young perch.

## Impacts

The principal impacts of the completed NCRAC yellow perch project have been the development and/or expansion of two of the NCR’s leading commercial perch aquaculture operations, the actual or planned start up of several new commercial perch aquaculture ventures, the utilization of the project’s newly developed knowledge and production procedures by a number of fish farmers, and the training of numerous graduate and undergraduate students at the participating institutions.

### *Specific examples*

The research done by MSU on the intensive flow-through culture of perch in tanks (objective 2) played a key role in the development and expansion of Bay Port Aquaculture Inc. of West Olive, Michigan. Similarly, all of the perch net-pen and pond production research (objective 2) and many of the perch fingerling production studies (objective 4) reported by the UW-Madison were done at Coolwater Farms of Dousman, Wisconsin, and directly involved Coolwater Farms’ personnel in these investigations. As a consequence, Coolwater Farms has greatly expanded both the scope and efficiency of its operations.

Private producers that are actually known to have recently started culturing perch largely as a consequence of the project, or who have made significant investments to soon start, include one in Iowa, three in Indiana, one in Michigan, two in Nebraska, two in Ohio, and two in Wisconsin. Other

perch aquaculture ventures in the region may have recently started or may soon become operational, but this cannot be presently documented.

The UW-Madison has reported that several “aquaculture endeavors in Iowa, Indiana, and Michigan have based their business plans on the pond culture of perch,”; that one in Iowa “is evaluating the use of net-pens,”; that “at least three commercial fish farms (one each in Wisconsin, Michigan, and Ohio) have begun to rear all-female perch,”; and that “at least three commercial perch aquaculture operations in the upper Midwest” have or are implementing the “egg hatching and fingerling production and training methods developed” during the project. The UW-Milwaukee has reported training fish farmers in its procedures for producing perch fingerlings intensively in tanks.

Purdue has indicated the partial training of two graduate students and three undergraduates, as part of the project; MSU reported training two graduate and several undergraduate students. In overview, all of the principal investigators and technical staff of the various laboratories and institutional programs participating in the project gained tremendous insights and new knowledge about the culture and biology of the yellow perch, and a better appreciation of the benefits of regional and inter-institutional collaboration.

## Recommended follow-up activities

Building on the results of these projects, NCRAC provided funding for another Yellow Perch project

which began on September 1, 1993, and will run for 2 years. The objectives of this new project are to: (1) determine the commercial scale feasibility and improve on the best intensive tank and pond culture practices for the production of yellow perch fingerlings and (2) determine the commercial scale feasibility of raising food-size yellow perch in flow-through raceways or tanks, open ponds, and large net-pens, comparing the best available formulated diets. A number of commercial fish farmers in the NCR have been named as major participants in this new project; many aspects of which will not be possible without their full cooperation and support.

The importance of UW-Milwaukee and OSU investigations on objective 4 is that they demonstrate that considerable additional research will probably be required to develop the procedures and diets necessary to successfully culture perch larvae intensively in tanks on a large scale. Based on experience with other species with small larvae, a long-term investment in selective breeding or in research on perch larval diet development might make such intensive culture technically feasible. Whether or not it would be commercially competitive with the improved methods developed in recent years for culturing young perch in ponds is unclear, particularly considering that continued improvements in the latter approach are likely.

## Publications, manuscripts, or papers presented

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

## Support

Years	NCRAC USDA funding	Other support				Total Support
		University	Industry	Other Federal	Other	
1989-91	\$162,680	\$213,435				\$213,435
1990-92	\$92,108	\$400,543				\$400,543
1991-93	\$99,997	\$165,394				\$165,394
<b>Total</b>	<b>\$354,785</b>	<b>\$779,372</b>				<b>\$779,372</b>
						<b>\$1,134,157</b>

## Yellow Perch

*Progress Report for the Period September 1, 1993 to August 31, 1996*

### **NCRAC funding level**

\$257,086 (September 1, 1993 to August 31, 1996)

### **Participants:**

Fred P. Binkowski, University of Wisconsin-  
Milwaukee, Wisconsin

Paul B. Brown, Purdue University, Illinois

Konrad Dabrowski, Ohio State University, Ohio

Donald L. Garling, Michigan State University,  
Michigan

Terrence B. Kayes, University of Nebraska-Lincoln,  
Nebraska

Jeffrey A. Malison, University of Wisconsin-Madison,  
Wisconsin

### ***Extension liaison:***

Donald L. Garling, Michigan State University,  
Michigan

### ***Non-funded collaborators:***

Harlan Bradt, etc., Coolwater Farms, LLC, Cambridge,  
Wisconsin

William Hahle, Pleasant Valley Fish Farm, McCook,  
Nebraska

John Hyink and John Wolf, Alpine Farms/Glacier  
Springs Trout Hatchery, Wisconsin

Dave Smith, Freshwater Farms of Ohio, Inc., Urbana,  
Ohio

Michael Wyatt, Sandhills Aquafarm, Keystone,  
Nebraska

Nebraska Game & Parks Commission, Calamus State  
Fish Hatchery, Burwell, Nebraska

Forrest Williams, Bay Port Aquaculture, Inc., West  
Olive, Michigan

### **Project objectives**

1. Continue to improve larval rearing techniques by developing and evaluating different starter diets in relation to size at transfer to formulated feeds under selected environmental conditions.
2. Continue to improve pond fingerling production through examination of in-pond feeding techniques using physical/chemical attractants and improved harvesting strategies for different sizes of

fingerlings from various types and sizes of ponds.

3. Continue development of extension materials and workshops emphasizing practical techniques coinciding with production events to meet the needs of established and potential yellow perch culturists through on-site presentations at two or more locations in different parts of the region.

### **Anticipated benefits**

This project addresses priority needs identified by the North Central Regional Aquaculture Center (NCRAC) Industry Advisory Council (IAC) for advancing yellow perch aquaculture in the North Central Region (NCR). The IAC has indicated that one major constraint that presently limits perch aquaculture is the lack of reliable methods of producing perch fingerlings habituated to formulated feeds. In addition, there is a continuing need to provide producer training on key aspects of perch aquaculture, and to transfer advances in perch culture technology to the public sector.

The information generated by these projects will greatly assist perch producers in their efforts to reliably raise the large number of perch fingerlings needed by the industry. Improvements in pond fingerling techniques will immediately increase the availability of fingerlings to the industry because almost all fingerlings currently available are produced in ponds. Research on the effect of spawner size on larval size and on starter diet formulation for yellow perch will improve intensive fry rearing techniques and decrease the dependence on live feeds. Laying the foundation for use of one of the more potent and proven legal flavor additives for fish requires quantifying two critical nutritional requirements for yellow perch; the total sulfur amino acid and choline requirements. These values alone are beneficial in terms of developing a diet for yellow perch and provide the foundation for evaluation of betaine as a flavor additive in diets.

Extension activities will continue to promote and advance yellow perch culture through expanded

outreach, education, and training programs. Additional extension materials (bulletins, fact sheets, and audiovisual materials) developed by the NCRAC Yellow Perch and Extension Work Groups and a series of hands-on workshops and field demonstrations will transfer current technology to established and potential fish farmers, and increase public awareness of the potential of yellow perch aquaculture as a viable agricultural enterprise in the NCR. In addition, this project will develop improved technologies for certain key facets of yellow perch aquaculture. Finally, the results of experiments incorporated into this proposal will immediately help fish farmers improve the production efficiency of yellow perch.

### **Progress and principal accomplishments**

As an integral component of this project, private producers have cooperated by providing facilities, fish, feed, day-to-day husbandry, and routine data collection. At its inception, this project included the participation of eight different private fish farms in various parts of the NCR. Participating university researchers provided project oversight on experimental design, advice or direct assistance with the technical set-up of any specialized experimental systems, supervision and assistance on critical end-point data collection, and analyses of results.

In year 1 of the project (September 1, 1993 to August 31, 1994), significant progress was made at certain sites at testing selected research-based production technologies. Accordingly, from an extension perspective, the project is successfully building and/or expanding working relationships between NCRAC researchers and certain regional fish farmers, testing various research-based technologies under practical production conditions, transferring knowledge from academia to the private sector, and identifying private producers who are both capable and willing to sustain a collaborative technology evaluation and demonstration effort. Several of the original private-sector collaborators have either met or have worked hard to meet their project commitments.

#### **Objective 1**

Researchers at Michigan State University (MSU) directed their efforts in 1996 towards studying the effects of female spawner size on the size of eggs and

fry. Spawning stock were collected from the outer Saginaw Bay, Lake Huron and transported to Bay Port Aquaculture, West Olive, Michigan. Bay Port workers held the fish until they could be manually spawned. Eggs were sampled from females divided into six size classes in 25 mm (1 in) increments from 200 to 350 mm (7.8 to 13.8 in).

Subsamples of eggs were collected from the ends and center of each ribbon. Approximately 1 gram of eggs from each subsample was weighed and fixed in Stockard's solution for subsequent measurements. The ribbon segments were fertilized and placed into specially designed incubator trays and incubated in well water at 11.5°C (52.7°F). Nine days after fertilization, measurements of larval mouth gape (height and width) and total length were taken using a dissecting microscope in conjunction with the Optimas imaging system, BioScan™. The data is currently being analyzed. Preserved egg samples were used to determine the number of eggs/g and 25 eggs were measured along the long axis of the egg outer diameter and the yolk membrane. Preliminary evaluation of egg size indicates a positive relationship with the length of the maternal parent.

A sulfur amino acid requirement study is underway at Purdue University (Purdue) and should be completed by December 1996. Through 4 weeks, fish fed 1.0% methionine are growing better than fish fed lower concentrations in the diet.

Studies at Ohio State University (OSU) have been designed to evaluate the use of pancreatic enzymes and a digestive tract neurohormone, bombesin, in the diets offered to 0.6 g (0.02 oz) yellow perch. Perch fry were raised initially in ponds (Ohio Valley Fisheries, Inc.) and were transferred to an indoor facility and accustomed to an artificial commercial diet (Ziegler). Studies on three experimental diets and one commercial diet fed to triplicate groups of yellow perch are being conducted using 40 L (10.6 gal) glass tanks at OSU. Experimental diet 1 is supplemented with either pancreatic digestive enzymes (PD), diet 2 with bombesin and PD, and diet 3 with nothing. Results indicated no significant differences between treatments. However, all experimental diets resulted in better growth of yellow perch than the commercial salmonid starter.

An accompanying study using the same batch of fish, the same commercial diet, and three different experimental diets was conducted at the Piketon Research and Extension Center. Four groups per treatment were used and a semi-purified, casein-gelatin diet (#1) was tested along with diets based on krill and squid meals (#2) or fish meals (#3). In a trial in Piketon, 4 weeks of feeding resulted in significantly lower growth rate of perch fed a semi-purified diet (gain 70+/-8%) than both experimental diets (105+/-11% - 115+/-15%) or a commercial diet (104+/-7%).

## Objective 2

An experiment was conducted by University of Wisconsin-Madison (UW-Madison) researchers at Coolwater Farms, LLC, to determine key parameters for producing yellow perch fingerlings habituated to formulated feeds and reared in ponds for an entire growing season, and to compare the performance of two types of pond lighting and feeding systems. Ponds are currently being harvested and production data are being collected. Observations made by Coolwater Farms culturists indicate that improvements in pond lighting and feeder design markedly reduced the labor needed for husbandry and system maintenance.

In the late spring and early summer of 1996, University of Nebraska-Lincoln (UNL) investigators compared the utility of different lighting systems, combined with a specially designed trap-net, to harvest photopositive young-of-the-year (YOY) yellow perch on a large scale from ponds. Previous research using similar capture gear had demonstrated that up to 38,000 young yellow perch could be captured per 30-min effort from heavily stocked, shallow (<1.25 m; 4.1 ft) earthen ponds of 0.4 ha (1 acre) surface area or less.

The 1996 trials compared the utility of different configurations of lights arrayed on rafts that could be easily pulled from an opposing pond shoreline to the trap-net. Trials were conducted at the Calamus State Fish Hatchery (near Burwell, Nebraska), in two plastic-lined 0.2 ha (0.5 acre) ponds that when full have an average depth of well over 1.25 m (4.1 ft). Both ponds were stocked with about 225,000 yellow perch fry, and managed by standard procedures used by the Nebraska Game and Parks Commission. Harvesting trials were initiated when the fish in each pond reached 19 mm (0.7 in) total length.

Two light-raft systems were tested. The lights on both could be turned on or off by remote control. One raft was equipped to broadcast a total of 250 W of omnidirectional light below water. The second was equipped to broadcast a total of 910 W of omnidirectional-submerged, directional-submerged, and directional-above-surface lighting. The directional lighting on the latter system was broadcast forward of the raft as it was pulled through the water.

The trap-net was fitted with a string of five, 75 W submerged lights that were turned off sequentially to draw fish into an open-top harvest pot, designed to facilitate the low-stress crowding and capture of small fish. The design of this trap-net has been proven effective at capturing large numbers of photo-positive young fish when used in combination with a variety of lighting systems in shallow earthen ponds.

The results of the 1996 UNL trials were that the 910 W light raft effected the capture of significantly greater numbers of yellow perch (about 5,000 fish per 30-min capture effort) than the 260 W light raft (about 3,800 fish per capture effort). The number of capture efforts made with each system were 14 and 17, respectively. One particularly noteworthy observation was that the numbers of yellow perch captured per unit effort in 1996 was significantly down from previous years (typically 10,000-20,000 fish per capture effort). This was attributed primarily to the fact that the Nebraska Game and Parks Commission added AquaShade® to the ponds to prevent excessive algae growth, and possibly to the greater depth of the ponds used in 1996. AquaShade® is a commercially available product that reduces light transmittance in water.

Extremely poor weather conditions, combined with budgetary shortfalls, precluded UNL testing of this or similar harvesting equipment at sites other than the Calamus State Fish Hatchery. Three years of research by UNL investigators on the use of light to harvest YOY yellow perch indicate that it is a very useful tool but can yield highly variable results, depending on a number of factors, e.g., pond depth and area, plankton concentrations, presence of aquatic vegetation, size, and age of fish.

## Objective 3

During 1996, two "Intensive Aquaculture of Yellow Perch in Conjunction with Recirculating Aquaculture

Systems” workshops were sponsored by the University of Wisconsin Sea Grant Institute, which included NCRAC Extension and Yellow Perch Work Group members. Alpine Farms (Sheboygan Falls, Wisconsin) personnel participated as aquaculture industry cooperators to provide their practical experience with, and knowledge of, yellow perch rearing in their recirculating aquaculture system (RAS) technology.

The program for the first workshop included a morning session with lecture presentations and an afternoon poster session during which small groups of attendees had the opportunity for direct contact with the presenters, having their specific questions answered and problems solved. In order to maximize personal contact with the presenters, the number of attendees at this workshop was limited to 75.

In the weeks following this workshop, small groups of workshop attendees were given the opportunity for additional direct hands-on advisory service concerning the technology for intensive rearing of yellow perch. These on-site activities were conducted at the University of Wisconsin System Aquaculture Institute in Milwaukee, and at Alpine Farms where they observed demonstrations on the intensive aquaculture of yellow perch in conjunction with a RAS.

A second 1-day workshop on the intensive culture of yellow perch with RAS was held in June 1996. The agenda for this workshop included lecture presentations on RAS operation and technology, water quality management in RAS, relevant aspects of yellow perch biology under intensive rearing, and the economic and business aspects of yellow perch culture. The format of this workshop was designed to focus on the most important topics and maximize the interaction between workshop attendees and aquaculture experts during an extended question/answer session. Eighty-five people attended this workshop.

Kayes of UNL conducted a workshop in Nebraska, part of which covered methods of harvesting yellow perch in ponds. In addition, progress was made on producing a videotape on the small-scale processing of yellow perch, in cooperation with videographers at Kansas State University.

## Work planned

### Objective 1

Preliminary studies were conducted at MSU to develop larval rearing tank designs similar to those that have been used successfully in raising larval walleye and mahi mahi. The initial design will be improved in 1996-97 and used in feed acceptance studies. Also in 1996-97, MSU researchers will use their findings from 1995-96 to select spawners from size classes that produce favorable hatchability and mouth size traits in their fry. The fry will be used for nutritional studies comparing live and formulated dry diets.

After completion of the methionine requirement at Purdue, the dietary choline requirement will be quantified, then the ability of betaine to supply part or all of the choline requirement will be determined. Work at OSU will continue to evaluate the use of pancreatic enzymes and a digestive tract neurohormone, bombesin, in the diets offered to young yellow perch.

### Objective 2

A second experiment on pond fingerling production will be conducted by UW-Madison researchers at Coolwater Farms, LLC. This experiment will evaluate strategies to maximize fingerling survival and crop uniformity in perch cultured throughout a growing season.

Nearly all the NCRAC funds allotted to UNL for research on objective 2 were exhausted in 1996. In 1996-97, UNL investigators will evaluate and compare the data collected over the past 3 years on harvesting YOY fish using light in preparation for submitting the findings to a peer-reviewed journal for publication, and as part of a NCRAC project termination report.

### Objective 3

A workshop demonstrating key facets of fingerling production and grow-out is being planned by UW-Madison researchers for June 1997.

The “Intensive Aquaculture of Yellow Perch in Conjunction with RAS Technology” workshops presented by University of Wisconsin-Milwaukee in 1996 provided the framework for the presentation of a hands-on workshop to be organized and presented in

1997. They intend to install a demonstration RAS at the University of Wisconsin System Aquaculture Institute in Milwaukee that can be directly used for hands-on participation and training of workshop attendees.

A NCRAC-sponsored conference and two workshops on yellow perch aquaculture will be held in Nebraska in 1996-97. Also, the videotape on the small-scale processing of yellow perch, which was proposed by Kayes of UNL, should be completed.

**Impacts**

Defining critical nutritional requirements for targeted species reduces feed costs and overall cost of production. These data will be important pieces of information for manufacturers of feed. This research provides strong evidence that commercial diets for salmonids need to be modified to meet nutritional requirements of yellow perch. These new diet formulations may significantly improve growth rate of yellow perch fry. Further, definite use of legal flavor additives may alleviate the problems of poor feed acceptance by larval and growout perch.

Studies on pond fingerling production by UW-Madison researchers have shown that research based production strategies can be used on a commercial scale to produce large numbers of yellow perch fingerlings at a relatively

low cost. Lights and automatic feeders used to habituate fingerlings to formulated feeds while they remain in ponds can be used throughout the first growing season, eliminating the need for a separate feed-training phase of production. Improvements in feeder design may increase reliability and decrease capital and operational costs.

The field trials conducted by UNL investigators have demonstrated both the utility and the limitations of using light to harvest YOY yellow perch. Present indications are that light is being used by increasing numbers of fish farmers to harvest young yellow perch (as well as other species) in several states including Ohio, Minnesota, and Wisconsin.

Workshops done on yellow perch aquaculture in the NCR have stimulated increased interest in this species among established fish farmers, potential fish farmers, and the general public. In the past year, requests for information on yellow perch aquaculture have increased significantly; for example, requests for yellow perch culture information from Kayes at UNL have increased by about 500%.

**Publications, manuscripts, or papers presented**

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

**Support**

Years	NCRAC USDA funding	Other support				Total	Total Support
		University	Industry	Other Federal	Other		
1993-94	\$75,000	\$87,240	\$30,000	\$10,000 <sup>a</sup>		\$127,240	\$202,240
1994-95	\$75,000	\$81,587	\$30,000	\$81,000 <sup>abc</sup>		\$192,587	\$267,587
1995-96	\$107,086	\$145,814	\$20,000	\$134,000 <sup>ac</sup>		\$299,814	\$406,900
<b>Total</b>	<b>\$257,086</b>	<b>\$314,641</b>	<b>\$80,000</b>	<b>\$225,000</b>		<b>\$619,641</b>	<b>\$876,727</b>

<sup>a</sup> Sea Grant/USDC/NOAA

<sup>b</sup> USDI, Bureau of Indian Affairs

<sup>c</sup> EPA



## Hybrid Striped Bass

*Project Component Termination Report for the Period May 1, 1989 to August 31, 1993*

### **NCRAC funding level**

\$232,960 (May 1, 1989 to August 31, 1993)

### **Participants:**

Terrence B. Kayes, University of Nebraska-Lincoln,  
Nebraska

Christopher C. Kohler, Southern Illinois University-  
Carbondale, Illinois

Jeffrey A. Malison, University of Wisconsin,  
Wisconsin

Robert J. Sheehan, Southern Illinois University-  
Carbondale, Illinois

### **Extension liaison:**

Joseph E. Morris, Iowa State University, Iowa

### **Reason for termination**

The objectives for this work on hybrid striped bass were completed.

### **Project objectives**

1. Obtain and maintain (in captivity) populations of spawning size white bass.
2. Define reproductive development in wild and captive white bass by characterizing seasonal changes in hormone titers and gonadal histology.
3. Evaluate the effects of selected photoperiod/temperature and hormonal manipulations on gonadal development and spawning in white bass brood stock.

### **Principal accomplishments**

Southern Illinois University-Carbondale (SIUC) researchers have successfully captured adult white bass, acclimated them to tank culture conditions, and trained them to accept formulated feed. Some fish have been held in captivity for over 3 years. This level of domestication is not known to have been achieved with white bass in any other laboratory or commercial enterprise.

Considerable numbers of white bass spawns have been accomplished using various hormonal/temperature/

photoperiod manipulations over the course of this project. Fish have been accelerated to spawn as early as January, and have had their spawning delayed to as late as October. Accordingly, techniques have been developed that allow successful spawning of white bass any season of the year. Moreover, female white bass that successfully spawned in October 1992 were successfully induced to spawn again in April 1993. Thus, it was demonstrated that white bass can be successfully spawned twice in a 7-month period. It was also shown that male white bass held at or above spawning temperatures (15°C; 59°F) produced viable sperm for at least 2 months. Average hatching rates have also been improved from 25% to 50%. These findings represent major steps toward the development of domesticated white bass brood stocks to be used for hatchery production of hybrid striped bass.

Injection levels of a synthetic luteinizing hormone-releasing hormone analogue (LhRha) and human chorionic gonadotropin (hCG) have been identified that greatly improve upon previous results at SIUC, and elsewhere, with respect to controlled spawning of white bass. Data indicate that hCG dosages considerably less than that traditionally used to induce final egg maturation are more useful in white bass. In addition to providing guidance for improved spawning performance, these data have positive implications toward eventual regulatory approval of hCG by FDA for spawning *Morone* species.

Annual rhythms of serum levels of estradiol-17 and testosterone, as well as gonadal growth and histology of the wild and the three captive populations of white bass were documented and correlated with actual spawning events.

### **Impacts**

#### *Domestication*

The development of a protocol to habituate adult white bass to captivity, including training to dry formulated feeds, allows for developing domesticated brood stock. Domesticated brood stock is clearly advantageous by:

- obviating need to collect brood stock from wild,
- resolving numerous regulatory issues regarding collection and hauling of wild brood stock,
- allowing for brood stock selection programs, and
- ensuring availability of brood stock when needed.

*Out-of-season spawning*

The development of efficacious procedures to manipulate sexual maturation and induce out-of-season spawning is important for optimal management of brood stock. It leads to:

- greater predictability of gamete production,
- reduced incidence of failed spawnings,
- reduced incidences of brood stock losses due to toxemia, and
- production of fertilized eggs and fry at predetermined times throughout the year.

*Hatching rates*

Improvements in hatching rates allows for increased hatchery production or reduction in brood stock needs.

*Hcg dosages*

Determination of the most efficacious hCG dosages not only improves spawning performance, but these data have positive implications toward eventual regulatory approval of hCG by the FDA for spawning *Morone*

species. As a direct consequence of this work:

- standard dosages of hCG are being tested for efficacy in a multi-state Investigational New Animal Drug (INAD) application being administered by Auburn University through sponsorship of Intervet Inc.;
- hCG will be tested for animal safety by SIUC under sponsorship of Intervet Inc.; and
- these projects will collectively provide FDA with necessary information to make a determination for approval of hCG for brood fish.

*Recommended follow-up activities*

NCRAC funded a follow-up study that is focused on developing procedures to intensively rear white bass larvae to a stage when they will consume formulated feed (see next Project Component Termination Report). A proposed study for the next NCRAC funding cycle will, among other topics, compare three strains of white bass in yield trials. Collectively, the results from these studies should pave the way to undertake a white bass brood stock selection program.

**Publications, manuscripts, or papers presented**

See the Appendix for a cumulative output for all NCRAC-funded Hybrid Striped Bass activities.

**Support**

Years	NCRAC USDA funding	Other support				Total	Total Support
		University	Industry	Other Federal	Other		
1989-91	\$136,410	\$93,436				\$93,436	\$229,846
1991-93	\$96,550	\$54,317				\$54,317	\$150,867
<b>Total</b>	<b>\$232,960</b>	<b>\$147,753</b>				<b>\$147,753</b>	<b>\$380,713</b>

## Hybrid Striped Bass

*Project Component Termination Report for the Period June 1, 1990 to August 31, 1996*

### **NCRAC funding level**

\$269,000 (June 1, 1990 to February 29, 1996)

### **Participants**

Fred P. Binkowski, University of Wisconsin-Milwaukee, Wisconsin

George G. Brown, Iowa State University, Iowa

Paul B. Brown, Purdue University, Indiana

Konrad Dabrowski, Ohio State University, Ohio

James E. Ebeling, Ohio State University, Ohio

Christopher C. Kohler, Southern Illinois University-Carbondale, Illinois

Jeffrey A. Malison, University of Wisconsin, Wisconsin

Robert J. Sheehan, Southern Illinois University-Carbondale, Illinois

Bruce L. Tetzlaff, Southern Illinois University-Carbondale, Illinois

M. Randall White, Purdue University, Indiana

#### ***Extension liaison:***

Joseph E. Morris, Iowa State University, Iowa

### **Reason for termination**

The objectives for this work on Hybrid Striped Bass were completed.

### **Project objectives**

1. Develop larval diets and economically feasible techniques to convert hybrid striped bass young from zooplankton to prepared diets.
2. Develop intensive hatchery production techniques for white bass and to "domesticate" white bass by producing brood stock originating from induced spawns.
3. Improve methods for storage and transport of striped bass and white bass gametes.

### **Principal accomplishments**

In a comparative study conducted at Southern Illinois University-Carbondale (SIUC), hatching rates for embryos incubated in Heath trays (28.2%) were equivalent to tannic acid-treated (150 mg/L water) embryos incubated in Heath trays (22.9%) or

McDonald jars (22.4%).

Facilities to intensively rear larval white bass were established at Ohio State University (OSU), SIUC, and the University of Wisconsin-Milwaukee (UW-Milwaukee). White bass larvae from three separate spawning trials were shipped by overnight freight to OSU and UW-Milwaukee. Attempts to rear larval white bass were minimally successful. Less than 1% survival rates were obtained by day 122 at UW-Milwaukee, day 45 at OSU, and day 24 at SIUC.

A group of white bass sac-fry shipped from SIUC to UW-Milwaukee was introduced evenly by volume into 12, 60 L (15.9 gal) flow-through aquaria. Each aquarium contained approximately 300 sac-fry. These fish were offered "green tank" water and the three experimental diets that were provided by Purdue University (Purdue). The length of the cylindrical food particles ranged from approximately 0.5 to 1.7 mm (0.02 to 0.07 in) and the diameter was 420-595 µm. White bass sac-fry are approximately 3.5 mm (0.14 in) in total length. The cross sectional diameter of the feed approximated the width of the entire head (550-630 µm) of white bass sac-fry, and was outside of the range of the width of the mouth. UW-Milwaukee researchers ground portions of the diets in a mortar and pestle and sieved it through a 150 µm mesh to obtain more suitable-size particles. From May 26-31, 1995, each of the three ground and sieved diets was offered to fry in triplicate aquaria along with "green tank" water. The controls received only "green tank" water. No feeding activity or interest by the fry in the formulated diets was observed. Mortality of the sac fry was heavy in all the tanks and by May 31 (within 6 days), less than a dozen fry were observed in any of the aquaria and more than half of them had only one or no living fry. At this point the trial was terminated.

Researchers at SIUC found that both hybrid striped bass crosses at a 2-5 g (0.07-0.18 oz) size range readily convert from zooplankton to formulated feed. Over 90% of the fish converted to formulated feed within 2

days as compared to 70-85% after 7 days for large-mouth bass which were trained in a "side-by-side" study. Preliminary results indicate that white bass and reciprocal-cross hybrids are equivalent in this regard and can make the switch between day 21 and 28 after hatch. Original cross hybrids can generally be switched at day 7 after hatch.

A problem facing hybrid striped bass aquaculturists is that hybrid fry are not always available. Gametes must be obtained from two species that may not be spawning simultaneously or are located in different geographical areas. Therefore to facilitate hybrid production, viable *Morone* semina need to be readily available when ripe eggs are available.

To aid in the solution of this problem, procedures for reliable short-term (refrigerated) and long-term (cryopreservation) storage of striped bass (*Morone saxatilis*) semina were developed. Initially, the characteristics of high quality spermatozoa were examined to determine methods for assessing sperm quality and developing effective sperm handling techniques. This led to the formulation of extenders for short-term (less than 21 days) refrigerated (1°C; 33.8°F) storage. The quality of stored seminal samples was tested by determining sperm motility percentages and developing a sperm quality index (SQI). Refrigerated extended seminal samples were routinely stored for 14 days with 50% sperm motility.

Cryopreservation procedures were developed and sperm quality of cryopreserved seminal samples of striped bass were assessed. Fertility tests with these samples were performed with white bass (*M. chrysops*) eggs and results were compared to those results when using (fresh) white bass semen.

Ten media containing dimethylsulfoxide (DMSO) were used to cryopreserve striped bass spermatozoa. Although all media successfully cryopreserved spermatozoa, the best motility (SQI 2.3: about 50%) was obtained with samples cryopreserved in the five media containing 4% DMSO. Using the criteria for high quality semen, the samples cryopreserved in media containing 4% DMSO with or without trehalose and bovine serum albumin gave the best motility results and were used in fertility tests with white bass eggs. Straws of the cryopreserved samples were transported from Florida to SIUC packed in dry ice. These were then

stored in liquid nitrogen until used in fertility tests. Striped bass spermatozoa were cryopreserved with relatively simple methods. This may partially be because of the small size of the sperm, causing damage by the freezing process to be minimal since the cryogenic medium penetrates the whole cell very rapidly and the actual freezing may be rapid enough to prevent damaging ice crystal formation.

In the hybrid cross, the study was pursued until the hatch of normal larvae. Although success with cryopreserved spermatozoa has previously been reported for striped bass results were determined on the basis of cleavage, which does not necessarily indicate the normal development of diploid embryos. Fertility was tested using striped bass semen cryopreserved in cryogenic media and white bass eggs. The percent fertilization based on the number of hatched, normal larvae was 6.2% for the cryopreserved semen and 2.5% for the eggs fertilized with fresh control white bass semen (dead and abnormal larvae were excluded). This represented a 251.2% hatch from cryopreserved semen related to control semen. No development was found in control eggs (unfertilized eggs) tested for parthenogenesis.

The motility intensity of thawed and activated cryopreserved spermatozoa was roughly equivalent to that of seminal samples activated after 14-21 days of refrigerated storage, indicating that cryopreservation of striped bass semen may be the best option when storage time exceeds 21 days.

Emphasis was also focused on developing refrigerated and frozen storage methods for white bass spermatozoa. Evaluations of sperm motility and nuclear magnetic resonance (NMR) were used as measures of success in developing methodologies. NMR was used to measure the availability of high energy phosphorus compounds to power flagellar movements in spermatozoa.

Sperm quality was best when seminal samples were extended prior to shipping and when they were transported in tissue-culture flasks which provided a larger air space than the microcentrifuge tubes which were also tested as shipping containers. Extenders with simple formulations, including one that was essentially only a saline solution, were as good or better than a more complex extender solution for maintaining sperm quality during refrigerated storage at 1°C (33.8°F). The

simple saline solution extender maintained good sperm quality for up to 1 month of refrigerated storage.

Declines in high-energy phosphorus compounds and increases in their breakdown product, as measured via NMR, corresponded with declines in sperm motility over time during refrigerated storage of semen. However, NMR detected differences in stored energy in spermatozoa among seminal samples when no such differences in sperm motility were detected, indicating that NMR may be a more sensitive measure of sperm quality.

It was found that a cryogenic solution consisting of a simple extender and DMSO as the cryoprotectant performed as well as more complex cryogenic media in sperm motility tests. Fertility was somewhat reduced using cryopreserved semen, as compared to semen which had been extended and stored at 1°C (33.8°F) for about 1 week. Cryopreservation reduced white bass sperm motility to 5 to 25% of motility in fresh semen samples, a reduction similar to that found in seminal samples which are extended and stored under refrigeration for about 4 weeks. It is recommended that refrigerated storage be used for white bass semen if storage times of 1 month or less are anticipated. Cryopreservation is the better option, if sperm storage is to exceed 1 month.

### Impacts

Studies by the Hybrid Striped Bass Work Group demonstrate that:

- improvements in hatching rates allows for increased hatchery production or reduction in brood stock needs;
- improvements in larval rearing techniques of white bass will allow “true” domestication;
- improvements in switching hybrid striped bass fingerlings from zooplankton to formulated feeds will increase production efficiency;
- *Morone* semen which is to be stored should be kept cold at all times subsequent to stripping;
- white bass injected with hCG once per month and held at 15°C (59.0°F) produced 2 to 3 times as many spermatozoa as compared to those either given hCG once per week or no hCG but otherwise treated similarly—using this approach allowed semen to be obtained from each fish once per week for several months;
- semen should be diluted with an extender prior to shipping and transported on ice;
- relatively simple extender solutions (saline solutions) are effective for refrigerated storage of *Morone* semen;
- tissue culture flasks proved to be better than microcentrifuge tubes for shipping white bass semen—this difference was attributed to the oxygen in the larger air space of the former;
- *Morone* semen can be extended and stored at 1°C (33.8°F) and good motility can be retained for 3 to 4 weeks;
- initial evaluations indicated that changes in NMR spectra of seminal samples are consistent with changes in sperm motility; however, NMR may provide a more sensitive measure of semen quality;
- cryopreservation reduced sperm motility by about 50%, as compared to extended semen;
- a relatively simple cryogenic medium (4% DMSO in a simple extender solution) was effective for storing *Morone* semen;
- excellent fertility in white bass eggs was obtained using cryopreserved striped bass semen, and good fertility was obtained using cryopreserved white bass semen;
- based on reductions in sperm motility, cryopreservation is the better option for *Morone* semen if it is to be stored for more than 3 to 4 weeks, whereas refrigerated storage is better for shorter storage times.

### Recommended follow-up activities

NCRAC is currently funding studies aimed at comparing different geographical strains of hybrid striped bass and white bass in ponds. These studies are incorporating spawning, sperm storage, and hatchery procedures developed in this project. The sperm storage protocols are also being tested in industry settings. Collectively, the results from past and current studies should pave the way to economically undertake hybrid striped bass culture in the NCR. Continued demonstration of the technologies developed need to be undertaken with industry partners.

### Publications, manuscripts, or papers presented

See the Appendix for a cumulative output for all NCRAC-funded Hybrid Striped Bass activities.

**Support**

Years	NCRAC USDA funding	Other support				Total Support
		University	Industry	Other Federal	Other	
1990-93	\$101,000	\$94,000			\$94,000	\$195,000
1993-96	\$168,000	\$119,440			\$119,440	\$287,440
<b>Total</b>	<b>\$269,000</b>	<b>\$213,440</b>			<b>\$213,440</b>	<b>\$482,440</b>

**Hybrid Striped Bass**

*Progress Report for the Period September 1, 1995 to August 31, 1996*

**NCRAC funding level**

\$90,270 (September 1, 1995 to August 31, 1996)

**Participants**

Fred P. Binkowski, University of Wisconsin-Milwaukee, Wisconsin

Michael L. Brown, South Dakota State University, South Dakota

Paul B. Brown, Purdue University, Indiana

Terrence B. Kayes, University of Nebraska-Lincoln, Nebraska

Christopher C. Kohler, Southern Illinois University-Carbondale, Illinois

Jeffrey A. Malison, University of Wisconsin, Wisconsin

Joseph E. Morris, Iowa State University, Iowa

Robert J. Sheehan, Southern Illinois University-Carbondale, Illinois

**Extension liaison**

Joseph E. Morris, Iowa State University, Iowa

**Non-funded collaborators**

Robert Lyons, Lyons Enterprises, Morocco, Indiana

Gary Shirley, Shirley's Fish Farm, Lafayette, Indiana

Mike Freeze, Keo Fish Farm, Inc., Keo, Arkansas

Scott Lindell, AquaFuture, Turners Fall, Massachusetts

**Project objectives**

1. Examine fry (phase 1) to fingerling (phase 2) production of three strains of white bass and three strains of hybrid striped bass (sunshine bass) in ponds with and without lights and vibrating feeders.

2. Conduct field testing of fingerling (phase 2) to advanced fingerling (phase 3) production of three strains of hybrid striped bass (sunshine bass) in various culture systems.
3. Extension component:
  - a. Coordinate selection of various culture systems and implement field testing (fingerling to advanced fingerling to food size).
  - b. Write an initial culture manual using the information generated by all the hybrid striped bass research sponsored by North Central Regional Aquaculture Center (NCRAC).
  - c. Produce associated fact sheets, bulletins, and videos for hybrid striped bass research in the North Central Region (NCR).

**Anticipated benefits**

The overall goal of this collaborative project is to enhance hybrid striped bass aquaculture in the NCR. Hybrid striped bass are consistently identified as a high priority species within the NCR and consistently ranked as a preferred species to eat (unpublished data from Purdue University). Out-of-season spawning of white bass has been achieved in an ongoing NCRAC-sponsored project. The development of intensive larval culture techniques for this species will allow for its full domestication. The development of techniques for semen storage (cryopreservation and extended) preclude the need for maintaining large numbers of male striped bass brood stock. The logical next step is to conduct field trials of several strains of white bass and hybrid striped bass in various culture systems.

Existing producers need to improve the economics of hybrid striped bass production by increasing stocking densities and improving feeds. The break-even production cost of hybrids grown in cages is reported to be \$2.29 to \$3.45/kg (\$1.04 to \$1.56/lb). Those values were based on a stocking density of 100/m<sup>3</sup> (2.8/ft<sup>3</sup>) and feed costs of \$0.55/kg (\$0.25/lb). As production of hybrids increases on a regional and national scale, market price will likely decrease. Thus, this research will help maintain current profit margins as production increases. The knowledge gained from this study should be of immediate use by the aquaculture industry. The extension component of the study will assure that research information gets to the industry in a user-friendly form. Although the proposed project is not directly interregional with respect to physical performance, lines of communications have and will continue to be maintained with the Hybrid Striped Bass Grower's Association and other researchers, specifically: Harrell, Woods, and Zohar at the University of Maryland; Smith and Jenkins at the South Carolina Department of Natural Resources; and Hodson and Sullivan at North Carolina State University.

### **Progress and principal accomplishments**

#### **Objectives 1 and 2**

*Southern Illinois University-Carbondale (SIUC)*

##### *Brood stock acquisition*

Adult white bass were acquired by SIUC researchers from three regions representing the extremes of white bass' native range: Arkansas, South Dakota, and Lake Erie. Arkansas white bass were collected by trap netting in the Arkansas River. The South Dakota stock of white bass was collected by South Dakota State University (SDSU) by angling in Lake Kampeska, South Dakota. Lake Erie white bass were collected by commercial fishermen in Sandusky Bay of Lake Erie. Brood fish were held at SIUC in recirculating systems in winter conditions (8°C [46.4°F] and 10 hours daylight) until all three stocks of fish were collected.

##### *Spawning of Brood Stock and Incubation of Larvae*

Once all three stocks of fish were acquired, temperature and number of daylight hours were gradually increased until 16°C (60.8°F) and 14 hours, respectively, were reached. During this warm-up phase brood fish were fed minnows on a daily basis. When spawning temperature and number of daylight hours were obtained, female white bass were injected with hCG at

a rate of 150 IU/kg (68.4 IU/lb) to induce ovulation. Males were injected at a rate of 100 IU/kg (45.5 IU/kg) to enhance semen production. Extended striped bass semen was obtained from Keo Fish Farm, Arkansas, so that sunshine bass could be produced.

At least 10 females of each stock ovulated, at which point the eggs were manually stripped and divided into two allotments. One allotment of the eggs was fertilized with white bass neat semen to produce pure white bass, while the other allotment of eggs was fertilized with extended striped bass semen to produce sunshine bass. Both allotments of eggs were treated with tannic acid to reduce the adhesiveness of the eggs. Eggs were then incubated in MacDonald jars until hatch. Hatch was complete at about 48 hours postfertilization.

##### *Enumeration and Stocking of Larvae*

At 4 days of age, the larvae were enumerated and subsequently stocked into ponds. In order to enumerate the larvae, ten samples of 100 mL (3.4 oz) were randomly drawn from each holding tank. The number of larvae in each sample was counted. From the 10 samples an average number of larvae per volume was calculated. This average value was used to extrapolate to the total volume of the holding tank. This procedure was repeated for all six stocks of fish.

Ponds used in this study are approximately 0.04 ha (0.10 acres); however, each pond's length and width were measured to determine its individual surface area. Larvae were transported from the holding tanks to the Touch of Nature pond facility in bags containing approximately one-third fish and water and two-thirds pure oxygen. Stocking of larvae began at dusk and continued after dark. Larvae were stocked at a rate of 500,000/ha (202,350/acre). Each stock of fish was stocked in quadruplicate.

##### *Pond Filling and Fertilization*

Ponds were filled 5-10 days prior to stocking and incoming water was filtered using a nylon "sock" with a mesh size of 500 mm. Ponds were fertilized using both cottonseed meal and 8-32-16 inorganic fertilizer. A single application of cottonseed meal was administered at 350 kg/ha (312.3 lb/acre) 4-5 days prior to stocking. The inorganic fertilizer was applied at 25 kg/ha (22.3 lb/acre) twice weekly for 5 weeks. An additional application of cottonseed meal was

administered once weekly at 25 kg/ha (22.3 lb/acre) starting in week 4.

#### *Feeding of Phase 1 Fingerlings*

Training the fish to accept prepared feed began 21 days poststocking. Fish were offered frymeal twice a day at 5-10 kg/ha/day (4.5-8.9 lb/acre/day). Once fish were observed accepting prepared feed, pellet size was increased as necessary and fish were fed to satiation. Feed amounts were recorded twice daily.

#### *Harvesting Phase 1 Fingerlings*

At 36-41 days of age phase 1 fingerlings were harvested by seining. Survival rate varied from pond to pond, but was generally poor. The highest survival rate for any pond was 21.3%, while the lowest survival rate was 0.0%. Survival rates were markedly higher for hybrid striped bass ponds versus white bass ponds averaging 12.8% and 2.6%, respectively. Average weight of an individual fish in any particular pond was inversely related to its survival rate; that is, if a pond had a high survival rate, then the average weight of an individual within that pond tended to be low. This trend is reflected in the relatively low average weights of hybrid striped bass (1.5 g; 0.05 oz) and the relatively high average weights of white bass (2.2 g; 0.08 oz). Average weights were calculated by weighing 120 individuals from each pond.

#### *Phase 2 Production*

Phase 1 fingerlings which were harvested were restocked for phase 2 production. Due to a lack of fish, all three white bass stocks were eliminated from this segment of the experiment. Both Arkansas and South Dakota hybrid striped bass stocks were restocked in triplicate, while Lake Erie hybrid striped bass were only restocked in duplicate. The stocking rate used for phase 2 production was 25,000 fish/ha (10,117.5/acre). Fish were offered feed two times per day. Fish were fed to satiation and feed amounts were recorded twice daily. One grass carp was stocked per pond to serve as a control on aquatic vegetation.

#### *Harvesting Phase 2 Fingerlings*

At the end of the growing season, phase 2 fingerlings were harvested by seining. Survival rates ranged from a low of 49.2% to a high of 85.8%. Survival rates for both Arkansas and Lake Erie hybrid striped bass were about 72%, while the survival rate for South Dakota hybrid striped bass was only 56.6%. The average

weight of individual fish also varied from stock to stock. The highest average weight was 90.2 g (3.2 oz) for Lake Erie hybrid striped bass, while South Dakota and Arkansas hybrid striped bass had average weights of 69.0 g (2.4 oz) and 58.4 g (2.1 oz), respectively. Average weights were calculated by weighing 50 individuals from each pond.

#### *South Dakota State University (SDSU)*

Two groups of hybrid striped bass fingerlings were transported from SIUC to SDSU to conduct strain comparison and density experiments. The two groups of offspring were produced either with female white bass collected from the Arkansas River, Arkansas, or Lake Kampeska, South Dakota, and stored striped bass spermatozoa maintained at SIUC, originating from Keo Fish Farm, Arkansas. These experiments, which began on August 16, 1996, will continue for approximately another 110 days.

The culture system for both experiments consists of 110 L (29.1 gal) glass aquaria connected as a closed freshwater recirculating system with a delivery rate of approximately 1 L/min (0.26 gal/min). Ammonia, nitrite, nitrate, pH, hardness, alkalinity, and carbon dioxide are measured every 2 days. Water temperature is maintained at 22°C (71.6°F) and dissolved oxygen is maintained near saturation by continuous aeration; both are monitored several times weekly. A light/dark cycle of 12 h light/12 h dark is maintained using incandescent lighting controlled by an automatic electric timer.

Initial mean weights were 3.6 g (0.13 oz) and 2.9 g (0.10 oz) for Arkansas and South Dakota hybrids, respectively. The diet (38% protein, 8% crude fat) used in both experiments was obtained from Southern States Cooperative, Inc. (Richmond, Virginia). All fish were conditioned for a 2-week period by feeding a #4 crumble *ad libitum* two times per day. Randomly selected fish from each strain group were then stocked in individual aquaria to provide four replicates. The feed was supplied to fish initially at a rate of 10% of body weight per day equally divided into four feeding periods. All feeding is done with belt feeders. The feeding rate will be progressively reduced to 3% of body weight during the experiment to minimize overfeeding while maintaining a level approaching satiation. Also, pellet sizes fed are periodically increased with graded changes in body size. Waste material is siphoned from each aquaria every other day.



Group and individual measurements are made at weekly intervals. Feed allotments are adjusted weekly. The same general protocol is being applied to the density experiment. Four replicates each of 5 (45/m<sup>3</sup>; 1.3/ft<sup>3</sup>), 15 (136/m<sup>3</sup>; 3.9/ft<sup>3</sup>), or 30 (273/m<sup>3</sup>; 7.7/ft<sup>3</sup>) South Dakota hybrids per 110 L (29.1 gal) aquaria are being maintained at present. Performance characteristics (e.g., growth, conversion, condition, survival) are monitored in both experiments.

### *Purdue University (Purdue)*

In the first year of the Purdue project, a private producer was going to provide fingerlings for the first evaluation, but none of the fish survived overwinter at the producer's site. A secondary supplier was identified and fish were brought to Indiana. However, most of those fish died due to the stress of a 15-hour haul. Cages were stocked at both field sites and will be harvested in November 1996. The tank loading study was initiated in late-summer 1996 and will be completed by December 1996. In a series of studies, soy products have been evaluated as a replacement for fish meal in diets. Maximum incorporation of raw soybeans was less than 20%, while roasted soybeans could be incorporated up to 20%. Solvent-extracted soybean meal could be incorporated up to 40% of the diet if sufficient mineral supplementation was provided.

### **Objective 3**

#### *Iowa State University and SIUC*

Kohler and Morris served as co-chairs for the NCRAC Hybrid Striped Bass Workshop that was held in November 1995, in Champaign, Illinois. The topics for the workshop included larval culture, cage culture, brood stock management, and an industry perspective; the 35 attendees were from Illinois, Iowa, Indiana, and Missouri. NCRAC-funded speakers included Chris Kohler, Sue Kohler, and Bob Sheehan (SIUC), George Brown and Joe Morris (Iowa State University), and LaDon Swann (Purdue). A hybrid striped bass fact sheet developed by Morris and Kohler is in press.

## **Work planned**

### **Objectives 1 and 2**

#### *SIUC*

Phase 2 fingerlings were redistributed at a rate of 4,940 fish/ha (2,000/acre) for phase 3 growout. There are five replicates for each stock of hybrid striped bass. Feeding will resume as early in spring 1997 as possible

and will occur twice a day until the end of the 1997 growing season.

Studies in aquaria are being conducted to compare the three strains of hybrid striped bass and the three strains of white bass. This study is being conducted as a "control" for the pond studies.

#### *University of Wisconsin-Madison and University of Nebraska-Lincoln*

Pond studies comparing survival of larval hybrid striped bass and white bass with and without lights and vibrating feeders will be carried out in 1997.

#### *SDSU*

During the summer of 1997, plans are to evaluate performance characteristics of three strains of hybrid striped bass (Arkansas, South Dakota, and Lake Erie female white bass sources) under flowthrough conditions. Phase 2 fish, supplied by SIUC, will be transported to SDSU and stocked in an indoor flow-through system comprised of 1,100 L (290.6 gal) rectangular tanks. Each strain will be stocked into a minimum of three replicate flow-through tanks at similar densities.

Commercial feed will be dispensed by belt feeders. General environmental conditions will be similar to those maintained during fall 1996. Trials will continue until each strain reaches a marketable size. Dressed and fillet proportions, and composition will be determined following harvest.

#### *Purdue*

Fish for the second year of the project have been acquired and will be reared at the Purdue University Aquaculture Research Facility. Thus, problems in acquisition and transport have been eliminated. Two commercial aquaculture facilities (Lyons Enterprises and Shirley's Fish Farm) will be stocked in April 1997 and fish grown until November of 1997. Stocking densities will be 100, 150, and 200 fish/m<sup>3</sup> (2.8, 4.2, and 5.7 fish/ft<sup>3</sup>). Tank stocking densities will range from 50 to 300 fish/m<sup>3</sup> (1.4 to 8.5 fish/ft<sup>3</sup>), with flow rates held constant at 1.0 L/min (0.26 gal/min).

### **Objective 3**

The culture manual will be written in 1997. One or more videos will also be developed.

**Impacts**

Much of the technology developed over the course of NCRAC-sponsored hybrid striped bass research was incorporated in the current project. For example, wild white bass brood stock were obtained from three distinct geographic locations and transported to SIUC where they were habituated to captivity and induced to spawn using hormones. Stored striped bass semen obtained from Keo Fish Farms, Arkansas, was then used to produce hybrid striped bass. Eggs were incubated using the jar technique and fry were stocked into newly fertilized ponds. Fingerlings were switched to formulated feed in the ponds and phase 2 production was carried out. Feed-trained fingerlings were also sent to SDSU for additional studies in aquaria and raceways. Identification of the maximum density of fish in cages

and tanks will allow maximum use of production space and resources; thus, increasing profitability of culture. Use of soybean products in diets decreases the cost of feed, while not sacrificing weight gain or health of fish. Further, these new formulations can be manufactured in the NCR. These studies, taken collectively, will not only meet the stated objectives of the project, but also will serve as a demonstration of the bulk of the technology developed by the NCRAC Hybrid Striped Bass Work Group since its inception.

**Publications, manuscripts, or papers presented**

See the Appendix for a cumulative output for all NCRAC-funded Hybrid Striped Bass activities.

**Support**

Years	NCRAC USDA funding	Other support				Total Support
		University	Industry	Other Federal	Other	
1995-96	\$90,270	\$85,117	\$55,019			\$140,136
<b>Total</b>	<b>\$90,270</b>	<b>\$85,117</b>				<b>\$140,136</b>

NCRAC has funded five Hybrid Striped Bass projects. This progress report is for the fifth project which is chaired by Christopher C. Kohler. The project continues and expands upon the first four projects. It began on September 1, 1995, and will conclude on August 31, 1997.

**Walleye**

*Project Component Termination Report for the Period, September 1, 1989 to August 31, 1993*

**NCRAC Funding level**

\$321,740 (May 1, 1989 to August 31, 1993)

**Participants**

Thomas G. Bell, Michigan State University, Michigan  
 Frederick W. Goetz, Jr., University of Notre Dame, Indiana  
 David E. Hinton, University of California-Davis, California  
 Anne R. Kapuscinski, University of Minnesota, Minnesota  
 Terrence B. Kayes, University of Nebraska-Lincoln,

Nebraska  
 Jeffrey A. Malison, University of Wisconsin-Madison, Wisconsin  
 Gary D. Marty, University of California-Davis, California  
 Robert J. Sheehan, Southern Illinois University-Carbondale, Illinois  
 Robert C. Summerfelt, Iowa State University, Iowa  
 Bruce L. Tetzlaff, Southern Illinois University-Carbondale, Illinois  
 Allan L. Trapp, Michigan State University, Michigan

## *Extension liaison*

Ronald E. Kinnunen, Michigan State University,  
Michigan

## *Non-funded collaborators*

Iowa Department of Natural Resources (DNR),  
Bellevue Research Station, Iowa  
Iowa DNR, Rathbun State Fish Hatchery, Iowa  
Iowa DNR, Spirit Lake State Fish Hatchery, Iowa  
Minnesota DNR, Devil's Track Hatchery, Minnesota  
Nebraska Game & Parks Commission, Calamus State  
Fish Hatchery, Burwell, Nebraska  
Ohio DNR, London State Fish Hatchery, Ohio  
U.S. Fish & Wildlife Service, Garrison Dam National  
Fish Hatchery, North Dakota  
U.S. Fish & Wildlife Service, Valley City National Fish  
Hatchery, North Dakota  
U.S. Fish & Wildlife Service, Gavins Point National  
Fish Hatchery, South Dakota  
U.S. Fish & Wildlife Service, Genoa National Fish  
Hatchery, Wisconsin

## **Reason for termination**

The objectives for this work on Walleye were completed.

## **Project objectives**

1. Develop baseline information on the mechanisms regulating the natural reproductive cycle of wild and pond-held walleye by characterizing seasonal changes in hormone titers and gonadal histology.
2. Develop methods for manipulating the annual reproductive cycle of walleye to induce out-of-season spawning.
3. Evaluate zooplankton seeding for pond culture of fingerling walleye.
4. Evaluate strategies for control of clam shrimp in ponds used for culture of fingerling walleye.
5. Determine the etiology of noninflation of the gas bladder of intensively cultured walleye fry.

## **Principal accomplishments**

### **Objective 1**

The endocrine and gonadal changes during the annual reproductive cycle of walleye were described for the first time. No differences were observed between the developmental patterns of wild walleye (captured primarily from the Mississippi River near Minneapolis, Minnesota) and walleye held in ponds in Carbondale, Illinois. Gonadal growth in wild male walleye began in August/September, and testes contained mature

spermatozoa by late fall (October/November). Mature spermatozoa could be expressed from males collected from January through April. Testosterone levels were low (<0.5 ng/mL) throughout the summer, rose in October to a plateau of >1.0 ng/mL from November through January, and peaked in March at 2.8 ng/mL. Serum levels of 11-ketotestosterone were <10 ng/mL from late April through January, and rose in March and early April to >35 ng/mL.

In wild female walleyes, oocytes increased in diameter from  $184.0 \pm 18.6$  mm in October to  $998.7 \pm 39.8$  mm in November. This increase occurred coincident with a marked rise in gonadosomatic indices (GSIs) and circulating levels of serum estradiol-17 (from 0.2 ng/mL to 3.7 ng/mL), the steroid responsible for stimulating hepatic vitellogenin synthesis in teleosts. Just prior to spawning in March, oocytes were approximately 1,500 mm in diameter. Following spawning, average GSIs in females declined from 15.3% to 1.5%. Data from *in vitro* cultures of walleye oocytes conducted at the University of Wisconsin-Madison (UW-Madison) suggested that 17,20-dihydroxy-4-pregnen-3-one (17,20-P) may be the steroid responsible for inducing final oocyte maturation in walleye. *In vivo* studies showed that 17,20-P levels rose very transiently to approximately 2 ng/mL coincident with final oocyte maturation. Taken together, these results suggest that vitellogenesis and spermatogenesis are at or near completion as early as mid-January, and that simple environmental and/or hormonal manipulations could be used to induce spawning from mid-January to late March.

### **Objective 2**

University of Nebraska-Lincoln (UNL) and UW-Madison investigators developed methods to manipulate the annual reproductive cycle and induce out-of-season spawning of walleye. Wild adult walleye were captured in autumn by the Nebraska Game and Parks Commission from Lake McConaughy, Elwood Reservoir, and Merritt Reservoir in Nebraska, and by the Iowa Department of Natural Resources (DNR) from the Mississippi River. The Nebraska fish were transported to the Calamus State Fish Hatchery near Burwell, Nebraska, where they were maintained in a lined pond. In early December, these fish were transferred to earthen ponds at the Gavins Point National Fish Hatchery near Yankton, South Dakota. Walleye captured from the Mississippi River were

transported directly to the Gavins Point Hatchery. All fish were fin-clipped to identify their origin, and transported by UNL personnel. At the Gavins Point hatchery, the walleye were separated by sex and overwintered in two 0.07 ha (0.17 acre) ponds stocked with forage fish.

In January, February, and March, 16-20 females and 4-5 males were recaptured from the Gavins Point hatchery ponds and transferred to the Calamus State Fish Hatchery, where they were placed in indoor tanks. There, the water temperature was gradually increased over a 10-d period to 10°C (50°F) and the photoperiod was set at 12-h light/12-h dark. Females were subject to one of four injection regimes: (1) saline on days 0 and 2; (2) human chorionic gonadotropin (hCG) on day 0 and day 2; (3) a synthetic luteinizing hormone-releasing hormone analogue (LHRHa) on day 0 and day 2; or (4) hCG on day 0 and 17,20-P on day 2. No difference in response patterns to these treatments was observed in walleye of different origins.

At each month, all hormone injection regimes successfully induced GVBD (germinal vesicle breakdown) and ovulation in at least some of the females, whereas none of the saline-injected fish underwent GVBD or spawned. In January, hCG was the most effective treatment for inducing ovulation. In February and March, egg survival was the highest in hCG-treated fish. At all times, the 17,20-P treatment resulted in very low egg survival and small egg size. The results demonstrate that appropriate hormone and environmental treatments can be successfully used to induce spawning in walleye from late January through March. The most effective hormone treatment in this regard was hCG. Regardless of when it was used, hCG at 150 and 500 IU/kg (days 0 and 2, respectively) (68 IU/lb and 227 IU/lb) induced spawning 6-8 days after the last injection.

### Objective 3

Investigators at Iowa State University (ISU) conducted experiments in 1989 and 1990 to evaluate zooplankton seeding (inoculation) for pond culture of fingerling walleye at the Valley City National Fish Hatchery (VCNFH), Valley City, North Dakota, and Garrison Dam National Fish Hatchery (GDNFH), Riverdale, North Dakota. Zooplankton for the inoculation experiments were collected with an air-lift pump and cage system or by filtering inflowing water to

raceways. In both cases, the zooplankton that were used for seeding the ponds were those large enough to be retained by a 0.5-mm (0.02-in) screen. All of the inocula consisted of cladocera (*Bosmina* and *Daphnia*) as well as cyclopoid and calanoid copepods. The zooplankton inoculation was done during the first week after fry stocking. The zooplankton inocula ranged from 0.4 to 28.8 kg/ha, or 70 to 990 organisms per m<sup>3</sup> based on pond volume. In 1989, 95 to 100% of the inoculum was *Daphnia pulex*; in 1990, 77% of the inoculum used at Valley City was *Daphnia pulex*, but at Garrison Dam, only 13.5% were *Daphnia* and 67% were copepods. Over 2 years, 22 ponds were inoculated with zooplankton, and 22 ponds served as the controls (i.e., without zooplankton inoculation).

In each year and at both hatcheries, the average yield (kg/ha, and number/ha) of walleye fingerlings from ponds that had received the zooplankton inoculation was lower than the control ponds. The yield of walleye fingerlings (number per acre) from ponds receiving a zooplankton inoculation at the VCNFH in 1989 was 21.8% less than the control ponds, and 22.4% less in 1990; at GDNFH, the yield from ponds receiving the zooplankton inoculation was 50.9% less in 1989 and 66.9% in 1990 than the control ponds. Also, data combined over both years and both hatcheries showed that smaller biomass yield of fingerlings (42.7 kg/ha; 38.1 lb/acre) was obtained from ponds that were seeded with zooplankton than the control ponds that were not inoculated (54.9 kg/ha; 49.0 lb/acre).

Overall, the findings indicate that zooplankton inoculation of culture ponds during the week fry are stocked had reduced fish production. These findings indicate that zooplankton inoculation as a pond management strategy must be undertaken with caution. Ponds should not be inoculated with large cladocera such as *Daphnia pulex* shortly after stocking walleye fry because the larger zooplankton in the inoculum have a competitive advantage over the smaller zooplankton *copepoda* nauplii and other smaller zooplankton that serve as important prey for first feeding larval walleye. This does not mean that an inoculation with smaller zooplankton or use of larger zooplankton will not be desirable, however, the findings demonstrate that the timing of such methods for biomanipulation need to be carefully evaluated. At these hatcheries, the normal inflowing water carried an abundance of zooplankton, but if zooplankton populations are not abundant in the

water used to fill the ponds (i.e., when ground water is used) or if zooplankton numbers decline during the culture interval, inoculation may be used to initiate or to sustain zooplankton populations. However, prior research on the effects and benefits of zooplankton inoculation is limited, and it has not been systematically studied in walleye fingerling culture.

### **Objective 4**

ISU investigators carried out studies on the ecology of clam shrimp at the GDNFH. In 1992, the studies were carried out on 23, 0.64 ha (1.58 acre) ponds during the culture season for northern pike, and 19 of the same ponds during the season for walleye. Ponds at the GDNFH were first used to raise northern pike. They were drained after 3 to 4 weeks to harvest the pike, then refilled to raise walleye. Adult clam shrimp were observed in 12 of the 23 ponds during northern pike culture, and 10 of the 19 ponds during walleye culture. Northern pike were cultured up to 29 days in ponds with clam shrimp, while pike were cultured a maximum of 22 days in ponds without clam shrimp. Survival and yield (number/ha and number/pond) of northern pike was significantly lower in ponds with clam shrimp compared to ponds without clam shrimp. Similar differences, although not significant, were seen in walleye culture ponds. Northern pike and walleye were cultured longer in ponds with clam shrimp, implying that fish growth is reduced in culture ponds with clam shrimp. The majority of large clam shrimp found during the walleye culture season were most likely hatched during northern pike culture. When ponds are used in tandem to raise northern pike and walleye, to prevent development of clam shrimp during the walleye culture the ponds should be thoroughly dried between culture periods.

### **Objective 5**

Studies on the etiology (cause for) of noninflation of the gas bladder (NGB) were carried out as a collaborative effort among ISU, Michigan State University (MSU), and University of California-Davis (UCD). The study objectives included development of methods for intensive rearing of larval walleye on formulated feeds (ISU), identification of pathological lesions that will indicate the etiology of non-inflation of the gas bladder (MSU), and a description of developmental histology of the gas bladder, pneumatic duct, and other tissues and glands (UCD).

Each year, researchers at ISU obtained 1- to 5-day posthatch walleye fry or eyed-eggs from at least three cooperating state and federal fisheries agencies. ISU personnel reared walleye fry in an intensive culture environment and fed the fish a formulated feed, "fry feed Kyowa" (BioKyowa, Inc.), sizes B-400 through B-700, for 21-30 days. Each lot of fish obtained each year was used to evaluate different intensive culture treatments. Culture conditions involved different tank design (cylindrical and square), single-pass and recycle water systems, and pH. These different culture systems have aspects of them that may influence feed particle density (i.e., feeding success affects survival) and water quality (i.e., surface films or pH) which, in turn, may affect gas bladder inflation.

The fry samples provided a progression in age and size of fish, some collected before and others after feeding, with and without the yolk sac, and fish with and without gas bladder inflation, from a variety of experiments in which environmental variables differed substantially. MSU investigators found degenerative changes in the gas bladders (i.e., hyperplasia and abundance of macrophages) which were indicative of an inflammatory disease, and preliminary evidence to suggest a microbial infection as a specific initiating process. The observation of bacteria in the macrophages suggested a bacterial infection, at least as a secondary invader.

UCD investigators found that inflation of the swimbladder began on the sixth day posthatch, coinciding with the time of yolk sac depletion and initiation of feeding. In larvae with noninflated swimbladders, the pneumatic duct was obvious and its diameter remained fairly constant (25-45  $\mu$ m) through the nineteenth day posthatch, but the pneumatic duct atrophied in larvae with inflated swimbladders. During the interval of swimbladder inflation, from the sixth to the 12<sup>th</sup> day posthatch, the common bile duct and pneumatic duct both opened to undifferentiated foregut where surfactant-like secretions from the common bile duct could affect fragmentation of large ingested air bubbles for transfer into the relative small-diameter pneumatic duct. After the 12<sup>th</sup> day posthatch, however, the pyloric sphincter developed and separated the common bile duct in the intestine from the pneumatic duct in the dorsal wall of the stomach. Thus, this finding indicates that differentiation of the foregut prevents inflation of larvae after 12<sup>th</sup> day posthatch.

The day for these events, however, will vary depending on water temperature.

## Impacts

### Objectives 1 and 2

These studies initially generated the basic knowledge of the reproductive cycle of walleye that was needed to begin efforts at controlling reproduction in walleye. This information was subsequently used to develop methods for inducing out-of-season spawning in walleye from late January through March.

The investigators also detailed techniques useful for synchronizing spawning in walleye, resulting in greater predictability of gamete production, and reduced incidence of failed spawning, gamete resorption and subsequent brood fish losses. These techniques can be used to increase hatchery efficiency and reliability.

Recently, UW-Madison personnel successfully led an effort to gain FDA-INAD approval to use hCG to induce spawning in walleye and yellow perch. This approval involves three regional private sector producers, and was done with the help of and in conjunction with the Iowa DNR.

Walleye producers (including the Iowa DNR) have used the technologies developed from these studies to produce walleye fry 9-12 weeks prior to the normal spawning season, and thereby greatly extended the period of time during which larval walleye can be reared intensively. This, in turn, has greatly increased the efficiency of existing intensive fry culture systems, facilitated research on the intensive culture of walleye fry, and aided hatcheries in their efforts to produce larger walleyes for stocking.

### Objective 3

Research on the use of zooplankton inoculation for pond culture of fish has not been systematically studied in walleye fingerling culture. In the present study, zooplankton inoculation of walleye culture ponds during the week walleye fry were first stocked reduced survival and yield. It was surprising to find lower survival and production in ponds supplemented with zooplankton because the method is believed to increase forage for fingerlings. However, basic studies by aquatic ecologists has long demonstrated the difficulties of precise prediction of zooplankton dynamics in ponds and lakes. Moreover, the use of zooplankton

inoculation is an unproven method for biomanipulation of aquatic ecosystems, even as small as fish culture ponds. Zooplankton inoculation may be beneficial in ponds filled with water which is devoid of planktonic life (e.g., well water), but at hatcheries which fill ponds with surface water there may be no benefit of adding zooplankton and it may actually be detrimental to production.

These findings indicate that zooplankton inoculation as a pond management strategy must be undertaken with caution. It seems, however, that ponds should not be inoculated with large cladocera shortly after stocking walleye fry because seeding ponds with zooplankton that are too large to be eaten by first feeding walleye may encourage a competitive advantage for the larger zooplankton over the smaller cladocera and copepods that are essential prey for first feeding larval walleye. In this study, the organisms used for seeding were generally larger than 2 mm (0.08 in) which is larger than first feeding walleye (about 9 mm; 0.35 in) can consume. It has been reported by others that the mean length of zooplankters in gut contents of first feeding walleye was 1.1 mm (0.04 in) at one study site and 0.8 mm (0.03 in) at another study site.

### Objective 4

Basic studies on the ecology of clam shrimp in culture ponds demonstrate that strategies for control of clam shrimp in culture ponds need to consider both the life history characteristics of clam shrimp and fish cultural practices. Clam shrimp life history information provided insight into pond management strategies to reduce the impacts of clam shrimp on fish production. The typical habitat of most North American clam shrimp species is small, ephemeral ponds. The key to clam shrimp survival in this habitat is their ability to produce eggs that are highly resistant to drying, mechanical injury, and freezing. Clam shrimp problems in fish culture ponds are persistent because the resting eggs are resistant to mechanical injury, sunlight, and desiccation. Clam shrimp resting eggs can survive long periods of direct sunlight and wind, which they encounter when culture ponds are drained for harvest. Control measures for clam shrimp include interruption of the wet-dry cycle in fish culture ponds, a fill-drain-and-fill strategy, biological control, and chemical control. A fill-drain-and-fill strategy would involve partial pond filling in the spring long enough for clam shrimp eggs to hatch, then drained to flush out

the newly hatched clam shrimp nauplii. The current tandem culture system at the GDNFH is a type of fill-and-drain strategy. At GDNFH, the northern pike culture season seems to end before clam shrimp reach sexual maturity, and many juvenile clam shrimp are flushed out before they were able to produce either summer or resting eggs. Also, many, but not all juveniles stranded on the pond bottom die before the ponds are refilled. These practices reduced the abundance of clam shrimp during the walleye culture season because clam shrimp that are hatched during the first week of northern pike season were unable to reproduce before they were washed out when the ponds were drained to harvest the northern pike. Although many of the clam shrimp were washed out, as observed in the catch basin when the ponds were drained, some clam shrimp are carried-over to the walleye culture season by surviving in the kettle and on the wet, pond bottom. Although these clam shrimp would be killed with a longer drying period, it is not possible to delay refilling (mean of 1.6 days in 1992) because hatching of these walleye has already been delayed to facilitate the double-cropping strategy. Biological control of clam shrimp with a predaceous fish does not seem to be effective because neither northern pike nor walleye culture feed on clam shrimp. Chemical control may be possible. Quicklime (calcium oxide, CaO) or slaked lime (calcium hydroxide, Ca(OH)<sub>2</sub>) is generally recognized as safe as a pond sterilant by FDA and can be applied at the rate of 1,500 kg/ha (1,338 lb/acre) as quick lime or 2,000 kg/ha (1,784 lb/acre) as slaked lime. Lime is often used as a pond disinfectant to kill infectious organisms and parasites, including fish, tadpoles, and insects. The toxicity of lime to clam shrimp resting eggs has not been evaluated, but it is a potential treatment for killing clam shrimp eggs if the lime is applied to the moist, pond bottom after it is drained at the end of each production season. In a hatchery such as the GDNFH, the best time to make a lime application would follow the walleye harvest. Previously, trichlorfon (commercially sold as Masoten™ or Dylox™) was widely used for control of clam shrimp, but is not registered for use in fish culture ponds. Other studies show that trichlorfon treatment may be detrimental to zooplankton and invertebrates.

### Objective 5

Culture tanks equipped with a surface spray, using about 10% of the inflow directed through a 90° nozzle to the water surface, removed the oil film, and feed and

bacterial growth from the tank surface, thereby greatly enhancing gas bladder inflation. Gas bladder inflation, which was 20-40% without a surface spray, was 80 to 100% with a spray. Circular tanks (cylindrical tanks) with black-painted side walls were found to be more effective culture vessels than cuboidal tanks or tanks with blue-colored side walls. A near neutral pH is a healthier environment for larval fish than one supplied with water with high pH (> 8.5).

Development of a successful intensive culture system is essential for use of out-of-season spawning of walleye when ponds are not available for stocking. The successful development of techniques for out-of-season spawning and intensive culture system for rearing larval walleye represent a major breakthrough in walleye culture, opening new opportunities of research and commercial culture.

## Recommended follow-up activities

### Objectives 1 and 2

Further efforts should be directed at developing techniques to induce out-of-season spawning in walleye throughout the year.

### Objective 3

The findings of negative effects from zooplankton inoculation suggest the need for further research to provide further explanation, and the need to define how and when (i.e., the timing) zooplankton inoculation may be used in pond culture of walleye. Distinction needs to be made between ponds receiving zooplankton from the water supply and those filled with well water and devoid of zooplankton. Likewise, little attention has been given to measuring the quantity and impact of zooplankton inoculation from the water supply of fish hatcheries using surface water sources.

### Objective 4

Strategies for control of clam shrimp in culture ponds with quicklime (calcium oxide, CaO) or slaked lime (calcium hydroxide, Ca(OH)<sub>2</sub>) need evaluation because these chemicals are approved for use in aquaculture.

### Objective 5

Non-inflation of the gas bladder has been a major constraint to successful mass culture of walleye. The development of tank design and a spray-system to remove surface contaminants was a major

breakthrough, however, survival is still typically less than 50% and further research would be beneficial to improve commercial feasibility. Research is especially needed on use of turbid water, optimizing light intensity, and feeding strategies for enhancing survival and growth of larval walleye.

## Publications, manuscripts, or papers presented

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

## Support

Years	NCRAC USDA funding	Other support				Total Support
		University	Industry	Other Federal <sup>a</sup>	Other <sup>b</sup>	
1989-91	\$177,517	\$127,535		\$17,511		\$145,046
1991-92	\$109,223	\$73,242		\$8,935		\$82,177
1992-93	\$35,000	\$26,475		\$9,424	\$40,990	\$76,889
<b>Total</b>	<b>\$321,740</b>	<b>\$227,252</b>		<b>\$35,870</b>	<b>\$40,990</b>	<b>\$304,112</b>

<sup>a</sup>University of Wisconsin Sea Grant Program/USDC/NOAA

<sup>b</sup>Nebraska Game and Parks Commission

NCRAC has funded six walleye projects. This project component termination report covers work undertaken for the first, third, and fourth projects. Robert Summerfelt chaired the first and third projects and Jeffrey A. Malison chaired the fourth project. The third project continued the first project for an additional year whereas the fourth project expanded upon earlier projects.

## Walleye

*Progress Report for the Period September 1, 1995 to August 31, 1996*

### NCRAC funding level

\$117,897 (September 1, 1995 to August 31, 1996)

### Participants

Jeffrey L. Gunderson, University of Minnesota, Minnesota  
 Terrence B. Kayes, University of Nebraska-Lincoln, Nebraska  
 Ronald E. Kinnunen, Michigan State University, Michigan  
 Jay A. Leitch, North Dakota State University, North Dakota  
 Jeffrey A. Malison, University of Wisconsin-Madison, Wisconsin  
 Marshall A. Martin, Purdue University, Indiana  
 Patrick D. O'Rourke, Illinois State University, Illinois  
 Jean R. Riepe, Purdue University, Purdue

Robert C. Summerfelt, Iowa State University, Iowa  
 David H. Wahl, Illinois Natural History Survey, Illinois  
*Extension liaison*

Ronald E. Kinnunen, Michigan State University, Michigan

#### *Non-funded Collaborators*

Larry Belusz and Greg Raisanen, Alexandria Technical College, Minnesota

Nebraska Game & Parks Commission, Calamus State Fish Hatchery, Burwell, Nebraska

### Project objectives

1. Evaluate growth, feed efficiency, and stress responses as functions of density, loading, temperature, and feeding regimes (feeding rate and frequency) under tank and open-pond rearing conditions for raising juvenile walleye to food size.



2. Characterize the economics and institutional aspects of the domestic market for walleye as food fish, fingerlings, and other intermediate products.
3. Offer several workshops in the North Central Region (NCR), using extension materials (fact sheets, videos, etc.) and other information that has or will be developed necessary to demonstrate the technology of culturing walleye and its hybrids.
4. Complete performance evaluations of walleye (sauger hybrids) to finalize research initiated during the 2-year project period of the June 1993 proposal - including studies on fillet yield, proximate analysis, and organoleptic properties.

### Anticipated benefits

This project is addressing priority needs identified by the North Central Regional Aquaculture Center (NCRAC) Industry Advisory Council, as well as specific objectives adopted by the NCRAC Board of Directors, to advance the development of commercial walleye aquaculture in the NCR. Two major lines of research are being pursued: first, to determine whether this species can be cultured to food size under practical conditions, at rearing densities and in a time frame conducive to commercialization; and second, to evaluate the nature and scope of the domestic market for walleye.

In addition, research is being completed to determine if one or more combinations of walleye and sauger genetic stocks can be used to produce hybrids that exhibit superior growth and performance characteristics, compared to purebred walleye. Collectively the data generated by research on both purebred and hybrid walleye will provide critical information to facilitate economic analyses of production costs, and provide extension professionals and private fish producers with new knowledge and training materials on key aspects of walleye aquaculture.

The research being done as part of this project on the production of walleye to food size is: (1) evaluating survival, growth, feed efficiency, and stress responses under various culture conditions; (2) examining methods of estimating growth and feeding rates under conditions that span the optimum temperature range for juveniles, with the goal of developing feeding tables for walleye; (3) adapting a bioenergetics model for use in projecting walleye growth and making feeding recommendations under various culture conditions; and

(4) completing studies comparing the growth, performance, and other characteristics of walleye hybrids and purebreds up to food size.

The research being done to investigate the domestic walleye market is documenting critical information about the historic and potential imports of Canadian walleye, and the potential negative price impacts such imports could have on a fledgling domestic walleye aquaculture industry. A clearer understanding of wild-caught supplies, market pricing systems, marketing channels, and institutional structures will assist walleye producers to position themselves better to achieve profitability; help plan production, financing and marketing strategies; and provide insights on the potential effect farm-raised walleye products will have on the domestic market for this species.

### Progress and principal accomplishments

#### Objective 1

Research in year 1 of the project on this objective was conducted by investigators from Iowa State University (ISU), the Illinois Natural History Survey (ILNHS), University of Nebraska-Lincoln (UNL), and the University of Wisconsin-Madison (UW-Madison). Much of the work done was preparatory to definitive research that will be completed in year 2.

Studies by ISU investigators were done using walleye hatched and raised to fingerling size at ISU in 1995. Final weight, percentage weight gain, and specific growth rate (percent weight gain per day) was greater for walleye cultured at 25°C (77.0°F) than at 20°C (68.0°F), but the difference was not statistically significant ( $P < 0.05$ ). At 25°C (77.0°F), growth (total percentage weight gain) and specific growth rate in fish of 250-300 mm (9.8-11.8 in) total length (TL), at feeding rates of 1.5 and 2.0 (percent body weight per day) were not significantly different.

Work by ILNHS investigators has thus far focused on modifying an existing bioenergetics model for walleye using more recently developed metabolic parameters and better measures of specific inputs. Determinations of caloric levels contained in individual whole walleye and formulated feeds (by Parr adiabatic bomb calorimetry) and feces (by microbomb calorimetry) are nearing completion. Preparations have been made to enter data into the model from tank experiments using

water temperature and food consumption as input variables.

The main focus of UNL investigators in year 1 of the project was to raise a large number of age 0 juvenile walleye for use in year 2 production trials aimed at culturing fish to market size under practical conditions. On June 6, 1996, UNL researchers harvested about 43,200 walleye of 28.5 mm (1.1 in) mean TL and 0.7 g (0.02 oz) mean body weight from 0.4 ha (1.0 acre) production ponds at the Calamus State Fish Hatchery near Burwell, Nebraska. Equal numbers of these fish (about 2,700) were assigned to 16 840 L (222 gal) cylindrical tanks, enclosed in a darkened Aquashelter® (Tuttle Industries, Friend, Nebraska).

All 16 tanks were equipped with in-tank lighting and 24-hour belt feeders (Zeigler Bros., Gardners, Pennsylvania), and supplied with Calamus Reservoir water run through packed columns for aeration. A feeding trial was conducted comparing a diet developed for juvenile walleye by Rick Barrows of the U.S. Fish and Wildlife Service (Bozeman, Montana) and “Silver Cup” salmon starter-series diet (Murrey Elevators, Murrey, Utah). Far more walleye were habituated to the Barrow’s diet than the Silver Cup diet. However, overall survival from the beginning to the end of the trial was extremely poor.

On July 12, 1996, less than 3,000 of the original 43,200 walleye remained alive, despite every effort to maximize survival. This poor survival was attributed primarily to facilities problems, though cannibalism was also a contributing factor. Significant disease problems were not observed. By October 9, 1996, only 973 walleye remained alive, though they were healthy and in excellent condition. Their mean total lengths and body weights were 161 mm (6.3 in) and 33.3 g (1.17 oz), respectively. On that date, the remaining walleye were placed in tanks supplied with 13.3°C (55.9°F) well water for overwintering.

Investigators at UW-Madison conducted experiments to measure changes in blood serum concentrations of cortisol, glucose, and chloride, following acute stress challenge tests of walleye acclimated to different water temperatures. Preliminary findings suggest that the stress-induced cortisol rise in walleye is far quicker and returns to baseline values faster than in rainbow trout, but peak values in the two species are comparable.

After being stressed, walleye held at 15°C (59.0°F) had lower peak cortisol levels, which took longer to return to baseline levels, than walleye held at 21°C (69.8°F). Holding walleye above their thermal optimum (25°C; 77.0°F) prior to being stressed accelerated the initial cortisol rise to peak levels and delayed the return to baseline - suggesting a stronger, more prolonged stress response.

## Objective 2

Investigators at Illinois State University completed a walleye market survey and an in-depth walleye fingerling culture and fish market literature review. The primary focus of the literature review was to identify any past research that might prove useful in describing the market for walleye fingerlings. Little of use was found. Information on the fingerling markets in Canada, the U.S., and the NCR was collected from research and extension experts, and from public and private suppliers and producers of walleye fingerlings using telephone interviews and mailed survey instruments.

Purdue University researchers conducted an in-depth literature review, which included the trade literature for food wholesalers, supermarkets, and restaurants; and developed a mailing list for those types of firms for the 12 states in the NCR. Supermarket and restaurant surveys were initiated in 2 phases. Phase 1 surveys differed for restaurants and supermarkets, and were limited to asking for general information on purchases and sales of fishery products. These surveys identified those restaurants and supermarkets where walleye was sold in 1996. The initial mailing was completed in the last week of August and the first week of September 1996.

North Dakota State University (NDSU) investigators have recently begun to collect published and secondary data on walleye exports from Canada to the U.S. This work got underway in August 1996.

## Objective 3

Two NCRAC-sponsored workshops on walleye aquaculture were held in 1996. The first, “Intensive Culture of Walleye: From Fry to Fingerlings on Formulated Feed,” was held on May 7, 1996, at the Max McGraw Wildlife Foundation, Dundee, Illinois. Robert Summerfelt and Richard Clayton of ISU were the principal speakers, and Tom Harder of the McGraw

Foundation provided a tour and detailed description of the McGraw fry culture facilities. The workshop covered nearly all aspects of walleye fry culture - including design of a large-scale culture system, fish husbandry techniques, and feeding. Terry Kayes of UNL, videotaped the workshop with the assistance of Ron Kinnunen of Michigan State University. Nineteen people attended the workshop; five from Illinois, three from Iowa, two from Minnesota, four from Michigan, one from Nebraska, and four from Pennsylvania.

The second NCRAC workshop, "Production of Advanced Fingerling Walleye: Growth of Minnows in Ponds and Intensive Culture of Formulated Feed," was held on June 18, 1996, at Spirit Lake, Iowa. This workshop was co-sponsored by the Iowa Department of Natural Resources. Techniques for the production of advanced (127-203 mm; 5.0-8.0 in) fingerling walleye were presented. Participants observed a partial harvest of Welch Lake, a 23 ha (56.8 acre) undrainable pond, with a large seine. A site visit was made to the Spirit Lake State Fish Hatchery to observe procedures for training walleye fingerlings to formulated feed. Fifteen people attended this workshop; four from Iowa, two from Michigan, four from Pennsylvania, and five from Wisconsin.

### **Objective 4**

Studies by UW-Madison investigators comparing hybrid and purebred walleye produced from several geographically different stocks of walleye and sauger were recently completed, but the data has not been fully analyzed. To date, hybrid walleye have exhibited superior growth to purebreds at all sizes up to food size. Food size Spirit Lake walleye (Mississippi River sauger gained 1.23 g/day (0.043 oz/day) compared to 0.45 g/day (0.016 oz/day) for purebred walleye. Significant differences in the growth and performance of walleye purebreds and hybrids from different geographic stocks were observed.

Organoleptic trials and proximate analyses of carcass composition revealed little or no difference between purebred and hybrid walleye. Taste panels expressed a high degree of consumer preference for these fish, describing them as firm, flaky, and tender, with an absence of any off-flavors. Proximate analyses indicated that fillets were very low in fat (1.1-1.7%).

## **Work planned**

### **Objective 1**

Investigators at ISU were unable to use a bioenergetics model to calculate feeding rate in year 1 of the project, because ILNHS collaborators were unable to provide measurements of energy values for walleye and formulated feeds until August 1996. Therefore, in year 2 a retrospective analysis will be undertaken using energetics data, combined with actual feeding and growth data, to estimate how the bioenergetics model can be used to determine feeding rates. Additional experiments with "feeding the gain" will be completed, and that procedure will be compared with the bioenergetics method, to estimate feeding rates for the production of food-size walleye.

In year 2, ILNHS researchers will complete their calorimetric studies and modification of the walleye bioenergetics model. Simulations with the model will evaluate potential growth and feeding rates of walleye of various sizes under different aquaculture conditions.

Studies by UNL investigators will focus on evaluating the effects of rearing density on culturing juvenile walleye to food size in tanks, as described in the original proposal. The overwinter survival and growth of juvenile walleye maintained in tanks on well water at 13.3°C (55.9°F) will also be examined. Because of the poor survival of fish in year 1 and the resulting small numbers of advanced fingerling walleye available, no studies on the production of food-size fish in ponds will be possible in Nebraska in year 2 of the project. A shortfall in funding also precludes this possibility.

Investigators at UW-Madison, however, will conduct a study to characterize the growth and performance of walleye cultured to food size in ponds in year 2 of the project. Also, a second experiment to study the special requirements of near-food-size walleye in tank culture systems will be conducted. Details on these investigations are outlined in the original project proposal.

### **Objective 2**

Investigators at Illinois State University will develop research and extension-oriented publications on the U.S. walleye fingerling market, based on data collected from public and private producers and suppliers, the research findings of NDSU, and other sources.

Researchers at NDSU will collect and evaluate secondary data on the export of walleye products from Canada to the U.S., as well as develop a report on the institutional components of the wild-capture fishery in Canada.

Purdue investigators will send phase 2 surveys to all those firms identified by phase 1 surveys as restaurants and supermarkets where walleye products were sold in 1996. The phase 2 surveys will ask targeted questions about walleye purchases and sales. Different surveys have been developed for restaurants and supermarkets. All other types of firms (e.g., fishery products wholesalers, brokers, and food service distributors) will be surveyed with the same, single survey instrument.

**Objective 3**

Two workshops on walleye aquaculture are planned for 1997. Both will be organized by Robert Summerfelt of ISU. The first workshop will be held in Ames, Iowa on February 25, 1997, and will cover the intensive culture of walleye fry. The second workshop will be held at Spirit Lake, Iowa on April 16-17, 1997, and will provide demonstrations of walleye brood stock collection, spawning methods, and egg incubation. In 1997, Kayes of UNL will edit and produce an introductory videotape on the intensive culture of walleye fry.

**Objective 4**

Investigators at UW-Madison will complete the analysis of all data collected under this objective in year 2 and will submit two manuscripts for publication, comparing the growth, performance, proximate analyses, and organoleptic qualities of hybrid and purebred walleye.

**Impacts**

**Objective 1**

The ongoing project will provide information that can be used to prepare guidelines and tables for predicting growth and determining appropriate feeding rates of juvenile to food-size walleye under different culture conditions, determine whether walleye can be raised to food size under practical production conditions, and help determine which culture techniques can be used to rear this species in a time frame and manner conducive to commercialization.

**Objective 2**

Research to date on this objective has generated no measurable economic impacts. But the research findings on this objective should produce valuable insights on the domestic markets for walleye as food fish, fingerlings, and other intermediate products.

**Objective 3**

The workshops on walleye aquaculture have provided the participants with conceptual information as well as demonstrations of important methods. This experience should enhance the ability of participants to learn from reading and doing, as well as undertake more advanced culture technologies.

**Objective 4**

The identification of hybrid walleye (sauger crosses) that have superior growth, performance, and other characteristics “put to use” should significantly reduce the time and costs required to produce food-size walleye.

**Publications, manuscripts, or papers presented**

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

**Support**

Years	NCRAC USDA funding	Other support				Total Support
		University	Industry	Other Federal	Other	
1995-96	\$117,897	\$143,355				\$143,355
<b>Total</b>	<b>\$117,897</b>	<b>\$143,355</b>				<b>\$143,355</b>

NCRAC has funded six walleye projects. This progress report is for the sixth project, which is chaired by Terrence B. Kayes. The project continues and builds upon the first five projects. It began on September 1, 1995, and will conclude on August 31, 1997.

## Sunfish

*Project Component Termination Report for the Period, June 1, 1990 to August 31, 1995*

### **NCRAC funding level**

\$280,577 (June 1, 1990 to August 31, 1995)

### **Participants**

Donald L. Garling, Michigan State University,  
Michigan

Robert J. Sheehan, Southern Illinois University-  
Carbondale, Illinois

Bruce L. Tetzlaff, Southern Illinois University-  
Carbondale, Illinois

#### *Extension liaison*

Joseph E. Morris, Iowa State University, Iowa

### **Reason for termination**

The objectives for this work on sunfish were completed.

### **Project objectives**

1. Determine the mechanisms of sex control in sunfish and to produce and evaluate polyploid sunfish and hybrids.
2. Determine optimal stocking densities and relationships between temperature and growth for *Lepomis*, *Lepomis* hybrids, and triploid *Lepomis*.

### **Principal accomplishments**

An evaluation of both cold and pressure shocks of varying magnitudes, initiation times (time after mixing egg and sperm), and durations to determine the optimum treatments to produce tetraploid (organisms with twice the number of normal chromosomes) bluegill (*Lepomis macrochirus*) has been completed at Michigan State University (MSU). Tetraploidy was induced in five of the 16 cold shock treatments tested. Maximum induction rates of 40% are comparable to those achieved in other species. Of the 10 pressure treatments examined, none were successful in producing tetraploids. Relative survival ranged from <1 to 34% for bluegill exposed to cold shock treatments or pressure shock treatments, respectively.

Twenty-seven combinations of pressure (41,369, 48,264, or 55,158 kPa), shock durations (2, 3, or 4 min), and post-fertilization shock initiation times (2, 3, or 4 min) were tested at Southern Illinois University-Carbondale (SIUC) to identify treatments which would most efficiently induce triploidy in green sunfish (*L. cyanellus*) male (bluegill female F<sub>1</sub> hybrids). Several of the shock treatments produced 100% triploids with at least 90% survival relative to controls. The two shock treatments which appeared to be most effective were: (1) 48,265 kPa for 4 min begun 2 min postfertilization and (2) 41,369 kPa for 2 min begun 3 min postfertilization. A paper based on this work appeared in the "Journal of the World Aquaculture Society;" it is the first publication on shock-induced triploidy in *Lepomis*.

Using starch gel electrophoresis, a diagnostic genetic technique, SIUC investigators found that they could distinguish among three species of sunfish, bluegill, green sunfish, and pumpkinseed (*L. gibbosus*). Furthermore, use of this technique made it possible to identify hybrids of these species; however, it did not allow for the identification of triploids.

Bluegill had a lower mean weight and poorer food conversion after 121 days of growth in a trial comparing bluegill, green sunfish, and male bluegill x female green hybrids. No significant differences were found between green sunfish and hybrids for final weight, specific growth rate, percent weight gain, or food conversion. Growth occurred over the entire range of temperatures tested, 8- 28°C (46-82°F) at 5°C intervals; 23°C (73°F) was optimum.

Male bluegill x green sunfish female triploid and diploid F<sub>1</sub> hybrid growth performance was compared in a 230-day trial at 23°C (73°F). Diploids showed larger final weight and better specific growth rate, percent weight gain, and food conversion. In a third growth trial at SIUC, diploid male bluegill/female green sunfish F<sub>1</sub> hybrids and green sunfish were compared to

triploid male green sunfish/female bluegill  $F_1$  hybrids over 235 days. Diploid taxa were selected on the basis of the results of the 121-day growth trial. No significant differences in weight, specific growth rate, percent weight gain, or food conversion were found. Green sunfish had lower dress-out weights than either hybrid. Gonadal somatic index was higher in the green sunfish than in the diploid and triploid hybrids. The vast majority of the green sunfish became sexually mature and were producing gametes over the range of tested temperatures, 8-28°C (46-82°F). Growth occurred at all temperatures; 18°C (64°F) was optimum. Lower growth rates and reduced optimum temperature were attributed in this trial to the use of fish larger than the ones used in the all-diploid growth trial.

Given the presumption of sterility and other potential advantages, triploids are a viable alternative for intensive food fish culture; they will not reproduce in culture units and will not cause genetic contamination of wild stocks. Male green sunfish/female bluegill  $F_1$  hybrid triploids and male bluegill/female green sunfish diploid  $F_1$  hybrids performed similarly in growth trials at SIUC and appeared to be the best candidates for food fish production.

The pressure-induced triploidy and allozyme species identification techniques derived at SIUC were used to produce gynogens (an organism with only maternal chromosomes) in a study to investigate the genetic sex determination system in bluegill. Heterologous (green sunfish) spermatozoa were irradiated, 15-360 sec, with 1500 uW/cm<sup>2</sup> of 254 nm wavelength UV light to deactivate the DNA. The irradiated spermatozoa were then used to activate bluegill eggs.

Control eggs which were not shocked but activated with irradiated sperm were all ( $N = 37$ ) haploid; controls which were fertilized with normal spermatozoa and not shocked were all diploid ( $N = 21$ ). Sperm irradiation times of 120, 150, or 180 sec plus the hydrostatic shock produced 48 diploids (gynogens) and no individuals with other ploidy levels or green sunfish loci, indicating 100% gynogen production efficiency.

Supposed gynogen larvae ( $N = 150$ ) were then produced and stocked into a pond. Seven sexually mature gynogens were recovered from the pond. All seven were pure bluegill, based on allozyme analysis, and female. The probability of obtaining seven females

from a 1:1 sex ratio population is only 0.008. This is strong evidence that the female is the homogametic sex and that an XX/XY genetic sex determination system occurs in bluegill.

This is the first study reporting induced gynogenesis and gynogen sex ratios in bluegills; this provides the foundation necessary for developing a technique for all-female production in bluegill. Sex reversal of gynogens would yield phenotypic males that would produce all-female progeny when crossed with normal females. This strategy could be used to eliminate reproduction in bluegill culture units, developing techniques for eliminating reproduction is one of the more important goals of the North Central Regional Aquaculture Center (NCRAC) Sunfish Research Effort.

Stocking densities for sunfish in cages and ponds were also evaluated at SIUC. Hybrid sunfish (bluegill male x green sunfish female) grew better at densities of 100 (3 fish/ft<sup>3</sup>) and 200 fish/m<sup>3</sup> (6 fish/ft<sup>3</sup>) than at 400 fish/m<sup>3</sup> (11 fish/ft<sup>3</sup>) in cages. Food conversion was best at the lowest density and it became worse as density was increased.

Growth of hybrid sunfish was directly related to stocking density in ponds at the tested densities of 7,410, 4,940, and 2,470 fish/ha (3,000, 2,000, and 1,000 fish/acre). Food conversion also improved as density increased in ponds. Food conversion (weight of food fed/weight gain) ranged from 2.5 to 5.3 in the highest and lowest density ponds, respectively.

## Impacts

### *Control of sunfish reproduction*

The development of protocols for reducing reproduction in these fishes allows for the potential of increased growth of these fish in aquaculture systems as opposed to unrestrained reproduction. The two shock treatments which appeared to be most effective were: (1) 48,265 kPa for 4 min begun 2 min post-fertilization; and (2) 41,369 kPa for 2 min begun 3 min post-fertilization.

### *Optimal stocking densities*

Information garnered from this work indicates the desirabilities of these fish for aquaculture in the NCR. The sunfish species and diploid and triploid hybrids which were evaluated showed optimum growth at temperatures of 18 to 23°C (64-73°F); all groups grew across a temperature range of 8 to 28°C (46-82°F).

Even at 8°C (46°F), the sunfish species, diploid and triploid hybrids, that were evaluated showed 150 to 200% weight gains over 121 days, and growth rates generally increased with increasing temperature. In light of results from this research project, the male bluegill x female green sunfish diploid F<sub>1</sub> hybrid and the male green sunfish x bluegill sunfish triploid F<sub>1</sub> hybrid appeared to be the best candidates for aquacultural production.

A stocking rate of 200 fish/m<sup>3</sup> (6 fish/ft<sup>3</sup>) is the recommended stocking density for sunfish in cages. In open pond culture, the recommended stocking density for sunfish is 12,355 to 17,297 fingerlings/ha (5,000 to 7,000 fingerlings/acre).

## Recommended follow-up activities

The male green sunfish x female bluegill triploid hybrid grew as well, or better than, the other four *Lepomis* taxa (green sunfish, bluegill, male bluegill male x female green sunfish hybrid, and male bluegill x female green sunfish triploid hybrid) evaluated. There is a need to evaluate growth in this triploid to a larger size, because

triploids, theoretically, have their greatest growth advantage over diploids after the diploids reach sexual maturity.

One of the major impediments to open pond culture of sunfish is uncontrolled reproduction. Monosex sunfish production would eliminate this problem. An important first step has been taken in the development of monosex stock production in sunfish. Induced gynogenesis in bluegill has been accomplished at SIUC. The mating of sex-reversed XX gynogens to normal XX females would produce all-female progeny. Although females did not grow as well as males in SIUC's studies, their growth performance could be considerably enhanced in the absence of males. There is a need to explore monosex stock production in sunfish to determine if this is a viable means for controlling reproduction in culture units.

## Publications, manuscripts, or papers presented

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

### Total support for the first two projects

Years	NCRAC USDA funding	Other support				Total	Total Support
		University	Industry	Other Federal	Other		
1990-92	\$130,758	\$96,710				\$96,710	\$227,468
1992-94	\$149,799	\$343,160	\$3,200 <sup>a</sup>		\$29,830 <sup>b</sup>	\$376,190	\$525,989
<b>Total</b>	<b>\$280,557</b>	<b>\$439,870</b>	<b>\$3,200</b>		<b>\$29,830</b>	<b>\$472,900</b>	<b>\$753,457</b>

<sup>a</sup>American Fishing Tackle Manufacturing Association

<sup>b</sup>Illinois Natural History Survey

NCRAC has funded three sunfish projects. This project component termination report covers work undertaken for the first and second projects. Bruce L. Tetzlaff chaired the first project and Robert J. Sheehan chaired the second project.

## Sunfish

*Project Component Termination Report for the Period, June 1, 1990 to August 31, 1996*

### NCRAC funding level

\$280,557 (June 1, 1990 to August 31, 1996)

### Participants

Michael L. Hooe, Illinois Natural History Survey,  
Illinois

Robert J. Sheehan, Southern Illinois University-  
Carbondale, Illinois

Bruce L. Tetzlaff, Southern Illinois University-  
Carbondale, Illinois

James R. Triplett, Pittsburg State University, Kansas

David H. Wahl, Illinois Natural History Survey, Illinois

#### *Extension liaison*

Joseph E. Morris, Iowa State University, Iowa

### Reason for termination

The objective for this work on sunfish was completed.

### Project objective

Determine optimum stocking densities and relationships between temperature and growth for crappie, crappie hybrids, and triploid crappie.

### Principal accomplishments

Hybrid and pure stock crappies were produced at the Sam Parr Biological Station during spring 1993 and 1994 (no black crappie were produced in 1994) by Illinois Natural History Survey (INHS) personnel with assistance from Southern Illinois University-Carbondale (SIUC) researchers. Diploid F<sub>1</sub> hybrid and triploid F<sub>1</sub> hybrid crappies were produced by crossing white crappie females with black crappie males. In spring 1994, ponds were drained and 1,300 - 1,500 fish of each stock (85-100 mm [3.3-3.9 in] total length) were provided for Pittsburg State University (PSU) and 400-500 of each stock were provided for SIUC. In early summer 1994, additional pure stock black and hybrid crappie were provided for SIUC (300-400 of each stock, 100-150 mm [3.9-5.9 inch] total length). Starch-gel electrophoresis for all brood fish confirmed genetic integrity of the fry.

#### *Observations derived from PSU research*

- Capture and post-transport mortalities were very high, but a small percent of the wild caught white crappie (2%) from summer 1993 survived and showed significant growth. These fish were moved indoors for further feeding trials in a recirculating system.
- Optimum stocking densities were not adequately determined for white crappie. In all cases (1.8 to 5.0 kg/m<sup>3</sup>; 0.1-0.3 lb/ft<sup>3</sup>) overall survivability in cages was poor. However, survival was high and growth was acceptable in indoor trials at densities of 4-5 kg/m<sup>3</sup> (0.2-0.3 lb/ft<sup>3</sup>).
- The high mortalities (57-98%) related to capture and transport of wild caught white crappie during 1993 were reduced to 0% in 1994. Approximately 4,200 fish were transported from the Sam Parr Biological Station in Illinois to the PSU Research Reserve in Kansas in two hauls of 8 hours (702 km; 436 mi) each, without any mortalities. The fish were handled and transported at night with temperatures less than 20°C (68°F) at 4-6 mg/L dissolved oxygen using oxygen diffusers and water treatments of 0.5% salt, PolyAqua™ (0.175-0.375 mL/L), and AmQuel™ (0.125 mL/L). Prior to handling for measurements, fish were anesthetized in Finquel™.
- White crappie, which were wild caught and fed in cages through the summer of 1993, were moved indoors in November and kept in two tanks in a recirculating system at a density of 4-5 kg/m<sup>3</sup> (0.2-0.3 lb/ft<sup>3</sup>) for nearly 18 months. During the six feeding trials, only 17 of the 71 fish died; 16 of these were killed accidentally by a single high chlorine event.
- Black crappie out-performed both white crappie and hybrid crappie in the second year of the cage culture trials. Black crappies showed the greatest growth rates, feed acceptance, uniformity, and survivability, with white crappies intermediate, and hybrid crappie showing poorest overall performance.
- Fish consumed and grew on 2.5 mm (0.1 in) Biodiet™ pellets in both cage trials and recirculating system trials. Examination of the abdominal cavity in all



cases revealed fatty livers and the cavity packed with fat.

- Observations of feeding activity in recirculating tanks suggested the formation of feeding hierarchies. Separate feeding experiments in aquaria during the summer of 1994 as part of a National Science Foundation (NSF) research training academy confirmed the presence of a dominance hierarchy.

A growth trial was conducted at SIUC using black, white, and hybrid crappie. White crappie used in the trial had been subjected to a pressure shock; about 66% of them were triploids. Ten 550 L (145.3 gal) circular tanks, each equipped with biofiltration, aeration, and heating and cooling systems, were used in the growth trial. The circular tanks were partitioned into three compartments, with each compartment receiving equal amounts of the inflow water. All three taxa were evaluated in each tank, one taxon per compartment constituted a replicate, 20 fish per replicate. Despite a protracted training period, feed acceptance was poor during the growth trial and none of the taxa grew well at any of the test temperatures. In most cases, test fish actually lost weight during the trial.

A second growth trial was designed so that growth of black and hybrid crappie would be evaluated against hybrid *Lepomis* sunfish (female green sunfish x male bluegill), a sunfish taxa known to be a good performer in recirculating systems under a variety of water temperatures. In this trial, a more protracted period of time was used to attempt to habituate black and hybrid crappies to prepared diets. The initial mean weight of the hybrid sunfish (60.1 g; 2.1 oz) was considerably greater than the black crappie (26.5 g; 0.9 oz) and hybrid crappie (30.4 g; 1.1 oz), but this was largely due to differences in body conformation and condition; there were only small differences in mean initial total length among the hybrid sunfish (14.7 cm; 5.8 in), black crappie (12.5 cm; 4.9 in), and hybrid crappie (13.0 cm; 5.1 in).

The growth trial was terminated at the end of 56 days when it became evident that hybrid crappie were not growing at some of the test temperatures. The extended training period appeared to be successful for black crappie in this trial. Black crappie grew at all test temperatures and had weight gains ranging from about 20 to 45%; hybrid sunfish had weight gains of 48 to 75% at 10 to 18°C (50.0 to 64.4°F). At test

temperatures of 10 and 14°C (50.0 and 57.2°F), the hybrid crappie lost weight and showed the poorest growth in comparison to either the black crappie or hybrid sunfish at the other test temperatures. The best growth during the trial was shown by the hybrid sunfish at 18°C (64.4°F). Percent weight gains for black crappie were the highest among the three taxa at 22 and 26°C (71.6 and 78.8°F). However, instantaneous growth rate for black crappie was not better than that for hybrid sunfish at the two highest tested temperatures. Mean survival rate was high for all three taxa with all of the hybrid sunfish and 97% of the other two taxa surviving the trial.

Hybrid sunfish showed their best growth at temperatures of 18°C (64.4°F) or less whereas black and hybrid crappie showed their best growth at temperatures of 18°C (64.4°F) or more. This may be significant, since farmers in our region would have more of an advantage over southern producers with culture animals that grow better at lower temperatures.

Although effective procedures for inducing triploidy in *Lepomis* are available (see the 1994-95 Annual Progress Report), methods developed for crappie have not proved to be as successful. Prior to this study, the best triploid induction rate obtained at SIUC with crappie, using pressure shocks similar to those effective in *Lepomis*, was 66%.

A study conducted at SIUC was designed to develop more effective methods for inducing triploidy in crappie and to test the hypothesis that the temperature at which fertilized eggs are incubated may influence the effectiveness of shocks. The approach used by SIUC researchers was to hold the magnitude (6,000 psi) and duration (3 min) of the pressure shock constant while varying post-fertilization shock initiation time (2 to 7 min, tested at 1 min intervals) and the incubation temperature (17, 20, and 23°C; 62.6, 68.0, and 73.4°F) of the developing embryos prior to and during the shock treatment.

Incubation temperature did not affect triploid induction rate but better triploid induction rates were obtained as post-fertilization shock initiation times were increased. The most effective shocks for producing triploids in *Lepomis* were initiated at 2 to 3 min post-fertilization. Based on frequencies of deformed larvae and triploidy induction rate, the longer post-fertilization times were

more successful with white crappie eggs. The highest triploidy induction rate SIUC researchers obtained (about 95%) occurred at a post-fertilization time of 7 minutes and at an incubation temperature of 20°C (68.0°F). This suggests that longer post-fertilization shock initiation times need to be investigated to optimize triploid induction procedures for white crappie.

### Impacts

- Findings from PSU indicate survivability in cages is a major problem for cage culture of crappie, but this may be a function of cage design. Consideration of capture and transport methods is vital to minimizing initial mortality losses. PSU researchers determined that black crappie were the most suitable species for cage culture.
- PSU researchers have developed capture, transport and handling techniques that can markedly reduce mortality problems associated with crappie in aquaculture settings.
- Hybrid sunfish had their best growth at temperatures of 18°C (64.4°F) or less whereas black and hybrid crappie had their best growth at temperatures of 18°C (64.4°F) or more. This may be significant, since farmers in this region would have more of an advantage over southern producers with culture animals that grow better at lower temperatures.
- Pressure shock procedures for inducing triploidy in white crappie were developed which yielded more than 90% triploids; it appears that pressure shocks for inducing triploidy in the white crappie need to be applied at a much later time after fertilization, as

compared to findings for *Lepomis*.

### Recommended follow-up activities

- Cage design needs to be modified and evaluated for crappie culture.
- Continue to evaluate black crappie in recirculating systems.
- Further study needed on transport and stress in crappie.
- Evaluate importance of acclimation in reducing stress.
- Develop feeding strategies that reduce the impact of feeding hierarchies and fat accumulation on growth.
- Re-evaluate density effects associated with stress conditions.
- Determine optimal temperatures for growth and feeding.
- *Lepomis* taxa have not required extended training periods to habituate them to prepared diets. Black, white, and hybrid crappie have been much more difficult to habituate to prepared diets, and they do not feed as aggressively, especially at lower temperatures. This is largely responsible for the poorer overall performance of crappies, as compared to *Lepomis* taxa, under tank culture conditions. There is a need to explore avenues to enhance the response of crappies to prepared diets.

### Publications, manuscripts, or papers presented

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

## Total support for the first two projects

Years	NCRAC USDA funding	Other support				Total	Total Support
		University	Industry	Other Federal	Other		
1990-92	\$130,758	\$96,710				\$96,710	\$227,468
1992-94	\$149,799	\$343,160	\$500 <sup>a</sup>	\$10,000 <sup>b</sup>	\$4,200 <sup>c</sup>	\$357,860	\$507,659
<b>Total</b>	<b>\$280,557</b>	<b>\$439,870</b>	<b>\$500</b>	<b>\$10,000</b>	<b>\$4,200</b>	<b>\$454,570</b>	<b>\$735,127</b>

<sup>a</sup>KOCH Industries - Koch Flexrings

<sup>b</sup>National Science Foundation - STARS Research

<sup>c</sup>\$3,000 from Kansas Department of Wildlife & Parks - white crappie and hauling tanks and \$1,400 from the City of Pittsburg Water Department - anthracite coal

NCRAC has funded three sunfish projects. This project component termination report covers work undertaken for the first and second projects. Bruce L. Tetzlaff chaired the first project and Robert J. Sheehan chaired the second project.

## Sunfish

*Progress Report for the Period September 1, 1994 to August 31, 1996*

### NCRAC funding level

\$174,999 (September 1, 1994 to August 31, 1996)

### Participants

Fred P. Binkowski, University of Wisconsin-Milwaukee, Wisconsin

Paul B. Brown, Purdue University, Indiana

Donald L. Garling, Michigan State University, Michigan

Robert S. Hayward, University of Missouri-Columbia, Missouri

Terrence B. Kayes, University of Nebraska-Lincoln, Nebraska

Christopher C. Kohler, Southern Illinois University-Carbondale, Illinois

Joseph E. Morris, Iowa State University, Iowa

Douglas B. Noltie, University of Missouri-Columbia, Missouri

#### *Extension liaison*

Joseph E. Morris, Iowa State University, Iowa

#### *Non-funded collaborators*

Denzil Hughes, Farmland Industries, Inc., Kansas

Fountain Bluff Fish Farms, Illinois

Illinois Department of Conservation, Little Grassy Fish Hatchery, Carbondale, Illinois

Jim Frey, Jim Frey Fish Hatchery, West Union, Iowa

Ron Johnson, Spruce Creek Fish Farm, Minnesota

Myron Kloubec, Kloubec Fish Farms, Amana, Iowa

Missouri Department of Conservation, Missouri

Tribal Council, Red Lake Band Chippewa, Wisconsin

National Biological Service, Midwest Science Center (formerly USFWS National Fisheries Contaminant Research Laboratory), Missouri

### Project objectives

1. Produce a production manual, accompanying videos and other information as necessary to demonstrate the technology for culturing centrarchids.
2. Determine the major nutritional requirements for centrarchids and to compare their growth and performance using available commercial feeds in laboratory and field settings.
3. Determine the best feeding management strategies for culturing centrarchids in laboratory and field settings.

### Anticipated benefits

At the 1993 Program Planning Meeting held in Madison, Wisconsin, the North Central Regional Aquaculture Center (NCRAC) Industry Advisory Council specifically requested the development of extension educational materials in the form of a production manual and accompanying videotapes, as a high priority need for demonstrating the commercial feasibility of centrarchid sunfish aquaculture in the region. Such information is needed to enable this industry to enlarge.

Defining the critical nutritional requirements for targeted sunfish will enable development of diets that meet, but not exceed, their requirements. Feed costs are typically the largest annual variable cost; thus, minimizing nutrient concentrations decreases costs without impairing weight gain or health of individuals. Protein requirements of sunfishes are poorly understood, which hinders their economic potential in food fish culture. Accurate estimates of protein requirements for hybrid sunfish that have sex ratios skewed towards males may prove useful in promoting maximal growth rates as well as minimizing feed costs.

Significant progress has been made with regard to sunfish brood stock development (bluegill and black crappie), spawning, acceptance of prepared diets, and good growth response. Most of the research and commercial production of sunfish has focused on utilizing pond systems (extensive aquaculture). However, to a lesser extent this same effort has been directed at intensive aquaculture. With a better understanding of the early life stage feeding strategies the aquaculture industry will be able to broaden the scope of sunfish aquaculture to include rearing these fish under intensive conditions.

### Progress and principal accomplishments

During the 1994-96 period University of Nebraska-Lincoln (UNL) researchers were to produce two 10-20-minute educational video tapes on selected topics covered in the new sunfish production guide. However, due to time constraints at UNL these videotapes are postponed until 1997. Michigan State University (MSU) and ISU personnel have completed drafts of the new sunfish culture guide. The individual chapters will be reviewed during winter 1996; the guide is scheduled for completion by summer 1997.

There have been numerous sunfish hybrids produced by both researchers and private aquaculturists; these hybrids have varying percentages of male offspring and growth rates. The hybrid sunfish used by NCRAC researchers is the F<sub>1</sub> offspring resulting from crossing a female green sunfish (*Lepomis cyanellus*) with a male bluegill (*L. macrochirus*).

At Southern Illinois University-Carbondale (SIUC), researchers used practical diets containing crude protein levels of 32, 36, 40, and 44% and compared their ability to promote growth of hybrid sunfish in two culture systems: recirculating culture system and culture ponds.

#### *Recirculating culture system*

Year 1 adult hybrid sunfishes (source: Fountain Bluff Fish Farms, Illinois; mean initial weight = 37.1 g; 1.3 oz) were stocked at a density of 28 fish per 300 L (79.3 gal) circular tank (three replicates per treatment). Flow rates were 30 L/min (7.9 gal/min) and water temperature was maintained at approximately 24°C (75.2°F). Feeding rates were 2%/day divided into two feedings during the 98-day growth trial. Survival ranged from 93 to 100% and did not differ significantly between treatments (P = 0.05). Weight increase and feed conversion efficiency were highest for the 44% crude protein diet and were significantly greater than the 36 and 32% diets (0.39 versus 0.33 and 0.27, respectively). These data indicate that optimal crude protein levels are likely to be in excess of 40% for hybrid sunfish in recirculating culture systems. The poor feed conversion efficiencies observed may be due to the experimental animals being sexually mature and directing considerable amount of their food intake towards gamete production and reproductive behavior. Proximate analysis of feeds and fish whole bodies is now under way.

#### *Pond culture*

Juvenile hybrid sunfish (mean weight = 12 g; 0.04 oz), were stocked (May 23, 1995) at a rate of 5,504 fish/ha (2,228 fish/acre), into 16 ponds averaging 0.04 ha (0.10 acre) (four treatments/four replicates per treatment). Ponds were supplied with one of four practical diet formulations containing crude protein levels of 32, 36, 40, or 44%. Feeding rate was initially 3% of the estimated biomass once a day except on days of sampling. All ponds exhibited nest building activities by June 6 and recruitment of F<sub>2</sub> hybrids in some ponds

was apparent by July 18. Feeding rates were reduced to 2% (August 15 through September 26, 1995) when a large amount of feed was noticed left from the previous feedings. This reduction in feeding activity coincided with high temperatures of 30°C (86°F). Resulting data was of limited use due to natural recruitment of F<sub>2</sub> offspring.

Year 1 adult hybrid sunfishes (source: Fountain Bluff Fish Farms, Illinois; mean initial weight = 40 g; 1.41 oz) were stocked April 16, 1996, into 16 ponds averaging 0.04 ha (0.10 acre) (four treatments/four replicates per treatment). Stocking density was 13,875 fish/ha (5,615 fish/acre). All ponds were limed and fertilized 2 weeks prior to stocking to promote plankton blooms. Feeding to apparent satiation was carried out two times per day except during times of rain and strong winds. Aeration to ponds with dissolved oxygen levels of less than 2.0 mg/L was applied with a tractor driven paddlewheel. Harvest is to be carried out October 29, 1996, following a complete draw down.

Researchers at MSU have empirically determined the optimal energy level for growth and protein retention in 125 mm (4.9 in) hybrid sunfish utilizing a saturation kinetics model for curve fitting. Results demonstrate the semi-purified diet developed for these trials is well accepted by these fish; this results in a slightly lower but comparable growth to that obtained using a commercial control diet. There were no significant differences in growth or net protein utilization (NPU) between the experimental diets and the control diet; hence the semi-purified diet is suitable for the remaining phases of these trials.

The whole body indispensable amino acid (IAA) profile of 50 and 125 mm (2.0 and 4.9 in) hybrid sunfish, green sunfish, and bluegill has been determined. The data obtained has been used for predicting the IAA requirements for these species using the A:E ratio ( $[\text{individual IAA content}/\text{total IAA content} + \text{Cys} + \text{Tyr}] (1000)$ ) of whole fish tissue. These predicted IAA requirements will be used in the preparation of diets for the remaining phases.

MSU researchers are currently beginning a trial evaluating growth, NPU, protein retention, and energy retention in 125 mm (4.9 in) hybrid sunfish fed graded levels of protein in isocaloric diets using the optimal energy level predicted in the previous trial. Diets have

been formulated to meet IAA requirements for hybrid sunfish determined by researchers at Purdue University (Purdue) with the unknown requirements incorporated at levels predicted by the A/E ratio. This trial will be completed the first of the year; results will be used to predict the optimal P:E ratio for 125 mm (4.9 in) fish.

Research at Purdue was initially focused on quantifying key nutritional requirements of hybrid sunfish. Through three separate studies with the hybrid sunfish, growth was relatively low despite offering a broad variety of diets. Prior to conducting the next series of studies on critical nutritional requirements, an evaluation of pure bluegill was conducted. Growth of pure bluegill was double the growth observed with hybrid bluegill. The studies were conducted in the same experimental systems in the same conditions with the same broad variety of feeds. There was also differential use of commercial diets. Results of those studies clearly indicated that diets formulated for trout and salmon were better than diets formulated for catfish. Further, there were clear distinctions within the trout diets. That is, all trout diets are not the same nor is the response in the hybrid sunfish comparable to the pure bluegill. Both the optimum lipid:carbohydrate ratio and quantitative phosphorus requirements are underway. The optimum lipid study was expanded to include both hybrid sunfish and bluegill. Results will be known by December 1996.

Researchers at the University of Missouri have examined the potential to increase growth rates of hybrid sunfish during growout by using feeding schedules that bring out these fishes' compensatory growth response (increased growth following a period of fasting). Hybrid sunfish were held individually in experimental enclosures submerged in larger water recirculation tanks. Water temperature was maintained at 24°C (75.2°F) as was a 15-h light/9-h dark photoperiod regime. Mealworms (*Tenebrio molitor*) were used as the food in these initial experiments so that daily consumption by individual fish could be accurately determined. Over the 105-day experiment, mean growth rates of hybrid sunfish in the 2 and 14 day no feeding cycle groups were 2.1 and 1.5 times faster than the controls that were fed *ad libitum* every day.

These results represent the first demonstration that fish can be grown significantly larger than daily-fed controls over identical time periods by eliciting the

compensatory growth response. Growth improvements from compensatory growth appeared to result from increases in both consumption rate and growth efficiency. While best results were observed for the shortest off/on feeding cycle, there was some suggestion from growth responses that longer off/on cycles (>14 days) may be of value.

The primary goal of the University of Wisconsin-Milwaukee (UW-Milwaukee) researchers was to utilize the early life stage feeding technology developed for yellow perch and apply this approach to centrarchids, specifically, black crappie. The researchers selected two early life stages as their starting points for the development of intensive aquaculture strategies. Young-of-the-year (YOY) Wisconsin pond-raised black crappie ( $N = 1,200$ ) were obtained in fall 1994. Under laboratory conditions these fish accepted adult frozen brine shrimp as a transitional food within 3 days and were habituated to commercial starter feed within 14 days. Survival to present was greater than 65%. In addition, UW-Milwaukee researchers obtained several hundred YOY black crappie from a commercial producer in Iowa. Initially these fish were fed "green tank" water organisms, which included copepods, ostracods, and smaller cladocerans. These organisms are all much larger than those fed to yellow perch at first feeding. Later on, brine shrimp nauplii (BSN) (*Artemia franciscana*) and a beef liver mixture was added to the feeding schedule. This group of black crappies habituated to a formulated starter diet within 26 days. This group of fish ( $N = 73$ ) was terminated on September 25, 1995; mean length and weight was 66.8 mm (2.63 in) and 3.92 g (0.14 oz), respectively.

Since the last report, UW-Milwaukee researchers have continued to expand their efforts to habituate YOY black crappie to formulated diets. Past efforts to spawn adults in the laboratory or to collect wild adults have not been successful. They have continued to maintain the group of YOY black crappies acquired in October 1994 for use as a captive brood stock. These fish were habituated to commercial formulated diet within 14 days of arrival and have been maintained on a rearing regime that is intended to promote gonadal development. It is anticipated that these fish will be fully mature and available for spawning in the spring of 1997.

As a back-up to their efforts to produce YOY from laboratory and wild spawns, UW-Milwaukee researchers obtained 2,741 pond-spawned YOY black crappies (mean length = 26 mm; 1.0 in; mean weight = 0.1-0.5 g; 0.004-0.018 oz) from the Gavin's Point National Fish Hatchery in Yankton, South Dakota. The fish were stocked into a circular flow-through rearing tank and the photoperiod was set at 13-h light. When offered BSN on the day of arrival approximately half the fish accepted the food. Trial feedings with formulated diets on the day of arrival were unsuccessful. These fish took longer to habituate to formulated diet than either the slightly larger YOY brought to the lab in October 1994, those habituated to a formulated diet within 14 days, or the larval crappies tested in July 1995 that habituated to formulated starter diet within 26 days. These results suggest that there is a strong preference for BSN, and that habituation is not readily achieved by merely offering the formulated diet along with the transitional live food. This group of YOY crappie was very reluctant to feed in the presence of observers. Although there was limited interest in formulated foods as early as 6 days after the beginning of the trial, the general population consumed mainly the BSN. Full habituation to formulated diet appeared to closely follow the forced restriction of the live food. Survival during the trial was excellent, 99% over a rearing period of 103 days. UW-Milwaukee researchers intend to continue rearing this group of fish to demonstrate the growth that can be achieved under intensive flow-through culture with formulated diets. Growth information has been obtained at 0 days (26 mm; 1.0 in), 12 days (34 mm; 1.3 in), 57 days (55 mm; 2.2 in), and 105 days (75 mm; 3.0 in) since the start of the trial.

One objective of the ISU researchers was to spawn sunfish out-of-season through temperature and photoperiod manipulation under laboratory settings (bluegill and hybrid sunfish). ISU researchers stocked adult fish at a ratio of two males to four females (170 g; 6.0 oz) per 640-L (169-gal) tanks in a recirculation system. After an acclimation period, temperature and photoperiod were maintained at 24°C (75.2°F) and 14-h light/10-h dark. They were able to spawn bluegills during a 6-month period (December 1994 - May 1995); 40 spawns averaging 20,000 larvae each were obtained from 24 females. Hybrid sunfish were successfully produced the following fall.

The second objective of the ISU study was to develop a procedure for tank-rearing larval bluegill and larval hybrid sunfish. In the first set of experiments, seven commercial diets were used for feeding larval bluegill from the onset of exogenous feeding to 28 days posthatch. Although all diets were consumed by the larvae, none were digested and survival was essentially zero. In the next set of experiments, bluegill larvae were able to digest commercial diets by feeding them BSN for an initial 7-day period and then switching to commercial feed over a 3-day period. Using this protocol, three feeds (Fry Feed Kyowa® B-250, Hatchery Encapsulon® Grade II, and Larval AP-100®) were compared over a 28-day interval. There were no significant ( $P < 0.05$ ) differences in growth (length and weight) among the three diets at the end of 28 days, but survival was significantly higher for fish fed Fry Feed Kyowa® B-250. In another experiment, Fry Feed Kyowa® B-250 was fed to larval bluegill after feeding them BSN for 3, 7, or 14 days with an additional 3-day weaning period with mixed feeding. Larvae fed BSN for 14 days had significantly higher growth and survival than did larvae in the 3-day and 7-day treatment groups. In a final experiment, Fry Feed Kyowa® B-250 was fed to larval hybrid sunfish after feeding them brine shrimp for 0, 3, or 7 days with an additional 3-day weaning period of mixed feeding. The larvae fed brine shrimp for only 0 or 3 days initially grew slower than did the larvae in the 7-day treatment; however, by the end of the experiment (28 days posthatch), there were no significant differences among lengths or weights in the three treatments. At 28 days posthatch, larvae fed brine shrimp for 7 days had a significantly higher survival rate than larvae in either the 0- or 3-day treatments. Results indicate that the protocol for tank-rearing larval bluegill and larval hybrid sunfish should include using brine shrimp prior to using a commercial diet. It appeared that larval hybrid sunfish could digest the commercial diet at the onset of exogenous feeding. However, without BSN much lower survival rates resulted. Survival rates of about 25 and 37% can be expected for bluegill and hybrids, respectively, by following this protocol.

### Work planned

UNL will produce videos in 1997 related to the upcoming sunfish culture guide. This guide will be completed during 1997.

Critical nutritional requirements for targeted species reduces feed costs and overall cost of production of fishes will continue to be defined by Purdue and MSU researchers. SIUC researchers will compile data from their recirculation and pond studies. These data will be important pieces of information for manufacturers of feed.

UW-Milwaukee researchers will attempt the laboratory spawning of their captive black crappie brood stock by manipulating temperature and photoperiod. If necessary they will use spawning induction substances in spring 1997. If successful, the YOY black crappie produced from this brood stock will be used in the new NCRAC sunfish project. Researchers at ISU will continue to do research into sunfish culture by growing hybrid sunfish up to food-size and to evaluate a sunfish hybrid produced by crossing a female redear sunfish (*L. microlophus*) with a male bluegill.

### Impacts

- Coupled with the NCRAC-sponsored development of improved intensive larval sunfish culture techniques at ISU under the direction of Morris, commercial fish farmers have the tools to establish stocks of polyploid sunfishes.
- NCRAC funding permitted SIUC to leverage funding from the American Fishing Tackle Manufacturing Association to evaluate benefits of triploid sunfish in recreational fishing ponds. The supply of triploids to recreational fisheries could provide a new market for regional producers.
- Developing diets specifically for targeted species results in maximum performance at the lowest possible cost. Purdue research directed at minimizing costs of feeds will help to maximize profit to the producer.
- It now appears that the intensive culture technology developed for yellow perch can be applied to black crappie. Also, YOY (30-60 day-old) pond-produced black crappie can habituate to prepared diets within 26 days; YOY (100 day-old) pond-produced black crappie can habituate to prepared diets within 14 days. The potential for the intensive culture of black crappie looks very promising.
- It is now possible to produce bluegills and hybrid sunfish in the laboratory out-of-season by manipulation of temperature and photoperiod

without the use of hormones. This protocol allows for the production of these fish, regardless of season, for both laboratory studies and aquaculture stocking.

- The potential for the intensive culture of black crappie will provide an alternative to seasonal pond rearing and could expand the growth and production to an annual basis in conjunction with recirculating

aquaculture system technology.

## **Publications, manuscripts, or papers presented**

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

## **Support**

Years	NCRAC USDA funding	Other support				Total Support
		University	Industry	Other Federal	Other	
1994-96	\$174,999	\$177,300	\$12,012 <sup>a</sup>		\$189,312	\$364,311
<b>Total</b>	<b>\$174,999</b>	<b>\$177,300</b>	<b>\$12,012</b>		<b>\$189,312</b>	<b>\$364,311</b>

<sup>a</sup>Farmland Industries, Inc.

NCRAC has funded three sunfish projects. This progress report is for the third project which is chaired by Donald L. Garling. The project continues and builds upon the first two projects. The 2-year third project began September 1, 1994.

## **Salmonids**

*Project Component Termination Report for the Period June 1, 1990 to August 31, 1996*

### **NCRAC funding level**

\$79,799 (June 1, 1990 to February 28, 1994)

### **Participants**

Anne R. Kapuscinski, University of Minnesota,  
Minnesota

James E. Seeb, Southern Illinois University-  
Carbondale, Illinois

Robert J. Sheehan, Southern Illinois University-  
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#### *Extension liaison*

Ronald E. Kinnunen, Michigan State University,  
Michigan

#### *Non-funded collaborator*

Hugo Kettula, Seven Pines Trout Hatchery, Lewis,  
Wisconsin

### **Reason for termination**

The objective for this work on Salmonids was completed.

### **Project objective**

Evaluate all-female diploids and all-female triploids, and use brood stock developed in the region to produce all-female diploid and all-female triploid trout populations.

### **Principal accomplishments**

Efforts culminated in a 265-day grow-out trial at Southern Illinois University-Carbondale (SIUC) in which the performance of all-female triploid, all-female diploid, and mixed-sex diploid rainbow trout were compared. The results of the growout trial vindicated the NCRAC interest in all-female and all-female triploid rainbow trout.



The growout trial was initiated with approximately 100 g (3.53 oz) fish. Progeny from three families of all-female triploid and progeny from three corresponding full-sib families of all-female diploid trout were used in the trial. The mixed-sex diploid trout were progeny of three families that were half-sibs of the corresponding all-female diploid and all-female triploid families. Trout used in the growout trial were from crosses made at the University of Minnesota (UM), where they were also reared to 10 to 20 g (0.35 to 0.71 oz) prior to shipping to SIUC for the growout trial.

A water re-use system and twelve raceways were used in the growout trial, four raceways per treatment. Each raceway was stocked with 25 trout, but stocking densities were reduced to 15 trout per raceway on day 180 of the trial. Mean initial weights were 93.5, 84.2, and 111.6 g (3.30, 2.97, and 3.94 oz) for the mixed-sex diploid, all-female diploid, and all-female triploid, respectively. Mean initial lengths and weights did not differ among the three groups.

Growth was linear during the growout trial. Absolute growth rate was highest for the all-female triploid, intermediate for the all-female diploid, and lowest for the mixed-sex diploid, 2.38, 1.78, and 1.58 g/day (0.08, 0.06, and 0.05 oz/day), respectively ( $P < 0.025$ ). Mean final weights were 520.5 g (1.15 lb) for the mixed-sex diploids, 567.5 g (1.25 lb) for the all-female diploids, and 748.9 g (1.65 lb) for the all-female triploids.

No significant differences ( $P < 0.05$ ) were found in survival, food conversion ratios, condition factor, liver somatic index, visceral fat weight, or dress-out percentage yield among treatments. By day 180 of the growth trial, most of the males in the mixed-sex diploid group were sexually mature, while the mixed-sex diploid females and the all-female diploids were still maturing. Based on subsamples of trout sacrificed at that time; mean gonadosomatic index for mixed-sex diploid males was 3.13, while values for the mixed-sex diploid females, all-female diploid and all-female triploid trout were 1.13, 1.86, and 0.38, respectively.

All-female diploid and all-female triploid trout show promise for practical trout farming. All-female trout production eliminates the problem of early maturation in males which leads to poor flesh quality and undesirable appearance, and results indicate that all-

female diploid trout grow better than mixed-sex diploid trout. All-female triploid trout, however, grew the fastest. Farmers should consider all-female triploid trout production, especially those targeting markets utilizing larger trout.

The all-female triploid trout grew faster than the all-female diploids and mixed-sex diploids in growth trials, but aquaculturists also need to know how triploids perform in other respects to make decisions regarding their production and use. Many culturists produce food fish, but fingerling production for recreational fish stocking programs provides another market outlet for cultured fish. Harvest, crowding, handling, and hauling are problems inherent to fish-farming as well as to fish stocking programs.

Survival of triploids was evaluated during simulated transportation in one experiment with 33 g (1.16 oz) chinook salmon, another with 14 g (0.49 oz) coho salmon, and a third with 1.5 g (0.05 oz) rainbow trout. Triploids were produced via heat shocks. Both diploids and triploids were stocked into replicate containers in each experiment at densities recommended for transporting salmonids. Mortality was recorded every 30 min. Diploids had been exposed to heat shocks in the chinook salmon experiment, but not in the other two experiments.

Triploid chinook salmon died faster than diploids ( $P < 0.005$ ). The maximum difference between mortality distributions ( $D_{max}$ ) was 21.7%. Coho salmon triploids also died faster than diploids ( $P < 0.005$ ).  $D_{max}$  occurred at 660 to 690 min, when 74% of the triploids were dead but only 47% of the diploids were dead.  $D_{max}$  in the rainbow trout experiment was only 6.7% ( $P < 0.05$ ), indicating no difference. These results indicate that triploid rainbow trout can tolerate extreme environmental conditions about as well as diploids.

The reduced survival found for triploid chinook and coho salmon indicates that survival may be lower for triploids of these two species under some aquacultural conditions, and survival may also be reduced after stocking. Diminished survival, however, does not necessarily preclude the use of triploids in situations where natural stock protection is an important consideration in stocking programs or in site-selection for an aquaculture installation.

Early growth and survival was also examined for mixed-sex diploid, all-female diploid, and all-female triploid rainbow trout at UM and SIUC during earlier periods of this research. Mixed-sex diploids and all-female diploids early growth and survival was also examined by Seeb at the Fort Richardson State Fish Hatchery, Anchorage, Alaska, under practical fish culture conditions.

Survival through the eyed stage was relatively high (83 to 97%) for mixed-sex diploid, all-female diploid, and all-female triploid eggs in the UM study. Survival through the eyed stage was significantly lower for all-female triploids, in comparison to mixed-sex diploids, but only by approximately 13%. All-female triploids survived as well or better than mixed-sex diploids and all-female diploids after hatching, and growth through 14 weeks did not differ between mixed-sex diploid, all-female diploid, and all-female triploid trout ( $P < 0.05$ ).

Cumulative mortality from fertilization through hatching, yolk-sac absorption, and up to 0.5 g (0.02 oz) was about 30% higher ( $P < 0.05$ ) for all-female triploids, as compared to mixed-sex diploid and all-female diploid trout in the SIUC trial. Differences in mortality appeared to primarily occur prior to hatching and, secondarily, during yolk-sac absorption. These results, in conjunction with those obtained at UM, suggest that the triploidy induction procedure, the heat shock, was the primary factor responsible for the increased mortality. Mortality did not differ ( $P < 0.05$ ) after hatching through growth to 0.5 g (0.02 oz) at SIUC.

A 240-day growth trial initiated with the mixed-sex diploid, all-female diploid, and all-female triploid trout once they reached 0.5 g (0.02 oz) was then conducted at SIUC. There were no mortalities in mixed-sex diploid, all-female diploid, and all-female triploid trout during the 240-day growth trial. This pattern of similar survival after yolk-sac absorption between diploid and triploid rainbow trout was confirmed in the UM study, in the simulated transportation experiment (above) and in the growout trial. The trout grew from 0.5 g (0.02 oz) to approximately 2.7 to 3.0 g (0.10 to 0.11 oz) during the trial. Growth did not differ between mixed-sex diploid, all-female diploid, and all-female triploid trout ( $P < 0.05$ ). Feed conversion efficiencies (wet weight of fish/dry weight of feed) did not differ during the 240-day growth trial; they ranged from 93% for the

mixed-sex diploids to 99% for the all-female diploids. Feed conversion efficiency was 96% for the all-female triploid trout.

Findings in the UM and SIUC studies show that survival in triploid trout is somewhat diminished prior to the onset of exogenous feeding. However, the economic loss associated with this additional mortality in all-female triploid trout prior to exogenous feeding is minor, since numbers of eggs generally are not limiting in rainbow trout culture, and relatively little investment in rearing costs occurs prior to feeding. The additional production costs associated with this early mortality is more than offset by the better growth during growout. All-female triploid trout do not undergo sexual maturation, so the retention of good flesh quality and appearance is reason enough for producing them.

The Fort Richardson State Fish Hatchery study confirmed no differences in survival between mixed-sex diploid and all-female diploid trout following yolk-sac absorption and through 349 days of age; survival for mixed-sex diploid and all-female diploid trout exceeded 90% during the trial. The growth trial was divided into three phases (83, 148, and 349 days) because numbers of trout per replicate were reduced twice as they grew. Mean weights for the mixed-sex diploid and all-female diploid trout, respectively, were 4.1 g (0.14 oz) and 3.7 g (0.13 oz) at 83 days; 32.4 g (1.14 oz) and 27.8 g (0.98 oz) at 148 days; and 81.0 g (2.86 oz) and 67.8 g (2.39 oz) at 349 days of age. Growth did not statistically differ between mixed-sex diploid and all-female diploid trout. Food conversion efficiency also did not differ between mixed-sex diploid and all-female diploid trout; it ranged from 65.3 to 73.7% during the first 2 phases.

The results of the Fort Richardson State Fish Hatchery study were consistent with findings in the UM and SIUC pre-maturation trials with rainbow trout. Prior to the onset of sexual maturation, mixed-sex diploid and all-female diploid trout differ little in survival and growth.

To determine why all-female diploid and triploid rainbow trout grow faster than mixed-sex trout during growout, two lines of investigation were pursued at SIUC. One investigation examined daily activity patterns and activity intensity in mixed-sex diploid and all-female diploid trout, and the other studied muscle

cell growth dynamics in diploid and triploid trout.

Although adult all-female diploid trout showed activity levels higher than mixed-sex diploids at lower water temperatures, the reverse was true at the higher temperatures (above 12.5°C; 54.5°F) at which this species is typically cultured. This means that all-female diploid trout have more dietary energy available for growth at culture temperatures, because they waste less energy on non-essential swimming. Another, and perhaps the most important, reason why all-female diploids outgrow mixed-sex diploids is that rainbow trout males mature and slow their growth earlier than females, due to the investment of energy into gonadal tissues and development of secondary sexual characteristics.

Muscle fiber growth dynamics in triploids is of interest, because whole-body growth occurs via two processes: (1) increased size of muscle fibers or hypertrophy and (2) increased numbers of fibers or hyperplasia. Fish show what has been referred to as indeterminate growth; i.e., they are capable of hyperplastic and hypertrophic growth even after adulthood, whereas postnatal growth occurs only by hypertrophy in other vertebrates. In fish, however, hyperplastic growth eventually ceases, but the longer a species is capable of hyperplastic growth, the larger its ultimate size and the faster its growth.

Muscle fiber growth dynamics were examined in triploid rainbow trout using both biochemical (RNA, DNA, and protein measurements) and histological (muscle fiber diameter sizes) approaches. It is believed that this is the first time that muscle cell growth dynamics has been investigated in any triploid animal.

Triploid trout less than 30 cm (11.8 in) in total length showed muscle fiber size distributions which differed from diploids. Specifically, triploid hyperplastic muscle fibers were larger than those of diploids. However, the difference in fiber size distributions diminished as the trout grew, and it disappeared in larger trout where hyperplasia plays only a small role in growth. This increase in hyperplastic muscle fiber size results in a decrease in the cellular surface area to volume ratio which may be unfavorable to metabolic exchanges between the cell and its external milieu. Poorer survival in triploids during early life may be linked to the increase in hyperplastic muscle fiber size.

Another potential disadvantage for triploids is that their muscle cells (which are multinucleate) appear to have fewer nuclei per muscle cell. This study also showed that larger diploid and triploid rainbow trout have similar growth capacities; i.e., they are capable of growing to the same maximal size and at the same rate, all else being equal. This suggests that the superior growth in all-female triploid trout is not due to any inherent differences in growth capacity. Rather, it is probably because triploid female rainbow trout do not direct dietary energy into gonadal growth and the development of secondary sexual characteristics. SIUC researchers also found that RNA concentrations did not differ between diploids and triploids growing at the same rate and that protein concentrations did not differ in diploid and triploid muscle tissues. This indicates that the rate of protein synthesis does not differ between diploids and triploids, despite the latter having fewer nuclei per cell. This further suggests that genes of the third set of chromosomes in triploids are expressed to compensate for the reduced number of nuclei in triploid muscle cells. Meiotic gynogenesis, followed by sex reversal, is an important initial step in the production of brood stock for all-female rainbow trout production, because it ensures all XX progeny. However, meiotic gynogens exhibit poor viability and growth, because they are highly inbred; a level of inbreeding roughly equivalent to several generations of full-sib matings. Gynogenesis followed by sex-reversal does ensure the production of 100% all-female progeny, but it is inefficient to use gynogenesis for the continuing production of brood stock. A far better approach is to sex-reverse all-female progeny produced from an outcross between an XX sex-reversed male gynogen and a normal XX female, because the outcross eliminates inbreeding depression. The progeny can thus be much more successfully and efficiently raised to sexual maturation.

SIUC researchers shipped about 500, 5 cm (2.0 in) sex-reversed XX males to the Seven Pines Trout Hatchery. Since these were the progeny from an outcross between XX Isle of Mann males and XX Seven Pines females, their viability should be excellent. However, only about 20 pairs of trout were used to produce the 500 progeny, substantially lower than the number of brood stock required to ensure sufficient genetic diversity for aquacultural purposes. Genetic diversity needs to be increased in the XX male brood stock at some future time before it can truly be said that a regional brood

stock for all-female production has been established.

## Impacts

Studies of all-female diploid rainbow trout demonstrate that:

- all-female diploid trout grow and survive as well as mixed-sex diploid trout during early life;
- declines in flesh quality and appearance due to sexual maturation occur earlier in mixed-sex diploid than all-female diploid trout;
- all-female diploid trout grow faster than mixed-sex diploids through grow out, and survival is similar;
- all-female diploid trout show reduced non-essential activity at culture temperatures above 12.5°C (54.5°F), possibly accounting in part for their better growth as compared to mixed-sex diploid trout.

Studies of all-female triploid rainbow trout showed that:

- all-female triploid trout show somewhat reduced survival through yolk-sac absorption; production of all-female triploids via crosses of tetraploids with diploids may reduce or eliminate this problem;
- survival beyond yolk-sac absorption in all-female triploid trout is similar to mixed-sex diploid trout under normal conditions, and it was also similar under adverse conditions in simulated transportation tests;
- all-female triploid trout showed the anticipated reduced gonadal growth;
- all-female triploid trout were clearly superior to mixed-sex diploids and all-female diploids during grow out through market size;
- studies of muscle cell growth dynamics indicate that there is no inherent difference in the capacity for growth between diploid and triploid rainbow trout; the superior growth in all-female triploid trout appears to be primarily due to their failure to undergo sexual maturation;
- all-female triploid trout production may be the best choice for regional farmers, given their superior growth over mixed-sex diploid and all-female diploid trout;
- all-female triploid trout production appears to be an especially strong option for farmers interested in producing a larger trout, since they grew faster than mixed-sex diploid and all-female diploid trout in

these studies, and all-female triploids should not show declines in flesh quality and appearance which accompany sexual maturation in mixed-sex diploid and all-female diploid trout.

## Recommended follow-up activities

All-female diploid and triploid rainbow trout show considerable promise for commercial aquaculture, especially in regions where breeding programs have not selected for stocks which mature at a larger size. Female rainbow trout mature at a larger size than males, so all-female diploid production reduces problems such as the declines in flesh quality and appearance prior to market size. All-female diploid trout also grew faster than mixed-sex diploid trout to market size in these studies. Producers interested in producing larger trout should give strong consideration to all-female triploid production, since the problems associated with sexual maturation appear to be forestalled indefinitely, and all-female triploid trout grew the best through growout in these studies.

Cost-effective all-female triploid and all-female diploid production in the NCR will necessitate farmers to develop brood stocks for producing all-female diploid and all-female triploid fry. This will require production of sex-reversed gynogens for all-female production and tetraploid production for crosses with diploids to produce triploids. Field trials which compare all-female triploid, all-female diploid, and mixed-sex diploid trout would enable farmers to determine the best choice for production stocks in commercial aquaculture settings. The following activities are suggested for follow-up:

- further production of sex-reversed gynogen brood stocks,
- production and evaluation of tetraploid brood stocks, and
- production trials for mixed-sex diploid, all-female diploid, and all-female triploid trout in commercial aquaculture settings.

## Publications, manuscripts, or papers presented

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

## Support

Years	NCRAC USDA funding	Other support				Total Support	
		University	Industry	Other Federal	Other		Total
1990-91	\$39,299	\$22,669		\$3,000 <sup>a</sup>		\$25,669	\$64,968
1991-92	\$20,500	\$13,265		\$5,000 <sup>b</sup>		\$18,265	\$38,765
1992-93	\$20,000	\$14,960				\$14,960	\$34,960
<b>Total</b>	<b>\$79,799</b>	<b>\$50,894</b>		<b>\$8,000</b>		<b>\$58,894</b>	<b>\$138,693</b>

<sup>a</sup>Seven Pines Trout Hatchery for time, use of rearing facilities, feed, and fish

<sup>b</sup>Alaska Fish and Game for time, use of rearing facilities, feed, and fish.

NCRAC has funded three salmonid projects. This project component termination report covers work undertaken for the first and second projects. Both projects were chaired by Paul B. Brown.

## Salmonids

*Progress Report for the Period September 1, 1994 to August 31, 1996*

### NCRAC funding level

\$200,000 (September 1, 1994 to August 31, 1996)

### Participants

Terence B. Barry, University of Wisconsin-Madison,  
Wisconsin

Paul B. Brown, Purdue University, Indiana

Konrad Dabrowski, Ohio State University, Ohio

Donald L. Garling, Michigan State University,  
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Terrence B. Kayes, University of Nebraska-Lincoln,  
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Jeffrey A. Malison, University of Wisconsin-Madison,  
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#### *Extension liaison*

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Y. Victor Wu, National Center for Agricultural  
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Michael Wyatt, Sandhills Aquafarm, Keystone,  
Nebraska

### Project objectives

1. Develop practical rainbow trout diets using regionally available feed ingredients, including fish meal analogs.
- a. Evaluate the effects of feed binders in diets formulated from locally available plant ingredients on trout performance and on the stability of trout feces to enhance the removal of solids from hatchery effluents.
- b. Evaluate the effectiveness of phytase treatment of plant feed ingredients on phosphorus and protein availability to trout.
- c. Develop and evaluate fish meal-free diets using regionally available feed ingredients.

2. Use the stress response as a selection tool for developing strains of trout having improved performance under conditions found in the North Central Region (NCR).
3. Use stress and performance responses in trout to evaluate culture system design and operation under practical conditions.

### Anticipated benefits

The development of less-polluting, lower-cost diets from regionally available ingredients will benefit existing aquaculturists facing stricter regulatory pressures to reduce waste nutrients in effluents, as well as new aquaculturists facing increasingly complex permitting processes. Using regionally available plant protein and animal by-product protein sources as substitutes for fish meal in trout diets should reduce the cost of feed manufacture (by reducing both ingredient and transportation costs) and help produce diets that are less polluting. The development of regional trout strains selected for superior growth and stress resistance when reared under the distinctive aquaculture conditions found in the NCR (i.e., relatively small-sized farms, low water flows, and variable water temperatures) will improve the overall production efficiency in both private and public sector facilities. In addition, sources of quality trout eggs from within the region will reduce the region's reliance on imported eggs, and help alleviate concerns about disease transmission. An increased understanding of how rearing density, loading, and water turnover rates influence fish growth, feed conversion, and disease resistance will improve overall production efficiency and help reduce effluent wastes. The improved feeds, fish strains, and rearing methods identified in this study will benefit private fish farmers, public sector hatchery managers, feed manufacturers, aquaculture facility designers, and other user groups.

### Progress and principal accomplishments

#### Objective 1

Investigators at Michigan State University (MSU) conducted research to determine if mineral and protein availability could be improved in plant-based rainbow trout diets by pre-treating dietary soybean meal and/or corn gluten meal with the enzyme phytase. Phytase hydrolyzes phytate, a molecule which binds minerals (such as Zn and P) and proteins in the intestine. Soy-corn gluten meal-based diets were or were not pre-

treated with phytase, and were or were not supplemented with 50 ppm zinc. The activities of the digestive proteases carboxypeptidase A (CPA) and B (CPB) were measured in pyloric fecal extracts because the enzymes contain a zinc atom at the active site. Intestinal alkaline phosphatase (ALP) activity was measured because ALP is also a zinc-dependent digestive enzyme and is associated with phosphorus digestion in vertebrates. Plasma was also collected for insulin assay because insulin is stored on a zinc-based crystal in the pancreatic cell. Insulin is important for protein utilization in fish. The feeding phase of the experiment was completed in July 1996. Tissue extracts were tested for CPA and CPB and ALP activities. Whole body, gill filament, and bone samples are currently being prepared for mineral analysis. Plasma samples have been sent to a collaborator, Dr. I. Navarro at the University of Barcelona, for insulin assay. The insulin results should be available by January 1997. Standard assays for CPA and CPB used with other fish species were modified for rainbow trout—the first CPA and CPB assay methods for this species. Preliminary results indicate that diets had no effect on fish moisture content, condition factor (k), or length-weight relationship. The evaluation of growth data was complicated by mortalities during the study; analysis of growth data is not yet available. Mortalities were not diet-related.

Researchers at Ohio State University (OSU) compared the growth rates of rainbow trout fed five different diets in which fish meal protein was replaced by an animal by-product mixture (i.e., replacement of 0, 25, 50, 75, and 100%). No differences were found among the five treatment groups in fish growth, dressing percentage, fillet quality, or gamete quality. Contrary to expectations, however, mineral analysis of fecal samples indicated that diets containing animal by-products were not less-polluting in terms of phosphorus levels.

Purdue University (Purdue) researchers found in the first year of this project that a fish meal-free diet containing soybean meal, corn gluten meal, and corn grain as the predominate ingredients could promote weight gains in rainbow trout within 90% of fish fed a control diet. In the second year, improvements were made in this diet. Lysine was identified as the first-limiting essential amino acid, meat meal was successfully incorporated into the diet, and a

combination of canola and fish oils were found to be better than either lipid source alone. A commercial astaxanthin product successfully masked the yellow pigmentation in the muscle of trout. Fish fed any of the fish meal-free diets were preferred by a trained taste panel over fillets from fish fed a commercial diet.

### Objective 2

University of Wisconsin-Madison (UW-Madison) investigators identified a physiological measure of stress that was well correlated with growth in rainbow trout—serum cortisol levels 3-hours following an acute handling stressor. Individual fish that consistently showed low 3-hour post-stress cortisol levels (i.e., fish that recovered rapidly from stress, defined as “low” fish) had a mean specific growth rate (SGR) of 0.54, as compared to a mean SGR of 0.41 in unselected fish. Fish with consistently high cortisol levels at 3-hours post-stress and a low SGR (“high” fish) were also identified. Selected fish were to be spawned in the autumn of 1995 and the offspring of selected and non-selected fish compared in terms of growth rates, stress responsiveness, and other indicators of performance. However, not enough selected individuals were available to complete the experiment, primarily because of a problem with tag retention. In December 1995, therefore, 160, 2-year-old fish were obtained from Seven Pines Trout Hatchery to begin a new round of selection. All fish were bled three times over a 6 month period to identify both “low” and “high” individuals. This selection process was much more efficient than that used previously, since only one physiological endpoint (3-hour cortisol level) was measured (compared to the nine endpoints evaluated in the earlier selection process). By September 1996, five female and five male “low” fish, and nine female and five male “high” fish had been identified. Spawning started in early October 1996. Sperm from two selected “low” males chosen at random was used to fertilize eggs from each selected “low” female, and likewise for the “high” fish. Eggs and milt from brood stock chosen randomly from the original Seven Pines population were fertilized in an identical manner to serve as non-selected controls. Five groups of larval fish from each population (“low,” “high,” and control) will be reared for subsequent performance evaluations.

### Objective 3

In 1996, a 10-week production-scale field trial was performed at the Calamus State Fish Hatchery by

University of Nebraska-Lincoln (UNL) researchers with help from personnel of the Nebraska Game and Parks Commission comparing the growth, performance, mortality rates, health, and stress responses of rainbow trout in raceways versus oxygen-supplemented cylindrical tanks. Six of the latter were each equipped with a sealed packed column supplied with oxygen, and assigned fingerling trout at a Piper rearing density of 0.45 or 0.9 (three tanks per treatment). Six raceways equipped with conventional packed columns were also each assigned fish at a Piper density index of 0.45 or 0.9 (three raceways per treatment). Turnover rates were kept constant between all four treatment groups. Parameters measured during the course of the study were dissolved oxygen, carbon dioxide, ammonia-nitrogen, pH, total dissolved gas pressure, P, and temperature. At the conclusion of the study, a stress challenge test and Goede health assessment were performed. Blood samples were collected and prepared for analyses of serum cortisol, glucose, and chloride levels.

### Work planned

MSU investigators anticipate completing all of their experiments by the end of February 1997. In 1997, UW-Madison investigators will be comparing the growth and performance of selected and control fish reared under identical conditions. The selected brood stock will also be kept and respawned in the fall of 1997 to evaluate the effects of the selection process on gamete quality as well as on subsequent offspring performance. UNL investigators will statistically evaluate the growth, production, Goede health-assessment, and water chemistry data collected during the 1996 field trial. The blood samples collected at the end of this field trial will be analyzed for serum cortisol, glucose, and chloride levels by UW-Madison investigators. The findings of all the Nebraska studies will then be compiled and submitted for publication in a peer-reviewed journal, and as part of a NCRAC project termination report.

### Impacts

- Trout diets devoid of fish meal can be used to produce marketable size rainbow trout, with no impact on fish quality. Basal diets can be formulated that use regionally-available feed ingredients. Growout fish fed that diet exhibited feed conversion ratios of 1.0-1.1 (fed to satiation). Ingredient costs of the diet were 15% less than a standard commercial trout diet

containing fish meal; price comparisons were based on 5-year average commodity prices. Thus, as fish meal prices rise, alternative diets have been identified that result in similar weight gain of trout.

- The availability of rainbow trout strains with improved growth rate, feed conversion, and disease resistance will greatly improve the production efficiency of private and public fish hatcheries throughout the NCR. The availability of quality trout eggs from within the region will help reduce the need that regional trout farmers currently have for importing eggs from the West Coast. The stress hyperresponsive, slow-growing fish identified in this study have characteristics typical of “wild” trout, and thus may have advantages for stocking recreational fisheries.

- The field trials conducted by UNL investigators, both in the present and past salmonid projects, have verified that rainbow trout can be readily produced under both laboratory and practical rearing conditions at much higher rearing densities than is traditionally recommended. The Nebraska studies have also demonstrated that by using pure oxygen supplementation, trout can be produced in cylindrical tanks at as high a rearing density as in raceways, but at a significantly lower water turnover rate than is normally used in the latter. These findings are particularly important to trout farmers in the NCR who are often constrained by limitations in water and rearing space.

### **Publications, manuscripts, or papers presented**

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

## Support

Years	NCRAC USDA funding	Other support				Total Support	
		University	Industry	Other Federal	Other		Total
1994-95	\$102,042	\$103,987		\$8,723 <sup>a</sup>	\$15,000 <sup>b</sup>	\$127,710	\$229,752
1995-96	\$97,958	\$104,096		\$9,867 <sup>a</sup>		\$113,963	\$211,921
<b>Total</b>	<b>\$200,000</b>	<b>\$208,083</b>		<b>\$18,590</b>	<b>\$15,000</b>	<b>\$241,673</b>	<b>\$441,673</b>

<sup>a</sup>University of Wisconsin Sea Grant

<sup>b</sup>International Collaborative Program for OSU to work jointly with the National Fisheries University of Pusan, Korea

NCRAC has funded three salmonid projects. This progress report is for third project which is a 2-year study that began on September 1, 1994. Ronald R. Rosati originally chaired the project until his departure from Illinois State University; after which Terence B. Barry became chair. The third project continues and builds upon the first two projects.



## North Central Region Aquaculture Conference

*Project Termination Report for the Period June 1, 1990 to March 31, 1992*

### NCRAC funding level

\$7,000 (June 1, 1990 to March 31, 1992)

### Participant

Donald L. Garling, Michigan State University,  
Michigan

### Reason for termination

The objective for this project was completed and proceedings of the Conference published.

### Project objective

To provide a forum for exchange of information and technology transfer between aquaculturists in the private and public sectors in the North Central Region (NCR) as well as surrounding states and provinces of Canada.

### Principal accomplishments

Funds provided for this project were used for the initial costs of planning and advertising the first NCR Aquaculture Conference that was held March 18-21, 1991, in Kalamazoo, Michigan. Approximately 240 people participated in the first NCR Aquaculture Conference which was co-hosted by North Central Regional Aquaculture Center (NCRAC); the Michigan Department of Natural Resources, Fish Division; Illinois Department of Conservation, Fish Division;

Michigan Fish Growers' Association; and Michigan Cooperative Extension Service. Proceedings from that conference were published in late 1991. Copies were sent to all registrants and distributed in accordance with the National Coordinating Council on Aquaculture's Publication Policy. Additional copies of the proceedings were made available at cost from the Michigan Department of Natural Resources, Wolf Lake State Fish Hatchery, Mattawan, Michigan.

### Impacts

Persons from both the public and private sectors were provided with the most recent information and technologies pertaining to aquaculture in the NCR.

### Recommended follow-up activities

Funds provided by NCRAC were to cover up-front expenses for planning and advertising this first regional aquaculture conference. These costs were to be recouped and revenues generated for additional regional conferences that would be held every 2 years thereafter at sites rotating throughout the region.

### Publications, manuscripts, or papers presented

See Appendix.

### Support

Years	NCRAC USDA funding	Other support				Total Support
		University	Industry	Other Federal	Other Total	
1990-92	\$7,000					\$7,000
<b>Total</b>	<b>\$7,000</b>					<b>\$7,000</b>

## Crayfish

*Project Termination Report for the Period, September 1, 1992 to August 31, 1995*

### NCRAC funding level

\$49,677 (September 1, 1992 to June 30, 1995)

### Participants

Paul B. Brown, Purdue University, Indiana  
 Harold E. Klaassen, Kansas State University, Kansas  
 Robert J. Sheehan, Southern Illinois University-  
 Carbondale, Illinois

#### *Extension liaison*

Jeffrey L. Gunderson, University of Minnesota-Duluth,  
 Minnesota

#### *Non-funded collaborators*

Carl Richards, University of Minnesota-Duluth,  
 Minnesota  
 Robert Wilkinson, Southwest Missouri State University,  
 Missouri

### Reason for termination

The objectives for this project were completed.

### Project objectives

1. Complete a study of the status of the crayfish industry in the North Central States, relative to its extent, culture operations in use, market characteristics, and problems which need to be addressed by research.
2. Complete a report on indigenous crayfish species appropriate for culture in the North Central Region (NCR), to include species life histories, ranges of distribution, economic assessment of appropriate culture production systems, a bibliography of pertinent literature, and a summary of critical information gaps.
3. Conduct preliminary trials evaluating the performance of several promising indigenous species in pond culture.

### Principal accomplishments

#### Objective 1

Within the NCR, 73 crayfish aquaculturists were identified by state extension contacts; they were sent a survey form and asked to respond to a series of questions. Based on the responses, crayfish production

in the region appears to be under 10,000 kg (22,046 lb) per year. It is felt that this may be an underestimate of total production as several of the larger producers did not respond despite numerous mailings of the survey. The majority of those who responded (71%) indicated they grew crayfish in polyculture with other finfish. The primary market for crayfish was bait, as a hard-shell product (78% of respondents). Respondents felt there was opportunity for expansion in both the bait (hard- and soft-shell) and human food market. They also indicated that the best return on investment was as tail meat or as a hard-shell bait product. The principal problem areas identified were markets for their products and growth rates of the various species native to the region.

#### Objective 2

A report on the life history and culture potential of four indigenous crayfish species (*Orconectes immunis*, *O. virilis*, *O. nais*, and *Procambarus acutus*) is nearing completion and will serve as an important source of information for new culturists interested in crayfish aquaculture in the NCR. Information is presented on the life history, biology, distribution, and an assessment of appropriate culture systems. A bibliography and summary of critical information gaps for each of the four species is also included.

#### Objective 3

Research was conducted at Purdue University (Purdue), Southern Illinois University-Carbondale (SIUC), and Kansas State University (KSU) to evaluate the growth, production, and survival of several indigenous crayfish species in various pond culture systems.

Research at Purdue was designed to compare growth of several of the region's native species of crayfish in side-by-side comparisons. In the first year of the study, *O. virilis*, *O. immunis*, *O. rusticus*, and *P. acutus* were evaluated. In the second year of the project, *O. virilis*, *O. propinquus*, and *O. longidigitus* were compared. *O. virilis* grew better than the other crayfish in both years and their yield was higher than the other crayfish

in the first year. However, yield was similar in the second year among all crayfish species evaluated.

Research at SIUC was conducted to compare the growth and production of three species of crayfish (*P. acutus*, *O. virilis*, and *O. immunis*) under polyculture conditions, and compare growth and production of crayfish under two production strategies: (1) artificial destratification/aeration, use of prepared feeds, perpetually filled ponds, and seining (first year) and (2) winter cover-crop production, fall-winter draw down, and harvest via baited traps (second year).

Four ponds (0.06 ha; 0.15 acre) were aerated and four were not in year 1. Each pond was stocked with about 8,340 young-of-the-year (YOY) crayfish. Only *O. immunis* and *O. virilis* were stocked in the first study year. All three species were examined in the second year. Heavy rains in November precluded planting in the fall prior to year 2, so cover-crop ponds were planted with Clark Wheat at a rate of 120 kg seed/ha (107 lb/acre) in April of year 2. The wheat reached a height of 20 cm (7.9 in) prior to flooding. The following were the specific findings over the 2 years of study by SIUC.

- Bottom mean dissolved oxygen (DO) concentrations were significantly lower in non-aerated ponds and in cover-crop ponds than in aerated ponds.
- Bottom temperatures were about 1°C higher on average in aerated versus non-aerated ponds, and the difference was significant.
- Average daily weight gain was significantly higher in aerated versus non-aerated ponds and cover-crop ponds.
- *P. acutus* grew faster than the other two species and *O. virilis* grew significantly faster than *O. immunis*. YOY crayfish began reaching harvestable size (70 mm total length [TL]; 2.76 in) in appreciable numbers by July.
- Mean weights were significantly greater in aerated (15.9 g; 0.56 oz) versus non-aerated ponds (11.8 g; 0.42 oz).
- Harvest from the cover-crop ponds was extremely low (8 kg/ha on average; 7.1 lb/acre) versus the aerated ponds (221 kg/ha on average; 197.2 lb/acre).
- *O. nais* appears to be a subpopulation of *O. virilis*, rather than a true species, based on starch-gel electrophoresis.
- The percent edible tail meat was higher for *O. immunis* (21.9%) than for *P. acutus* (19.3%),

*O. virilis* (19.6%) and a sample of *P. clarkii* (16.1%) that had been obtained.

At KSU growth, survival, and harvest of the crayfish *O. nais* were evaluated in three 0.20 ha (0.5 acre) farm ponds. The water quality of these ponds varied considerably but was typical of many Kansas farm ponds.

Each pond was to be stocked in mid-summer with a low density (3/m<sup>2</sup>; 0.3/ft<sup>2</sup>) of YOY crayfish to allow for maximum growth rate. Ponds were not fed or aerated. Growth, survival (both summer and winter), and harvest were evaluated through two growing cycles, 1993-94 and 1994-95. Prior to stocking, the ponds were to be poisoned to remove existing crayfish. Due to unusually wet weather during 1993, only one pond was poisoned and stocked at the low density. The other two ponds were intensively seined and trapped; crayfish that remained in the pond were used for the study. During 1994-95 all three ponds were poisoned and stocked as proposed.

### *Edible size crayfish*

The size of crayfish considered edible or the minimum marketable size varied somewhat among the three research groups. Crayfish were judged to be edible size in the KSU study if they were larger than 38 mm (1.5 in) carapace length (CL) (approximately 76 mm TL; 3.0 in). This is somewhat larger than the 70 mm (2.8 in) TL (approximately 11-12 g or 38-41 crayfish/lb) judged as edible size in the SIUC research. Crayfish exceeding 47 mm (1.9 in) CL (approximately 94 mm TL; 3.7 in) were designated jumbo size in the Kansas State study. Crayfish weighing 15-18 g (approximately 25 to 30 crayfish/lb) were considered minimum marketable size in the Purdue study.

Crayfish did not generally reach edible size during their first growing season, but attained edible and jumbo size by the following June. At the end of the growing season (both years), average CL of YOY crayfish varied significantly among ponds and ranged from 23 to 41 mm (0.9 to 1.6 in).

During June of 1994 and 1995 all three ponds were intensively trapped with minnow traps over a 2-week period. The catch was high at first, but fell off rapidly. Generally, after ten trap nights at least 90% of the harvested crayfish had already been captured. Ponds

with larger crayfish trapped out more quickly. The weather was cool during the beginning of June 1995 and trapping success was low, but success increased rapidly as water temperatures warmed. Size of yearling crayfish harvested in June varied from pond to pond and year to year and ranged from 8% edible size (no jumbo) to 100% edible size (87% jumbo).

Crayfish survival was variable. Summer survival (stocking time to fall) ranged from 12% to 78%. Winter survival (fall to spring harvest) ranged from 3% to 55%. Winter survival was consistently lower than summer survival.

**Impacts**

- The crayfish producer survey was the first attempt at defining the status of crayfish aquaculture in the NCR, the potential for expansion, and the current crayfish culture problems/impediments.
- A manuscript is being written that succinctly summarizes the biological characteristics and examines the aquaculture potential of four native crayfish species. The document will be a valuable tool for aquaculturists and extension personnel in the region.
- Growth of several species was compared and *O. virilis* appears to be the best of those studied when reared in pond monoculture.
- Several species grew to minimum marketable size for human consumption in one growing season and many attained jumbo size by the following June.
- Aeration improves growth and production in crayfish ponds, but providing a cover crop did not.

- All three species evaluated at SIUC have their advantages: *P. acutus* reaches harvestable size early in the production season, *O. virilis* exhibited good growth and survival, and *O. immunis* had the highest percentage of edible tail meat.
- YOY crayfish can be successfully stocked.
- Most of the marketable-size crayfish can be harvested from small ponds within 2 weeks.
- Survival is quite variable and dependent on weather and pond conditions. Winter is a critical period. Aeration would improve survival.

**Recommended follow-up activities**

The two primary problems identified by current aquaculturists raising crayfish in the region were market assessment and development and crayfish growth rates. Marketing studies for freshwater crustaceans are lacking and are needed for developing business plans. Numerous factors can effect growth and virtually all need to be explored with crayfish. Crayfish exhibit density-dependent growth and survival, that is, as density increases, growth and survival decrease. This happens with various species of fish as well, but is usually solved by in-depth studies. There is also a need for a biocide registered for use on crayfish which would allow for more active management of production ponds.

**Publications, manuscripts, or papers presented**

See Appendix.

**Support**

Years	NCRAC USDA funding	Other support				Total Support
		University	Industry	Other Federal	Other Total	
1990-95					\$61,960 <sup>a</sup>	\$61,960
1992-95	\$49,677	\$58,049				\$107,726
<b>Total</b>	<b>\$49,677</b>	<b>\$58,049</b>			<b>\$61,960</b>	<b>\$169,686</b>

State of Indiana, Business Modernization and Technology Center, through the Purdue University New Crops Center

This project was chaired by Paul B. Brown.

## National Aquaculture Extension Workshop

*Project Termination Report for the Period, October 1, 1991 to September 30, 1992*

### **NCRAC funding level**

\$3,005 (October 1, 1991 to September 30, 1992)

### **Participants**

Carole R. Engle, University of Arkansas at Pine Bluff,  
Arkansas

James T. Davis, Texas A&M University, Texas

#### ***Collaborators***

Donald W. Webster, University of Maryland, Maryland

Joseph E. Morris, Iowa State University, Iowa

Fred Conte, University of California Davis, California

Paul Olin, University of Hawaii, Hawaii

### **Reasons for termination**

The objectives for this project were completed.

### **Project objectives**

1. Improve Extension's information and delivery system for aquaculture in concert with industry areas of high priority.
2. Enhance the development of new state Extension educational programs in aquaculture by highlighting innovative programs.
3. Examine the scope of Extension efforts in aquaculture at the state, regional, national, and international levels.
4. Evaluate the role and effectiveness of Extension activities within the USDA Regional Aquaculture Centers (RACs) program and pursue actions of enhancement.
5. Identify opportunities and needs to enhance the development of regional and interregional Extension programs in aquaculture.
6. Strengthen communication networks and exchanges of materials, programs, and expertise among aquaculture Extension staff.
7. Examine team building and multidisciplinary approaches to Extension programming in aquaculture.
8. Address national and regional issues impacting educational programs and state and federal legislation.

9. Examine major trends in commercial aquaculture and emerging opportunities.
10. Explore use of new communication technology and innovative reporting techniques.

### **Principal accomplishments**

Specialized training was provided for over 90 extension scientists from at least 43 states during the National Extension Aquaculture Workshop held March 3-7, 1992, at the 4-H Center in Ferndale, Arkansas. Featured were speakers from many agencies and organizations that impact on aquaculture production, marketing, and federal policy in the United States.

This training was jointly sponsored by the five RACs and the states involved. In addition to speakers from within the group, representatives from several universities, the U.S. Departments of Agriculture, Commerce, and Interior, U.S. Army Corps of Engineers, and the Food and Drug Administration presented papers and led discussions. "Networking with other Professionals," "Use of Therapeutants," "Aquaculture Waste Management," and "The National Aquaculture Information Center" occupied participants on the first day of the workshop. There was also time for other discussion groups and visits to a very well stocked and maintained media center. Many participants got an opportunity to discuss points of view with experienced extension scientists throughout the workshop. In addition, they were exposed to many resource materials of immediate use as well as becoming acquainted with other extension specialists to contact for information when it is needed.

On the second day, the discussion switched to "Fish and Shellfish Inspections," "Marketing and Processing," "Production and Marketing Economics," and an excellent panel discussion on "You and the Law." Later a discussion of how to interface with extension efforts of the Fish and Wildlife Service alerted many of the participants to the possibilities of funds available and other joint efforts. Though this day was concerned with economics rather than biology, the learning

opportunities were tremendous. “Time Management,” which is a concern for all professionals, led off the discussion on the workshop’s third day. This session was followed by a discussion of “Environmental Impacts, Endangered Species, and Non-tidal Wetlands.” Facility design covered ponds, raceways, and recirculating systems in a manner that all non-engineers could easily follow. Next on the agenda was a session on “Improved Communications” with discussions ranging from electronic information networks to teleconferencing. Guidance on the use and misuse of these systems was particularly enlightening.

The final day was spent in group discussions on how the RACs can better serve their clientele, followed by a tour of two premier aquaculture installations. One of these, Anderson Minnow Farms, is reputed to be the largest such installation in the world. The other, Keo Farms, allowed many to see first hand some of the complexities of commercial spawning and rearing of hybrid striped bass and triploid grass carp. Over 60 of the participants stayed for an additional day to receive in-depth training in production and marketing of either hybrid striped bass or bait minnows. These sessions featured speakers from the commercial sector as well as researchers and extension specialists.

Evaluations from the participants were tabulated, and the workshop was rated 4 on a scale of 1 to 5. Two objectives of the workshop were for participants to receive training in current subject matter and to understand new information transfer capabilities. These received very high evaluation scores, as did an improved understanding of national initiatives and guidance in enhancing coordination with other

agencies. When asked, “What did you get from the program?”, 41 of 42 respondents indicated that they got the names of other people to contact for help in difficult situations, and 40 said they received new, usable resource material. Over one-half stated that they received answers to questions, ideas that they could try immediately, and most indicated a better understanding of RAC programs. As this was the first of these ever held, these indicate that the participants received excellent training as well as taking part in a varied and intensive program.

**Impacts**

The total effect of this workshop will not be realized for 5 to 10 years. At this stage it is known that there has been increased cooperation across regional lines in planning of projects and better communication between specialists. Knowing aquaculture specialists in other parts of the United States has enabled many specialists to secure assistance with specialized problems.

**Recommended follow-up activities**

Participants rated the need for future workshops at 4.6 out of a possible 5.0 after considering the time and travel costs involved, and most preferred 2 or 3 years between workshops. A few of the participants commented that the program was too intense and too long, but even these indicated that if it was shortened and the format modified they would recommend the workshop to others.

**Publications, manuscripts, or papers presented**

See Appendix.

**Support**

Years	NCRAC USDA funding	Other support				Total Support
		University	Industry	Other Federal	Other	
1991-92	\$3,005				\$12,020 <sup>a</sup>	\$15,025
<b>Total</b>	<b>\$3,005</b>				<b>\$12,020</b>	<b>\$15,025</b>

<sup>a</sup>Each of the four other Regional Aquaculture Centers contributed \$3,005 for the workshop.

## Baitfish

*Project Termination Report for the Period September 1, 1992 to September 30, 1995*

### **NCRAC funding level**

\$61,973 (September 1, 1992 to September 30, 1995)

### **Participants**

Frederick A. Copes, University of Wisconsin-Stevens Point, Wisconsin

Daniel W. Coble, University of Wisconsin-Stevens Point, Wisconsin

Leroy J. Hushak, Ohio State University, Ohio

#### ***Extension liaisons***

Daniel A. Selock, Southern Illinois University-Carbondale, Illinois

Joseph E. Morris, Iowa State University, Iowa

#### ***Non-Funded Collaborators***

Charles Berry, Jr, South Dakota Coop. Fishery & Wildlife Unit, South Dakota

Carl Gollon, Gollon Brothers Fish Farm, Wisconsin

Dirk Peterson, Minnesota Department of Natural Resources, Minnesota

Charles Rabeni, Missouri Cooperative Fishery & Wildlife Unit, Missouri

### **Reasons for termination**

The objectives for this project were completed.

### **Project objectives**

1. Conduct a comprehensive survey of the status of the baitfish industry in selected North Central states to determine: (a) species used; (b) sizes of species marketed; (c) sources of species; (d) seasonal availability; (e) shortfalls in supplies; (f) relative value of various fish and nonfish species; and (g) common problems of the industry that may need to be addressed by research.
2. Estimate the costs of culturing bait species commonly used in the North Central Region (NCR) in selected types of production facilities, e.g., extensive and intensive pond culture, tanks, and raceways.
3. Estimate the economic contribution (output, employment, and income) generated by the bait industry to selected state economies.

4. Assemble a list of rules and regulations for each state affecting the baitfish culture industry.
5. As time permits, summarize biological life cycle information for several underused or unused species that have culture potential and which may match needs of the regional industry.

### **Principal accomplishments**

#### **Objective 1**

The many species and sizes used were identified; the most important baitfish was the fathead minnow and non-fish bait was the night crawler. About two-thirds of baitfish were harvested from the wild; the rest were cultured. Non-fish bait was about 50:50 wild vs. cultured. Availability varied seasonally and shortages were identified. Values of various fish and non-fish baits were estimated; baitfish composed 64%, and non-fish bait, 36% of the estimated value of bait. Bait mortality was a problem for 50% or more of wholesalers and retailers. Better temperature control and handling and transport would probably reduce mortality.

#### **Objective 2**

The 107 respondents who reported growing baitfish on the 1990 survey of fish growers in the Economics and Marketing Project were resurveyed about their baitfish enterprises, the sales of baitfish, and the costs of producing those baitfish during 1993. After three mailings and numerous follow-up telephone calls, a total of 33 surveys were completed, of which only 10 were useable. The remaining respondents were no longer in the baitfish business or handled only wild-caught species. Even for the 10 useable responses, the data provided was not sufficient for detailed budget analysis. Four of 10 reported sales of less than \$10,000 during 1993 while only one reported baitfish sales in excess of \$40,000. Nearly all operations could reach break even, i.e., cover their reported costs, within the sales class they reported.

**Objective 3**

The value of the industry was estimated in six state economies. For all six combined, the total minimum estimated value in 1992 was about \$165 million for baitfish and \$92 million for non-fish bait.

**Objective 4**

A list of rules and regulations affecting the bait industry was assembled for the 12 states in the NCR.

**Objective 5**

Several species of important baitfish that are harvested from the wild were identified for investigation of potential for fish culture. At least one, the emerald shiner, will be studied with funding from another source.

**Impacts**

- Identification of the most important baitfish species, fathead minnows, and non-bait, night crawlers, was accomplished. Protection and research may be needed in the future.
- Identification of supply shortages indicates species for which increasing the supply would aid the industry. Baits commonly in short supply include: fathead minnows, lake shiners, golden shiners, night crawlers, leeches, and crayfish.
- Identification of disease and handling problems indicates areas for fruitful future research and

extension efforts.

- Estimates of economic value, \$165 million for baitfish and \$92 million for non-fish bait for six states, emphasize the importance of the industry to those state economies.
- Inconsistent state regulations identified as problematic to the industry.
- Study has generated more than 50 inquiries on baitfish culture and markets.
- Aquaculture shortcourse offered, March 1993.
- Copes served as moderator of afternoon session of the Governor's Conference on Agriculture: Wisconsin Aquaculture 1994, University of Wisconsin-Stevens Point, February 1994.

**Recommended follow-up activities**

- Study problems identified by the survey respondents and increase extension educational information on proper bait handling procedures.
- Investigate culture methods for important non-propagated species and potentially valuable unused species.
- Study ways to alleviate supply shortages.

**Publications, manuscripts, or papers presented**

See Appendix.

**Support**

Years	NCRAC USDA funding	Other support				Total	Total Support
		University	Industry	Other Federal	Other		
1992-95	\$61,973	\$44,482	\$2,000 <sup>a</sup>	\$16,132 <sup>b</sup>	\$62,614	\$124,587	
<b>Total</b>	<b>\$61,973</b>	<b>\$44,482</b>	<b>\$2,000</b>	<b>\$16,132</b>	<b>\$62,614</b>	<b>\$124,587</b>	

<sup>a</sup>Various bait dealers

<sup>b</sup>U.S. Fish and Wildlife Service and National Biological Service (Wisconsin Cooperative Fishery Research Unit)

This project was chaired by Daniel W. Coble.



## Wastes/Effluents

*Project Termination Report for the Period September 1, 1992 to August 31, 1996*

### **NCRAC funding level**

\$153,300 (September 1, 1992 to February 29, 1996)

### **Participants**

Fred P. Binkowski, University of Wisconsin-Milwaukee, Wisconsin

James M. Ebeling, Ohio State University, Ohio

Konrad Dabrowski, Ohio State University, Ohio

Reginald D. Henry, Illinois State University, Illinois

Kyle D. Hoagland, University of Nebraska-Lincoln, Nebraska

Terrence B. Kayes, University of Nebraska-Lincoln, Nebraska

Joseph E. Morris, Iowa State University, Nebraska

Ronald R. Rosati, Illinois State University, Iowa

#### ***Extension liaison***

LaDon Swann, Purdue University, Illinois/Indiana

#### ***Non-funded collaborators***

John Hyink, Glacier Springs Trout Hatchery/Alpine Farms, Wisconsin

Iowa Department of Natural Resources (DNR), Fairport State Fish Hatchery, Iowa

Iowa DNR, Rathbun State Fish Hatchery, Iowa

Myron Kloubec, Kloubec's Fish Farm, Iowa

Bill Johnson, Rushing Waters Fisheries, Wisconsin

Dave Smith, Freshwater Farms of Ohio, Inc., Ohio

John Wolf, Glacier Springs Trout Hatchery/Alpine Farms, Wisconsin

Michael Wyatt, Sandhills Aquafarm, Nebraska

### **Reason for termination**

The objectives for this project were completed.

### **Project objectives**

1. Characterize aquaculture effluents from four types of aquaculture production systems: pond culture, flow through culture (raceway), cage culture, and recirculating systems.
2. Generate a data base from these four types of production systems to help promote a reasonable choice of effluent discharge regulations by government agencies.

### **Principal accomplishments**

#### **Objective 1**

##### *Pond production systems*

#### **Fairport State Fish Hatchery, Iowa**

Water quality was monitored in four culture ponds stocked with channel catfish (*Ictalurus punctatus*), fingerlings at Fairport State Fish Hatchery near Muscatine, Iowa during 1993. Data were collected during the culture season and at harvest to analyze pond and effluent water quality. During the course of the growing season, water temperature, nitrates, and total suspended solids levels decreased while dissolved oxygen (DO), ammonia, un-ionized ammonia, and 5-day carbonaceous (organic) biological oxygen demand (CBOD<sub>5</sub>) increased.

Analysis of data collected at harvest revealed that total phosphorus and total solid levels increased substantially in the pond effluents compared to those within the ponds. Towards the latter stages of fish harvest, CBOD<sub>5</sub> levels significantly increased within the ponds; effluent quality significantly deteriorated, having increased levels of total phosphorus, total nitrogen, CBOD<sub>5</sub>, total solids, and total suspended solids. Fish biomass was a positive influence on CBOD<sub>5</sub>.

#### **Kloubec Fish Farm, Iowa**

Samples were obtained from Kloubec's channel catfish and hybrid sunfish ponds in early and late October 1993. During the sampling period, two ponds had elevated levels of nitrites and three ponds had elevated levels of nitrates compared to earlier sampling periods. However, CBOD<sub>5</sub> levels decreased in all ponds during this sampling period. The two ponds with the highest levels of CBOD<sub>5</sub> at this time had been harvested the previous month. The act of seining probably resulted in direct increase in CBOD<sub>5</sub> levels compared to those ponds that had not been harvested.

Ponds at Kloubec's Fish Farm had higher CBOD<sub>5</sub> levels than did the flow-through situation at the Rathbun State Fish Hatchery, but were similar to pond levels at the Fairport State Fish Hatchery. However, the nitrogenous

compounds levels were low. The two ponds with the highest feed levels had the highest CBOD<sub>5</sub> levels.

### *Raceway (flow-through) production systems*

#### **Rathbun State Fish Hatchery, Iowa**

The effects of a flow-through aquaculture facility, Rathbun Fish Hatchery, Iowa, were assessed in 1993. Significant differences ( $P < 0.10$ ) were determined in both water quality and invertebrate parameters at six sample sites. Sites closest to the culture facility had elevated levels of several nitrogenous and phosphorus compounds compared to sites at the water intake and Chariton River. Main production factors influencing water quality parameters at sites were those taking place within the main hatchery building (flow, fish biomass, feed quantity, and quality). Invertebrate groups, both zooplankton and other macroinvertebrates, did not differ between the upstream and down-river stations.

The overall conclusion concerning this data set is that the effects of aquaculture effluents from this hatchery are minimal at best on both chemical and biological factors. High flows resulting from flood conditions caused increased dilution of aquacultural effluents. The 1993 field season had the worst flooding in the state's history. Thus, data collected during this period may not be representative of a typical year where some hatchery effects may have been seen under more normal conditions.

#### **Sandhills Aquafarm, Nebraska**

The goal of the University of Nebraska-Lincoln (UNL) research was to monitor key water quality parameters above and below Sandhills Aquafarm, a modern trout production facility on Whitetail Creek in Western Nebraska. Whitetail Creek is a spring-fed, first order stream with relatively constant flow and good water quality. Sandhills Aquafarm consists of 12, 2.4- 33.5 m (8-100 ft) raceways with total flows of 23.5 m<sup>3</sup>/min (6,200 gpm) and annual production rates of rainbow trout of 77,100 kg/year (170,000 lb/year). Four sites were established above the facility and four below to obtain reliable, representative physicochemical measurements and water samples for laboratory analyses.

It was clear that several water quality parameters continued to differ consistently above versus below the aquaculture facility, particularly DO, pH, ammonium-

nitrogen, total nitrogen, orthophosphate, and phosphorus. Total suspended solids and turbidity showed no consistent trends. While temperature and biochemical oxygen demand (BOD) appeared to exhibit relatively little difference above and below the facility (although even these differences were consistent), downstream decreases in DO and pH, and increases in ammonium-nitrogen, total nitrogen, total phosphorus, and orthophosphate were evident. These data clearly indicate that water quality was altered downstream from the facility in both 1993 and 1994.

#### **Rushing Waters Fisheries, Wisconsin**

Rushing Waters Fisheries is one of the most productive commercial rainbow trout hatcheries in Wisconsin. It has earthen raceways and ponds with a total flow approximately half that of the Nebraska Sandhills operation. As such, it is representative of the more typically-sized private trout production facilities in the North Central Region (NCR). This facility is supplied by groundwater wells and springs of moderate conductivity (between 400-600  $\mu$ S/cm) and is located at the head of a small creek that is a tributary to Blue Springs Creek in Jefferson County, Wisconsin.

Alterations in water quality occurred in the effluents of the three chains of raceways as compared to the source waters entered at the head of each raceway chain, and the water quality of the combined effluent in the creek leaving the property. Increases in BOD, total suspended solids, total ammonia nitrogen, nitrite-nitrogen, soluble reactive phosphorus, and total phosphorus were evident. Under typical production conditions these changes were slight, but on at least one occasion raceway cleaning activities created more elevated conditions of total suspended solids and total phosphorus in the creek leaving the property.

The effluents from the earthen production raceways had slightly lowered levels of nitrate-nitrogen compared to the source water. It seems reasonable that the natural primary and secondary productivity in the earthen bottomed rearing units would utilize nitrate. Dissolved oxygen levels in the groundwater well sources tended to be slightly lower than in the effluents from the raceways. Use of aerating devices in the rearing units kept DO levels high, and the level in the newly pumped well water probably had not yet had enough contact with the atmosphere to reach full saturation before sampling. Source water samples were taken from an

open reservoir rather than from groundwater wells, and water from this reservoir had slightly higher levels of solids, ammonia, and phosphorus than the well water sources. This difference was slight, however, in comparison to the general differences between the source waters and the effluents.

### **Alpine Farms, Wisconsin**

Tank rearing of yellow perch and whitefish at Alpine Farms, Sheboygan Falls, Wisconsin was investigated to characterize the effluents produced by alternative regional aquaculture species. Yellow perch and whitefish tank effluents produced changes in water quality parameters similar in direction and magnitude to those of the other flow through rearing situations. Differences appeared to be controlled by production conditions (water exchange, loading, and ration level) rather than by the species reared.

### **Glacier Springs Hatchery, Wisconsin**

The intended opportunity to examine the changes in effluent water quality during the renovation of a former hatchery that had been inactive for over a decade did not materialize during the project period, due to changes in the owner's plans. A representative set of before renovation water quality data was gathered, which would be suitable for comparison if future renovation occurs. Emphasis was shifted to the Rushing Waters Hatchery study when plans for renovation were delayed.

### *Cage culture production systems*

#### **Trout culture**

Freshwater Farms of Ohio's trout cage culture facility is located near Urbana, Ohio in an abandoned quarry. The site consists of four separate quarry lakes, two of which discharge into a third, which together with the fourth, discharge into the Mad River. A total of 10 sampling sites were monitored, including spring inflow into two lakes, the cage culture site at two depths, the discharge from the production lake into a settling lake, the discharge into the Mad River from the settling pond, discharge from an unused lake into the Mad River, and the Mad River upstream and downstream from the discharges.

For all measured parameters, there were no significant differences. There was no negative impact of Freshwater Farms of Ohio's trout cage culture operation on the water quality of the quarry lakes or the receiving

water of the discharge. In fact, in most cases significant improvement occurred due to the diluting effect of the quarry discharges.

### **Channel catfish culture**

The Piketon Research and Extension Center (PREC-OSU) has a small demonstration cage culture operation in a 1.8 ha (4.4 acre) reservoir located at the facility. The cage culture operation reflects what a small farmer could easily build in a farm pond for the production of channel catfish and for trout grow out in the winter months. The system was lightly stocked over the spring months with trout and yellow perch fingerlings and then heavily stocked with channel catfish (850 kg; 1,874 lb) in mid-summer.

The impact of the small scale cage catfish cage culture operation at PREC-OSU is not easily characterized due to the input from the Center's wastewater treatment plant. Still most water quality parameters were typical of catfish production ponds.

### *Recirculating systems*

The facilities studied at Illinois State University included two recirculating aquaculture systems (RAS) stocked with Nile tilapia (*Oreochromis niloticus*). The first system consisted of a 18,927 L (5,000 gal) culture tank, a settling tank particle filter, a vertical screen submerged media biofilter, and an oxygen column. The second system consisted of a 170,343 L (45,000 gal) culture system, a drum microscreen particle filter, a submerged media biofilter, and oxygen columns. The second system is similar in design, although smaller in scale, when compared to operating commercial systems found in the private sector. The second system was producing 226.8-453.6 kg (500-1,000 lb) of live tilapia each week during the time of these trials.

Data collected on two different RASs demonstrate that RAS effluents contain elevated levels of total solids, settleable matter, BOD, forms of nitrogen (excluding non-ionized ammonia), phosphorus, and reduced concentrations of DO, which agree with previous RAS studies.

### **Objective 2**

The combined data sets from these investigations have been tabulated and attached, along with an extensive bibliography concerning aquaculture effluents, as appendices to the Project Termination Report Part II.

This data set and references provide a single source overview of effluent water quality from representative regional aquaculture production facilities.

## Impacts

Data from the recirculating system study has already been used by a private sector aquaculturist in developing a new large recirculating system that meets USEPA compliance. It is anticipated that as this database is made available to the industry, many more actual applications will occur. Aquaculturists can use this data to take a proactive stance in helping environmental regulatory agencies compose practical aquaculture discharge regulations. Aquaculturists may also use the data collected as baseline values in research to determine the efficiency of newer effluent treatments and best management practices (BMPs) designed to reduce the impact of effluent discharges. Practices that alleviate effluent problems may result in more efficient operation of the hatchery facility and economic savings, and also decrease environmental impacts. This information will help protect the quality of water resources and may alleviate the fears of the general public as to the perceived polluting aspects of aquaculture systems.

## Recommended follow-up activities

This work has measured the levels of solids produced by representative regional facilities as settleable solids, total suspended solids, and dried solids. The more dramatic changes in water quality from aquaculture operations are associated with clean out events for removal of settled aquaculture waste materials. Solid wastes are most easily removed from culture systems by conventional water treatment processes, while nutrients, once they become dissolved, require treatment technologies that are cost prohibitive. Strategies to improve commercial fish foods from the perspective of waste management need to be evaluated. Better understanding of the influence of commercial diet formulations on the integrity of fecal solids and the consequent impact on nutrient release, holds promise of reducing the release of phosphorus into aquaculture effluents. This is one of the aspects of greatest regulatory concern due to its potential role in the eutrophication of receiving waters. The addition of fiber to the diet can potentially influence fecal durability in water and permit easier removal. The practicality of this strategy needs to be investigated and

demonstrated.

In recirculating aquaculture systems the size distribution of suspended solids particles shifts to smaller sized particles that are the most difficult to remove, and more information is needed on the impact of such particulate solid matter on fish health and the performance of recirculating systems. Recycle systems also produce expectedly more concentrated waste, but they also permit more opportunities for innovative reuse or disposal. Efficient solids removal and management provides an avenue for the utilization of waste by-products as potentially beneficial resources in the context of integrated resource management plans.

Wise resource management calls for finding beneficial use for these concentrated aquaculture by-products. These types of practices can promote aquaculture as a beneficial or at least a benign influence on water resources when good stewardship is practiced. In this regard, comparison of real-life aquaculture production situations to other common land and water use practices will be helpful in arguing for realistic and just regulatory and permit situations. Comparing aquaculture waste production within the context of other contemporary land use practices with regard to impact on regional water quality, should be useful for demonstrating that current and prospective regional aquaculture is relatively benign environmentally.

Using the results of this project, a report needs to be prepared that contrasts the potential impact of regional aquaculture development with other contemporary agricultural, municipal, industrial, and natural resource land uses. There is a need for this information to be organized into an easily understood format so that normal aquaculture operating conditions can be viewed against the existing general background of water quality fluctuation and environmental impact. This report should also review and evaluate current research on alternative beneficial uses of aquaculture by-products, emphasizing integrated resource management and aimed at developing sustainable aquacultural practices. Options to be examined should include constructed wetlands, irrigation uses, hydroponics, and even biogas production strategies. Often the situations and examples of such practices, as presented in the existing literature, deal with species, climates, and situations, which may or may not be applicable to the environmental and regulatory situations in our region.

## North Central Regional Aquaculture Center (NCRAC)

There is a need to review and present this information in relation to its relevance and application to aquaculture in the NCR.

### Publications and papers presented

See Appendix.

### Support

Years	NCRAC USDA funding	Other support				Total Support
		University	Industry	Other Federal	Other	
1992-93	\$77,064	\$54,427	\$15,000 <sup>a</sup>		\$69,427	\$146,491
1993-94	\$76,236	\$43,261	\$20,000 <sup>a</sup>	\$10,000 <sup>b</sup>	\$73,261	\$149,497
<b>Total</b>	<b>\$153,300</b>	<b>\$97,688</b>	<b>\$35,000</b>	<b>\$10,000</b>	<b>\$142,688</b>	<b>\$295,988</b>

<sup>a</sup>Glacial Springs Hatcheries

<sup>b</sup>Archer Daniels Midland

This project was chaired by Fred P. Binkowski.

## National Aquaculture INAD/NADA Coordinator

*Progress Report for the Period September 1, 1992 to August 31, 1996*

### NCRAC funding level

\$17,000 (September 1, 1993 to May 14, 1997)

### Participants

Ted R. Batterson, Michigan State University, Michigan  
Henry S. Parker, USDA/CSREES/PAPPP, Washington, DC

Robert K. Ringer, Michigan State University, Michigan  
Rosalie A. Schnick, Michigan State University, Wisconsin

### Project objectives

1. Ensure effective communications among groups involved with Investigational New Animal Drug/ New Animal Drug Applications (INADs/NADAs), including Canada.
2. Serve as an information conduit between INAD/ NADA applicants and the U.S. Food and Drug Administration's Center for Veterinary Medicine (CVM).
3. Identify and encourage prospective INAD participants to become involved in specific investigational studies and NADA approval-related research.
4. Seek the support and participation of pharmaceutical sponsors for INAD studies and NADAs and coordinate with INAD/NADA sponsors to achieve CVM approval more quickly.
5. Guide prospective and current INAD holders on the format for INAD exemption requests and related submissions to CVM.
6. Identify existing data and remaining data requirements for NADA approvals.
7. Review, record, and provide information on the status of INADs and NADAs.
8. Encourage and seek opportunities for consolidating the INAD/NADA applications.
9. Coordinate educational efforts on aquaculture drugs as appropriate.
10. Identify potential funding sources for INAD/ NADA activities.

### Anticipated benefits

Investigation and approval of safe therapeutic and production drugs for use by the aquaculture industry are some of the highest priorities currently facing the industry. At present, only a few approved compounds are available to the industry and further development of the aquaculture industry is severely constrained by a lack of approved drugs essential for treating more than 50 known aquaculture diseases. CVM has afforded the aquaculture industry throughout the U.S. with a “window of opportunity” to seek approval of legal drugs to be used in their production practices. The need for additional drugs is great, but securing data necessary to satisfy the requirements of CVM for drug approval is time consuming, costly, and procedures are rigorous. The INAD/NADA process is the one method that allows the industry to provide CVM with data on efficacy and also aids producers in their production practices.

Coordination and educational efforts directed toward potential INAD/NADA applicants will save time and effort for both the industry and CVM. The National Coordinator for Aquaculture INADs/NADAs serves as a conduit between an INAD/NADA applicant and CVM. The National Coordinator helps to alleviate time demands on CVM staff, thus allowing more time to process a greater number of applications, as well as increasing the breadth of research endeavors within the industry. The grouping of INAD applicants should help to alleviate redundancy, amalgamate efforts, and increase the amount of efficacy data, all of which should result in greater progress toward developing available, approved therapeutic, and production drugs.

### Progress and principal accomplishments

In September 1992, Ringer, Professor Emeritus of Michigan State University, was hired on a part-time basis as National Coordinator for Aquaculture INAD Applications. He served in that capacity through August 31, 1994.

As National Coordinator for Aquaculture INADs Ringer participated with CVM in educational workshops on INAD procedures and requirements. These workshops were conducted throughout the U.S. This included workshops held in conjunction with the U.S. Trout Farmers Association, Boston Seafood Show, and Aquaculture Expo V in New Orleans. The work-

shop at the Boston Seafood Show was videotaped and is now available on cassette from the Northeastern Regional Aquaculture Center. In addition to the workshops, talks were presented on aquaculture drugs at the request of several organizations, including the World Aquaculture Society.

Ringer also helped in the preparation of a letter that CVM used in requesting disclosure information from those holding aquaculture INADs. By law, CVM cannot release any information about an INAD without such permission. A table containing information about these disclosures was made available to the general public. This included the names and addresses of the INAD holders as well as the drug and species of fish intended for use of the drug. It is intended that this table will be periodically updated after additional disclosure permissions have been obtained.

On May 15, 1995, Schnick, recently retired Registration Officer from the National Biological Service’s Upper Mississippi Science Center (UMSC), was hired on a three-quarter time basis as National Coordinator for Aquaculture New Animal Drug Applications (National NADA Coordinator). On May 15, 1996, her position was increased to a full-time basis.

As National NADA Coordinator, she organized and coordinated a major INAD/NADA workshop in November 1995, under sponsorship of CVM, that led to increased communications between INAD coordinators, better coordination of the data generation for each drug, and consolidation of several INADs.

#### New INAD/NADA sponsors

Schnick helped gain a new INAD/NADA sponsor for amoxicillin (INAD #9659) and met with Vetrepharm Limited (United Kingdom) in May 1996 in Fordingham, UK, to discuss an action plan for the development of the INAD/NADA on their broad spectrum antibacterial product. Schnick also helped obtain and is working with INAD/NADA sponsors for hydrogen peroxide (microbicide, INAD #9671), luteinizing-hormone releasing hormone (spawning aid, INAD #9318), common carp pituitary (spawning aid, INAD #9728), and AQUI-S (anesthetic, INAD #9731).

#### Progress on therapeutic drugs

Schnick and representatives of the Upper Mississippi Science Center (UMSC), La Crosse, Wisconsin, held a

special session at the Mid-continent Warmwater Fish Culture Workshop in February 1996 to consider label claims and identify potential pivotal study sites for chloramine-T under the federal-state drug approval partnership program (a project of the International Association of Fish and Wildlife Agencies = IAFWA Project).

Based on residue and environmental data, CVM determined on July 11, 1996, that there are no human food or environmental safety concerns over the use of copper sulfate as a therapeutant, thus making approval relatively easy. Two meetings were held in July and August 1996 with a potential NADA sponsor and CVM to discuss the data requirements for approval and develop an action plan needed to obtain approval of copper sulfate as a therapeutant.

On July 18, 1996, CVM accepted the data and conclusions of a target animal safety study on the toxicity of formalin to warm- and coolwater fish eggs that was submitted along with a proposed formalin label by UMSC in December 1995. CVM will soon issue a notice in the Federal Register inviting sponsors to amend their labels to include the extended claims for both the fungicide (based on UMSC studies) and parasiticide uses (based on studies at Auburn University, Auburn, Alabama). These extensions of the formalin NADA to additional species will remove the need for INADs on formalin for these claims.

### **Progress on anesthetics**

Two meetings in June and August 1996 were held with representatives of Aqui-S™, an anesthetic approved for use on fish in New Zealand, to discuss the potential for development of Aqui-S™ in the United States. Aqui-S™ is approved in New Zealand with a zero withdrawal time and offers a potential alternative to benzocaine. UMSC decided to evaluate the comparative efficacy and regulatory requirements needed for approval on both benzocaine and Aqui-S™. Work on benzocaine through the IAFWA Project has been put on hold until the new anesthetic, Aqui-S™, can be evaluated. After an evaluation has been made on efficacy and regulatory requirements, UMSC will decide along with its state partners in the IAFWA Project and U.S. Fish and Wildlife Service whether to pursue Aqui-S™ or benzocaine for approval as an anesthetic/sedative.

### **Progress on hormones**

A meeting was held at CVM headquarters on April 11, 1996, with Stoller, users of common carp pituitary (CCP), and researchers to determine a course of action for gaining approval of CCP. As a follow-up to that meeting, CVM coordinated a conference call on May 15, 1996, that covered: (1) the identification of researchers and the design of target animal safety studies; (2) the writing of the environmental assessment through the National Research Support Program Number 7 (NRSP-7); and (3) potential funding sources of the target animal safety studies.

The National NADA Coordinator contacted all the holders of disclosed INADs on human chorionic gonadotropin (hCG) at the urging of CVM to send all the data to the sponsor, Intervet, Inc., that was incorporated in a February 1996 Intervet submission to CVM. CVM ruled on February 12, 1996, that enrollment in an INAD will not be required to use hCG as a spawning aid. CVM will defer regulatory enforcement if used by or on order of a veterinarian. Any hCG product may be prescribed, but CVM strongly encourages the use of Intervet's product, Chorulon®.

Schnick worked with CVM, Auburn University, Rangen, Inc., and tilapia producers to develop INAD #9647 on 17- methyltestosterone (MT) for tilapia (obtained January 25, 1996) and then worked to obtain authorization from CVM and permission from Auburn University to allow the use of MT on yellow perch under Auburn's INAD (obtained February 22, 1996). NCRAC provided \$25,000 to Southern Illinois University-Carbondale and the University of Wisconsin-Madison to conduct a target animal safety study on MT with walleye and has requested \$5,000 for Auburn University to conduct a literature review of the environmental data on MT for NADA submission to CVM.

### **Progress on the IAFWA project**

Several meetings were held at UMSC in May and June 1996 to review the whole IAFWA Project related to the following topics on each of the 10 study plans: (1) remaining data requirements; (2) tasks and jobs; (3) assignments for each job; (4) a time table for completing each assigned task; (5) budget projections by study plan and year; (6) budget shortfalls for the original IAFWA Project; and (7) assessment of the potential products at the end of the IAFWA Project.

UMSC has reprogrammed its effort and direction under the IAFWA Project due to changes in requirements and circumstances for benzocaine, chloramine-T, hydrogen peroxide, oxytetracycline, and sarafloxacin. Efforts were made to save the entire IAFWA Project during government downsizing and budget reductions. Based on the assessment of the remaining data requirements and the funds available, UMSC determined that the IAFWA Project was short a total of \$1.4 million and 2 years of effort.

**Work planned**

The National NADA Coordinator developed an action plan that centers on coordinating all drugs of high priority for aquaculture toward NADAs through the INAD process. In particular, Schnick plans to: (1) develop a major initiative on amoxicillin to obtain approval for its use as a broad spectrum antibacterial in all fishes; (2) determine the potential of fumagillin to control or prevent whirling disease in salmonids and Hamburger Gill Disease in catfish and pursue an INAD/NADA if feasible; (3) help determine the potential for approval of two anesthetics, benzocaine and AQUI-S™; (4) assist the efforts of the NRSP-7 to complete the approval process for sarafloxacin to control enteric septicemia in channel catfish; (5) identify potential funding sources for INAD/NADA activities; and (6) continue to coordinate efforts to obtain approvals for all 19 high priority aquaculture drugs.

Several meetings and workshops are planned that will benefit aquaculture drug approvals. A meeting will be held in Kansas City, Missouri, on November 7-8, 1996, to discuss the protocols and select the pivotal study

sites for chloramine-T. The National NADA Coordinator arranged the agenda and speakers for a special session entitled “Partnerships for Aquaculture Drug Approvals: Models for Success” to be held at World Aquaculture ‘97, Seattle, Washington, February 19-23, 1997. An International Harmonization Workshop for Aquaculture Drugs/Biologics is scheduled as part of World Aquaculture ‘97 to be held in Seattle, Washington on February 24, 1997, that will create an international forum, identify potential actions, and develop implementation strategies in cooperation with other countries to facilitate approvals of aquaculture drugs.

**Impacts**

Establishment of the National NADA Coordinator position in May 1995, has resulted in coordination, consolidation, and increased involvement in the INAD/NADA process on 17 of the 19 high priority aquaculture drugs and activities on two new drugs of interest to aquaculture. Six new INAD/NADA sponsors have initiated new INADs and progress has been made toward unified efforts on existing and new INADs/NADAs.

This enhanced coordination will help gain extensions and expansions of approved NADAs and gain approvals for new NADAs. In fact, data on formalin have recently been accepted by CVM and amended NADAs are expected soon from the three current NADA sponsors of formalin.

**Publications, manuscripts, papers presented, or reports**

See Appendix.

**Support**

Years	NCRAC USDA funding	Other support				Total Support
		University	Industry	Other Federal	Other	
1992-93				\$17,000 <sup>a</sup>		\$17,000
1993-94	\$2,000			\$12,180 <sup>b</sup>	\$4,000 <sup>c</sup>	\$18,180
1994-95	\$5,000		\$23,750 <sup>d</sup>	\$70,000 <sup>e</sup>	\$10,000 <sup>f</sup>	\$108,750
1995-96	\$10,000		\$20,000 <sup>g</sup>	\$56,920 <sup>h</sup>	\$5,000 <sup>i</sup>	\$91,920
<b>Total</b>	<b>\$17,000</b>		<b>\$43,750</b>	<b>\$156,100</b>	<b>\$19,000</b>	<b>\$235,850</b>



## North Central Regional Aquaculture Center (NCRAC)

<sup>a</sup>USDA funding through a Cooperative Agreement with NCRAC

<sup>b</sup>USDA funding through a Cooperative Agreement with NCRAC (\$8,500) and FDA's Office of Seafood Safety (\$3,680)

<sup>c</sup>Northeastern Regional Aquaculture Center (\$2,000) and Southern Regional Aquaculture Center (\$2,000)

<sup>d</sup>American Pet Products Manufacturers Association (\$7,500), American Veterinary Medical Association (\$10,000), Catfish Farmers of America (\$2,000), Fish Health Section of AFS (\$1,000), Florida Tropical Fish Farm Association, Inc. (\$500), Natchez Animal Supply (\$1,000), National Aquaculture Council (\$1,000), and Striped Bass and Hybrid Producers Association (\$250)

<sup>e</sup>USDA funding through a Cooperative Agreement with NCRAC (\$23,000), CVM (\$22,000), and USDI/NBS International Association of Fish and Wildlife Agencies Project (\$25,000)

<sup>f</sup>Northeastern Regional Aquaculture Center (\$5,000) and the Center for Tropical and Subtropical Regional Aquaculture (\$5,000)

<sup>g</sup>American Pet Products Manufacturers Association (\$1,000), Catfish Farmers of America (\$10,000), Fish Health Section of AFS (\$1,000), Florida Tropical Fish Farms Association, Inc. (\$1,500), Striped Bass & Hybrid Producers Association (\$1,500), Simaron Fresh Water Fish, Inc. (\$2,500), and Abbott Laboratories (\$2,500)

<sup>h</sup>CVM (\$18,400) and USDI/NBS International Association of Fish and Wildlife Agencies Project (\$28,520)

<sup>i</sup>Center for Tropical and Subtropical Aquaculture (\$5,000)

Ted R. Batterson serves as the facilitator for this multi-year project interacting with Henry S. Parker and a steering committee in overseeing the Coordinator's activities.

## Appendix

### Extension

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- 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)

North Central 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)

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)

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)

Aquaculture in the Age of the Information Highway. Special session, World Aquaculture Society, San Diego, California, February 7, 1995. (LaDon Swann)

North Central Aquaculture Conference, Minneapolis, Minnesota, February 17-18, 1995. (Jeffrey L. Gunderson, Lead; Fred P. Binkowski, Donald L. Garling, Terrence B. Kayes, Ronald E. Kinnunen, Joseph E. Morris, and LaDon Swann, Steering Committee)

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## Walleye

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## Sunfish

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