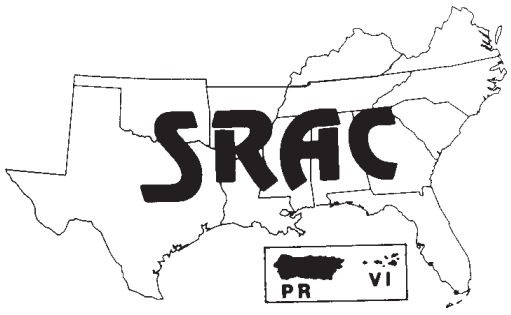
SOUTHERN REGIONAL AQUACULTURE CENTER



NINETEENTH ANNUAL PROGRESS REPORT

For the Period Through August 31, 2006

December, 2006

Southern Regional Aquaculture Center
P.O. Box 197
Stoneville, Mississippi 38776
Phone: 662-686-3285
Fax: 662-686-3320
E-mail: srac@drec.msstate.edu
http://www.msstate.edu/dept/srac

In cooperation with the U.S. Department of Agriculture, Cooperative State Research, Education, & Extension Service

Work summarized in this report was supported in part by Grant Nos. 2002-38500-11805, 2003-38500-12997, 2004-38500-14387 and 2005-38500-15815 from the United States Department of Agriculture sponsored by the Cooperative State Research, Education, and Extension Service.

Nineteenth Annual Progress Report

SOUTHERN REGIONAL AQUACULTURE CENTER
Dr. Craig S. Tucker, Director
P.O. Box 197
Stoneville, Mississippi 38776
Phone: 662-686-3285
Fax: 662-686-3320
E-mail: srac@drec.msstate.edu
http://www.msstate.edu/dept/srac

TABLE OF CONTENTS

PREFACE

In 1980, Congress recognized the opportunity for making significant progress in domestic aquaculture development by passing the National Aquaculture Act (P.L. 96-362). The Act established USDA as the lead agency for aquaculture coordination and called for development of a National Aquaculture Plan. The next year, Congress amended the National Agricultural Research, Extension, and Teaching Policy Act of 1977 (P.L. 95-113) by granting, in Title XIV, Subtitle L, Sec. 1475(d) of the Agriculture and Food Act of 1981 (P.L. 97-98), authority to establish aquaculture research, development, and demonstration centers in the United States.

Congress envisioned the Centers as focal points in a national program of cooperative research, extension, and development activities that would be developed in association with colleges and universities, state Departments of Agriculture, federal facilities, and non-profit private research institutions with demonstrated excellence in aquaculture research and extension. Eventually, five such Centers were established—one in each of the northeastern, north central, southern, western, and tropical Pacific regions of the country. Funding for the Centers was reauthorized in subsequent Farm Bills (the Food, Agriculture, Conservation, and Trade Act of 1990 [P.L. 101-624]; the Agriculture Improvement and Reform Act of 1996 [P.L. 104-127]; and the Farm Security and Rural Investment Act of 2002 [P.L. 107-171]).

Projects that are developed and funded by the Regional Centers are based on industry needs and are designed to directly impact commercial aquaculture development in all states and territories. The Centers are organized to take advantage of the best aquaculture science expertise, education skills, and facilities in the United States. Center programs insure effective coordination and a region-wide, team approach to projects jointly conducted by research, extension, government, and industry personnel. Inter-agency collaboration and shared funding are strongly encouraged.

ACKNOWLEDGMENTS

The Southern Regional Aquaculture Center (SRAC) acknowledges the contributions of the Project Leaders and Participating Scientists involved in the projects reported in this Nineteenth Annual Progress Report. Members of the SRAC Board of Directors, Industry Advisory Council, and Technical Committee have provided valuable inputs to the successful operation of SRAC during the past year. We particularly appreciate the assistance of the chairs of our Board, IAC, TC and those serving as Administrative Advisors.

We also thank the scientists and aquaculturists from across the country who contributed their expertise and valuable time to review SRAC project proposals and publications. Without their help, it would be impossible to maintain the high quality of this program.

INTRODUCTION

Nearly 70% of the United States $1 billion aquaculture crop is produced in the southeastern states. Aquaculture is an important part of southeastern agriculture and its success has come with relatively little private sector support for research and development. The larger, more developed agricultural sectors—such as poultry, cotton and soybeans—are supported by a vast infrastructure of agribusinesses that conducts most of the research needed to sustain commodity growth. Aquaculture, on the other hand, receives little private-sector R&D support, relying instead almost entirely on public-sector funds for technology development.

Although government agencies, particularly the United States Department of Agriculture, have provided significant support for aquaculture research and development, much of that funding is earmarked for specific use by specific institutions. The USDA-CSREES Regional Aquaculture Center program is the only funding activity with the flexibility to stay abreast of industry development, identify problems on a region-wide scale, and implement cooperative, interstate projects to solve those problems.

Since its inception in 1987, the Southern Regional Aquaculture Center has become the centerpiece of aquaculture research and extension in the southeastern United States. In its 19 years of operation, the Center has disbursed more than $12 million to fund multi-state research and extension projects. More than 185 scientists from 30 institutions in the southeast have participated in Center projects.

In the past year, four research projects funded at almost $2 million were in progress. The Center's "Publications" project is in its eleventh year of funding and is under the editorial direction of faculty and staff at Texas A&M University. Eight publications were printed this year, and three more were in various stages of production. To date, the "Publications" project has generated more than 175 fact sheets with contributions from 164 authors from throughout the region.

The most important measure of the impact of projects funded by the Southern Regional Aquaculture Center is the extent to which the results have influenced or improved domestic aquaculture. For example, many people believe that the hybrid catfish produced by crossing the female channel catfish with the male blue catfish is a superior fish for catfish aquaculture, which is by far the largest aquaculture industry in the United States. However, hybrid eggs and fry are difficult to produce and breeding technologies need to be improved to allow the promise of this fish to be realized. Nine scientists at five institutions are conducting research to improve the efficiency of hybrid production and allow economical delivery of the hybrid technology to the catfish industry. At the beginning of this project, only about 4 to 5 million hybrid catfish fry were being hatched per year. Research results from this project have been important in increasing hybrid catfish production to more than 25 million fry hatched in 2006.

Beginning with the first projects funded by the Southern Regional Aquaculture Center, interest among aquaculture research and extension scientists in Center activities has been excellent. We are pleased with the participation by our research and extension scientists in the Southern Region in ad hoc Work Groups and Steering Committees, and their willingness to serve as Project Leaders and Principal Investigators for the projects. We believe this broad-based representation has resulted in strong, cooperative research that will be of long-lasting

benefit to aquaculture producers and consumers, and to the growth of the aquaculture industry in the Southern United States.

This Ninetheenth Annual Progress Report of the Southern Regional Aquaculture Center covers the activities of the Administrative Center during the past year. Progress reports on the four multi-year research and extension projects supported by Southern Regional Aquaculture Center during this reporting period cover the life of the projects from their initiation date through August 31, 2006.

ORGANIZATIONAL STRUCTURE

The Agriculture Acts of 1980 and 1985 authorized establishment of aquaculture research, development and demonstration centers in the United States. With appropriations provided by Congress for the 1987 and 1988 FYs, efforts were undertaken to develop the five Regional Aquaculture Centers now in existence. Organizational activities for SRAC began in 1987, with the first research and extension projects initiated in 1988.

Research and extension problem areas for the southern region are identified each year by the Industry Advisory Council (IAC), which consists of fish farmers and allied industry representatives from across the region. The Technical Committee (TC), consisting of research and extension scientists from all states within the region, works with the IAC to prioritize problem areas. The two groups then work together to develop "Problem Statements" describing objectives of work to solve problems with the highest priority. Using inputs from industry representatives, regional Work Groups of the most qualified research and extension scientists are formed. The Work Groups then plan and conduct the work in conjunction with an Administrative Advisor appointed by the Board. Regional aquaculture funds are allocated to participants in SRAC projects approved by the Board and CSREES. Reviews of project proposals, progress reports, and recommendations for continuation, revision, or termination of projects are made jointly by the TC and IAC and approved by the Board.

The thirteen states and two territories represented by SRAC are Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Mississippi, North Carolina, Oklahoma, Puerto Rico, South Carolina, Tennessee, Texas, U.S. Virgin Islands, and Virginia.

ADMINISTRATIVE CENTER

The Administrative Center is located at the Delta Research and Extension Center, Stoneville, Mississippi. Mississippi State University serves as the Host Institution. All necessary support services for the Board, IAC, TC, Steering Committees and project Work Groups are provided by the Administrative Center. This includes monitoring status and progress of projects, preparing and executing Letters of Agreement, tracking administrative and project expenditures, reviewing progress reports, and assisting Project Leaders and participating institutional Grants Offices as needed.

Operation and funding are approved by the Board for inclusion in the Grant Application submitted annually to USDA/CSREES. The Center staff also prepares and submits to USDA/CSREES an Annual Plan of Work covering Center activities and projects to be funded. Following final approval, Letters of Agreement are prepared and executed with all participating institutions. The Center acts as fiscal agent to disburse and track all funds in accordance with the provisions of the grants. Additional Administrative Center responsibilities are detailed in the "Administrative Activities" section of this report.

BOARD OF DIRECTORS

The Board is the policy-making body for SRAC. Membership provides an appropriate balance among representatives from State Agricultural Experiment Stations, Cooperative Extension Services, 1890 Institutions, and the Administrative Heads Section (AHS) of the Board on Agriculture Assembly (BAA) of the National Association of State Universities and Land Grant Colleges (NASULGC).

The structure of the Board is as follows:

Three members of the 1862 Southern Extension Service Directors Association
Three members of the 1862 Southern Experiment Station Directors Association
One member of the 1890 Association of Research Administrators
One member of the 1890 Association of Extension Administrators
One AHS administrator from the host institution

Members of the Board are:

Sam Fowler, Alabama Cooperative Extension System
Gaines Smith, Alabama Cooperative Extension System
Ivory Lyles, Arkansas Cooperative Extension System
Greg Weidemann, University of Arkansas
Harold Benson, Kentucky State University
David Morrison, Louisiana State University
Vance Watson, Mississippi State University, Chairman

Ex-officio Board members are:

Chair, Industry Advisory Council
Vice-chair, Industry Advisory Council
Co-chair for Extension, Technical Committee
Co-chair for Research, Technical Committee
Director, SRAC

The Board is responsible for 1) overall administration and management of the regional center program; 2) establishment of overall regional aquaculture research and extension goals and allocations of fiscal resources to ensure that the center develops strong programs in both research and extension; 3) establishment of priorities for regional aquaculture research and extension education activities based on inputs from the TC and IAC and guidance from the National Aquaculture Development Plan; 4) review and approval of annual plans of work and accomplishment reports; and 5) final selection of proposals for funding by SRAC.

INDUSTRY ADVISORY COUNCIL

The IAC, which meets at least annually, is composed of representatives of state and regional aquaculture associations, federal, territorial and state agencies, aquaculture producers, aquaculture marketing and processing firms, financial institutions, and other interests or organizations as deemed appropriate by the Board of Directors.

The IAC provides an open forum wherein maximum input from private and public sectors can be gained and incorporated into annual and ongoing plans for SRAC. The chairman serves for two years and is elected by IAC members.

Members of the IAC are:

Neal Anderson, AR
Lynn Blackwood, VA
Bill Cheek, LA
Jane Corbin, TN
Richard Eager, SC
Theop Inslee, OK
Austin Jones, MS
Shorty Jones, MS
Joey Lowery, AR
Robert Mayo, NC
Sandy Miller, GA
Steve Minvielle, LA
Steve Price, KY
Fernando Rodriquez, PR
Brent Rowley, TX
Robert Schmid, TX
Dan Solano, FL
Marty Tanner, FL
Rafe Taylor, AL
David Teichert-Coddington, AL

IAC members serve up to four-year appointments having staggered terms with options for reappointment.

The IAC 1) identifies research and extension needs; 2) works with the TC to prioritize research and extension needs; 3) works with the TC to develop problem statements and recommend funding levels for projects addressing priority research and extension needs; 4) reviews project proposals, progress reports, and termination reports; and 5) recommends to the Board, jointly with the TC, actions regarding new and continuing proposals, proposal modifications and terminations.

TECHNICAL COMMITTEE

The TC consists of representatives from participating research institutions and state extension services, other state or territorial public agencies as appropriate, and private institutions. Membership of the TC includes research and extension scientists representing essentially all states in the region. The TC meets as needed, but at least annually, and has a co-chairman for research and a co-chairman for extension. Co-chairmen serve for two years and are elected by TC members.

Members of the TC for research are:

David Brune, SC
Frank Chapman, FL
Louis D'Abramo, MS
Jason Danaher, VI
Allen Davis, AL
Patricia Duncan, GA
Carole Engle, AR
Delbert Gatlin, TX
Conrad Kleinholz, OK
John Kubaryk, PR
Tom Losordo, NC
Ray McClain, LA
Steve Mims, KY
Mike Oesterling, VA
Larry Wilson, TN

Members of the TC for Extension are:

Jimmy Avery, MS
Ron Blair, TN
Gary Burtle, GA
Jesse Chappell, AL
Dennis Delong, NC
David Heikes, AR
George Luker, OK
Greg Lutz, LA
Michael Masser, TX
Mike Schwarz, VA
Craig Watson, FL
Saul Wiscovich Teruel, PR
Jack Whetstone, SC
Forrest Wynne, KY

Technical Committee members serve up to four-year appointments having staggered terms with options for reappointment.

The TC 1) works with the Industry Advisory Council to prioritize research and extension needs; 2) works with the Industry Advisory Council to develop problem statements and recommend funding levels for projects addressing priority research and extension needs; 3) reviews proposals, progress reports, and termination reports; and 4) recommends to the Board, jointly with the IAC, actions regarding new and continuing proposals, proposal modifications and terminations.

PROJECT CRITERIA

Projects developed within SRAC should meet the following criteria:

- Addresses a problem of fundamental importance to aquaculture in the Southern Region;
- Involves participation by two or more states in the Southern 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 3 years);

PROJECT DEVELOPMENT PROCEDURES

The IAC initiates the project development process by identifying critical problems facing aquaculture in the region. The TC and IAC then jointly prioritize problem areas and recommend the most important research and extension needs to the Board. Writing teams selected from the TC-IAC membership develop "problem statements" for each of the selected priority areas. Problem statements briefly describe the problem area and general objectives of the work to be conducted. The problem statement also includes a recommended funding level and project duration. Draft problem statements are then forwarded to the Board for approval to release project development funds.

Once an area of work has been approved, the Executive Committee (the SRAC Director, the co-chairs of the TC, and the chair and vice-chair of the IAC) appoints a Steering Committee to develop the "Call for Statements of Interest" and oversee development of the project proposal and the conduct of the regional project. The "Call for Statements of Interest" is distributed to state, territorial or federal institutions and private institutions within the Southern Region with demonstrated competence in aquaculture research and development. Interested parties respond by submitting a "Statement of Interest" to the SRAC Adminstrative Office. After careful review of the Statements of Interest, the Steering Committee recommends a Work Group consisting of selected project participants and the Steering Committee. The Work Group is responsible for preparing the regional project proposal and conducting work outlined in the proposal.

Project proposals are reviewed by the Steering Committee, IAC, TC, all project participants and designated peer reviewers from within the region and from outside the region. The SRAC Director submits the project proposal and peer reviews to the Board of Directors for review and approval. Proposals not approved by the Board are returned for revision or eliminated from consideration.

The Director prepares an annual plan of work, including all project proposals approved by the Board, and submits the plan to CSREES for approval. Pending a successful review of the project plan and budget, CSREES notifies SRAC of final approval. Letters of Agreement (subcontracts) between SRAC and participating institutions are then prepared and forwarded for approval and execution by the authorized institutional official. At that point, formal work on the project begins.

ADMINISTRATIVE ACTIVITIES

The SRAC administrative staff consists of the Center Director and Administrative Assistant. A wide variety of support functions for the various SRAC components, including the Board, TC, IAC, Steering Committees and project Work Groups are provided:

- Center Director serves as an ex-officio member of the Board, TC, and IAC.
- Monitor research and extension activities sponsored by SRAC.
- Solicit and receive nominations for memberships on the TC and IAC.
- Coordinate submission of written testimony to the House Agriculture, Rural Development, and Related Agencies Subcommittee on Appropriations regarding RAC support.
- The Director of SRAC serves as a member of the National Coordinating Council for Aquaculture which consists of the Directors of the five Regional Centers and appropriate USDA/CSREES National Program staff.
- Prepare and submit Grant Application to USDA/CSREES entering into funding agreement for each fiscal year, Annual Plan of Work and Amendments.
- Develop and execute appropriate Letters of Agreement with participating institutions in each funded proposal for the purpose of transferring funds and coordinating and implementing projects approved under each of the grants.
- Serve as fiscal agent to review and approve invoices and distribute funds to participating institutions as approved under the grants and as set forth in the Letters of Agreement.
- Prepare budgets for the Administrative Center, track administrative expenditures, and obtain USDA/CSREES approval for project and budget revisions.
- Prepare budget reports for the Board of Directors, tracking expenditures and status of funded projects and the Administrative Center.
- Assist Steering Committees and Work Groups with preparation and revision of proposals for technical and scientific merit, feasibility and applicability to priority problem areas.
- Solicit and coordinate national reviews of project proposals.
- Distribute fact sheets and videos to research and extension contacts throughout the Southern Region, other RACs, and USDA personnel.
- Produce and distribute the "SRAC Annual Progress Report," which includes editing and proofreading the project reports and producing camera-ready copy.
- Produce and maintain the web site for SRAC which provides downloadable copies of all SRAC fact sheets, the Operations Manual and Annual Reports, as well as lists of other research publications and extension contacts in the Southern Region.
- Prepare and distribute Calls for Statements of Interest to research and extension directors and other interested parties throughout the Southern Region.
- Respond to requests from aquaculture producers, the public, and research and extension personnel for copies of fact sheets, research publications and videos produced by SRAC and the other Centers, as well as requests for general aquaculture-related information.

PROGRESS REPORTS

The following cumulative reports detail the progress of research and extension work accomplished for the duration of the respective projects through August 31 of the current year. These reports are prepared by the Project Leaders in conjunction with the institutional Principal Investigators.

PUBLICATIONS, VIDEOS AND COMPUTER SOFTWARE

Reporting Period
April 1, 1995 - August 31, 2006

Funding Level

Year 1	$ 50,000
Year 2	60,948
Year 3	45,900
Year 4	60,500
Year 5	67,000
Year 6	77,358
Year 7	82,205
Year 8	77,384
Year 9	84,113
Year 10	78,700
Year 11	78,115
Total	$762,223

Participants Texas A&M University System serves as Lead Institution, with Dr. Michael Masser as Project Leader. Participants in this project include authors and co-authors from all states in the region as shown in the listing of publications at the end of this report.

PROJECT OBJECTIVES

1. Review and revise, as necessary, all SRAC extension printed and video publications.

2. Establish an ongoing project location to develop and distribute new SRAC educational publications and videos for Southern Region aquaculture industries. This project will be responsible for preparation, peer review, editing, reproduction, and distribution of all Extension and popular-type publications for all SRAC projects.

3. Place current, revised, and new publications in electronic format (e.g., Internet or compact disk) for more efficient use, duplication, and distribution.

ANTICIPATED BENEFITS

The direct benefit from this project to the aquaculture industry is the widespread and ready availability of detailed information on production and marketing of aquacultural products. SRAC fact sheets, videos, and other publications are distributed worldwide to a diverse clientele.

Extension Specialists. When this project was initiated, fewer than half the states had educational materials covering the major aquacultural species in their state. The concept of using the SRAC program to produce timely, high-quality educational materials is based upon the benefits of centralizing the production process while using a region-wide pool of expertise to develop materials. Distribution is then decentralized through the nationwide network of Extension Specialists and County Agents. This process assures an efficient publication process that makes use of the best available talent in specific subject areas. The result is widespread availability of high-quality educational material for scientists, educators, producers, and the general public.

Educators. Many colleges and universities in the United States use SRAC technical fact sheets as reference material in aquaculture and fisheries courses. Educational institutions at the elementary and secondary level use SRAC extension materials in the classroom to make students aware of aquaculture production and associated trades as a possible vocation.

Consumers. Information is readily available for consumers who are seeking background information on aquaculture.

Producer. Information on the use of therapeutants, pesticides, methods of calculating treatment rates, and possible alternative crops and marketing strategies is in constant demand by aquaculturists. Videos that demonstrate such techniques are a ready source of "how-to" information.

Potential investors. Detailed information on production and marketing constraints and ways to alleviate or manage those constraints are particularly helpful to people making decisions about entering the aquaculture business. Economic information is used by lending agencies and potential investors, as well as established producers who use the information to help make day-to-day decisions on farm management.

Internet access. Availability of SRAC publications via the Internet and compact disk makes access faster and easier, facilitates searching for needed information, and reduces storage space requirements for printed documents.

Results at a glance...

- ☆ *164 authors from across the United States have contributed to SRAC's publication projects.*
- ☆ *Eight fact sheets and a video were completed this year with three fact sheets in progress.*
- ☆ *Sixteen scientists from across the Southern Region contributed to publications completed by SRAC this year.*
- ☆ *SRAC has now published 179 fact sheets and species profiles, 4 project summaries, 19 research publications, and 20 videos.*
- ☆ *Educators in schools and colleges use SRAC publications in classrooms throughout the U.S. and the world.*

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

During this current project year, eight new fact sheets were completed and the Aquaplant web site updated. All publications have been distributed throughout the Southern Region and to interested Extension Specialists in other regions. Three fact sheets are in some stage of writing, production, or revision. Five fact sheets have currently not had drafts submitted. Three project summaries have also not yet been submitted. One DVD on Crawfish Aquaculture and one on Water Quality are in our review process. All SRAC publications are based on research conducted within the region or in surrounding areas.

Research funding from universities within the region, as well as funding from private sources, has been used to support the work on which the fact sheets are based. Copies of all SRAC fact sheets are available at <http://www.msstate.edu/dept/srac> and <http://srac.tamu.edu>.

WORK PLANNED

During the next project year, four fact sheets will be revised and five new fact sheets/species profiles, two project summaries, and a DVD on crawfish aquaculture will be produced. The new fact sheets will address 1) hard clam hatcheries, 2) sperm cryopreservation, 3) softshell crab shedding techniques, 4) new seining technologies, and 5) in-pond grading techniques.

The four fact sheets to be revised are: 1) three fact sheets on forage species, and 2) one fact sheet on aquatic herbicides.

Final project summaries from the projects "Management of Environmentally Derived Off-Flavors in Warmwater Fish Ponds" and "Optimizing Nutrient Utilization and Waste Control through Diet Composition and Feeding Strategies" will be developed.

A DVD on crawfish aquaculture will also be developed.

Results at a glance...

Titles of some recent SRAC publications:

- *Pond Production of the Freshwater Prawns in Temperate Climates*
- *Economics of Freshwater Prawn Farming in the United States*
- *Queen Conch,* Strombus gigas
- *Yellow Perch,* Perca flavescens
- *Aquatic Weed Management: Herbicides*
- *Crawfish Aquaculture - Marketing*
- *Managing Hatch Rate and Diseases in Catfish Eggs*
- *Pond Aeration*

IMPACTS

This is a highly productive project with significant regional, national, and international impact. Fact sheets and videos are requested and used by clientele in all 50 states on a regular basis. Within the Southern Region, more than 80 fact sheets and six videos are distributed on request daily. Fact sheets generated within the Southern Region are also widely distributed by RACs and extension personnel in

other regions. An average of 5 to 20 SRAC fact sheets and three videos are distributed daily from each of the other four regions. This means that about 20,000 fact sheets and 3,200 videos per year are used by interested producers or consumers. In addition to direct requests for printed material, fact sheets and other informational materials are accessed daily from the SRAC web site by people searching for technical information. In the period January through September of 2006, more than 85,000 fact sheets were downloaded (printed or saved) off the SRAC web site. Since the fact sheets are also accessible through numerous other university research and extension web sites, the total usage and impact is undoubtedly several times greater.

Publications and videos produced by SRAC are increasingly used in educating high school and college students about aquaculture. In recent years there has been a rapid expansion of aquaculture curricula in high schools. These programs heavily utilize our publications and videos for educational purposes but usage is impossible to measure because many people access the information from Internet sites. Aquaculture and fisheries courses taught at many colleges and universities also use SRAC technical fact sheets as part of their course reference material.

Another important impact is the education of local, state, and federal regulators about the aquaculture industry. This impact is difficult to measure but feedback from personnel in two states indicates that the fact sheets are recommended reading for all new employees dealing with aquaculture water quality, exotic species, and other permitting duties. This should be a positive influence toward making aquaculturists better understood and the development of more enlightened regulations.

The impact on consumers of aquaculture products is also likely significant, although it has not been quantified. Consumers are primarily interested in a wholesome, safe, and inexpensive product, and it has been reported that the consumer-oriented fact sheets and videos developed within SRAC have generated more interest than the producer-directed materials. The fact sheets are in demand in both the English and Spanish versions and, as more information becomes available, extension materials on food safety will be in increased demand by health conscious consumers.

Results at a glance...

- ☆ *In the months from January through September 2006, more than 85,000 fact sheets were downloaded from the SRAC web site.*
- ☆ *All fact sheets completed by this project to date are available on the Internet at <http://www.msstate.edu/dept/srac> and <http://srac.tamu.edu>.*

PUBLICATIONS, MANUSCRIPTS OR PAPERS PRESENTED

Fact Sheets Completed (9/1/05 - 8/31/2006)

D'Abramo, Louis R., James H. Tidwell, Mack Fondren and Cortney L. Ohs. Pond production of the freshwater prawns in temperate climates. SRAC Fact Sheet 484 (revision).

Dasgupta, Siddhartha. Economics of freshwater prawn farming in the United States. SRAC Fact Sheet 4830.

Davis, Megan. Species Profile: Queen conch, *Strombus gigas*. SRAC Fact Sheet 7203.

Hinshaw, J. M. Species Profile: Yellow perch, *Perca flavescens*. SRAC Fact Sheet 7204.

Masser, Michael P., Tim R. Murphy and James L. Shelton. Aquatic weed management: herbicides. SRAC Fact Sheet 361 (revision).

Romaire, Robert P., W. Ray McClain, Mark G. Shirley and C. Greg Lutz. Crawfish aquaculture–marketing. SRAC Fact Sheet 2402 (replaces SRAC Fact Sheet 242).

Small, Brian C. Managing hatch rate and diseases in catfish eggs. SRAC Fact Sheet 1804.

Tucker, Craig. Pond aeration. SRAC Fact Sheet 3700 (replaces SRAC Fact Sheets 370 and 371).

Manuscripts in review

Minchew, C. Douglas, Mack W. Fondren and Edwin H. Robinson. Advances in catfish harvesting gear: seines and live cars.

Mischke, Charles C., Joseph E. Morris and Ryan L. Lane. Species profile: hybrid sunfish.

Rakocy, James E., Michael P. Masser and Thomas M. Losordo. Recirculating aquaculture tank production systems: aquaponics–integrating fish and plant culture. SRAC Fact Sheet 454 (revision).

Minchew, C. Douglas, Mark W. Fondren, Edwin H. Robinson, John W. Watson, and Charles W. Taylor. Advances in catfish harvesting gear: seines and livecars.

DVD in review

Benedict, Linda, W. Ray McClain, Robert P. Romaire, Greg Lutz, Mark Shirley, and Craig Gautreaux. Crawfish: Louisiana's Culinary Crustacean.

Masser, Michael P. Water Quality Testing and Management.

On-going project

Updating of the AQUAPLANT web site on aquatic weed management. Michael Masser.

◆ ◆ ◆

INNOVATIVE TECHNOLOGIES AND METHODOLOGIES FOR COMMERCIAL-SCALE POND AQUACULTURE

Reporting Period
March 1, 2004 - August 31, 2006

Funding Level	Year 1	$314,409
	Year 2	$287,451
	Year 3	$213,168
	Year 4	$170,096
	Total	$985,124

Participants	Auburn University (Lead Institution)	Claude Boyd
	Clemson University	David Brune, T. E. Schwedler
	Louisiana State University	John Hargreaves, Robert Romaire
	Mississippi State University	Doug Minchew, Charles Mischke, Filip To
	University of Arkansas at Pine Bluff	Carole Engle, David Heikes
	USDA/ARS (Stuttgart)	Bart Green
	USDA/ARS (Stoneville)	Les Torrans

Administrative Advisor
Dr. Kenneth J. Roberts, Associate Vice Chancellor
LSU Ag Center
Baton Rouge, Louisiana

PROJECT OBJECTIVES

1. Evaluate new or improved production systems for channel catfish.
    a. Continuous production and inventory control with the partitioned aquaculture system.
    b. Installation of low-cost, semi-confinement systems in commercial-scale, earthen ponds.
    c. Fry and food fish production using in-pond raceways with the option for culturing supplemental species in open-pond areas.
    d. High-intensity production in heterotrophic-based culture units.
2. Improve equipment to enhance culture.
    a. Motor-powered U-tube aerator for commercial-scale channel catfish ponds.
    b. Low-head, low-speed paddlewheel aerator for crawfish ponds.
    c. Low-power, electrically-enhanced seine to harvest market-sized channel catfish from commercial-scale ponds.

3. Assess energy, material, and economic efficiency of production systems.

   a. Quantify energy, protein, and water use in traditional systems for channel catfish culture.
   b. Develop and evaluate economic and financial models of existing and improved production practices and technologies.

ANTICIPATED BENEFITS

Aquaculture operations in the southeastern United States find it increasingly difficult to maintain profitability as production costs increase and farm gate prices remain relatively low. Solutions to the problem are complex and multifaceted, but improved production efficiency can decrease production costs and improve the prospects for profitability. This project will provide new technology for production systems, aeration and harvesting techniques, and use of energy, materials, and capital. These technologies will be valuable in improving the profitability of aquaculture in the southeast.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Objective 1. *Evaluate new or improved production systems for channel catfish.*

Objective 1a. *Continuous production and inventory control with the partitioned aquaculture system.*

Clemson University. Studies are being conducted at Clemson University on continuous production with inventory control in partitioned aquaculture systems (PAS). On June 10, 2005, channel catfish fry were stocked in six cells (1.83 m × 3.05 m × 1.22 m deep) located within the 0.81-ha PAS system (Figures 1, 2, and 3). The specific experiments for the 2005 research focused on 1) physical holding and handling of fry and fingerlings, 2) stocking density and required water flow rates, 3) feed presentation and food consumption, and 4) growth response under raceway culture conditions as opposed to an "accelerated" fingerling culture pond.

Six, 5.7-m^3 PAS cells were stocked with 5,000 fry (in three cells) and 10,000 fry (in three cells) on June 10. The fry were held in bins (46 cm × 76 cm × 30 cm deep) with 0.16-cm mesh screens for 1 week and then transferred to bins with 0.32-cm

Results at a glance...

- *In 2005, channel catfish fry were stocked into 5.7-m^3 PAS cells equipped with adjustable depth net-pens with feed applied with automated feeders for a period of 120 days. After only 4 months of growth the fingerlings were harvesting at average sizes ranging from 122 to 158 g each. A preliminary feed uptake relationship was developed as: Percent Body Wt Fed = 0.3223 X -0.551 where X = fish weight (g). The coefficient of determination (R^2) was 0.846. Additional growth trials (in 2006) are currently underway to determine optional feed application rates, and maximum fingerling carrying capacity per acre.*

Figure 1. Overview of the 0.8-ha Clemson PAS unit with fingerling production cells.

Figure 2. Six, 4.5-m^2 fingerling production cell with aerator-driven water flow.

Figure 3. Individual fingerling production cell with aerator-driven water flow

mesh screens for an additional week. After having reached an average size of 1.2 to 1.4 g, fingerlings were released into the 0.63-cm mesh net-pens held within the 5.58-m^2 PAS cells. Each cell was supplied with water delivered by a 0.56-kW submerged aerator providing between 280 to 720 L/min to individual cells (Figures 2 and 3). After initial stocking, fish were fed starter feed of 52% to 56% protein supplied using automated feeders (Figure 4).

After 6 weeks, fingerlings had reached 11 to 14 g and hand feeding was initiated. At 7 weeks, fish in cells containing 10,000 fingerlings had reached 14 to 15 g, and were moved to grow-out raceways in the 0.1-ha PAS units (2.1 m × 9.1 m × 1.22 m deep). At the end of 8.5 weeks, fingerlings had reached 27 to 32 g in units stocked at 5,000 per cell, and 20 to 22 g in cells stocked at 10,000 per cell.

On October 10, 2005, fingerlings in the cells, raceways and control ponds were harvested, sorted, counted and weighed (Table 1; Figure 5). After 120 days of culture, the fingerlings grew from an initial weight of 0.10 g/fish (3 to 7 days old) to an average harvest weight of 122 to 158 g (Figure 6). Feed uptake for the pooled fingerlings was fit to a power law (Figure 7) with the result being Percent Body Weight Fed = $0.3223\ X^{-0.551}$ where X = fish weight (g). The coefficient of determination (R^2) was 0.846.

In addition to the fingerling culture trials conducted within cells and raceways, experiments were initiated to study the possibility of using PAS cells and raceways to provide an initial growth acceleration before stocking and grow-out in conventional fingerling ponds. A conventional, 0.20-ha fingerling culture pond was stocked with 34,000 fry (0.03 g/fish), while 17,000 fry of the same cohort were held in bins for 2 weeks until reaching 1.4 g. They then were stocked into a conventional, 0.12-ha fingerling culture pond. To date, the fingerlings in both ponds are of similar size. This suggests the importance of converting fry or fingerlings to floating feed as quickly as possible. Growth response in the "accelerated" pond was delayed as a result of slow response of the fish to hand feeding after being stocked in the pond.

On June 1, 2006, channel catfish fry were stocked

Figure 4. Automatic feeders used to feed fingerlings during initials stages of culture.

Table 1. Average fingerling weight, density (in cells and ponds) and feed application rates in 2005.

Unit #	Size (g/fish)	Density (kg/m^3)	Loading/ feed(kg/ha)
Cell 1	122	112.9	2,576/63
Cell 2	139	136.7	2,576/63
Cell 3	124	111.3	2,576/63
Cell 4	158	47.7	2,576/63
Race 1	73	28.6	3,696/112
Race 2	77	28.6	9,072/215
Race 3	56	19.1	9,072/215
Pond 1	38	0.95	5,600/78
Pond 2	49	0.64	3,808/56

in nine, 1.21 m × 3.66 m × 1.22 m deep cells located within the Clemson 0.8-ha PAS system. The specific experiments for the 2006 system are focused on 1) physical holding and handling of fry and fingerlings, 2) required water flow rates, 3) optimum food application rate, and 4) growth response under cell as compared to raceway conditions.

Nine, 5.7 m^3 cinder block cells were stocked with 3,000 fry in each cell on June 1, 2006. The fry were held in 930-cm^2 bins for 2 weeks, transferred to 3,710-cm^2 bins for two additional weeks, and after having reached 1.8 to 2.0 g in size, the fingerlings were released into a 0.32-cm mesh net-pens held within the cells. Each cell was supplied with water flow delivered from two, 0.56-kW submerged aerators providing between 280 to 946 L/min flow to the individual cells. From the initial stocking, the fish were fed starter feed supplied with automatic feeders. After 8 weeks the fingerlings had reached 14-17 g in size. At 8 weeks, 14 to 15 g fingerlings were stocked into the 0.12-ha PAS raceways units

Figure 5. Fry stocking and fingerling harvest sizes.

Figure 6. Final harvest fingerling size distribution in cell 2 (average wt = 139 gm) in 2005.

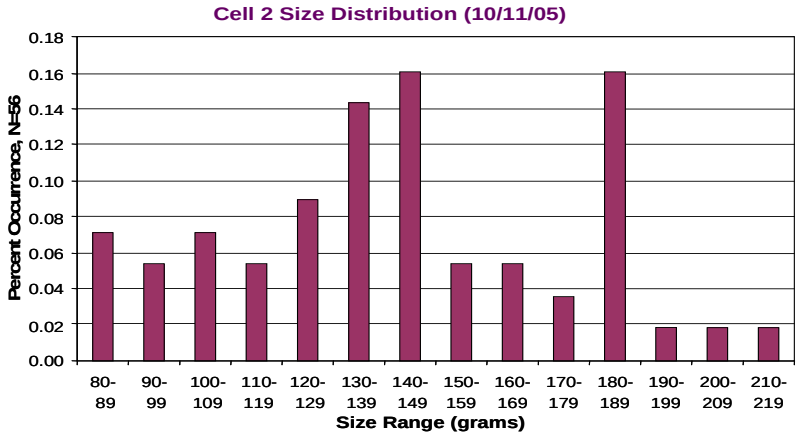
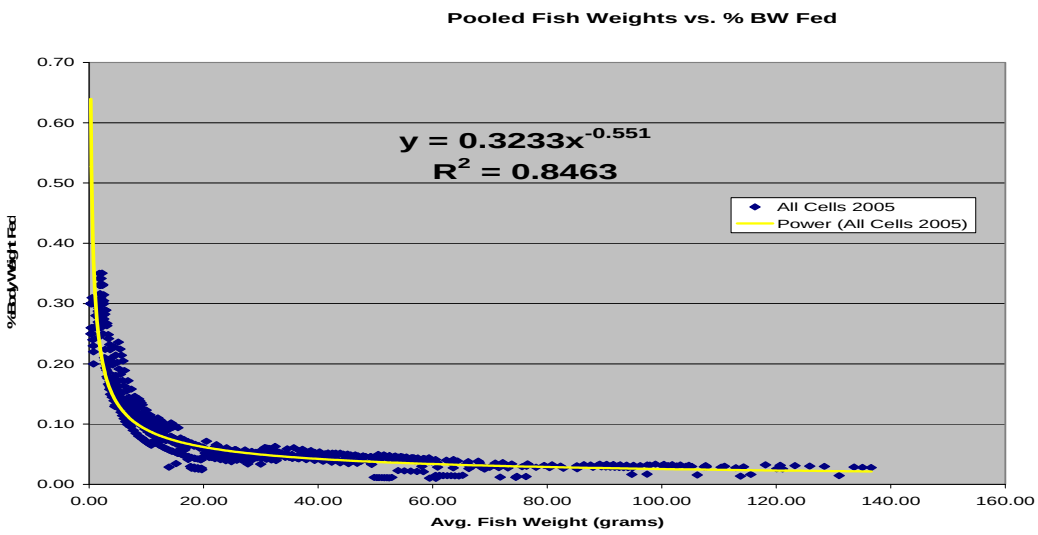


Figure 7. Observed, pooled fry/fingerling feed application rate vs fingerling wt. in 2005.



(15.2 m × 2.1 m × 1.22 m deep) at a stocking rate of 74,130 fingerlings/ha.

Mississippi State University. The PAS as currently configured in the Clemson system consists of an extensive, shallow algal growth basin (representing about 95% of the total system water surface area), and an intensive fish-confinement area in which fish are crowded at about 20 to 40 times the density of traditional ponds. In this objective, a modified PAS system has been constructed that confines fish at a much lower density than the Clemson system. The "extensive PAS" was built with a lower proportion of the total system area in the algal growth basin (about 80% of the total area) and a higher percentage of area in the fish-holding area (fish will be held at only 5 times the density of traditional ponds). The overall concept is to take advantage of the fish confinement benefits of the PAS (facilitation of inventory, harvest, health management, and protection against predation) while avoiding the need for intensive system management.

In year 2004, one system was constructed in an existing 0.324-ha earthen pond at the National Warmwater Aquaculture Center, Stoneville, Mississippi. A 2-m-high earthen levee was constructed to separate the pond into two sections: a 0.227-ha algal basin and 0.073-ha fish confinement area. Two, 3-m concrete-block sluiceways were constructed at either end of the cross-levee. One sluiceway was equipped with a six-bladed, 3-m long paddlewheel to induce water flow out of the fish confinement area and into the algal basin. The paddlewheel is 2 m in diameter and was installed to provide minimal clearance (less than 3 cm) with the sluiceway bottom and side walls. The paddlewheel can be operated at 1 to 6 rpm via a variable-speed, 3.7-kW hydraulic motor. The other sluiceway accommodates return flow from the algal basins into the fish confinement. Both sluiceways were fitted with double barriers of 2.54-cm expanded metal to prevent fish escape out of the confinement area. Aeration in the fish confinement area is provided by eight, highly efficient deep-water release membrane diffusers. Air to the diffuser array will be provided by a 3.7-kW blower through a manifold of PVC pipe. The aeration system is designed to provide a

field oxygen transfer rate of approximately 4.5 kg oxygen/hour at a water temperature of 30° C and 2 mg/L ambient dissolved oxygen. That rate should be adequate to meet the respiratory needs of at least 8,000 kg of fish.

The system was stocked with approximately 4,000 kg of catfish to optimize operating parameters. A paddlewheel speed of 1 rpm resulted in a water flow of 15.2 m^3/minute through the fish-confinement basin. This flow rate was adequate to prevent accumulation of waste ammonia in the fish-confinement area at fish feeding rates of 150 kg/ha per day. At fish feeding rates of 175 to 200 kg/ha per day, total ammonia concentrations did not exceed 0.5 mg/L and dissolved oxygen concentrations remained above 3 mg/L.

In spring 2005, the system was stocked with 7,400 stocker-sized catfish (24,710 fish/ha). Fish grew from an initial average weight of 0.08 kg/fish to an average of 0.78 kg/fish in a 6-month growing season. Total harvest weight was 5681 kg (18,940 kg/ha), for a net fish production of 5089 kg (16,960 kg/ha). Fish survival was 99% at a feed conversion efficiency of 1.87 kg of feed/kg of fish produced.

In spring 2006, the system was stocked with 11,100 fish (37,065 fish/ha). As of August 23, 2006 fish averaged 0.43 kg/fish, giving a standing crop of approximately 16,000 kg/ha.

Two more earthen PAS systems are under construction, including one full-sized, 2-ha system. All systems should be operational in summer of 2007.

Objective 1b. *Installation of low-cost, semi-confinement systems in commercial-scale, earthen ponds.*

University of Arkansas at Pine Bluff. Final results have been compiled and analyzed for the study outlined last year where five confinement systems were installed in research ponds at the UAPB Aquaculture Research facility to determine whether physically separating fish by size group with a pond confinement system would result in improved yield, survival, FCR and growth compared to normal multiple-batch culture. This study consisted of ten, 0.1-ha ponds; five of these were control ponds and did not have barriers. The five treatment ponds had a 1.27 cm × 2.54 cm PVC-coated wire mesh barrier that partitioned off a third of the pond. In the treatment ponds, fingerling catfish were reared in the smaller portion of the pond and larger carryover fish were stocked in the remaining larger portion of the pond. The fish in the control ponds were allowed to co-mingle as in traditional multiple-batch culture. Ponds were seined every 2 months during the growing season and average weights were calculated to estimate growth. After harvest, survival and FCR were calculated. The facility was stocked on April 28, 2005 and the study terminated on October 18, 2005. Net yield, total feed fed, mean daily feeding rate, feed conversion ratio and survival were compared among treatments and controls with t-tests (Table 2).

Mean net yield of the fingerlings, total feed fed (kg/ha), and mean daily feeding rate (kg/ha/d) were greater in the confinement system ($P>0.05$) than in the control ponds. However, there were no differences ($P>0.05$) in net yield of carryover fish, pond feed conversion ratio, or survival of either size of fish in the confinement system as compared to the control ponds. There were no significant differences ($P>0.05$) in TAN, unionized ammonia, nitrite, nitrate, total nitrogen, and total phosphorous. The confinement system appears to offer potential to increase yield of fingerling catfish because of greater feed consumption in the system when the barrier is used to separate size classes.

Table 2. Selected data of fingerling and carryover fish in control and confinement ponds. Values with the same letter in the row are not significantly different. All values are mean ± SD.

	Control[1]	Confinement
Net Yield (kg/ha)		
Fingerlings	1,788 ± 448a	2,391 ± 158b
Carryover	4,882 ± 490a	4,712 ± 679a
Total Feed Fed (kg/ha)	11,095 ± 541a	12,189 ± 579b
Mean Daily Feeding Rate (kg/ha/d)	62 ± 3a	67 ± 3b
Feed Conversion Ratio	1.67 ± 0.2a	1.68 ± 0.1a

Another study was initiated in the spring of 2006 to compare production of catfish within the barrier system to open pond culture. This will help determine if there are any potential culture advantages to confining catfish to 1/3 of the total pond area. This study is currently under way and final results will be available after the final harvest in October 2006.

Figure 8. Construction of a commercial-scale confinement system in a 6-ha earthen pond in Chicot County, Arkansas.

In order to evaluate scale-up issues, a commercial size barrier system is currently under construction on a catfish production facility in Chicot County, Arkansas (Figure 8). The barrier system was constructed in a 6-ha earthen pond that was under renovation. Construction of the barrier system should be completed by mid-October 2006 and we anticipate stocking this demonstration pond by mid-November.

Objective 1c. *Fry and food fish production using in-pond raceways with the option for culturing supplemental species in open-pond areas.*

Louisiana State University. A study will be conducted to evaluate the technical and economic performance of an in-pond raceway system for producing both fry and foodfish-sized channel catfish in raceways and various combinations of other species in the open-pond areas. Because the

cooperating commercial producer ceased his catfish farming operation, this objective will be conducted at the Aquaculture Research Station, LSU AgCenter, in Baton Rouge, in larger ponds located on the station. An existing 0.3-ha demonstration PAS system is currently being renovated for use in this study. The levee and baffles are being re-built and a new water quality monitoring system is being installed. Two, 0.4-ha experimental earthen ponds are being retrofitted as modified PAS ponds. Engineering design plans are being drafted for the 0.4-ha ponds and construction should be completed in spring 2007.

Objective 1d. *High intensity production in heterotrophic-based culture units.*

Louisiana State University. Louisiana State University (LSU) could not conduct objective 1(c) in Year 2 because the commercial cooperator ceased operation. Therefore, the SRAC work plan was modified and LSU participated in objective 1(d) "high intensity production in heterotrophic-based culture units". The performance of a heterotropic-based "biofloc" system consisting of eight 1.5-m^3 tank mesocosms stocked with tilapia (3.0 kg/m^3, 41 g/fish) was investigated in an indoor wet laboratory. Vigorous diffused aeration was provided to maintain solids in suspension, provide oxygen, and remove carbon dioxide. Settleable solids concentration was measured daily in each tank and maintained at eight different nominal concentrations (5, 10, 15, 20, 25, 50, 75, 100 mL/L) through intermittent operation of 80-L settling columns and removal of solids. The range of settleable solids concentration was equivalent to a range of total suspended solids concentration of about 250 to 1,000 mg/L. Daily feeding rate was increased weekly by 25 g/m^3 and water quality was measured weekly before feeding rate adjustments. The biofloc system operated effectively within arbitrarily established water quality limits for ammonia, nitrite, carbon dioxide, and dissolved oxygen concentrations across a broad range of solids concentration and feed loading. After 11 weeks at a daily feeding rate of 275 g/m^3, total ammonia concentration exceeded the pre-established criterion of 2 mg N/L in all tanks. For these indoor tank mesocosms, the sustainable maximum daily feeding rate is about 200 g/m^3. At daily feeding rates greater than 200 g/m^3, control of solids concentration became more difficult and water quality became more variable. Process instability was related to the development of filamentous bacteria that produced severe foaming associated with flocs with poor settling characteristics. As solids concentration increased, water respiration rate, nitrification rate, and solids retention time increased, and hydraulic retention time decreased. Increases in water respiration and nitrification rates also were related to increases in daily feeding rate. There was no effect of solids concentration on specific growth rate (1.27 %/d), final biomass density (9.8 kg/m^3), and feed conversion ratio (1.83). After a cumulative feed loading of about 12 kg/m^3 and a cumulative feed burden of about 130 kg/m^3, tilapia in all tanks displayed signs of respiratory distress and stopped feeding. All tilapia in one tank died. This loading limit was independent of solids concentration. Hypotheses offered to explain this effect include combined metal toxicity related to low hardness, nitrate toxicity, or some factor associated with the accumulation of dissolved organic matter. Within three days of a 50% dilution of tank volume, fish resumed feeding, indicating that dilution sufficiently reduced the concentration of the factor that caused cessation of feeding.

United States Department of Agriculture-Pine Bluff. An intensively-managed, microbial-based production system has been used successfully to culture penaeid shrimp and tilapia, and appears to have potential application in growing catfish. When used for penaeid shrimp or tilapia production, the

microbial floc that develops in the culture unit serves as a sink for ammonia-nitrogen and as a supplemental food source for the culture species. While it is unlikely that catfish will derive nutritional benefit from the microbial floc, bacterial control of ammonia-nitrogen may permit increased catfish stocking and feeding rates.

Nine raceways (4.6 m × 9.2 m × 0.9-m water depth) with semi-circular ends that are equipped with a center divider and lined with HDPE were filled with well water on 6 April 2005 and each fertilized with 0.32 kg 18-46-0 fertilizer. Stock salt (5 kg/tank) was added on 12 April 2005 and 12 August 2005. Each raceway was equipped with a 0.37-kW electric paddlewheel aerator that operated continuously. Well water was added periodically to replace evaporative losses.

In 2004, stocker channel catfish (un-vaccinated) stocked in the raceways suffered high mortality from ESC (*Edwardsiella ictaluri*). Channel × blue hybrid catfish were stocked in 2005 because they appear more resistant to ESC. Hybrid catfish (mean weight 0.085 kg/fish), obtained from the ARS Catfish Genetics Research Unit, Stoneville, Mississippi, were stocked on 13 April 2005 at 25, 50, 75, 100, 125, 150, 175, 200, or 225 fish/raceway. A stocking error was detected for the 125-fish treatment, so that treatment was excluded. Fish were fed a 32% protein floating feed daily to apparent satiation. Beginning on 14 July 2005, white flour (0.7 kg/kg feed), as a flour-water slurry, was added daily as an additional source of carbon to raceways. Agricultural limestone (250 mesh) was added to raceways as needed beginning in mid-August to mitigate low water pH. All raceways were harvested by draining on 17 October 2005.

Hybrid catfish survival after 188 days ranged from 61.3 to 79.1%, with an average of 71.0% (Table 3). Mean individual weight at harvest appeared independent of stocking rate up to a stocking rate of 100 fish/raceway (2.4 fish/m^2), and decreased linearly ($y = -0.0017x + 0.6988$; $R^2 = 0.9835$) at stocking rates of 100 to 225 fish/raceway (2.4 to 5.5 fish/m^2) (Table 3; Figure 9). Fish biomass at harvest increased linearly with stocking rate (Figure 10). Feed conversion was variable, ranging from 1.8 to 6.3, and averaged 2.8. Daily feed rates ranged from 13 to 331 kg/ha. The feeding response by hybrid catfish in the raceways was variable and appeared unpredictable.

Table 3. Mean weight at harvest, gross and net yields, and survival of channel × blue hybrid catfish after 188 days. At stocking, mean fish weight was 0.085 kg/fish.

Fish/Raceway	Mean Weight (kg/fish)	Yield (kg/m^3)		Survival (%)
		Gross	Net	
25	0.53	0.22	0.18	64.0
50	0.50	0.47	0.38	78.0
75	0.54	0.66	0.52	61.3
100	0.54	0.86	0.67	77.0
150	0.44	0.94	0.66	70.7
175	0.40	1.16	0.84	73.7
200	0.35	1.08	0.69	64.0
225	0.34	1.34	0.86	79.1

Figure 9. Mean individual weight at harvest of channel × blue hybrid catfish stocked at 25 to 225 fish in 41-m^2 raceways.

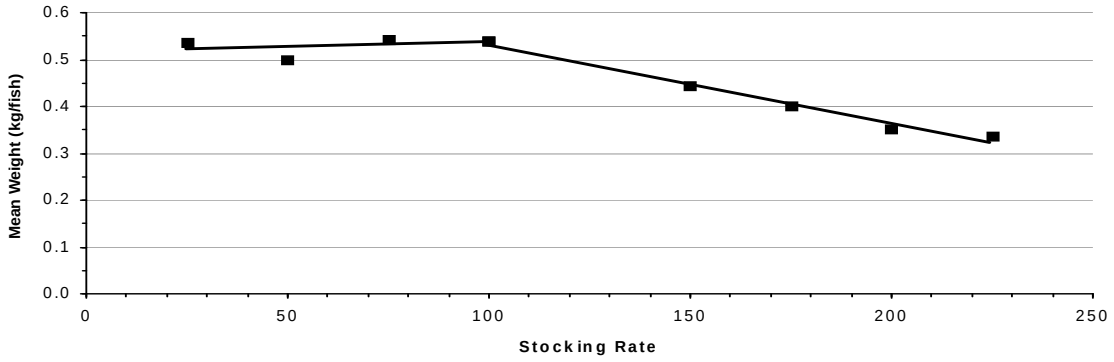
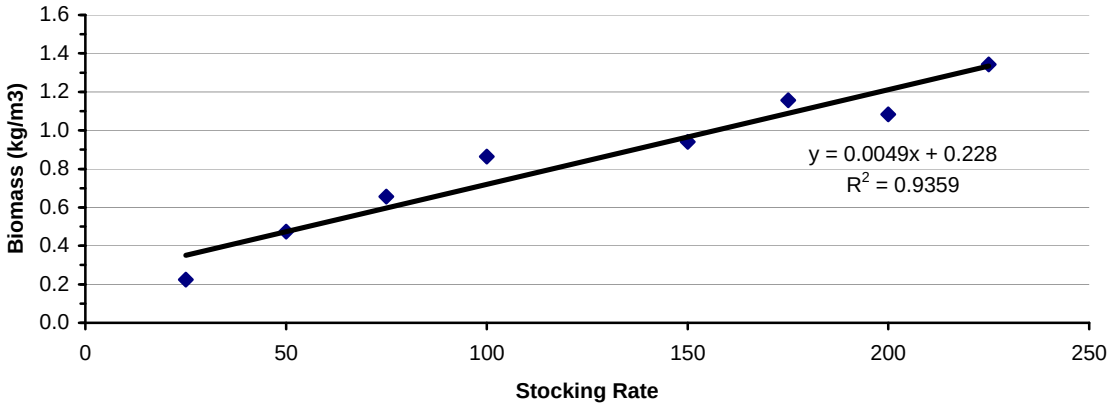


Figure 10. Relationship between final biomass and stocking rate of channel × blue hybrid catfish in 41-m^2 raceways.



Mean weekly nitrite-nitrogen concentrations were low and independent of fish stocking rate (or the total amount of feed fed; Table 4). Nitrite-nitrogen concentrations remained low throughout the experiment except between days 60-80 when concentrations spiked as high as 5.78 mg/L NO_2-N. Mean weekly nitrate-nitrogen concentrations were high and increased as fish stocking rate increased (Table 4). Concentrations of NO_3-N were 0.30 mg/L or less through about day 60, after which concentrations increased. Mean weekly total ammonia-nitrogen (NH_3-N) concentrations were low and independent of fish stocking rate (Table 4). There were several spikes in total NH_3-N concentration, generally in the raceways stocked with greater than 150 fish. The concentration spikes were short-lived and likely inconsequential to stocked fish because pH values were

Table 4. Mean weekly concentrations of dissolved inorganic nitrogen and phosphorus, total nitrogen and phosphorus, organic nitrogen, pH, and chlorophyll *a* in raceways stocked with 25 to 225 channel × blue hybrid catfish.

Fish/ Raceway	NO_2-N	NO_3-N	NH_3-N	Total N	Organic N	PO_4-P	Total P	pH	Chlorophyll *a*
				mg/L					mg/m^3
25	0.390	13.88	0.03	30.91	16.60	0.26	1.07	7.30	1,241.6
50	0.056	7.19	0.29	22.57	15.03	0.16	0.93	7.50	908.1
75	0.197	9.66	0.01	25.77	15.90	0.08	0.74	7.29	1,264.4
100	0.108	12.31	0.01	32.08	19.65	0.24	1.03	7.28	803.2
150	0.225	16.83	0.02	43.13	26.06	0.27	1.38	7.07	1,223.8
175	0.289	18.75	0.03	45.23	26.15	0.15	1.11	7.01	1,126.8
200	0.106	21.60	0.35	53.08	31.04	0.57	1.50	6.74	1,461.0
225	0.205	28.82	0.38	67.43	38.02	0.76	2.00	6.64	1,454.5

less than 7.9, and often less than 7.0. A maximum of about 5% of the total NH_3-N would be present as un-ionized ammonia at the water temperatures when the concentration spikes were observed. Mean weekly total nitrogen (N) and organic N concentrations were high and each increased linearly as fish stocking rate increased ($R^2 = 0.855$ and $R^2 = 0.909$, respectively; Table 4). Total N and organic N concentrations increased throughout the experiment. Organic N was, on average, 59% of the total N concentration.

Mean, weekly soluble reactive phosphorus was low and independent of fish stocking rate (or total amount of feed fed) below about 150 to 175 fish/raceway (Table 4). Above 150 to 175 fish/raceway mean weekly concentrations increased linearly with increased fish stocking rate. Total phosphorus mean weekly concentrations ranged from 0.93 to 2.00 mg/L PO_4-P and increased linearly as stocking rate increased ($R^2 = 0.809$; Table 4).

Mean weekly early morning pH was 7.50 or less and decreased linearly as fish stocking rate increased ($R^2 = 0.869$; Table 4). During the first 100 days, mean, weekly early morning pH values were similar among raceways, and ranged from pH 7 to 8. After day 100, weekly early morning means became more variable and trended lower at stocking rates 150 fish/raceway and greater. Afternoon pH generally was 0.5 to 1.0 pH units greater than the morning pH.

Mean, weekly chlorophyll *a* concentrations were high and increased linearly ($R^2 = 0.425$; Table 4). Chlorophyll *a* concentrations increased throughout the experiment in all raceways, attaining concentrations of 1,000 to 2,500 mg/m^3 at the end of the experiment. A combined photoautotrophic-autotrophic bacteria system appeared to control raceway water quality. Phytoplankton (photoautrotrophic) removed dissolved inorganic nitrogen and inorganic carbon as alkalinity or carbon dioxide. Autotrophic bacteria involved in nitrification oxidize ammonia to nitrate in a two-step process mediated by bacteria of two distinct genera. The populations of nitrifying bacteria appear to have become established in two stages beginning with increasing populations of ammonia oxidizing bacteria around days 60 to 80 that produced a spike in nitrite-nitrogen concentrations. Populations of nitrite oxidizing bacteria lagged slightly, with concentrations of nitrate beginning to increase around day 80. Total ammonia-nitrogen remained low throughout the experiment.

Nitrification results in decreased pH values, which were more apparent in raceways with the higher stocking rates. Applications of agricultural limestone were necessary in all raceways to mitigate the decrease in pH.

The 2006-2007 trial continued to investigate the effect of stocking rate on production of catfish in heterotrophic-based raceway culture units. Three stocking rates were selected based on the 2005-2006 results.

Nine raceways (4.6 m × 9.2 m × 0.9-m water depth) with semi-circular ends are equipped with a center divider and lined with HDPE. They were filled with well water on 19 March 2006. On 20 March, each raceway was fertilized with 0.32 kg of 18-46-0 fertilizer and 1.14 kg white (wheat) flour. Each raceway received a total of four applications of chemical fertilizer. Flour application (1.14 kg/raceway) continued on a daily basis. Salt was added to each raceway to ensure chloride concentration exceeded 100 mg/L. Each raceway was equipped with a 0.37-kW electric paddlewheel aerator that operated continuously. Well water was added periodically to replace evaporative losses.

On 28 March 2006, raceways were stocked with stocker hybrid channel × blue hybrid catfish obtained from the ARS Catfish Genetics Research Unit, Stoneville, Mississippi. Stocking rate was 100, 300, or 500 fish/raceway. Treatments were assigned randomly to raceways. At stocking, fish averaged 0.069 kg/fish. Fish were fed daily to apparent satiation with a 32% protein floating feed. Dissolved oxygen, temperature and pH are measured on a daily basis, and water quality variables are measured on a weekly basis. Harvest is planned for October-November 2006.

Objective 2. *Improve equipment to enhance culture.*

Objective 2a. *Motor-powered U-tube aerator for commercial-scale channel catfish ponds.*

United States Department of Agriculture-Stoneville. A prototype U-tube was constructed and installed in a 0.4-ha pond at the National Warmwater Aquaculture Center, Stoneville, Mississippi. The U-tube was fabricated from a 91-cm-diameter, corrugated, galvanized culvert that was installed vertically in a 6-m deep bore hole made in the pond bottom. The unit was powered by a 240-volt, 3-phase, 3.72-kW, helical-geared Flender motor. The motor was vertically mounted on a 91-cm-diameter culvert elbow that was attached to the tube with a 25-cm band clamp. The motor turned a three-vane impeller attached to a 61-cm long × 5-cm diameter unsupported, steel shaft. Water level was maintained at the top of the elbow. The impeller speed was controlled by an in-line, general purpose, open-loop vector, AC-drive (Safetronics Model GP10). With an impeller speed of 150 rpm at 60 Hz, the motor drew 12.7 amps and produced 3.99 kW with a water output of 30.6 m^3/min (Table 5).

Pump efficiency increased as impeller speed decreased, but both total output and water velocity decreased. It was determined that the higher velocity was necessary to entrain the volume of air needed to optimize performance. Air was provided by a 3.7-kW, 3-phase blower to diffusers located at or below the mouth of the "down-leg" of the U-tube, which was level with the pond bottom and approximately 1.5 m below the water surface.

Oxygen transfer efficiency tests were conducted using a variety of diffuser types and configurations.

Table 5. Operational data for a prototype motor-powered U-tube aerator.

Impeller (rpm)	Motor Amperage	Volts	kW	Water Velocity (m/sec)	Water Output (m^3/min)	Pump Efficiency (m^3/kW·hr)
150	12.7	230	4.00	0.78	30.6	459
125	10.6	205	2.55	0.60	23.8	560
100	8.1	148	1.43	0.45	18.1	759

The optimum conditions produced an increase in dissolved oxygen of 2.3 mg/L (outflow DO minus inflow DO) and a standard aeration efficiency of 1.01 kg O_2/kW·hr. These results were encouraging but less than desired for commercial application.

Two problems were noted during testing of the initial prototype during Year 1. First, it was desired to eliminate obstructions in the tube to enhance water flow. Thus, the impeller shaft was kept relatively short because it had no lateral support near the end. This resulted in the impeller being located slightly above the bottom of the horizontal (discharge) end of the elbow. As the air:water ratio increased, backflow from the pond through the mouth of the discharge was observed. This decreased water flow through the tube, and at higher air:water ratios, flow through the tube ceased entirely. Second, using this design, the water level is critical. If the water level dropped below the top of the discharge elbow, flow rate decreased. If the water level rose more than 15 cm above the top of the elbow, the motor could be damaged. For commercial application, the unit should have at least a 60 cm "freeboard" to allow for normal variations in pond water level.

During Year 2 (1 August 2005 – 31 August 2006), two major design modifications were introduced to eliminate the problems identified in Year 1. First, the shaft length was increased to 91 cm. The only concern was the potential for instability with a longer, unsupported shaft. This was not observed. The longer shaft was stable and apparently eliminated the "backflow" problem seen with the shorter shaft. Second, a 60 cm diameter × 41 cm insert was built and installed in the "down" leg of the tube. This did provide a faster water velocity in the "down" leg, allowing for a greater input of air into the system. Tests are now underway to quantify the impact of these modifications. While funding through SRAC ended in July 2006, work on this project is continuing under USDA/ARS funding.

In addition to further testing of the completed modifications, three additional design changes are being considered. First, a submersible motor placed in the mouth of the "down" tube would allow for larger pond water level fluctuations. This would be desirable in commercial applications. Suitable motors are being examined. Second, a venturi will be examined as a means of introducing gas into the water, eliminating the need for a blower. This would both reduce the overall horsepower requirements (increasing efficiency) and eliminate a motor that is a potential cause of failure. Third, the use of pure oxygen (instead of air) will be examined. While the economics may not justify this for routine aeration, the use of pure oxygen in an emergency situation could eliminate the need for a tractor-powered aerator.

Objective 2b. *Low-head, low-speed paddlewheel aerator for crawfish ponds.*

Louisiana State University. A low-speed paddlewheel mixer is being designed and a horizontal circulator/aeration unit was acquired for evaluation in two, 1.5 to 2 ha experimental crawfish ponds at the Aquaculture Research Station in Baton Rouge. Baffle levees are being constructed to configure the ponds so that water can be recirculated. Mixing patterns and water quality will be monitored during the 2006-2007 crawfish production cycle.

Objective 2c. *Low-power, electrically-enhanced seine to harvest market-sized channel catfish from commercial-scale ponds.*

Mississippi State University. The primary objectives for the first year of research were to design, manufacture, and test the electrical components required to build the individual modules that will power the electrically-enhanced seine. Three models of the power supply and electrical circuitry were designed, manufactured, and tested during this year. Through this process, the total weight of the electrical components needed to build an electrical module has been reduced over 60%. The power supply and electrical circuitry were miniaturized to fit on a 7.6 cm × 12.7 cm circuit board. A safety circuit designed to switch off the electrical power to a panel as it comes out of the water was added to the circuit board of the latest model of the system. The results of tests conducted in concrete vats indicate that the low powered electrical system (electrodes with no net) will repel adult catfish away from the attached electrodes. However, the system currently appears to be underpowered.

The miniature fish stimulator and power supply module was redesigned based on results of the first year. The system was further reduced in weight while maintaining the developed safety features. The output transformer was redesigned to operate at a higher frequency. In addition, the operating power was reduced from 60 to 10 watts. The results of vat tests using production-sized catfish indicated that the unit is only moderately effective as constructed. It was recently determined that the redesigned output transformer had a lower voltage than specified because of a tooling error by the manufacturer. Efforts are currently underway to get the manufacturer to correct this problem. The power supply and electrical circuitry will be re-tested once properly manufactured parts are obtained.

Objective 3. *Assess energy, material, and economic efficiency of production systems.*

Objective 3a. *Quantify energy, protein, and water use in traditional systems for channel catfish culture.*

Auburn University. During 2005, data on electricity and fuel use were obtained monthly from four catfish farmers. However, in early 2006, the graduate student unintentionally caused slight, cosmetic damage to some equipment on one of the farms. All four farmers subsequently refused to cooperate further.

Data on the cost of electricity and fuel used on catfish farms are now being sought by other means.

Twelve farmers, two hatchery owner-operators, one processing manager, and one seining crew manager agreed to participate in the study. In early summer of 2006, questionnaires about energy use were sent to the new cooperators. We have received full responses from four farmers, one seining crew manager, one processor, and no hatchery operators. Data collection was delayed by the onset of the peak of the farming season, and will continue again in the fall when people are more accessible. We anticipate full participation except for the two farmers that have been unresponsive.

The questionnaires include items on total use of electrical power and petroleum specific to each aspect of raising channel catfish. The questionnaires make sure that the respondent lists only energy use for catfish. The questionnaires also include items on fish survival, food use, and yields where appropriate.

Investigations of water use require definitions of water use terminology. Total water use should refer to the amount of water applied to an aquaculture system in rainfall, runoff, and other natural processes and by management intervention, such as water added by pumping or other mechanical means.

Consumptive water use should represent the reduction in surface runoff caused by an aquaculture facility on a watershed. Less runoff equates to less stream flow for downstream water users. In addition, all freshwater withdrawn from aquifers by wells should be included as a consumptive use, because this water would not be available to other users of ground water in the area. Although ground water is re-charged by infiltration, it sometimes is removed by wells at a rate exceeding recharge. This diminishes the amount of water available from wells in the area. Ground water depletion usually is more serious in arid than in humid climates, but even in humid climates, availability of water from wells may be reduced during the dry season and especially during droughts. Consumptive water could be determined as follows:

> Consumptive water use = Reduction in stream flow + Water withdrawn from wells

The amount of ground water pumped or derived by artesian flow from wells should be indicated as a separate variable for ground water use. This is a major issue in many regions. Spring flow should not be included, for springs discharge onto the land surface naturally.

Non-consumptive water use should refer to water that passes through aquaculture facilities and is still available to other users downstream. It could be calculated as follows:

> Non-consumptive water use = Total water use – Consumptive water use

A water use index relating the amount of water used in an aquaculture facility to production could be useful. Although this index could be calculated for both total and consumptive water use, the consumptive water use index would be most meaningful. The index could be calculated as shown below (mt = metric tons):

> Water use index (m^3/mt) = consumptive water use (m^3) ÷ production (mt)

An index of the economic value of water used in aquaculture should be available. This variable could be determined with the following equation:

> Water value index ($/$m^3$) = [production (mt) × crop value ($/mt)] ÷ consumptive water use (m^3)

Studies of protein use in catfish farming also will require some indices of protein and fish meal use. The following indices have been developed based upon the feed conversion ratio (FCR):

Protein conversion ratio (PCR), an index of the amount of feed protein needed per unit of production: PCR = FCR × [feed protein (%) ÷ 100]

Protein equivalence (PE), the ratio of feed protein to aquaculture protein produced: PE = FCR × [Feed protein (%) ÷ protein concentration in live culture species (%)]

Fish meal conversion ratio (FMCR), the ratio of fish meal in feed to aquacultural production: FMCR = FCR × [fish meal in feed (%) ÷ 100]

Live fish equivalence of fish meal (LFE), the ratio of live fish needed for the fish meal in feed to aquacultural production: LFE = FMCR × 4.5

Objective 3b. *Develop and evaluate economic and financial models of existing and improved production practices and technologies.*

University of Arkansas at Pine Bluff. Cash flow budgets were developed for five farm sizes: 24 ha, 103 ha, 147 ha, and 407 ha. Validation tests were conducted against cash flow budgets of commercial catfish farms. The effect of varying equity levels, from 0% to 100% was measured across the five farm sizes. Schedules of cash flow and cash flow risk were developed in 10% increments from 0% to 100% equity for each farm size. With 100% equity, monthly cash flows were positive for all months except February for all farm sizes. Cash flow risk ranged from 0.28 to 0.31 when compared with total cash inflow and from 0.39 to 0.44 when compared against operating expenses. With 100% financing, only the larger farm sizes cash flowed, but at very high levels of risk (0.0008 compared to cash inflow and 0.0012 compared against operating expenses).

A survey is underway to gather data from lenders with portfolios in catfish, row crop, and livestock loans. A total of 80 banks (6 in Alabama, 23 in Arkansas, 36 in Mississippi, and 15 in Louisiana) have been included in the sample. Of these, 32 have catfish loans and 48 have agriculture, but not catfish loans. Data obtained from the survey will be used to identify the range of lending programs, structures, and repayment plans commonly used for catfish loans as well as those commonly used in other types of agriculture. These financial lending scenarios will then be applied sequentially to the cash flow budgets to assess the effects on cash flow and repayment capacity.

Two mathematical programming economics models have been developed that incorporate grow-out and fingerling production activities. The models maximized net farm income subject to constraints that include: quantity of operating capital, the number of ponds available, farm size, appropriate balance and transfer rows, and non-negativity conditions. Fingerlings were produced either with or without thinning at different stocking densities. Results showed that the optimal size of fingerling to under-stock was 12.7 cm. On-farm production of fingerlings was selected across all farm sizes but the fingerling production technique selected varied with farm size. Models of larger farm sizes began to thin fingerling production ponds, while models

Results at a glance...

- *Indicators of the efficiency of resource use in aquaculture have been developed and are being used by several environmental advocacy groups in assessing the sustainability of aquacultural production.*

of smaller farm sizes produced fingerlings only without thinning. When farm size was treated as endogenous, the optimal size of a catfish farm was 404-water ha. Sensitivity analyses suggested that net returns were sensitive to changes in the key parameters of the model, whereas the optimal size of fingerling to under-stock was robust to variations in the model's parameters. In multiple-batch production, profits were maximized with on-farm production of 12.7-cm fingerlings.

We developed a second multi-period mixed integer-programming model that included six different types of stockers (stockers produced from 6.7-cm fingerlings stocked at 50,000, 100,000, and 150,000/ha, and from 9-cm, 11-cm and 13-cm fingerlings stocked at 100,000/ha) and three different sizes of fingerlings (7.6-cm, 12.7-cm, and 17.8-cm). The results revealed that nearly one-third of the area available for catfish grow-out production should be allocated to foodfish production from fingerlings and two-thirds from stockers. Profits were maximized with on-farm production of 12.7-cm fingerlings, and stockers produced from 9-cm and 11-cm fingerlings stocked at 100,000/ha (Table 6). Sensitivity analyses suggested that the results were sensitive to varying levels of operating capital in that a decrease in the availability of operating capital would result in an increase of foodfish production from fingerlings and a decrease in foodfish production from stockers. Increased availability of operating capital increased on-farm production of stockers for subsequent use in foodfish grow-out. Results of this analysis provide guidelines for farmers related to trade-offs between the use of fingerlings and stocker catfish on farms.

Table 6. Results of simulations of farm size and pond allocation to stockers and fingerlings.

	Pond Allocation			Fry Stocking Rates (fry/ha)		Stockers	
				No Thinning		Fingerlings Stocking Rate (100,000/ha)	
Farm Size	Stockers	Fingerlings	Fingerlings Stocked	543,334	1,398,990	9-cm	11-cm
(ha)	(%)	(%)	(cm)	(%)	(%)	(%)	(%)
40	71	29	12.7	0	100	96	4
80	71	29	12.7	0	100	100	0
120	71	29	12.7	0	100	99	1
160	70	30	12.7	0	100	95	5
200	66	34	12.7	1	100	81	19
240	62	38	12.7	25	75	70	30

IMPACTS

Studies of PAS aquaculture systems at Clemson University and Mississippi State University revealed that fingerling growth can be accelerated in the system. The PAS systems also allowed excellent grow-out of fingerlings to harvestable size. Thus, the system should be evaluated further for its commercial potential. The semi-confinement units tested at the University of Arkansas at Pine Bluff also increased the yield of fingerling catfish in ponds.

Studies at Louisiana State University and University of Arkansas at Pine Bluff revealed that channel catfish can be produced in super-intensive, heterotrophic, biofloc systems similar to those used for marine shrimp production. These findings justify further investigations on commercial-scale catfish production in biofloc systems.

The prototype, motor-powered, U-tube aerator being developed by United States Department of Agriculture-Stoneville can move up to 759 m^3/water/kW · hr, but the oxygen transfer efficiency must be improved for commercial application.

Studies at Auburn University have resulted in a number of indicators of sustainable aquaculture that are already being used by the Global Aquaculture Alliance and the World Wildlife Fund in evaluating the ecological efficiency of different production systems. Economic analyses done at University of Arkansas at Pine Bluff revealed that the optimum size of a catfish pond was about 400 ha of water surface area.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

Publications in print

Boyd, C. E. 2005. Ground water and wells. Global Aquaculture Advocate 8(3):62-63.

Boyd, C. E. 2005. Water use in aquaculture. World Aquaculture 36(3):12-15, 70.

Boyd, C. E. 2006. Estimates of bottom soil and effluent load of phosphorus at a semi-intensive marine shrimp farm. Journal of the World Aquaculture Society 37(1):41-47.

Minchew, C. D., S. D. To, K. K. McDill, and R. V. Beecham. 2004. Harvest of channel catfish with an electrically-enhanced seine. Journal of the World Aquaculture Society 35(4):34-36.

Pearson, P. R., S. E. Duke, B. W. Green, C. D. Minchew, R. V. Beecham, and J. M. Kim. 2006. Evaluation of a diffused oxygen aeration system under on-farm harvest conditions. North American Journal of Aquaculture 68:47-52.

Presentations

Beecham, R.V., C. D. Minchew, and G. R. Parsons. 2005. Non-horizontal swimming performance of blue and channel catfish fingerlings. America Fisheries Society Annual Meeting, September 11-15, Anchorage, Alaska.

Bouras, D. and C. R. Engle. 2006. Optimal size of fingerling to understock in multiple-batch catfish farms. Abstract. Annual Meeting of the U.S. Aquaculture Society, A Chapter of the World Aquaculture Society, Las Vegas.

Boyd, C. E. 2006. Indicators for assessing environmental performance of aquaculture. AQUA 2006, May 9-13, Florence, Italy.

Chambers, J. P., D. Minchew, and R. Beecham. 2004. AquaScannerTM Catfish Sonar: Acoustic Instrumentation for Aquaculture. Mississippi Intellectual Property Forum, November 30-December 1, Jackson, Mississippi.

Minchew, C. D., R. V. Beecham, P. R. Pearson, B. W. Green, and J. M. Kim. 2005. The effects of harvesting and

hauling on the blood physiology of adult channel catfish, *Ictalurus punctatus*. American Fisheries Society, Mississippi Chapter Meeting, February 2005, Philadelphia, Mississippi.

Minchew, C. D., R. V. Beecham, and G. R. Parsons. 2005. Design and calibration of a tilting tunnel respirometer developed to study non-horizontal swimming in fishes. America Fisheries Society Annual Meeting, September 11-15, 2005, Anchorage, Alaska.

Pearson, P. R., B. W. Green, C. D. Minchew, R. V. Beecham, J. M. Kim. 2005. Evaluation of the sock saver aeration unit under commercial channel catfish farming conditions. Aquaculture America 2005, the Annual Meeting of the United States Aquaculture Association, January 17-20, 2005, New Orleans, Louisiana.

To, S. D. F., C. D. Minchew, E. H. Robinson, J. Doler, Y. Y. Chi, and Said. 2005. Personal in-farm information system (PIFIS). Precision Agriculture Conference, Info Ag Midsouth, February, 7-9, Tunica, Mississippi.

◆ ◆ ◆

IMPROVING REPRODUCTIVE EFFICIENCY TO PRODUCE CHANNEL × BLUE HYBRID CATFISH FRY

Reporting Period
April 1, 1995 - August 31, 2006

Funding Level

Year 1	$118,390
Year 2	$111,610
Year 3	$123,000
Year 4	$123,000
Total	$476,000

Participants

Auburn University (Lead Institution)	Rex Dunham, Allen Davis, Ron Phelps
Louisiana State University	Terrence Tiersch
Mississippi State University	Lou D'Abramo
University of Memphis	Charles Lessman, Bill Simco
USDA/ARS	Brian Bosworth, Brian Small

Administrative Advisor

Dr. John Jensen
Special Advisor to the President
Auburn University, Alabama

PROJECT OBJECTIVES

1. Develop brood stock selection and management protocols to optimize channel × blue hybrid embryo production.
    a. Determine minimum cold period required and rates and patterns of application of thermal changes to promote synchronous gonadal development and spawning.
    b. Improve hybrid embryo production by determining the best nutritional regime to maximize fecundity and hatch rate from induced channel catfish females and blue catfish males.
    c. Improve hybrid embryo production via genetic enhancement.
2. Develop induced spawning techniques and management strategies to optimize gamete collection and storage.
    a. Develop procedures to predict ovulation of channel catfish.

b. Conduct pivotal protocol studies for determining dosage rates and timing of application of luteinizing hormone releasing hormone (LHRHa), carp pituitary extract and catfish pituitary extract to maximize ovulation, hatch rate and fry production.
c. Improve hybrid embryo production via pheromonal manipulation of channel catfish males and blue catfish males for improved ovulation, spermiation, egg quality, hatch and fry production.
d. Develop extended refrigerated storage and cryopreservation of sperm.

3. Develop techniques to identify, assess and improve gamete quality.

a. Develop criteria for standardizing and classifying egg quality prior to injection and after manual stripping and describe the morphological and physiological condition of channel catfish eggs including evaluation of morphological changes of oocytes during oocyte maturation in female catfish.
b. Determine the profile of estradiol hormone from serum plasma of 2-year-old female channel catfish over a 12-month period, determine changes in oocyte maturation during vitellogenesis and identify the different cathepsins that are responsible for vitellogenin degradation and oocyte maturation in female catfish.
c. Develop in vitro assays to evaluate sperm quality and evaluate their predictive ability in relation to fertilization and hatch.

4. Develop economically viable standardized hatchery procedures and fertilization protocols to optimize hatching rate of hybrid embryos.

a. Determine optimal sperm (fresh, frozen and refrigerated)-to-egg ratios for fertilization and hatch.
b. Determine the effects of commonly used therapeutics on hatching success.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Objective 1. *Develop brood stock selection and management protocols to optimize channel × blue hybrid embryo production.*

Objective 1a. *Determine minimum cold period required and rates and patterns of application of thermal changes to promote synchronous gonadal development and spawning.*

Louisiana State University and University of Memphis. Water temperature is the primary environmental factor affecting the spawning of channel catfish. Spawning begins when water temperatures consistently remain above 21°C at some locations such as Louisiana and west Mississippi. The spawning season at the Aquaculture Research Station of the Louisiana State University Agricultural Center was lengthened by heating ponds through addition of geothermal water (36°C). This study attempted to use degree-days to describe and quantify the total heat requirement for

channel catfish to initiate spawning, which should also indicate the same requirement to initiate artificial spawning to produce hybrid embryos. Degree-days were calculated for 153 spawns between 1999 and 2004. Ponds from 1999 to 2002 had four available spawning sites (cans), and in 2003 and 2004 the ponds had six sites. Degree-days needed to obtain the first four (1999-2002) or six (2003-2004) spawns were calculated to prevent spawning site limitations effects on the degree-day values.

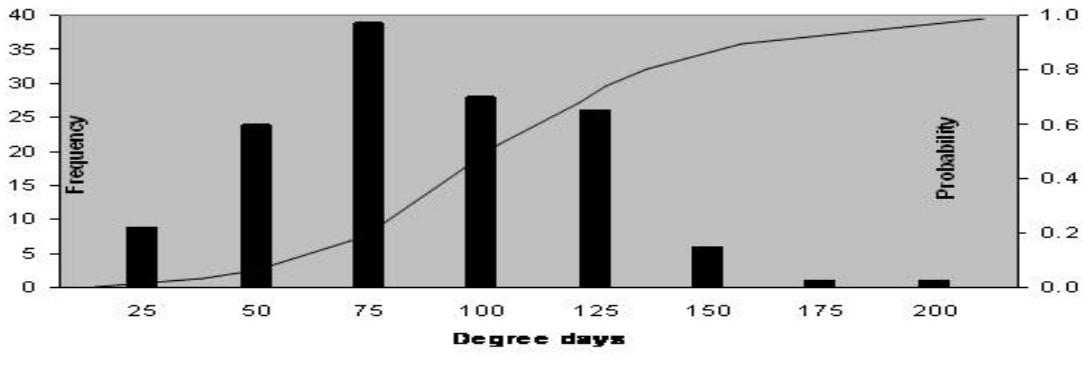
In 2004, three heated ponds were maintained at three different temperatures. Degree-day values were calculated for 18 spawns using three threshold temperatures as the starting point to calculate the degree-days (Table 1). The 21°C threshold yielded a constant value of 98 ± 4°D for the heat requirement of channel catfish to initiate spawning.

Degree-days were also calculated using the 21°C threshold for 135 spawns collected during the early spawning and regular spawning periods between 1999 and 2003. The average °D-value above the 21°C threshold was 97 ± 33°D. Spawning probabilities and frequency of spawns were plotted against degree-day values (Figure 1). The probability that a fish will spawn after 100°D was 50% and increased to 93% after 150°D. Fifty percent of spawns occur between 75°D and 125°D and ninety percent between 50°D and 150°D. These results concur with the literature that 21°C is the minimal water temperature needed to initiate the reproductive process in channel catfish.

Table 1. The average degree day value for spawns above three thresholds from ponds maintained at different temperatures. Values in the same column followed by the same letter do not differ significantly ($P < 0.05$).

		Threshold		
Target temperature	Actual temperature	18°C	21°C	24°C
21°C	23.1 ± 1.5°C	234 a	95 a	8 a
24°C	23.1 ± 2.6°C	203 ab	98 a	22 b
27°C	24.6 ± 3.0°C	184 b	102 a	41 c

Figure 1. Spawning probabilities and spawning frequency at different degree-day values above the 21°C threshold.



Additionally, degree-days above 21°C may be useful as a management tool to predict channel catfish spawning times in heated ponds, and the correct time to initiate artificial spawning for hybrid embryo production.

Results at a glance...

☆ *A water temperature of 21°C is the minimal temperature needed to initiate the reproductive process in channel catfish. Tracking degree-days above 21°C may be useful as a management tool to predict channel catfish spawning times and will be especially useful as a tool to predict best times to initiate artificial spawning for hybrid embryo production.*

In 2005, the temperature data results from previous years was used to compare reproductive performance of channel catfish females induced to spawn before and during the natural spawning season. The goal was to extend the documented time female channel catfish could be induced to spawn before the natural spawning season without affecting reproductive performance.

In December of 2004, channel catfish broodstock (1.17 ± 0.38 kg; 48.1 ± 4 cm) were purchased from Haring Fish Farms, Inc., a commercial fingerling producer in northern Louisiana. Broodfish were stocked in nine, 0.04-ha earthen ponds at the Aquaculture Research Station of the LSU Agricultural Center. Six of the ponds were stocked with 30 females and 10 males. The remaining ponds were stocked with 30 females only. Geothermal water (36°C) was added to three of the ponds (two mixed-sex and one all-female) beginning on January 13, 2005. Initial pond temperatures were approximately 20°C and were increased 2°C/day until the temperature reached 28°C. Ponds were heated in sets of three until natural spawning occurred. After six spawns (egg masses, 20% spawning) were collected from spawning containers in the ponds, fish were collected by seining and brought indoors for induced spawning.

Broodfish were acclimated in an indoor recirculating system for 48 to 72 hours. Females were evaluated using ultrasound imaging of ovaries and oocytes to assess reproductive readiness. Selected females were weighed, measured, marked for identification, and placed into eight, 120-L fiberglass tanks. Pairings were made based on length and weight to minimize size differences within the tanks. Four tanks held one male and one female (mixed-sex pairs); the other four tanks contained two female fish (female pairs). Each female was given a single injection of leutenizing hormone-releasing hormone analog at a dosage of 100 μg/kg. Temperature was maintained at 27°C and spawning behavior was monitored every 2 hours. After females began releasing eggs they were anesthetized (tricaine methane sulphonate, MS-222) and manually stripped.

There was no significant difference in total number of eggs produced per female or in latency (time from hormone injection to spawning) between fish spawned in 15 heated ponds before the natural spawning season and fish spawned in 27 unheated ponds during the natural spawning season. Fertilization for eggs from females from heated (35%) and unheated (43%) ponds were similar. Likewise, hatching rate for eggs from heated (38%) and unheated (34%) females did not differ. There was no significant difference in number of eggs produced per female or latency between fish induced to spawn 63, 47, and 12 days before the natural spawning season. This study showed that channel catfish females can be induced to spawn as early as 63 days (02/01/05) before the start of the (4/12/05) natural spawning season without affecting reproductive performance.

Further studies were conducted during 2005-2006 to better characterize the effects of temperature on spawning of female channel catfish before and during the natural spawning season. To determine the patterns of application of thermal changes to promote synchronous gonadal development and spawning, three temperature requirements were evaluated: 1) minimum number of cold degree-days (degree-days calculated for temperatures below 21°C); 2) minimum number of warm degree-days (degree-days calculated for temperatures above 21°C) before the natural spawning season, and 3) minimum number of warm degree-days during the natural spawning season.

In November of 2005, channel catfish brood stock were purchased from Haring Fish Farms, Inc., a commercial fingerling producer in Northern Louisiana. Twelve, 0.10-acre geothermal ponds at the Aquaculture Research Station of the LSU Agricultural Center were stocked with broodfish at a ratio of 4 females to 1 male. The ponds were heated three at a time and temperatures were controlled using geothermal water. Ultrasound was used to classify females during the accumulation of cold degree-days and throughout the subsequent warm degree-days. The first channel catfish to spawn this past research year (by hormonal induction in the laboratory) was recorded on December 24, 2005. In January pond spawning became possible and after six spawns (egg masses) were collected from spawning containers in the ponds, fish were collected by seining and brought indoors for hormone-induced spawning.

Broodfish were acclimated in an indoor recirculating system for 48 to 72 hours. Females were evaluated using ultrasound imaging of ovaries and oocytes to assess reproductive readiness. Selected females were weighed, measured, marked for identification, and placed into eight 120-L fiberglass tanks. Pairings were made based on length and weight to minimize size differences within the tanks.

Results at a glance...

☆ *Early spawning can be accomplished by heating water prior to the natural spawning season without any difference in success compared to the natural spawning season. When 100 degree-hours are reached ovulation and fertilization should be successful. If warm water is available, channel catfish can be successfully spawned in early January.*

Four tanks held one male and one female (mixed-sex pairs); the other four tanks contained two female fish (female pairs). Each female was given a single injection of leutenizing hormone-releasing hormone analog at a dosage of 100 μg/kg. Temperature was maintained at 27°C and spawning behavior was monitored every 2 hours. After females began releasing eggs they were anesthetized (tricaine methane sulphonate, MS-222) and manually stripped. All of the spawning and degree-day data for this experiment are being analyzed.

Objective 1b. *Improve hybrid embryo production by determining the best nutritional regime to maximize fecundity and hatch rate from induced channel catfish females and blue catfish males.*

Auburn University. Since nutrition can play a key role in maturation, as well as egg and fry quality, proper nutrition could be a key factor in the development of hybrid rearing technologies. Hence, the

primary goal of this component of the project is to improve hybrid embryo production through nutrient manipulations.

Protein Level and Feed Frequency

The first subobjective was to evaluate the influence/interaction of dietary protein level and feeding rate on egg production of channel catfish. As there are numerous interactions and it is difficult to obtain brood stock, a holistic approach was initially used to help identify important factors to control.

A total of 495 females were stocked in 16 ponds, using four ponds per treatment. The females were divided in three strains considering previous spawning behavior (high spawning, low spawning, and NWAC103), and based on that characteristic they were assigned proportionally in a randomized manner to each pond. Fish were pit-tagged and heat-branded for identification. The fish were stocked on February 13, 2004 in 0.04-ha ponds at a density of approximately 1,500 kg/ha, followed by an acclimation period of approximately one month. During the acclimation period, fish were offered a commercial floating feed (32% protein) diet three times a week at 1.5% of their body weight. The two test diets were a 32% typical practical catfish feed and a 42% high fish meal practical catfish feed, and the feed was offered either three or six times a week to apparent satiation. A fifth treatment utilized 32% protein 3 days per week with supple-mental feeding of liver 2 additional days per week at a rate similar to the dry feed. Females were spawned in three periods (early, middle and late spawning periods). Dietary protein level and feeding rate treatments were evaluated using the following indicators: egg mass, number of eggs, fecundity (number of eggs per kilogram female), egg diameter, and fertilization rate 48 hours after fertilization.

Results for this experiment are presented for "High" and "Low" spawning strains, as strain 103 had a low number of individuals per treatment per spawning period. High spawning and low spawning females had a survival of 92% (414 females), of which 63.5% spawned.

Based on logistic analyses by strain, the following results were determined. For strain High, the dietary treatment did not have a significant effect on spawning percentage, but age and spawning period had a significant effect on spawning percentage. For this strain, the odds of females that were 5 years old spawning were 9.4-times higher than females that were 3 years old and 8.4-times higher than females that were 4 years old. The odds of spawning in the early spawning period were 10.6 times higher than for the late spawning period; while the middle spawning period had 5.1-times higher odds of spawning than late spawning period.

With respect to strain Low for spawning percentage, dietary treatment did not have a significant effect overall, however when comparisons between treatments were performed, the odds of spawning for treatment 4 (32% protein feed, 3 times/week) were 2.5-times higher than for treatment 3 (32% protein feed, 6 times/week). The other two variables, age and spawning period, had a significant effect on spawning percentage. For this strain, the odds of females that were 5 years old spawning were 5.5-times higher than females 3 years old, and was not different than for 4-year-old females. The odds of spawning in the early spawning period were 13.4-times higher than for the late spawning period; and there was no difference between the middle spawning period and the late spawning period. In general, the two strains exhibited different responses. These results indicate that brood stock management must adjust to strain variations and age.

Although using a holistic approach has disadvantages, we were able to evaluate the effects of strain, age and spawning period on spawning success and egg production. In general, each strain responded differently with respect to dietary treatments and spawning period. It appears that the high-protein diet when offered six times per week resulted in reduced fecundity. With respect to the other

treatments there were few differences due to dietary treatments.

Comparison of the response of fish maintained on the 32% protein diet with 3 feedings per week with those that also received liver, there were no notable improvements in egg production. As with the other treatments strain and age influenced the response.

When fry production data for the various ages, strains and spawning period are pooled to represent only the dietary treatments, overall production can be determined. Table 2 presents the combined data for the five dietary treatments for groups of fish that could be followed and that received LHRHa injections (a single 20 μg/kg priming injection and a 100 μg/kg resolving injection). Based on mean separation there were significant differences between treatments with fish maintained on the 32% protein diet offered six times per week, producing more fry/kg than those maintained on the 32% protein diet fed three times per week with a liver supplement twice a week as well as fish offered the 42% protein diet fed six times per week. The treatment with the highest observed mean was 32% protein diet offered six times per week although there were no significant differences between fish offered the 32% protein diet three or six times per week or those offered the 42% protein diet three times per week. For unknown reasons, supplementation with liver was detrimental to fry production.

Lipid Source and Ratios (n3:n6)

The second subobjective was to evaluate different ratios of polyunsaturated fatty acids, and their influence on fecundity and on egg quality. A total of 190 females were stocked in eight ponds, using two ponds per treatment. All females were 4-year-old Kansas strain from Harbin Farms in Anthony, Kansas. The fish, which were in relatively poor condition, were stocked on January 7, 2005, giving an acclimation period of approximately 2 months. The trial period was 73 days to 87 days depending on the spawning period. Female brood stock were maintained in 0.04-ha ponds at a density of approximately 600 kg/ha. They were offered a commercial floating feed (32% protein, 5% lipid) diet three times a week at 1.5% of their body weight. Water temperature and dissolved oxygen were measured daily in the early morning and after sunset. After the acclimation period, test diets were offered.

The four test diets were based on a commercial

Table 2. Effect of nutrition, (% protein-number of feedings per week) 42-3, 42-6, 32-3, 32-6 and 32-3 plus 2 days of liver, on hybrid fry/kg female body weight for channel catfish females fertilized with blue catfish sperm. Females were ovulated individually in bags within tanks. GMP grade LHRHa injections (20 μg/kg priming dose with a 100 μg/kg resolving dose). A mixture of low and high producing lines of channel catfish was utilized. Means followed by different letters are significantly different ($P < 0.05$).

Treatment	N	Fry/kg
32-6	51	1,732 a
42-3	48	1,505 ab
32-3	54	1,476 ab
42-6	54	1,032 b
32-3-2L	54	969 b

Results at a glance...

☆ *Feeding standard 32% protein floating catfish feed six times per week for 2 months prior to spawning gives equal or better fry production compared to high protein diets. Supplementation of broodfish diets with menhaden fish oil, DHA and ARA 2 months prior to spawning can increase hybrid fry output up to 100% depending upon previous preparation of the fish. Availability of forage fish, even at low levels, can have a positive impact on hybrid fry production.*

catfish feed containing 5% lipid that was top-coated with an additional 2% lipid. Three test diets used a combination of vegetable oil sources proportioning different 18:3n3, 18:2n6 ratios. The lipid sources were mixed in the following ratios: Diet 1 contained soybean oil and linseed oil in a ratio of 0.90:1.00; Diet 2 also contained soybean oil and linseed oil, but in a ratio of 7.00:1.00; Diet 3 contained linseed oil only. The fourth diet was based on fish oil and high DHA and ARA oil sources with an n3:n6 ratio of 3:2. Diet 4 contained menhaden fish oil, high DHA, and high ARA in a ratio of 2:1:1. Thus diets contained a range of n3 and n6 fatty acid combinations (Diets 1-3) as well as one diet (Diet 4) which contained HUFA supplements. The n3:n6 ratios of the oil supplements were approximately: 1:1, 1:4, 4:1, and 3:2, respectively.

Dietary lipid treatments were evaluated using the following indicators: total number of eggs produced, fecundity (number of eggs per kilogram female), and fry production, fry per kg female and overall fry survival. Biochemical analysis will also be conducted on egg samples as indicators of egg quality. Data for treatments evaluating lipid source and different ratios between essential fatty acids (Table 3) is restricted to the first spawning period (early), since there was a hatchery problem for the second spawning period, with a minimum of fertilization across all the treatments. Additionally, one pond of fish evaluated in treatment four during the second spawning period were stressed (due to a broken water-supply line) at their harvest causing around 30% mortality.

Based on initial analyses of the data there are few indications that the dietary treatments had a significant effect on the percentage of fish spawning or the number of eggs produced. However, the various lipid combinations did have a strong effect on fry production. Using lipid supplement with a 4:1 ratio of 18:3n3, 18:2n6 (linseed oil, Diet 3) resulted in very poor fry production. The use of menhaden

Table 3. Egg and fry production in the early spawning period from broodfish fed four diets containing different lipid sources. Diets are described in the text.

Diet	Female weight (kg)	No. females spawned	No. eggs	Eggs/kg female	No. fry	Fry/ kg female	% Hatch
1	1.91	12	198,679	8,680	23,901	1,044	12
2	2.15	10	201,949	9,397	27,841	1,296	14
3	1.86	8	111,294	7,500	7,522	507	7
4	1.64	13	186,056	8,731	47,045	2,208	25

fish oil with DHA and ArA supplements and a ratio of 3:2 for n3:n6 fatty acids (Diet 4) produced the best results. Fish maintained on this diet produced almost twice the fry as fish maintained on diet supplemented with lipids (primarily18:3n3 and 18:2n6) producing n3:n6 ratios of 1:1 (Diet 1) or 1:4 (Diet 2). These results indicate that highly unsaturated fatty acids, HUFA, are probably a key factor in proper brood stock nutrition.

Lipid Source and Ratios (n3:n6) and Forage Fish

A second evaluation of different ratios of polyunsaturated fatty acids, and their influence on fecundity and on egg quality was conducted in 2006. Additionally, the effect of supplementation with forage fish was examined. A 10-week trial was conducted in ponds in Auburn, Alabama. In March, 219 female channel catfish broodfish (Kansas Select from Holland Fish Farm in Mississippi) were stocked into nine, 0.04-ha ponds, for an approximate stocking rate of 1,332 kg/ha. Fish were in good condition when stocked. Three dietary treatments were randomly assigned and fish were offered feed based on their observed response to floating feeds. Diet 1 was a standard 32% crude protein, 6% lipid floating catfish feed. Diet 2 was the same feed supplemented with forage fish (bluegill and fathead minnows) at approximately 28 kg/ha. Diet 3 was the same catfish feed, but top-coated with 2% lipid (1% menhaden fish oil, 0.5% high-DHA oil and 0.5% high-ARA oil). The DHA and ARA oils contained approximately 40% of the designated HUFA. Biochemical analyses of the feeds indicate that the standard and top-coated diets contained 9.4% and 10.4%, moisture; 32.9% and 32.2% protein, 5.95% and 7.58% fat, 5.06% and 4.64% fiber and 7.05% and 6.74% ash, respectively.

In May, females were harvested and those with good spawning characteristics were selected for hormone injection. Selected females were placed individually in soft mesh bags and transferred to holding tanks supplied with continuous flow-through water. Total length and body weight were recorded. Hormone injections were administered in two doses, a priming injection of 30 μg/kg LHRHa, followed 12 hours later by resolving dose of 150 μg/kg. Twenty-four hours after the second injection, females were examined for ovulation. Females with released eggs were removed from holding tank and anesthetized in buffered 250 mg/L tricaine methane sulfonate (MS-222). Females were then stripped and eggs were collected in metal pans previously lubricated with vegetable shortening. Females that did not express eggs were returned then rechecked later. Stripping of gametes ceased when all females had been stripped or attempts to strip them had been made. Eggs were subsampled for later biochemical and visual analyses; the remaining mass was weighed and then fertilized with blue catfish sperm and incubated in paddle wheel troughs. Viable fry were estimated by assessing individual egg masses 24 hours pre-hatch. Pooled fry counts were also determined once the egg masses hatched.

Broodfish fed the top-coated, high lipid diet displayed an increase in percent spawn, general fecundity, mean egg weight, grams of eggs spawned, embryo viability at 24 hours pre-hatch and overall fry production per kg of broodfish stocked (Table 4). The availability of forage fish at this density did not result in any statistically significant effects, however, there was a trend of increased spawning percentage, grams of eggs obtained, and increased fry production.

Blue Catfish Males and Lipid Source and Ratios (n3:n6)

A study was conducted in 2006 to evaluate the effect on sperm characteristics from fatty acid enrichment of the male blue catfish diet. Rio Grande strain male blue catfish were fed for six months either a non-enriched commercial catfish diet or an enriched diet where DHA and arachidonic acid was

Table 4: Summary of induced spawning data for female channel catfish crossed with male blue catfish after being maintained on various dietary treatments over a 10-week period. Values represent the means of three replicate ponds per treatment. Means in the same row with different superscripts are significantly different ($P < 0.05$).

	Standard Feed	Feed and Forage Fish	Fatty Acid Supplemented Feed
kg females/hectare	1358	1460	1457
% spawn	65.2	74.5	77.3
Mean egg weight (mg)	18.9b	19.1b	21.4a
Number of eggs/kg female weight	7435	7141	7266
g eggs /kg female spawned	138.3b	135.3b	155.9a
g eggs/total kg brood fish harvested	87.3	99.8	112.8
Fry/kg brood fish harvested	1,787	2,146	2,370
Fry/kg brood fish that spawned	2,817	2,909	3,392
% viable pre-hatch fry	40.6	41.6	47.4
Total fry	291,117	376,067	433,583

added. In May 2006, half the males on each diet were given a 50-µg LH-RH implant followed two weeks later with a 100-µg implant. Within 3 days after the second implant, brooders from each diet and hormone combination were paired in pens with a channel catfish female and allowed to spawn naturally. Other males were used as sperm donors to fertilized channel catfish eggs obtained by induced spawning and manual stripping. All males were described by weight gain, body proportions (length, head width and girth) and photographed. Gonadosomatic index (GSI), relative percentage of anterior testis, sperm count and motility time were recorded for each sacrificed male.

Diet had no effect on testes development or sperm count, but the motility time of sperm from brooders given the enriched diet was longer. Fertilization rates (% viable eggs at 48-hours post-fertilization) were similar for each diet and implant combination.

Objective 1c. *Improve hybrid embryo production via genetic enhancement.*

Auburn University.

Strain Effects

The channel × blue catfish hybrid grows faster, has more efficient feed conversion, has a higher tolerance for low dissolved oxygen concentrations, and better survival compared to channel catfish. However, economic production of hybrid embryos is problematic. Some strains of channel catfish

females or blue catfish males may have reproductive characteristics more suited for production of channel catfish female × blue catfish hybrid catfish embryos than others.

AU-1 channel catfish females produced greater numbers of hybrid fry than AU-7 for 5 consecutive years (Table 5). AU channel catfish lines 1, 3, 5, and 11 consistently produced high numbers of hybrid fry compared AU lines 6-10, 12, and strain 103 over a 3-year period (Tables 6 and 7). AU lines 4 and 13 were only evaluated 2 years, and were high performers one year and low another. Fry/kg for 103 was very low the first 2 years, but improved to average performance levels the third year.

Results at a glance...

☆ *Utilization of the appropriate genetic line of channel catfish female can double and triple hybrid fry output. Strain of blue catfish male impacts hatch rate of hybrid embryos and sperm production.*

Strain of male blue catfish also affected hatching rate of hybrid embryos and sperm production. Mean sperm/g of testes ranged three-fold among strains one year and was almost 33% different another year. The total ml of sperm/kg body weight and sperm number/kg body weight also varied three-fold among strains of blue catfish. Percent hatch was different for hybrid embryos produced by different strains of blue catfish males. Percent hatch for hybrid embryos fertilized with sperm from, different blue catfish strains were: AU-1, 2.6%; AU-2, 10.4%; AU-3, 10.9%; AU-4, 26.1%, and AU-5, 1.3%. The percent hatch using AU-4 blue catfish, males was significantly higher than with the other strains. In another year, AU-1 males had higher hatch than AU-2 males,15% and 10%, respectively. Genotype-environment interactions were also observed for sperm production. Utilization of genetic variation has the potential to double efficiency and productivity of hybrid embryo production.

The percentage of channel catfish females that were gravid, culled, ovulated and spawned when injected with LHRHa also varied among strains (Table 8). Differences for these four traits were less pronounced when analyzing the data within 3-year-old, 4-year-old and 5-year-old females, with the differences being the lowest among 5-year-old females. Mean latency periods ranged from 40.9 to 48.4 hours and differed among 17 strains of channel catfish females, but on a relative and biological basis, differences are slight, with less than 10 percentage points difference among strains. When the latency data were analyzed by week, and temperature and temperature degree-days are accounted for, these small differences for latency period become

Table 5. Fry/kg for AU-1 and AU-4 channel catfish female when injected with LHRHa and hybridized with blue catfish males over a 5-year period.

	Fry/kg female body weight				
Genotype	2001	2002	2003	2004	2005
AU-1	4,598	4,300	1,857	3,105	5,552
AU-7	2,638	2,550	693	1,045	959

Table 6. Percentage of females ovulating, fecundity, and fry/kg for channel catfish female strains when injected with LHRHa and hybridized with blue catfish males in 2003.

Channel catfish female	% ovulation	fecundity (eggs/kg)	fry/kg
AU-1	100	11,047	1,857
AU-2	75	7,133	2,154
AU-3	100	11,997	1,283
AU-4	82	6,545	1,005
AU-5	100	8,790	858
AU-7	73	10,179	693
AU-8	100	9,122	625
AU-9	75	9,438	492
AU-6	90	7,814	395
103	80	7,425	257
AU-10	45	9,575	163

Table 7. Fry/kg for different lines channel catfish female when injected with LHRHa and hybridized with blue catfish males over a 3-year period.

	Fry/kg female body weight		
Line	2002	2003	2004
AU-11	5,570	-	3,421
AU-5	8,500	858	3,136
AU-1	4,300	1,857	3,105
AU-13	3,500	-	3,042
AU-3	4,800	1,283	2,844
103	679	257	2,680
AU-6	-	395	2,570
AU-12	3,550	-	2,566
AU-8	-	625	2,427
AU-4	-	1,005	2,332
AU-9	4,500	492	2,178
AU-10	-	163	1,902
AU-7	2,550	693	1,045

Table 8. Percentage of channel catfish females gravid, culled, ovulated and spawned by strain during 2004 when induced to spawn with 20 and 100 µg/kg (priming and resolving dose) luteinizing hormone-releasing hormone analog (LHRHa). Significant differences existed among strains for % culled and % spawned.

Genotype	N	% Gravid	% Culled	% Ovulation	% Spawned
103	48	81.3	18.8	82.1	66.7
Forks Albino	19	94.7	5.3	83.3	79.0
Low	5	100.0	0.0	60.0	60.0
AU-1	35	74.3	25.7	61.5	45.7
AU-1 Control	60	61.7	38.3	78.4	48.3
AU-3	52	86.5	13.5	77.8	67.3
AU-4	36	86.1	13.9	87.1	75.0
AU-5	46	78.3	21.7	77.8	60.9
AU-6	21	81.0	19.1	94.1	76.2
AU-7	24	79.2	20.8	84.2	66.7
AU-7 Control	28	82.1	19.9	87.0	71.4
AU-8	57	89.5	10.5	90.2	80.7
AU-9	29	79.3	20.7	87.0	69.0
AU-10	16	81.3	18.8	61.5	50.0
AU-11	13	84.6	15.4	100.0	84.6
AU-12	10	90.0	10.0	100.0	90.0
AU-13	10	100.0	0.0	80.0	80.0

insignificant among strains. Mean egg quality scores did not differ among strains of females. However, replication may not have been inadequate to show true differences among egg quality scores.

Effect of Selection for Body Weight on Hybrid Embryo Production

Six lines of channel catfish females that had been selected for increased body weight for 6 or 7 generations were compared to randomly bred controls for channel catfish female × blue catfish hybrid embryo production. There was no indication of either a negatively correlated response or inbreeding depression for % of females gravid, % culled, ovulation %, spawning %, latency period, egg quality, fecundity, and hatching %. In some cases, there appeared to be a positive correlated response between direct selection for body weight and certain traits. For example, when the select AU-4 line was compared to its randomly bred control line, the select line outperformed the control line for % gravid females (86.1% vs 68.2%), 5 culled females (13.9% vs 31.8%) and % females spawned (75.0% vs 55.7%). With regards to the interrelated traits hatch % and fry/kg (Table 9), the selected line AU-6 (29.2% and 2,849 fry/kg) showed more than over two-fold increase compared with the randomly bred control AU-7 (10.9% and 993 fry/kg). Also, similar results were seen when comparing the selected line AU-3 (30.4% and 2,313 fry/kg) to AU-7 control (10.9% and 993 fry/kg).

In one case, hybrid embryo production exhibited

inbreeding or negatively correlated response to selection for increased body. As 3-year-olds (Table 9), AU-1 had the same hatch, but reduced observed fecundity and fry/kg compared to their randomly bred control. As 4-year-olds (Table 10), they had both reduced hatch and fry/kg. In individual years, these differences were not significantly different, but over time, they were different, as the decrease in fry/kg was almost exactly 50% each year. Several possible explanations exist. Selection for body weight decreased reproductive performance and the ability to generate hybrid fry via artificial fertilization techniques and/or reduced the age of first sexual maturity as 3-year-olds. Alternatively, these effects could be attributed to inbreeding depression from mass selection in the select lines that may be accumulating in these relatively small research populations. The results for the 4-year-olds would not likely be due to a reduced age of sexual maturity.

The adverse effects of mass selection for body weight on reproductive performance may be more severe than what these results indicate. Early research at Auburn University indicated that in early generations of individual selection for body weight, positively correlated responses occurred for reproductive traits. In cases where the select lines are not different from controls or have decreased reproductive performance compared to controls, the reduction in performance is even greater if the select lines once had better reproductive performance than randomly bred controls.

Selection for increased body weight appears to have variable effects on reproduction and hybrid fry production when channel catfish females are induced to ovulate with LHRHa and are strip-spawned. Increased, decreased, or no change in reproductive output was observed in our trials. This variable result may have been partially caused by the genetic diversity of the founder population of the select line; however, additional experimentation is needed to confirm this hypothesis. The variable results prevent making a specific recommendation on the use of lines that are mass-selected for body weight for production of hybrid fry. However, breeding history must be considered as utilizing fish from a narrow genetic base did have an adverse effect on channel × blue hybrid fry production.

Effect of crossbreeding

The main problem preventing the widespread use of the channel catfish × blue catfish hybrid catfish has been inadequate production of commercial numbers of fry in an efficient manner. One method of overcoming this barrier is the use of genetic enhancement programs and, in particular, crossbreeds. In the current experiment, the crossbreed AU-7 × AU-1 was compared to its parental lines to determine if any reproductive benefits were achieved through the use of the crossbreed when forming hybrid catfish. The crossbreed actually exhibited lower ovulation % (52.1%) than the parental lines (AU-7 = 83.3%; AU-1 = 63.2%), suggesting negative heterosis by the crossbreed for age at maturity (Tables 11 and 12). For egg quality, and fecundity, there was no maternal heterosis exhibited by the crossbreed, with parental lines performing as well or better than the crossbreed. With regards to hatch % and fry/kg, the crossbreed exhibited performance equal to (4 years of age; 27.4% and 3,393 fry/kg) or less than (3 years of age; 8.4% and 880 fry/kg) the best performing parental line. There was no advantage to using this crossbred line to form hybrid, since the best overall 2-year performance was exhibited by parental line AU-1.

The lower ovulation % and spawning % obtained from the crossbreed could be the result of negative heterosis for reproductive performance, or delayed sexual maturation. This is a surprising result as previous work showed the opposite effect for intraspecific spawning in pens within ponds when ancestors of these lines were compared to their crossbreed. In the previous work, the crossbreed exhibited positive heterosis for early sexual maturation, but there was no difference among genotypes at 4

Table 9. Mean egg quality, fecundity, hatch %, and fry/kg for selected strains and their randomly bred controls that were spawned during all weeks combined, and each individual spawning week. Each strain couplet represents a different selected line (top) compared to its randomly bred control (C). An asterisk following the entry indicates that there are significant differences in that trait between the select and control lines for that strain.

	Egg Quality		Fecundity (Eggs/kg)		Hatch %		Fry/kg	
Genotype	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
AU-1	13	4.0 ± 0.9	16	6,575 ± 2,863	16	33.5 ± 24.8	16	2,173 ± 1,739
AU-1C	29	4.0 ± 0.6	29	8,138 ± 3,275	29	36.7 ± 34.9	29	3,066 ± 3,141
AU-7	16	3.9 ± 0.8	16	8,150 ± 2,617	16	10.8 ± 9.8	16	801 ± 793
AU-7C	18	3.6 ± 1.1	20	9,130 ± 3,922	20	10.9 ± 15.1	20	993 ± 1,169
AU-3	32	3.9 ± 0.6	35	9,207 ± 3,490	35	30.4 ± 44.9	35	2,313 ± 2,473*
AU-7C	18	3.6 ± 1.1	20	9,130 ± 3,922	20	10.9 ± 15.1	20	993 ± 1,169*
AU-6	16	3.8 ± 0.5	16	9,479 ± 2,398	16	29.2 ± 24.5*	16	2,849 ± 2,670*
AU-7C	18	3.6 ± 1.1	20	9,130 ± 3,922	20	10.9 ± 15.1*	20	993 ± 1,169*
AU-4	25	3.8 ± 1.0	27	8,841 ± 3,273	27	22.5 ± 28.2	27	1,870 ± 2,067
AU-1C+AU-7C	47	3.8 ± 0.9	49	8,543 ± 3,548	49	26.1 ± 31.0	49	2,220 ± 2,712
AU-8	45	3.7 ± 0.8	46	10,202 ± 3,120*	46	22.9 ± 35.0	46	2,124 ± 2,400
AU-1C+AU-7C	47	3.8 ± 0.9	49	8,543 ± 3,548*	49	26.1 ± 31.0	49	2,220 ± 2,712

Table 10. Ovulation %, mean fecundity (eggs/kg), mean hatch %, and mean fry/kg for AU-1 select and control lines spawned during the 2005 spawning season using a 100 ug implant of luetinizing hormone-releasing hormone analog (LHRHa). Couplet represents a different selected line (top) compared to its randomly bred control (bottom).

	Ovulation %		Fecundity (Eggs/kg)		Hatch %		Fry/kg	
Genotype	N	%	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
AU-1	13	69.2	9	10,581 ± 2,366	9	32.5 ± 31.4	9	3,772 ± 3,443
AU-1 Control	18	61.1	11	10,360 ± 3,776	11	58.2 ± 59.5	11	5,552 ± 4,650

Table 11. Percentage of three-year-old females gravid, culled, ovulated, and spawned for AU-1, AU-7, and the crossbreed AU-7 female × AU-1 male channel catfish during the 2004 spawning season when induced to spawn with 20 and 100 µg/kg (priming and resolving dose) luteinizing hormone-releasing hormone analog (LHRHa). Ovulation % and spawning % were significantly different among genotypes. Strains were not different for % culled.

Genotype	N	% Gravid	% Culled	% Ovulation	% Spawned
AU-1	34	73.5	26.5	60.0	44.1
AU-7	24	79.2	20.8	84.2	66.7
AU-7 × AU-1	47	68.1	31.9	46.9	31.9

Table 12. Ovulation %, mean latency period, egg quality score, fecundity (eggs/kg), hatch %, and fry/kg for AU-1, AU-7 and the crossbreed AU-7 female × AU-1 male channel catfish females when induced to spawn during the 2004, 2005, and 2004 and 2005 spawning season combined. Means followed by the same letter are not different (*P*>0.05) within each column. Ovulation % was different among genotypes for three-year-old and when data from three-year-old and four-year-old females are pooled. Ovulation % was not different for four-year-old females.

	Ovulation%		Egg Quality		Fecundity (Eggs/kg)		Hatch%		Fry/kg	
Genotype	N	%	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
Three-Year Old-Females										
AU-1	25	60.0	12	4.0 ± 1.0a	15	6,633 ± 2,954a	15	32.5 ± 25.3a	15	2,135 ± 1,794a
AU-7	19	84.2	16	3.9 ± 0.8a	16	8,150 ± 4,829a	16	10.8 ± 9.8b	16	801 ± 793b
AU-7× AU-1	22	46.9	13	3.7 ± 0.6a	15	7,317 ± 4,829a	15	8.4 ± 15.5b	15	880 ± 1,591b
Four-Year-Old Females										
AU-1	13	69.2	2	5.0 ± 0.0a	9	10,581 ± 2,366a	9	32.5 ± 31.4a	9	3,772 ± 3,443a
AU-7	5	80.0			4	12,579 ± 3,196a	4	9.9 ± 15.9a	4	1.592 ± 2.773a
AU-7 × AU-1	16	62.5	5	4.6 ± 0.9a	10	12,230 ± 3,038a	10	27.4 ± 26.3a	10	3,393 ± 3,002a
Pooled Years										
AU-1	38	63.2	14	4.1 ± 0.9a	24	8,113 ± 3,327a	24	32.5 ± 27.1a	24	2,749 ± 2,595a
AU-7	24	83.3	16	3.9 ± 0.8a	20	9,036 ± 3,213a	20	10.7 ± 10.8b	20	959 ± 1,348b
AU-7 × AU-1	48	52.1	18	3.9 ± 0.8a	25	9,282 ± 4,806a	25	16.0 ± 21.0b	25	1,885 ± 2,537ab

years of age. The negative heterosis for early sexual maturity in the current study is the more likely explanation since the same females spawned as 4-year-olds during the 2005 spawning season, and had ovulation % intermediate to the parental lines, AU-7 and AU-1. Six generations of selection for body

weight in the parental lines has apparently altered their genotypes such that combining ability for reproductive performance has been altered. Alternatively, this may be a genotype × environment interaction for reproductive performance as the original study was intraspecific spawning in pens, whereas the current study was induced spawning and artificial fertilization to produce interspecific hybrids.

Regardless of spawning period there was no effect of genotype on latency period when induced to spawn with a 20 μg/kg priming dose of lueitenizing hormone-releasing hormone analog (LHRHa) followed by a resolving dose of 100 μg/kg of LHRHa at 3 years of age. It appears that the water temperature is the major determinant for latency period. As water temperature increases, naturally the latency period from the time of priming dose of LHRHa becomes shorter. Another factor that may cause shorter latency periods during the latter portion of the spawning season is the accumulation of temperature degree-days which causes maturation of ova and thus would shorten latency periods.

However, in 2005 when the fish were older and a 100-μg implant of the ovulating agent LHRHa was used to induce spawning, there was a significant effect of genotype on latency period. The crossbreed had an intermediate latency period (65.7 hours) that was between those of the parental lines (AU-1 = 73.4 hours and AU-7 = 55.1 hours) and, there was a much wider variation in latency periods for each of the genotypes. This increased variation in latency period occurs when using implants of LHRHa during the first half of the spawning season. Apparently, there is either a genotype × age, genotype × delivery method or genotype × temperature interaction for latency period in channel catfish females.

Egg quality scores of the crossbreed AU-7 × AU-1 and its parental lines were high both years. The eggs in 2005 could have been of higher quality because females used were older. Alternatively, egg quality could have been improved due to the use of an implant of LHRHa instead of priming and resolving doses of LHRHa. Additionally, the 3-year-old females had to compete with older females during preparation whereas the 4-year-old females were prepared by themselves.

Genotype of female did not have an effect on fecundity during either of the two spawning seasons nor when the data from the spawning seasons are combined. Fecundity of the genotypes during the 2004 spawning season varied between 6,600 and 8,200 eggs/kg of female body weight. In 2005, the fecundity of females belonging to all genotypes was higher (range: 10,600 to 12,600 eggs/kg) than that seen during the 2004 spawning season. Either increased sexual maturity or some environmental difference was the cause of this increase as relative fecundity usually decreases with size. Other researchers, with strains of similar breeding history, have also found that there has been no improvement in fecundity when using a crossbreed. However, the same researchers found that when using the crossbreeds AR and ARMK, there was an improvement in fecundity over the parental lines. Crossbreeding has variable effects on fecundity as it does for virtually all traits.

Hatch % and fry/kg were interrelated. During the 2004 spawning season, there was a significant effect of genotype on the hatching % of eggs with parental strain AU-1 having significantly higher hatches than either the parental line AU-7 or the crossbreed. Hatch % was not significantly different during 2005, but ranged from a low of 9.9% (AU-7) to a high of 32.5% (AU-1). The observed result was that AU-1 had three times the hatch of AU-7 both years, triple that of the crossbreed when 3 years old, but very similar to the crossbreed at 4 years of age.

When the data from 3-year-old and 4-year-old females was combined, there was also an effect of genotype on hatch. The parental line AU-1 had a hatch % between two and three times that seen by

either the parental line AU-7 or the crossbreed AU-7 × AU-1.

Results were essentially parallel for fry/kg. Three-year-old females belonging to the genotypes AU-7 (801 fry/kg) and AU-7 × AU-1 (880 fry/kg) produced significantly fewer fry/kg of female body weight than genotype AU-1 (2,135 fry/kg). No statistical significant differences in fry production were observed during the 2005 spawning season once all females had achieved 4 years of age. During this spawning season, fry per kilogram of female body weight varied from a minimum of 1,600 (AU-7) to a maximum of 3,800 (AU-1). Similar to hatch %, the observed result was that AU-1 had two to three times the fry production of AU-7 both years, triple that of the crossbreed when 3 years old, but very similar to the crossbreed at 4 years of age. Also, similar to hatch %, when the data is pooled for 3-year-old and 4-year-old females, AU-1 produces approximately three times as much fry/kg than AU-7, and about two times as many as AU-7 x AU-1.

One explanation for the difference in hatch % and fry production among the genotypes is a genetic maternal effect with regards to these reproductive traits. The crossbreed and the maternal genotype, AU-7, produced virtually identical hatching % and fry production values during the 2004 spawning season. However, the maternal effect may only be evident at younger ages, since once females achieved 4 years of age, all genotypes examined have statistically identical hatching % and fry production.

Reproductive traits often exhibit heterosis and can be improved with crossbreeding; however, there was no heterosis observed in this experiment. At 3 years of age, there was the possibility of dominance from the poorer performing AU-7 parent, but this genetic mechanism was not evident at 4 years of age.

The parental lines were potentially inbred and may have been exhibiting inbreeding depression for reproductive performance. The data from the 3-year-olds does not support this hypothesis as means for the crossbreed are the same as the poorest parent, and crossbreeding should correct inbreeding depression. If AU-7 was experiencing inbreeding depression, data from the 4-year-old broodfish would support correction of the inbreeding depression for AU-7, but not for AU-1. Contradictory to what was observed, the effects of heterosis and inbreeding are usually more dramatic at younger ages.

If we consider the results from both years and the data from combined years, there is no benefit for hybrid fry production by using crossbred channel catfish females as dams. The best 2-year performance would be obtained from using the AU-1 line as dams.

During 2006, the early sexual maturity of 4-year-old blue catfish male crossbreeds (AU-1 × AU-2) was examined. These fish exhibited no signs of early sexual maturity, and had extremely small testes.

Mississippi State University. Groups of nine, 2-year-old female channel catfish brood stock obtained from each of four different strains/sources were tagged and stocked into four, 0.04-ha earthen ponds (36 fish per pond, 9 fish per strain) in April. Blood and egg samples were collected from twelve fish in each pond (3 fish/strain) every month for 11 and 9 months for blood and eggs, respectively. No individual fish within a strain was subject to sampling more than once every four months. Plasma estradiol, plasma testosterone, cathepsins, protein content of eggs and egg size were measured. No noteworthy differences in the mean values of the physiological indices monitored were observed among the four strains during each month.

Objective 2. *Develop induced spawning techniques and management strategies to optimize gamete collection and storage.*

Objective 2a. *Develop procedures to predict ovulation of channel catfish.*

Auburn University. Hybrid channel × blue catfish can be obtained by induced spawning and artificial fertilization but with variable results. A threshold degree of maturity must be reached before broodfish can be induced to spawn, but selection of such fish can be very subjective. Temperature of the surrounding environment affects the rates of physiological processes in fish. Response time to applications of induced spawning hormones such as LHRHa is thought to be related to water temperature.

Female broodfish (Marion strain channel catfish) were given a subjective ranking of poor, fair or good as well as measurements of body weight, total body length, body width and girth were taken. Broodfish were held at 24, 26, and 28°C in 60-gallon aquaria and injected with LHRHa at 20 μg/kg as a preparatory injection followed 12 hours later with 100 μg/kg. Fish were monitored hourly as ovulation approached, and the time of the first egg deposit and when approximately 100 eggs were found were recorded. Approximately half the females were manually stripped soon after the first egg was observed, and the other fish were stripped 4 to 6 hours after the first egg was observed. Eggs were artificially fertilized with blue catfish sperm and incubated. For each egg mass, the percentage of viable embryos at 24 hours after fertilization, the percent hatch, and percent survival at swim-up was determined.

The overall mean degree-hour response time (temperature in °C multiplied by the time in hours to first egg release) was 1,156 ± 275. The mean degree hour response time was 1,416 ± 107 at 24°C, 1,228 ± 211 at 26°C and 981 ± 278 at 28°C. The percentage of females that ovulated were 58, 62.5 and 87.5% at 24, 26, and 28°C, respectively. The majority of females which did ovulate did so between 58 to 64 hours at 24°C, 48 to 52 hours at 26°C and 24 to 40 hours at 28°C with the fish classified as "good" spawning sooner than the "poor" classification at all temperatures. When only the good quality females were considered, the weight of eggs released/kg female varied by water temperature. At 24°C an average of 70 ± 60 g were obtained/kg, at 26°C 126 ± 41, and at 28°C 154 ± 34. The number of eggs/g of eggs also varied by temperature, 71 ± 11, 53 ± 6, and 48 ± 10 at 24, 26 and 28°C respectively. Egg quality varied with how soon eggs were taken after the first egg was released. For females at 28°C, when eggs were taken within 2 hours of being observed the % viable embryos averaged 76 ± 13% and the % hatch was 31 ± 16%. When eggs were taken at 4 or more hours of being observed, the % viable embryos averaged 66 ± 19% and the % hatch was 9.7 ± 6.6%. When a female was stripped within 2 hours after the first eggs were released, a lower weight and total number of eggs/kg (107.3 ± 46.6 and 5,739.8 ± 2174) were obtained relative to fish stripped 4 or more hours after the first eggs were released (147.7 ± 36 and 7,724 ± 2,120, respectively).

Proper selection of broodfish for induced spawning can help insure a high rate of spawning success and good egg quality. However, the brood selection is often subjective based on general appearance of the fish and the culturist's experience. Appropriate quantitative criteria can reduce individual bias and assists the less experience biologist in brood selection. Five trials were conducted using 3- and 5-year-old channel catfish females where the physical characteristics of total length, weight, and width were measured and ratios calculated. Development and pulsation of the genital papilla was also used as

a point of evaluation. Females were induced spawned using LHRHa at 120 µg/kg and manually spawned. Eggs were artificially fertilized with blue catfish sperm. Spawning success, and egg production characteristics were evaluated as to their relationship to brood stock characteristics.

Results at a glance...

☆ *Hatch rate of hybrid embryos is improved if LHRH-injected channel catfish females are stripped within 2 hours of first observation of egg release. Waiting longer will increase the number of eggs stripped, but this is more than offset by much lower hatch rate.*

Age of broodfish had a significant effect on spawning success. Of the 5-year-old fish, 91% spawned while only 26% of the 3-year-old fish spawned. The two age groups also differed in average weight which was a factor influencing spawning success. Fish weighing over 3 kg had an average spawning rate of 80% while fish weighing 3 kg or less averaged 20%. Fish that were 60 cm, or more, in total length, had a spawning rate of 80%. Fish with a length (cm)/weight (kg) ratio less than 15 also averaged an 80% spawning rate. The length (cm)/width (cm) ratio did not exhibit a well-defined pattern, however, fish with a ratio less than 5 had a 60% success rate. Fish with a width (cm)/weight (kg) ratio less than 4 have 75% chance of spawning. Whether or not the genital papilla was pulsating at the time of the first LHRHa injection had no relationship to spawning success. Brood age affected egg characteristics. Younger fish had more eggs per gram of eggs. The mean number of eggs/kg for 3-year-old fish was 8274 ± 2868, while the mean for 5-year-old females was 4842 ± 1130. The 3-year-old fish ovulated, on average, 9.7 hours later than 5-year-old fish. In general, variations in other brood stock descriptors were not associated with variations in eggs per gram, number of eggs per kilogram body weight, time of ovulation, or viability. However, egg diameter was related to length (cm)/weight (kg) ratio with larger fish producing larger egg diameters than smaller fish.

In this study, brood stock age was the most important consideration for spawning success. Related to age were brood weight and length and their effect on spawning success. Ratios of body proportions also were related to spawning success with fish having a width (cm)/weight (kg) ratio of less than 4 having a greater than 70% success rate. To obtain the best induced spawning success broodfish should be selected to be 5 years old, with a weight of over 3 kg, and a length greater than 60 cm. Such fish should give a 94% spawning rate.

Results at a glance...

☆ *Brood stock age is important for spawning success. Other indicators of spawning success are broodfish weight, length, and ratios of body proportions. Broodfish having a width (cm)/ weight (kg) ratio of less than 4 have a high spawning success rate. To obtain the best success in induced spawning to produce hybrids, broodfish should be 5 years old, with a weight of over 3 kg (6 to 7 pounds), and a length greater than 60 cm (24 inches).*

Objective 2b. *Conduct pivotal protocol studies for determining dosage rates and timing of application of luteinizing hormone releasing hormone (LHRHa), carp pituitary extract and catfish pituitary extract to maximize ovulation, hatch rate and fry production.*

USDA-ARS. The effectiveness of catfish pituitary extract, carp pituitary extract, and LHRHa for inducing spawning in female channel catfish and subsequent production of channel catfish × blue catfish hybrid fry was compared. Mature female catfish (3 to 5 years old) were injected with carp pituitary extract (N = 66), catfish pituitary extract (N = 51), or LHRHa (N = 58). Catfish pituitaries were collected in March and April at a commercial catfish processing plant from fish larger than 3 pounds, dried in acetone, and ground to a powder. Carp pituitary and LHRHa were purchased from commercial vendors (Stoller Fisheries, Spirit Lake, Iowa and Syndel International, Inc., Vancouver, British Columbia, Canada, respectively). Injection regimes were 2 mg/kg female body weight (BW) initial injection and 8 mg/kg 20 hours later for carp and catfish pituitary extract or 40 μg/kg female BW initial injection followed by 80 μg/kg 20 hours later for LHRHa. Females were checked for ovulation 24 hours following the final injection. Ovulating females were tranquilized and eggs were manually stripped into Hank's Balanced Salt Solution (HBSS). Eggs were weighed and then fertilized with blue catfish sperm. Blue catfish sperm was prepared by macerating testes from 4 to 5 blue catfish males and pooling the sperm in HBSS. Approximately 25 mL of sperm-solution was used to fertilize each 400-g sample of eggs. Egg masses were placed in hatching troughs following fertilization and percent viable embryos was determined at 48 hours post-fertilization. Fry numbers at hatch were estimated volumetrically. Data collected for each treatment included: weight of females injected, percent of injected females that ovulated, fecundity (number of eggs/kg female body weight), percent viable embryos at 48 hours, fry/kg body weight of all females, fry/kg body weight of ovulated females, and total fry.

There were no differences among treatments for any of the variables measured (Table 13). Results demonstrate that catfish pituitary extract was as effective as carp pituitary extract or LHRHa for inducing ovulation in channel catfish females. Catfish pituitary is readily available from commercial catfish processing facilities, although regulatory issues associated with using it to induce spawning in fish are not known.

Eggs from females ovulated with LHRHa flowed much easier and more completely, but it seemed their time-frame for ovulation was wider. The LHRHa may have done better if a longer period of time would have been allowed for ovulation. The pituitary-treated fish seemed to ovulate more synchronously, but never flowed as well as a good LHRHa fish. This observation that CPE-treated fish ovulate more synchronously has been confirmed at Auburn University. Latency time for LHRH-treated fish is longer, and the observations observed at USDA are consistent with observations at other locations.

Auburn University. The 2004 research was conducted with Good Manufacturing Practices (GMP) grade LHRHa for injections and research grade LHRHa for implants at Auburn University. The dose 30/150 (μg/kg priming/resolving dose) was the most effective injection treatment confirming earlier results with research grade LHRHa. (All doses are reported as total micrograms of product injected. The peptide content of the product is 82% LHRHa. Thus, a 100 μg dose of ingredient is actually 82 μg of LHRHa.). This peptide content is the same for all experiments for all institutions conducting research in this SRAC project. The efficacy of the 30/150 injection and the 100-μg implant were not different. Females that were not hormone induced

Table 13. Comparison of catfish pituitary extract, carp pituitary extract, and LHRHa for inducing spawning in channel catfish females and production of channel catfish × blue catfish fry.

Treatment	# of females injected	Mean weight of females (kg)	% females ovulating	Eggs/kg female BW	% viable embryos	Fry/kg BW all females	Fry/kg BE ovulated females	Total fry
Carp PE	66	2.9	71.0	6,482	55.5	1,348	1,788	239,100
Catfish PE	51	2.8	68.0	6,767	64.1	1,128	1,600	190,100
LHRHa	58	3.0	65.0	6,482	66.3	1,527	1,999	254,100
Standard Error		0.2	8.4	720	8.6	344	350	

and held in ponds or for short periods in tanks did not ovulate. All LHRHa treatments were effective yielding a minimum of 64.7% ovulation.

The highest observed means for ovulation percentage were the 125-µg implant and the 20/100 injection (Table 14). However, the treatments yielding the most fry/kg female body weight were the 100-µg implant and the 30/150 injection.

Early in the spawning season implants gave more consistent results than injections, but the best injection regime (30/150) was not different from the best implant regime (100 µg) (Table 15). Ovulation of females in individual units was more effective than in a communal group, and in absence of conspecific males was more effective than in the presence of conspecifics males. In terms of gamete release, all treatments were highly effective with ovulation % ranging from 66.6 to 100.0% with a grand mean of 86.1%. However, gamete quality differed among treatments as indicated by the variation in fry/kg (Table 15).

During the peak spawning season, the ovulation rates decreased (Tables 15 and 16). In terms of gamete release, most treatments were effective with ovulation % ranging from 28.6 to 71.4% with a grand mean of 54.9%. However, gamete quality differed among treatments as indicated by the variation in fry/kg. Again, 30/150 injection and the 100-µg implant were the most effective treatments and not different from each other. Fry output, approximately 1700 fry/kg, of these two better treatments was similar to the output in the early spawning period. Results were similar for the early and peak spawning (Tables 15, 16 and 17).

In two additional studies during the peak spawning season, 100-µg implants resulted in about triple the number of fry/kg compared to other treatments (Tables 18 and 19). Additionally, a single injection of 150 µg/kg of liquid LHRHa was no more effective than 50-µg implants.

During the late spawning period, the injection regime with the highest ovulation rate was 10/50 at 100% using high-line females (Table 20). The implant with the highest ovulation rate was 75 µg at

Results at a glance...

☆ *The dose of 100 µg/kg LHRHa implants results in the most consistent hybrid fry production. At the end of the spawning season this dose needs to be reduced to 75 µg/kg.*

85.7%, again for the high-line females. No hatch was obtained in the last spawning period probably because of poor quality sperm or an error in sperm preparation. Genetics had an impact on ovulation. High-line females had higher ovulation than low line females. However, egg quality data was obtained (Tables 21-25).

A second experiment was conducted during the late spawning period comparing 75-µg implants with 10/50 injections. Ovulation rates were not different, 91.4% and 91.6%, for implants and injections, respectively. Hatch, 29%, was much higher for 75-µg implant than for 10/50 injection, 12% (Table 26). In a third run, 28 out of 32 individual fish (87.5%) implanted with 75-µg ovulated, with 75.6% hatch.

Egg quality data was measured subjectively on a 5-point scale (Tables 21-25). Traits included overall quality or % good, bloodiness, whiteness and clumpiness, the latter three being negative traits. Early in the season there was little difference in egg quality obtained from fish with different dosages of injections or implants. There was a relationship between subjective egg quality and hatch. Early in the season the correlation between egg quality and hatch was 0.23. By the peak spawning period differences in egg quality emerged. Egg quality was higher for implants than injections. The correlation between egg quality and hatch increased to 0.67.

In the late spawning season differences in egg quality still existed. Again implants tended to have higher mean values than injections. Genetics impacted the results. High-line females had higher observed means than low lines.

Implanted fish had a more variable time of ovulation, but females that ovulated up to 48 hours later than the average female gave high quality eggs, whereas late ovulating injected females give over ripened eggs. The advantage of the implants is greatest late in the spawning season.

Latency period after initial injection was longer in the early season at lower temperatures. Higher doses tended to give shorter and more uniform latency periods. Injections produced shorter latency

Table 14. Ovulation percentage and hybrid fry/kg female body weight for channel catfish and females fertilized with blue catfish sperm. Females were ovulated communally in tanks, individually in bags within tanks or individually in aquaria with or without channel catfish males. GMP grade LHRHa injections (priming dose/resolving dose, µg/kg) or research grade LHRHa implants (µg/kg) were used. Dose 0 is primarily from females held in ponds and some in tanks.

LHRH Dose	N	Ovulation %	Fry/kg
0	555	0.0	-
10/50	24	75.0	500
20/100	500	76.3	1,260
30/150	24	66.6	1,750
75 implant	32	68.9	580
100 implant	34	64.7	1,728
125 implant	10	100.0	955

periods than implants. As the season progressed and the water warmed, these differences diminished and the overall latency period shortened (Table 27). By the late season, virtually no difference in latency existed among treatments, including no differences between injections and implants.

Table 15. Early season ovulation percentage and hybrid fry/kg female body weight for channel catfish females fertilized with blue catfish sperm. Females were ovulated communally in tanks, individually in bags within tanks or individually in aquaria with or without channel catfish males. GMP grade LHRHa injections (priming dose/resolving dose, µg/kg) or research grade LHRHa implants (µg/kg) were used. The hatching environment was extreme as fungal infection was extremely heavy. Low-producing line of channel catfish was utilized.

LHRH Dose	Environment	N	Ovulation %	Fry/kg
10/50	tank	10	80	0
20/100	tank	10	100	0
20/100	bag	9	78	2,293
30/150	tank	10	90	1,727
75 implant	tank	10	80	492
100 implant	aquaria w/male	7	71	1,650
100 implant	aquaria no male	6	67	1,831
125 implant	tank	10	100	955

Table 16. Peak season ovulation percentage and hybrid fry/kg female body weight for channel catfish females fertilized with blue catfish sperm. Females were ovulated communally in tanks, individually in bags within tanks or individually in aquaria with or without channel catfish males. GMP grade LHRHa injections (priming dose/resolving dose, µg/kg) or research grade LHRHa implants (µg/kg) were used. Low-producing line of channel catfish was utilized.

LHRH Dose	Environment	N	Ovulation %	Fry/kg
10/50	bag	7	71.4	800
20/100	tank	7	28.6	502
20/100	bag	7	71.4	855
30/150	bag	7	57.1	1,736
75 implant	tank	7	42.8	858
100 implant	bag	7	71.4	1,704
75 implant	bag	7	42.8	536

Table 17. Comparison of early and peak season hybrid fry/kg female body weight for channel catfish, *Ictalurus punctatus*, females fertilized with blue catfish sperm. GMP grade LHRHa injections (priming dose/resolving dose, µg/kg) or research grade LHRHa implants (µg/kg) were used. Low-producing line of channel catfish was utilized.

LHRH Dose	Fry/kg Early	Fry/kg Peak
10/50	0	800
20/100	0	855
30/150	1,740	1,736
75 implant	492	858
100 implant	2,000	1,704

Table 18. Mean percent ovulation, mean latency period, hatching percentage, fecundity, fry per kilogram of female, and egg quality of channel catfish females by experiment after injection (priming dose (µg/kg)/ resolving dose)or implantation (single implant, µg/kg) with luteinizing hormone releasing hormone agonist, LHRHa when hybridized with blue catfish males. Water temperature averaged 27.5ºC. Means in a column followed by different letters are significantly different (*P*<0.05).

Treatment	Females	Ovulation %	Latency (hrs)	Fry/kg	Egg Masses	Egg Quality	% Hatch
10/50	7	71.4	44.6ab	862b	21	2.5c	29.9
20/100	7	71.4	40.9b	1,341b	26	3.4b	49.7
30/150	7	43.0	43.5ab	1,120b	16	3.9ab	44.1
75 implant	5	40.0	45.2a	634b	10	3.7ab	28.0
100 implant	5	80.0	42.8ab	3,394a	15	3.9ab	39.4

Table 19. Mean percent ovulation, mean latency period, hatching percentage, fecundity, fry per kilogram of female, and egg quality of channel catfish females by experiment after injection (priming dose/resolving dose, µg/kg) or implantation (single implant, µg/kg) with luteinizing hormone releasing hormone agonist, LHRHa when hybridized with blue catfish males (mean ± SD). Water temperature averaged 24.9 ºC.

Treatment	Females	Delivery Method	Mean Percent Ovulation	Latency Time	Fecundity	Fry per Kilogram	Egg Masses	Egg Quality	Percent Hatch
50	10	Implant	100	83.2a	12,452	1,500b	34	3.6	19.1a
100	10	Implant	90	78.0ab	12,198	3,846a	34	3.7	34.3a
150	10	Injection	60	69.2b	10,178	831b	22	3.4	19.0a

Table 20. Late season ovulation percentage for channel catfish females fertilized with blue catfish sperm. Females were ovulated individually in bags within tanks without channel catfish males. GMP-grade LHRHa injections (priming dose/resolving dose, μg/kg) or research-grade LHRHa implants (μg/kg) were used. Low- or high-producing lines of channel catfish were utilized.

LHRH Dose	Line	N	Ovulation %
100 implant	high	7.0	71.4
75 implant	high	7.0	85.7
30/150	low	7.0	28.6
20/100	low	8.0	25.0
10/150	low	8.0	25.0
100 implant	low	7.0	42.8
75 implant	low	8.0	25.0
10/50	high	4.0	100.0
10/50	low	3.0	33.3

Table 21. Egg quality and hybrid fry/kg female body weight during the early season for channel catfish females fertilized with blue catfish sperm. Females were ovulated communally in tanks. GMP grade LHRHa injections (priming dose/resolving dose, μg/kg) or research-grade LHRHa implants (μg/kg) were used. Low-producing line of channel catfish was utilized. R = 0.23 ($P > 0.05$) between egg quality and fry/kg.

HRH Dose	N	Egg Score	Fry/kg
10/50	10	3.7	0
20/100	10	4.3	0
30/150	10	4.3	1,727
75 implant	10	4.2	492
100 implant	13	4.0	1,831
125 implant	10	3.6	955

Table 22. Egg quality and hybrid fry/kg female body weight during the peak season for channel catfish females fertilized with blue catfish sperm. Females were ovulated individually in bags. GMP grade LHRHa injections (priming dose/resolving dose, μg/kg) or research-grade LHRHa implants (μg/kg) were used. Low- producing line of channel catfish was utilized. R=0.67 ($P < 0.05$) between egg quality and fry/kg.

LHRH Dose	N	Egg Score	Fry/kg
10/50	7	2.5	800
20/100	7	3.3	855
30/150	7	3.9	1,736
100 implant	7	3.9	1,704
75 implant	7	4.1	850

Table 23. Egg quality during the late season for channel catfish females fertilized with blue catfish sperm. Females were ovulated individually in bags. GMP-grade LHRHa injections (priming dose/resolving dose, µg/kg) or research-grade LHRHa implants (µg/kg) were used.

Dose	Line	N	Egg Score
20/100	low	4	3.0
30/150	low	5	3.4
75 implant	low	5	3.8
75 implant	high	18	4.1
100 implant	low	12	3.2
100 implant	high	20	3.6

Table 24. Percentage of eggs good or bloody during the early (E), peak (P) and late (L) season for channel catfish females fertilized with blue catfish sperm. GMP-grade LHRHa injections (priming dose/resolving dose, µg/kg) or research-grade LHRHa implants (µg/kg) were used.

Dose	Line	% Good			% Bloody		
		E	P	L	E	P	L
10/50	low	30	16	-	63	63	-
10/50	high	-	-	0	-	-	0
20/100	low	40	13	25	40	81	75
30/150	low	37	33	20	57	58	100
75 implant	low	46	44	60	58	56	80
75 implant	high	-	-	40	-	-	55
100 implant	low	39	29	42	47	37	75
100 implant	high	-	-	40	-	-	35
125 implant	low	32	-	-	50	-	-

Table 25. Percentage of eggs either "good" or "bloody" during the early (E), peak (P) and late (L) season for channel catfish females fertilized with blue catfish sperm. GMP-grade LHRHa injections (priming dose/resolving dose, µg/kg) or research-grade LHRHa implants (µg/kg) were used.

Dose	Line	% White			% Clumps		
		E	P	L	E	P	L
10/50	low	0	47	-	10	89	-
10/50	high	-	-	0	-	-	0
20/100	low	0	0	50	0	72	75
30/150	low	0	0	40	8	42	20
75 implant	low	4	0	0	4	28	40
75 implant	high	-	-	10	-	-	15
100 implant	low	0	0	25	26	50	5
100 implant	high	-	-	0	-	-	25
125 implant	low	0	-	-	35	-	-

Table 26. Effect of LHRH implants on the late season ovulation rate and hatch rate for hybrid catfish embryos. Means differed significantly.

Dose	N	Delivery	Ovulation (%)	Hatch (%)
75	59	implant	91.4	29.0a
10/50	25	injection	91.6	12.0b

Table 27. Mean latency period (hours ± standard deviation, SD) for female body weight for channel catfish females fertilized with blue catfish sperm. Females were ovulated communally in tanks, individually in bags within tanks or individually in aquaria with or without channel catfish males. GMP-grade LHRHa injections (priming dose/resolving dose, µg/kg) or research grade-LHRHa implants (µg/kg) were used. No resolving dose treatments are implants. Batch = nutrition or injection (dose) experiments.

Date	Batch	Priming	Resolving	N	Latency	SD
5/20	Injection	20	100	3	45.72	5.56
5/20	Injection	30	150	1	43.80	
5/21	Injection	10	50	8	55.81	3.35
5/21	Injection	20	100	14	51.61	1.01
5/21	Injection	30	150	8	51.17	0.31
5/21	Injection	75	0	8	57.45	0.5
5/21	Injection	100	0	9	56.42	3.77
5/21	Injection	125	0	10	56.51	0.98
5/26	Nutrition	20	100	75	43.65	1.58
5/27	Nutrition	20	100	69	40.80	1.74
6/3	Nutrition	20	100	55	50.27	1.98
6/11	Injection	10	50	5	44.06	2.14
6/11	Injection	20	100	7	40.89	4.36
6/11	Injection	30	150	3	40.43	3.52
6/11	Injection	75	0	5	43.50	3.86
6/11	Injection	100	0	4	41.75	2.2
6/16	Nutrition	20	100	64	42.35	3.17
6/17	Nutrition	20	100	57	39.85	2.1
6/18	Nutrition	20	100	41	38.88	2.72
6/23	Injection	10	150	2	40.35	4.17
6/23	Injection	20	100	2	39.35	0.07
6/23	Injection	30	150	2	43.55	2.76
6/23	Injection	75	0	8	39.8	2.81
6/23	Injection	100	0	8	38.09	1.36
6/24	Injection	10	50	5	50.28	2.42

In general, the higher doses yielded a higher number of fry per kilogram of female until late in the season when the lower doses provided maximum effectiveness. The LHRHa implants were more effective than the injections at producing channel catfish female × blue catfish male hybrid embryos. Two treatments, 100 μg/kg implants and 30/150 μg/kg injection, yield the greatest number of fry/kg. Of those two, 100 μg/kg implants was the most consistent treatment, and had the maximum mean fry/kg. Late in the spawning season 75 μg/kg implanted females had both higher ovulation rate and egg quality compared to females implanted with 100 μg/kg of LHRHa. Latency time decreased with increasing temperatures.

A study was conducted in the 2006 season to evaluate the value of LHRHa implants for blue catfish male reproductive characteristics. Males were fed either a control diet or a fatty acid enriched diet. In May 2006, males were given a 50-μg LH-RH implant followed two weeks later with a 100-μg implant. All males were described as to their weight gain, body proportions (length, head width and girth) and photographed. GSI, relative percentage of anterior testis, sperm count and motility time were recorded for each sacrificed male.

The use of LHRHa implants had no effect on testicular development but sperm count/mL was greater from implanted males as was motility time. Fertilization rates (% viable eggs at 48-hours post-fertilization) were similar for each diet and implant combination.

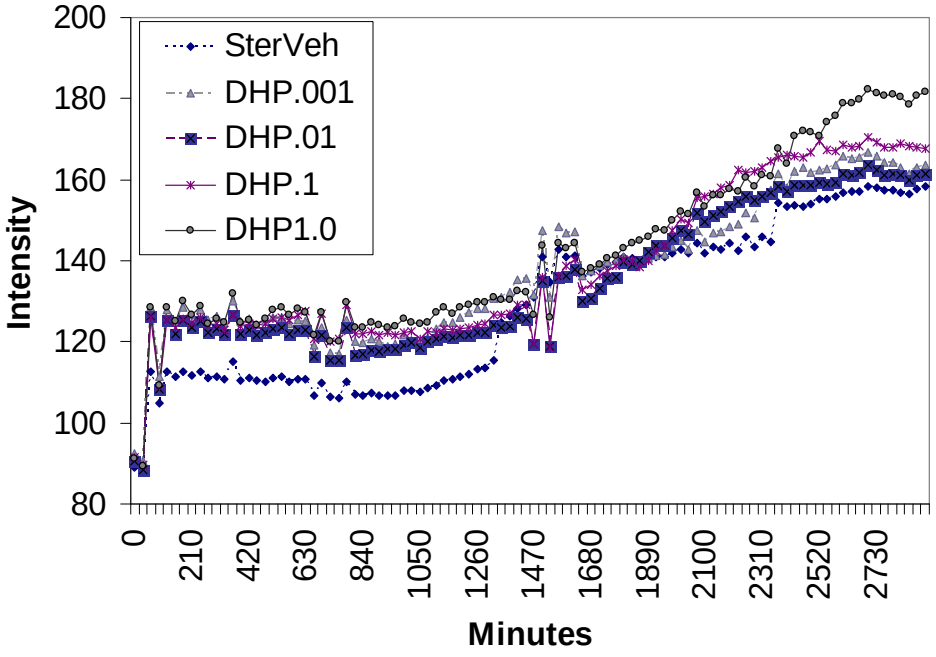
In a second experiment, different strains of blue catfish males were given a 100-μg/kg implant and then sacrificed after 48 hours. Results were not consistent across strains. Some strains had increased sperm production, but most did not. Although not statistically significant, in 4 of 5 strains there was a trend of increased hatch rate for embryos from implanted males.

University of Memphis. Channel catfish ovarian follicles were treated in vitro with 17α, 20β-dihydroxyprogesterone (DHP) and human chorionic gonadotropin in vitro. Initial efforts have focused on screening for potentially effective hormones to influence oocyte maturation and ovulation. Evaluations have included various culture media, hormonal concentrations, and the timing of the application of hormones. Methods are being investigated to adequately evaluate the oocyte response to various treatments. Such findings will hopefully be applicable to the evaluation of gonadotropins used to induce spawning of eggs of high quality from channel catfish brood stock.

Trials were conducted of in vitro oocyte maturation in early June 2006 on several ovaries. The largest size classes of oocytes were dissected from the ovaries as ovarian follicles (i.e. with surrounding follicle wall and placed into Cortland's balanced fish saline. Groups of 5 follicles were placed into wells of a 24-well plate containing 2 mL of Cortland's per well. Steroids were administered in ethanol:propylene glycol (1:1). Trials in previous seasons used an incubation time up to 24 hours; this year we extended the incubation for an additional day and found indications of successful oocyte maturation (Figure 2). There was a dose-related increase in translucency over time with most of the ooplasmic clearing occurring in the second day of incubation.

While the greatest change in translucency occurred at the highest doses of DHP, the vehicle alone also showed increased translucency indicating a high basal level of spontaneous maturation (Figure 2). Animation of image stacks revealed the quiescent nature of oocytes during maturation (no equivalent cytoplasmic movements that we have seen in activated eggs and embryos). Nevertheless, some favorably positioned oocytes that underwent clearing did show what appeared to be blastodisc formation (ooplasmic streaming to one pole).

Figure 2. Effect of 17α, 20β dihydroxyprogesterone (DHP) on in vitro channel catfish oocyte maturation as determined by computer-aided meiotic maturation assay (CAMMA). Dose of DHP in µg/ml. Temperature on scanner bed 28°C. Each treatment represents 20 follicles (4 replicate wells with 5 follicles each).



Objective 2c. *Improve hybrid embryo production via pheromonal manipulation of channel catfish males and blue catfish males for improved ovulation, spermiation, egg quality, hatch and fry production.*

Auburn University. Reducing handling and stress of channel catfish females may be key factors for effective production of channel catfish female × blue catfish hybrid catfish embryos. Females were either left free in tanks or confined in bags or aquaria. Confinement increased hybrid fry production and reduced labor involved in the production protocol. Exposure to the scent of conspecific males increased, decreased or did not affect hybrid fry production (Tables 28, 29, and 30). Method of exposure appears to have an effect. Positive effects on hybrid fry production were obtained when water from tanks containing males is introduced, whereas visual or actual contact appears to have negative effects (Tables 29 and 30).

These experiments were repeated. Direct exposure of females to the scent of conspecific males appeared to have a positive effect on fry production (Tables 31 and 32). Indirect exposure had a stronger and positive effect on fry production. Curiously, females that were directly exposed to males had an increased latency of about 6 hours.

Table 28. Mean eggs/kg female body weight (BW), hatching percentage, fry/kg female body weight and egg quality of channel catfish females exposed or not exposed to channel catfish male after injection with luteinizing hormone releasing hormone, LHRHa, when hybridized with blue catfish male (mean ± SD) in 2001. Means followed by the same letter are not different (*P*>0.05) within each column.

Treatment	Spawning Percentage (N=24)	Egg/kg Female BW	Hatching Percentage	Fry/kg Female BW	Latency Time (hour)	Egg Quality
Unexposed	90a ± 30	6,822a ± 2,268	31.1a ± 6.7	2,246a ± 652	31a ± 5	3.3a ± 0.2
Exposed	100a ± 0	7,358a ± 1,756	40.5b ± 1.6	3,031b ± 1,028	30a ± 5	3.7b ± 0.1

Table 29. Mean spawning percentage, egg/kg female body weight (BW), hatching percentage, fry/kg female body weight and latency time at 29EC for channel catfish females injected with luteinizing hormone releasing hormone, LHRHa, with different exposures to channel catfish males (mean ± SD) in 2002. Means followed by the same letter are not different (*P*>0.05) within each column.

Treatment	Spawning Percentage (N =10)	Egg/kg Female BW (N =10)	Hatching Percentage (N = 10)	Fry/kg Female BW (N = 10)	Latency Time (hour) (N = 10)
30 + 150 low male	80a ± 42	9,368a ± 1,519	14.4a ± 0.64	1,351a ± 219	31a ± 0.10
30 + 150 no male	80a ± 42	8,288a ± 2,671	52.9b ± 0.45	4,384b ± 1413	31a ± 0.10
30 + 150 high male	90a ± 31	8,211a ± 3,882	23.2c ± 0.11	1,901a ± 899	32b ± 0.52

Table 30. Ovulation % and fry/kg female body weight for channel catfish receiving 100-Fg LHRHa implants either in direct contact with or not exposed to conspecific males and fertilized with blue catfish sperm.

LHRH Dose (µg/kg)	Environment	N	ovulation %	Fry/kg
100	aquaria w/male	7	71.4	1,650
100	aquaria no male	6	66.6	1,831
100	bag no male	7	71.4	1,704

Table 31. Percent ovulation, mean latency period, and fry per kilogram of female for channel catfish females receiving 100-mg LHRHa implants when hybridized with blue catfish males males (mean ±SD). Females were not exposed to males (N), directly exposed to males (YD), or indirectly exposed (YI) to males. Means followed by the same letter are not significantly different (*P*>0.05).

Year	Male Exposure	N (Females)	Mean Percent Ovulation	Latency	Fry per Kilogram of Female
2004	N	170	62.4	47.2b± 6.8	534 ± 1289
	YD	8	62.5	53.1a± 2.7	1449 ± 0
2005	N	165	62.2	62.6a± 14.5	1,350 ± 1,660
	YI	10	50.0	62.8a ± 9.7	4,322 ± 853
	YD	32	78.1	69.0a± 10.5	2,188 ± 2,440

Table 32. Percent ovulation, mean latency period, fecundity, fry per kilogram of females, and percent hatch for female channel catfish receiving 100-mg LHRHa implants when hybridized with blue catfish males (mean ± SD). Females were not exposed (N), directly exposed (YD) or indirectly exposed (YI) to males in aquaria. Means followed the sames letter are not significantly different (*P*>0.05).

Treatment	N (Female)	Delivery Method	Mean Percent Ovulation	Latency	Fecundity	Fry per Kilogram of Female	N (Egg masses)	Egg Quality	Percent Hatch
N	5	Implant	80	67.3	13,793	5,321	22	NA	46.1
YD	32	Implant	78.1	69.0	10,676	3,232	101	4.3	41.5
YI	10	Implant	50	62.8	11,944	6,649	25	3.8	62.0

Objective 2d. *Develop extended refrigerated storage and cryopreservation of sperm.*

Louisiana State University. Knowledge of sperm concentration is essential for standardization of protocol for gamete cryopreservation and for optimizing fertilization in artificial spawning. Currently there is a lack of information regarding sperm concentration and how it relates to cryopreservation and fertilization in essentially all species including channel catfish. Practical methods for evaluation of sperm concentration in channel catfish are needed. The specific objectives of this study were to evaluate: 1) the use of a spectrophotometry in determining sperm concentrations; 2) sperm concentrations relative to gonad composition, and 3) optimal sperm concentration for fertilization during artificial spawning.

Channel catfish were seined from ponds during the months of April and May 2004. The males were killed and total lengths and weights were recorded. Testes were surgically removed and were suspended in calcium-free Hanks' balanced salt solution (C-F HBSS) at 290 mOsmol/kg. The testes were cleaned by removing adherent blood and tissue, separated into visually estimated anterior and posterior sections, weighed, and crushed in C-F HBSS (1 g/20 mL) to release sperm.

Sperm concentrations and motility estimates relative to gonad composition are summarized in Table 33. Sperm concentrations varied in relation to gonad composition.

Auburn University. Research on the production of channel catfish × blue catfish hybrid embryos and on other genetic improvement programs would be enhanced by more effective refrigerated storage of sperm. Bacterial growth is one of the main causes of death for sperm during refrigeration. Using antibiotics to improve sperm condition while refrigerated could increase sperm concentrations and ultimately the number of fry produced or would allow for more complicated mating designs. Blue catfish sperm were stored 4 days with and without gentamyacin. When antibiotics were applied to the sperm the percent hatch increased 2.5 times. In another experiment sperm refrigerated with gentamyacin gave high hatch rates after 21 days of storage. Antibiotics could allow refrigeration to become a way to store viable sperm for several days without the sperm dying from bacterial infections.

Table 33. Summary of sperm concentrations and motility from whole testis and posterior and anterior sections. Means in a column with different letters were significantly different ($P<0.05$, n=21).

	Concentration (/mL)	Total Concentration	Sperm/g Testis	Motility (%)
Intact	$1.73 \times 10^8 \pm 9.4 \times 10^7$a	$1.78 \times 10^{10} \pm 2.0 \times 10^{10}$a	$3.52 \times 10^9 \pm 1.89 \times 10^9$a	35±4.5a
Posterior	$1.06 \times 10^7 \pm 2.7 \times 10^7$b	$1.41 \times 10^8 \pm 2.37 \times 10^8$b	$2.09 \times 10^8 \pm 5.4 \times 10^8$b	23±4.6a,b
Anterior	$3.13 \times 10^8 \pm 1.18 \times 10^8$c	$1.42 \times 10^{10} \pm 1.5 \times 10^{10}$c	$5.74 \times 10^9 \pm 2.24 \times 10^9$c	41±4.6b

Objective 3. *Develop techniques to identify, assess and improve gamete quality.*

Objective 3a. *Develop criteria for standardizing and classifying egg quality prior to injection and after manual stripping and describe the morphological and physiological condition of channel catfish eggs including evaluation of morphological changes of oocytes during oocyte maturation in female catfish.*

University of Memphis. Initial images of catfish oocytes and embryos were made by automated transparency scanners. Automated transparency scanners imaged catfish oocytes and embryos during oocyte maturation and embryogenesis, respectively. This technology was developed for analysis of motility mutants in zebrafish (Computer-Aided-Screening, CAS) and is being adapted for analysis of catfish oocytes and embryos. Initial trials indicate that CAS may be used to follow catfish embryos throughout their 6- to 7-day period of development to hatching. The CAS system worked quite well in spite of the prolonged development time for catfish embryos (6 to 7 days compared with 2 days for zebrafish).

Animations of time-lapse image stacks in ImageJ revealed a surprising amount of cell movement in cleavage stage embryos. Other details of embryonic development included gastrulation/epiboly, neurulation, initiation of motility and hatching. Arrested development and subsequent cytolysis of abnormal embryos could also be clearly documented, including the developmental events prior to arrest and death.

In collaboration with Dr. Terry Tiersch at LSU, broodfish in ponds were subjected to elevated water temperatures early in the year in an attempt to induce early gonadal maturation and spawning in 2005. Eggs were stripped from female channel catfish and fertilized from sperm obtained from blue catfish testes. Observations were made on development and survival of the progeny produced. Several hybrid and channel spawns were obtained and imaged by Computer-Aided Screening (CAS).

The initial analysis of two parallel runs (trials 1 and 2) showed a time window of mortality that corresponded to approximately 2000 to 2900 minutes post-fertilization (33 to 48 hours post-fertilization). Initial analysis of the CAS images revealed embryos undergoing cytolysis as expected (Figures 3 and 4). However, upon closer examination, development was arrested in some embryos and they failed to gastrulate, yet they continued to survive. Cleavage-arrested embryos continued to show these movements in spite of failed development. Developmental arrest is not necessarily followed immediately by cytolysis and death. We are currently examining this surprising finding in more detail. Lambert, Small and Chatakondi have also observed this window of critical development, and treatment of embryos at this developmental stage is discussed in Objective 4. During this same time period, mortality occurs in channel catfish embryos exposed to antisense contructs designed to disrupt dorsal-lateral orientation. The cause of this developmental arrest needs to be ascertained and corrected.

Embryo densities of 30 or more per well were found to be deleterious to embryos imaged by the CAS system even with flows greater than 10 mL/hour. While embryo densities of 16 or less embryos per well allowed complete development to hatch in the CAS system.

In addition to cytolysis, developmentally-arrested embryos may continue to survive and superficially appear normal and viable. However, careful imaging of groups of hybrid embryos at various times after fertilization revealed cleavage stage embryos persisting until at least 72 hours post-fertilization

Figure 3. Viability of hybrid catfish and channel catfish embryos (trial 1) using CAS.

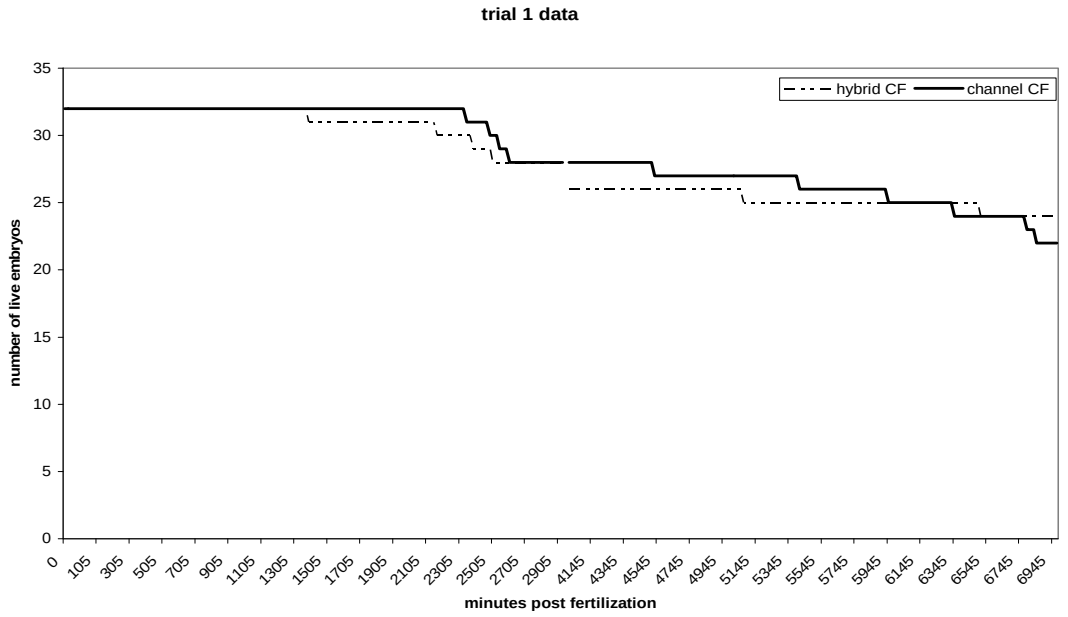
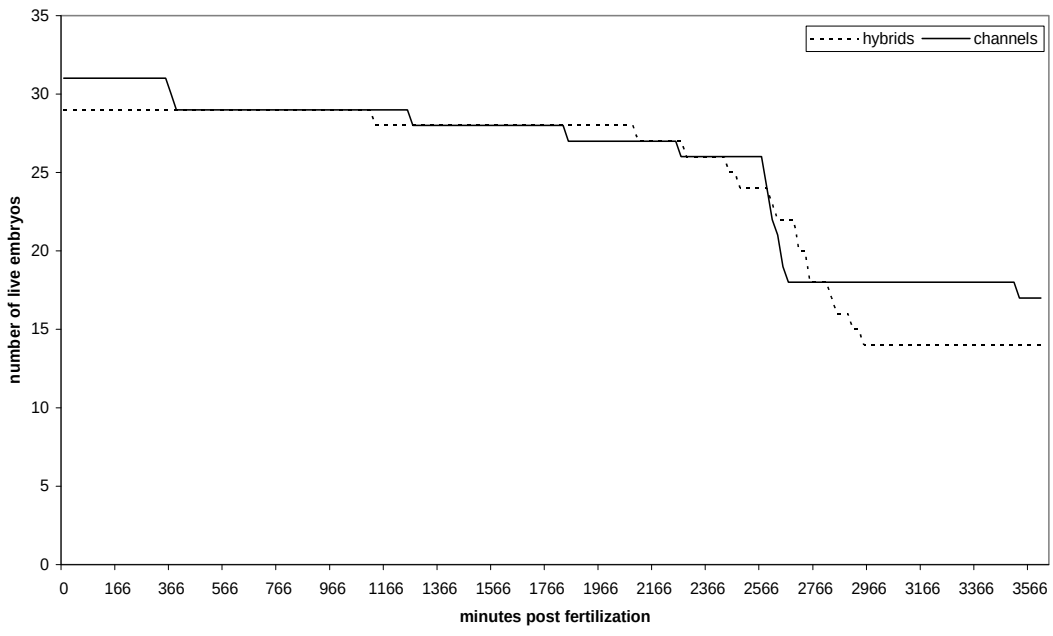


Figure 4. Viability of hybrid catfish and channel catfish embryos (trial 2) using CAS.



(Figure 5). This surprising result may explain some of the variability observed in hatch rates for hybrid catfish embryos.

Large ovarian follicles were obtained from a single channel female and were tested for germinal vesicle (GV) position and response to progestogen. Application of 5% acetic acid elicited clearing of the ooplasm and visualization of the GV. The dynamics of the clearing process was determined and imaged using CAS (Figure 6). GV identity was verified by presence of numerous nucleoli upon microscopic examination (Figure 7).

Additional images of catfish oocytes and embryos were made by automated transparency scanners. Daily use of 20-FL 4% formalin per well provided protection from parasitic infestations that prevented complete development of catfish embryos within the CAS system previously. We have begun high resolution imaging of fixed embryos to develop an embryo staging table for channel and hybrid catfish.

A test of 0.5% bovine serum albumen (BSA) as an egg extender in Hank's salts was found to be less effective than Hank's salts alone for hybrid catfish from the USDA-ARS Stoneville, Mississippi, facility. The CAS system worked quite well in spite of the prolonged development time (6 to 7 days).

Cytoplasmic movement and hence viability may occur without complete or continued embryonic development in both hybrid and channel catfish embryos at least for hours to days. We wanted to determine 1) if unfertilized, but water activated channel eggs produced these cytoplasmic movements and 2) to what extent cleavage occurred in the absence of sperm. Channel catfish eggs from brood females at the USDA Catfish Genetics Research Unit, Stoneville, Mississippi, were obtained and divided into several batches. Some batches were placed directly into well water to activate the eggs without sperm. Other batches were placed into Hank's balanced saline solution and subsequently fertilized with blue catfish sperm or activated with water alone. Computer aided screening (CAS) was used to follow changes in eggs and embryos over time. Activated, but unfertilized, eggs showed the characteristic movements seen previously in normally fertilized embryos. In addition, blastodisc enlargement and protrusion also took place mimicking normal development (Figure 8). None of the activated, unfertilized eggs underwent gastrulation. To determine if these eggs did in fact undergo cleavage, groups were fixed in 4% formalin, dehydrated in Methanol and stained with DAPI to visualize DNA and cleavage furrows, as described previously for fertilized eggs. No nuclei or cleavage furrows were evident compared to normally fertilized eggs prepared in the same way (Figure 9).

Results at a glance...

- ☆ *Water activated, but unfertilized, eggs showed the characteristic movements seen previously in normally fertilized embryos. Blastodisc enlargement and protrusion also took place mimicking normal development, however, none of the activated, unfertilized eggs underwent gastrulation or cleavage.*

To determine the effect of Hank's balanced saline solution on subsequent early development of hybrid catfish embryos, eggs from two channel females were fertilized with blue catfish sperm after eggs had no pre-incubation, 2, 3 or 4 hours of Hank's saline treatment. The results of initial scoring for blastodiscs containing normal and abnormal cleaving blastomeres (Figure 10) is shown in Table 34.

The preliminary results presented in Table 34

Figure 5. Digital photomicrographs of dechorionated, formalin-fixed hybrid catfish embryos. Embryos may arrest development in the cleavage stage but not immediately cytolyze.

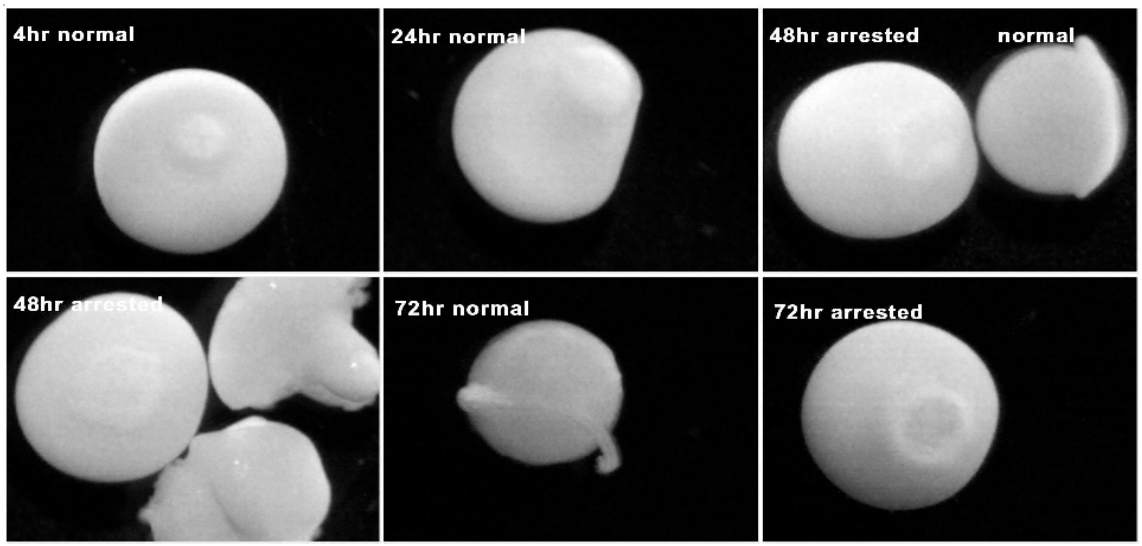
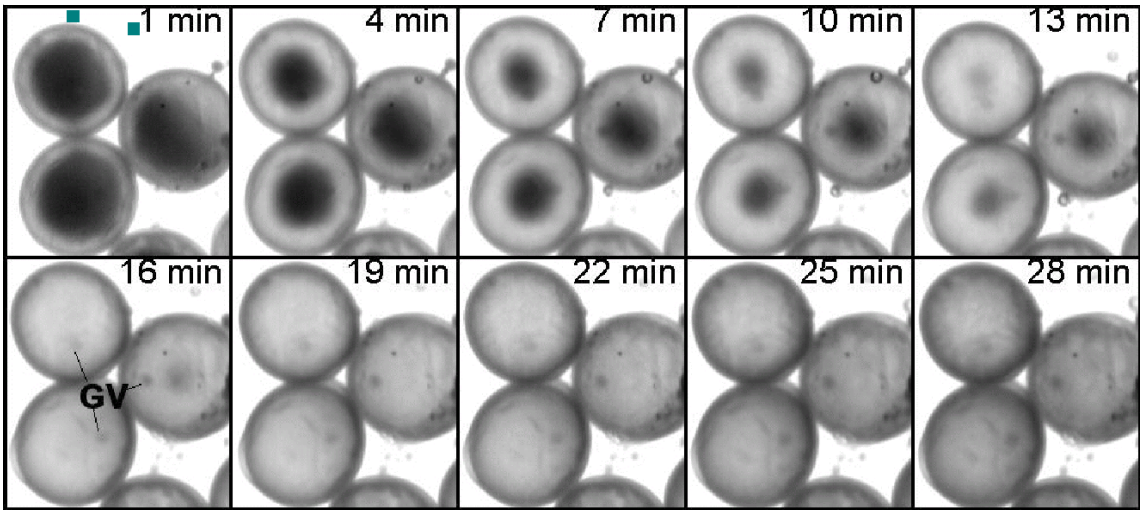


Figure 6. Channel catfish oocytes imaged by CAMMA and treated with 5% acetic acid. This treatment clears the yolky ooplasm and reveals the presence and location of the oocyte nucleus or germinal vesicle (GV).



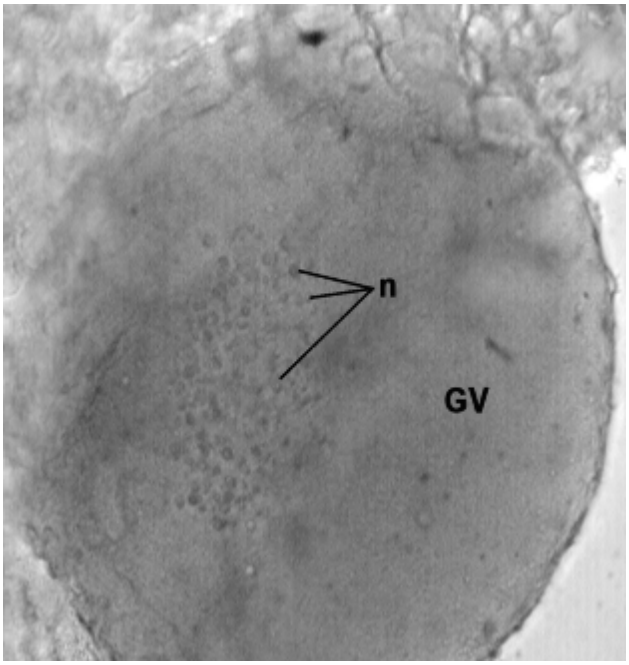


Figure 7. Isolated channel catfish oocyte germinal vesicle (GV) after 5% acetic acid fixation. Note the large number of characteristic nucleoli (n), a definitive set of landmarks for the GV.

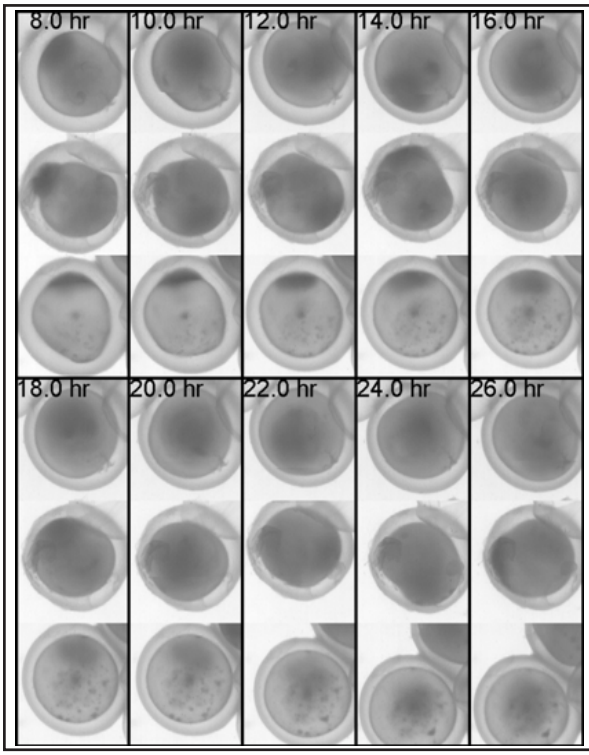


Figure 8. Three representative activated, unfertilized channel eggs imaged by CAS. Time after water addition shown at the top of each panel. Note the dark blastodisc found at one end of each egg in the top, left panel. Also note the change in egg outline indicating cytoplasmic movements or contractions. None of these underwent gastrulation.

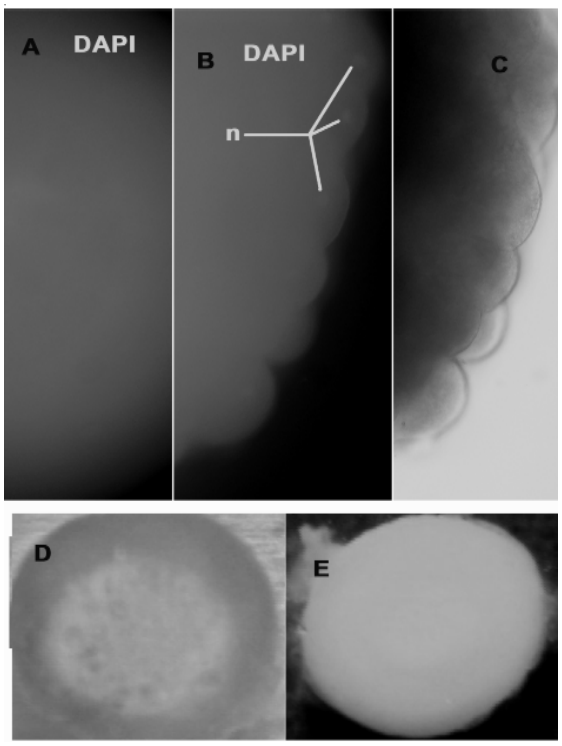


Figure 9. Comparison of activated, unfertilized channel eggs (A & D) to hybrid catfish embryos B, C & E) at 5.5hr post activation or insemination, respectively. DAPI DNA staining revealed blastomere nuclei (n) in fertilized egg blastodisc (B) but not in activated, unfertilized egg blastodisc (A). Brightfield (C) shows rounded blastomeres with prominent cleavage furrows in the fertilized egg. The corresponding dissecting microscope view (E) of the fertilized egg shows a uniform blastodisc with a faint halo of clear ooplasm, by contrast, the blastodisc of the activated, unfertilized egg (D) seems riddled with crater-like holes and lacks a halo around the irregular blastodisc.

Figure 10. Comparison of normal (B) and abnormal (A) cleavage stage hybrid catfish embryos. Embryos were fixed in 4% formalin, dehydrated in MeOH and manually dechorionated to allow direct inspection of the cleaving blastodisc. Normal embryos have a smooth blastodisc with a regular outline, often with an apparent halo of clear ooplasm. Abnormal embryos have an irregular, pock-marked blastodisc without the halo.

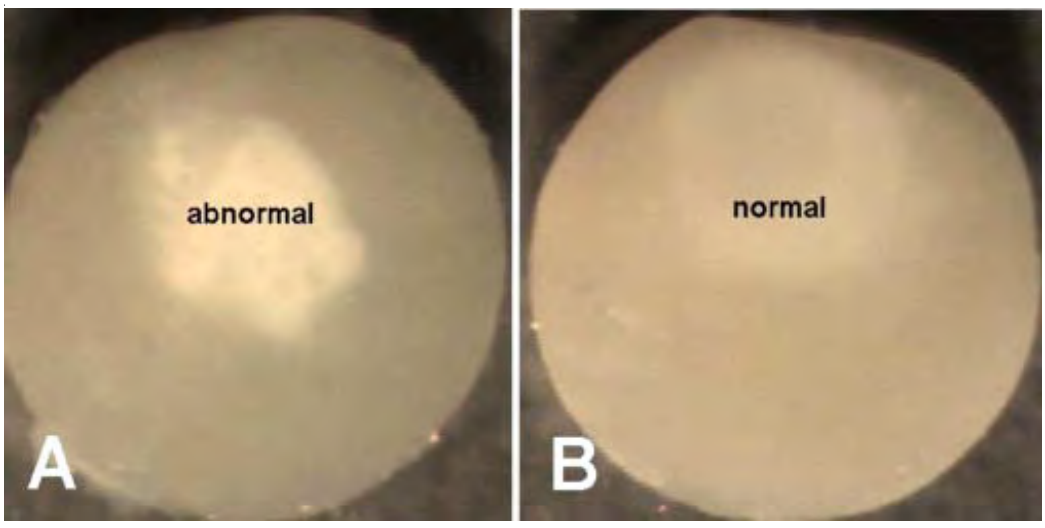


Table 34. Effect of channel egg incubation in Hank's balanced salt solution on development to early and late cleavage stages after insemination with blue catfish sperm. Data presented as number of embryos fixed and manually dechorionated presenting smooth blastodiscs with halos of clear ooplasm (scored as normal), all others were deemed abnormal or arrested.

	Hanks hrs	5/25/06 6pm normal	early cleavage total	% normal	5/26/06 10am normal	late cleavage total	% normal
female 1	0	8	11	73	2	7	29
	2	1	8	13	5	10	50
	3	10	16	63	1	11	9
	4	5	10	50	2	10	20
female 2	0	8	14	57	4	10	40
	2	12	14	86	nd	nd	nd
	3	11	12	92	7	10	70
	4	13	15	87	8	13	62

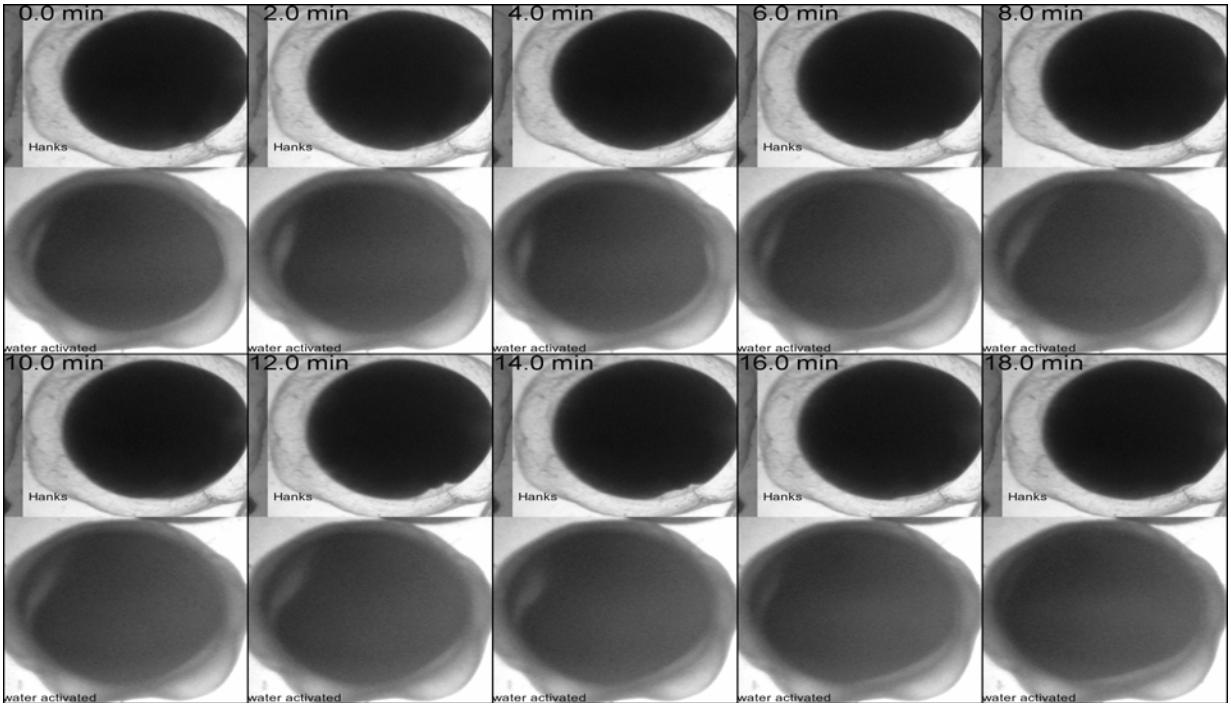
indicate that channel eggs remain fertilizable in Hank's balanced saline for at least 4 hours and can develop at least through late cleavage. Nevertheless, there does seem to be a decrease in development from early to late cleavage in these samples, suggesting some progressive developmental arrest.

Since the results presented in Table 34 indicated that channel catfish eggs may remain viable and fertilizable for an extended period, eggs incubated for 24 hours in Hank's were imaged using CAS while either remaining in Hank's or after being transferred to fresh, dechlorinated water (activation conditions). The results (Figure 11) indicate that eggs in Hank's after 24 hours are already undergoing cytoplasmic contractions, albeit reduced, and that activation by fresh water did not affect the egg significantly. This surprising result suggests that refinement of a saline based on Hank's saline may allow short-term (hours to days) storage of eggs, thereby expanding possible husbandry practices with hybrids.

We are currently extracting data from stacks of scanned images from 1) catfish oocytes treated with various media and hormones, and 2) analysis of embryonic development. We also plan to screen catfish oocyte and ovary extracts for reaction with cell-cycle control protein anti-bodies (e.g., anti-cyclin B1) that may prove useful in our studies of oocyte maturation in catfish. We are carrying out large scale dechorionations and imaging of the samples fixed at various times post insemination, especially comparing the early developmental time course of channel × channel, blue sperm × channel (hybrids), and unfertilized, but activated channel eggs. In addition, these samples will compare eggs pre-incubated in Hank's saline versus eggs used directly from the female.

Louisiana State University. Ultrasound is a user-friendly technology capable of creating ultra-clear images that can be captured as movies or still images. Ultrasound has been used extensively in human medicine and livestock species, but has had limited application in finfish. This non-invasive technique has been used with female livestock to monitor follicular growth through ovulation. It has also been used as a tool for sex identification and carcass evaluation in several species of fish (e.g.,

Figure 11. Channel catfish eggs stored in Hank's saline for 24 hours then imaged with CAS. Images were taken every 2 minutes (at top of each panel). The egg at the top (darker) remains in Hank's saline, note the contractions of the egg cortex from one time slice to the next. The water activated egg (lighter) is somewhat swollen (hydrated) and shows similar cortical contractions.



Atlantic salmon, Atlantic halibut, striped bass, shovelnose sturgeon, and barfin flounder). The objectives of this study were to evaluate visibility of gonads at different life stages, ovarian development in strip spawned and non-spawning females, oocyte diameter, compare ultrasound measurements to physical measurements, classify females prior to hormone injection, identify the time to strip eggs after injection, and determine the efficacy of stripping by use of ultrasound in channel catfish.

During February through June, 2004, channel catfish gonads were evaluated at three different life stages: fingerlings (under 0.4 kg), market-sized food fish (0.4 to 0.8 kg), and brood stock (more than 1.5 kg). Fish were scanned using a linear ultrasound probe (3 to 10 mHz), and gonadal sex was verified by dissection. To evaluate ovarian development, 12 females were given injections of artificial luteinizing hormone-releasing hormone. Of these, five were strip-spawned. Fish were scanned daily to monitor gonadal development.

Gonads were correctly identified as testis or ovary for fingerlings (57%), food fish (90%), and brood stock (86%). Immature gonads were difficult to distinguish from surrounding tissues. Mature testes were partially visible, but we could not quantify their development due to lack of contrast with surrounding tissues. Unlike testes, mature ovaries were easily distinguished and their development quantified by measuring ovarian diameter, calculating the ratio of ovarian diameter to body wall diameter (OD:BD), and measuring oocyte diameter. There were no

significant differences in ovarian diameter or in OD:BD between strip-spawned and non-spawning females (Table 35). Strip-spawned females had significantly larger oocyte diameters than non-spawning females on days 3 and 4 after injection. The results indicate that ultrasonography could be a useful tool for monitoring ovarian development in channel catfish. This could be used in artificial spawning of large groups of females, such as in production of hybrids of channel catfish females and males of blue catfish.

Results at a glance...

☆ *Ultrasound may be used to ascertain ovulation in channel catfish females and the appropriate time for stripping of eggs. Spectrophotometric assays can be used to determine sperm concentrations from crushed testis of catfish. Utilization of these tools should result in more efficient use of sperm and more consistent fertilization rates.*

During January to June of 2005, 234 channel catfish females were scanned *in situ* using a 3 to 10 mHz linear probe on a laptop ultrasound (TELAVET 1000, Classic Medical, Tequesta, Florida). Ultrasound measurements were compared with physical caliper measurements of the body cavity and oocytes diameters for 15 female catfish. Conditioned females (N = 210) were seined from ponds, scanned by ultrasound, and classified as "already spawned," "poor ovarian development," or "good ovarian development." Seventy-two females (6 sets of 12) classified as having "good ovarian development" were given injections of luteinizing hormone-releasing hormone analog (Peninsula Laboratories Inc., San Carlos, California). Females were scanned at 2 to 8 hour intervals to identify the proper time to strip eggs. After the fish were stripped, scanning was repeated to determine the efficacy of stripping.

Ultrasound measurements were not significantly different from the physical measurements (Table 36). With ultrasound, a trained technician could classify fish prior to injection within 10 seconds. Identifying the time to strip females involved several factors including a decrease in the space between the body cavity and the ovaries and an increase in ovarian edema (black areas) that were coincidental to ovulation of eggs into the lumen of the ovary.

Using these criteria, a trained technician could identify when to strip eggs during induced spawning by classification of eggs as "under-ripe," "ripe" and "overripe." After fish were stripped, ultrasound determined if a second stripping was necessary. The ability to identify proper timing of collection of ripe eggs from females would increase efficiency of hybrid catfish production by improving egg quality and possibly increasing numbers of fry produced.

During 2006, ultrasound was tested on a production scale. The goal of this study was to evaluate ultrasonography as a non-invasive production tool to improve the efficiency of artificial spawning in channel catfish females. The objectives were to use ultrasound to: 1) classify females prior to hormone injection; 2) identify the time after injection to strip eggs; and 3) determine the efficacy of stripping.

Between April and May 2006, approximately 800 channel catfish females were scanned in situ using a 3-10 mHz linear probe on a laptop ultrasound (TELAVET 1000, Classic Medical, Tequesta, Florida) at Baxter Land Company (Watson, Arkansas). Females were classified before injection on a scale of 1 to 5, with 5 indicating full ovarian maturity. Fish classified as 3 (fair), 4 (good), or 5 (excellent) (N = 84) were injected with carp pituitary extract, and were scanned periodically to identify the proper time to strip eggs. After the fish were

Table 35. Ovarian and oocyte development of strip-spawned (N = 5) and non-spawning (N = 7) females after hormone injection. Daily means for strip-spawned and non-spawning fish within each variable that share letters were not significantly different (*P*<0.01).

	Ovarian Diameter (mm)		OD:BD*		Oocyte Diameter (mm)	
Day	Strip-spawned	Non-spawning	Strip-spawned	Non-spawning	Strip-spawned	Non-spawning
1	54.7 ± 5.8a	54.2 ± 7.4a	0.87 ± 0.03a	0.84 ± 0.05a	1.8 ± 0.4a	1.8 ± 0.5a
2	65.8 ± 5.1a	50.4 ± 9.1a	0.89 ± 0.03a	0.85 ± 0.04a	1.9 ± 0.4a	1.9 ± 0.5a
3	70.6 ± 6.6a	63.9 ± 7.4a	0.89 ± 0.03a	0.88 ± 0.05a	2.2 ± 0.5a	1.8 ± 0.4b
4	60.6 ± 0.0a	64.3 ± 11.3a	0.90 ± 0.00a	0.87 ± 0.06a	2.0 ± 0.4a	1.8 ± 0.5b

*Ovarian diameter : Body wall diameter

Table 36. Ultrasound and physical diameter measurements (mean ± SE) for channel catfish females (N=15). Means sharing a letter within each variable were not significantly different (*P*>0.05).

Diameter	Ultrasound measurement (mm)	Physical measurement (mm)	% Difference *
Body cavity	70.4 ± 1.0a	68.4 ± 2.0a	-3
Oocyte	1.7 ± 0.1a	1.9 ± 0.1a	9

*[(Physical measurement – Ultrasound measurement)/ Physical measurement] x 100

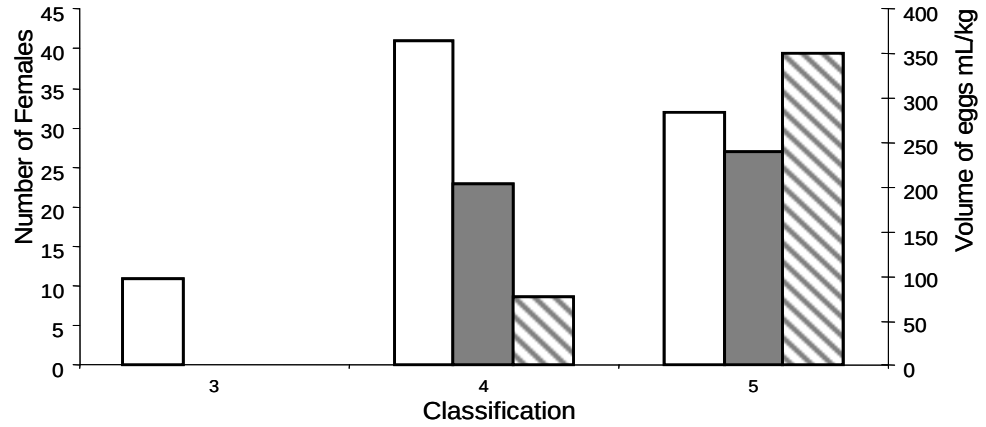
stripped (N = 50), scanning was repeated to determine the efficacy of stripping.

For the three classifications of fish that were injected, there was a significant difference in the percent of females that released eggs and in the quantity of eggs produced by the females (Figure 12). A trained technician identified the period when stimulated females could be stripped with 79% accuracy. Ultrasound techniques were able to determine the effectiveness of the stripping procedure. Because of the increase in the percentage of females spawned and the quantity of eggs produced per kilogram of female, ultrasound classification of females prior to injection could be used as a tool to increase the efficiency of hybrid catfish production.

Auburn University. The variation in the timing of egg collection and the act of manually collecting eggs may impact the quantity and quality of eggs obtained. The ovulation process and factors that might affect egg quality was examined.

Female channel catfish were held individually in aquaria and induced to spawn using LHRHa at 120 μg/kg body weight. Fish were monitored hourly as ovulation approached. The time of the first egg release into the aquaria was noted. Fish

Figure 12. Ultrasound was used to classify females as 3, 4 or 5. Selected fish were injected (white bars), stripped (gray bars), and the volumn of eggs was recorded (crosshatched bars).



releasing eggs were randomly assigned to one of four treatments: stripped at 1 hour post-egg release, sacrificed at 1 hour and the ovary collected, stripped at 4 hours post-egg release, and sacrificed at 4 hours and the ovary collected. An additional set of injected females were paired with male channel catfish and allowed to spawn naturally. Data on egg quantity and quality was collected for each female including number of eggs/g of eggs, egg diameter, fecundity and viability 48 hours post-fertilization. Samples of un-ovulated eggs and ovulated eggs from fish manually stripped at different times were collected and frozen for biochemical analyses.

The gonadosomatic index (GSI) index of females at the time of first egg release averaged 21.31%. The time of stripping after the first egg release had little effect on the quantity or quality of eggs obtained. Fish stripped at approximately 2 hours after the first egg release had a mean fecundity of 6,374 ± 2,111 eggs/kg compared to fish stripped at 4 hours of 7,086 ± 3,330 eggs/kg. The number of eggs/g egg mass (42.5 ± 9.4 and 41.1 ± 12.9) were the same for the two times of stripping groups. Egg diameter averaged 3.51 mm for both groups. Likewise egg viability at 48 hours post-fertilization did not differ between strip times, 85.1 ± 12.6 and 87.0 ± 9.4 respectively. No differences were seen in the characteristics of the first set of eggs stripped from a female and the last set of eggs stripped. Fecundity of same-age females induced to spawn and either manually stripped or which spawned naturally were similar, 7,804 ± 2,954 and 6,166 ± 4,570, respectively.

Proper selection of broodfish for induced spawning can help insure a high rate of spawning success and good egg quality. However, the brood selection is often subjective based on general appearance of the fish and the biologist's experience. Appropriate quantitative criteria can reduce individual bias and assists the less experienced biologist in brood selection. In 2005, five trials were conducted using 3- and 5-year-old channel catfish females where the physical characteristics of total length, weight, and width were measured and ratios calculated and egg samples taken for fatty acid profiles. Percent lipids of eggs collected in 2005 were compared. Eggs collected during the first stripping had lower percent lipids than those collected during the third stripping, 4.34± 1.28 and 5.59 ± 0.76 respectively. The time at which eggs were collected post-ovulation had some effect on % lipids with eggs collected at 1 hour

after egg release having 4.28 ± 1.57 % lipid and those collected at greater than 4 hours having 5.03 ± 1.25%. Three-year-old and 5-year-old females had similar percent lipids in ovulated eggs. Fatty acid profile of the above egg samples has been analyzed and the results are being processed.

A study was conducted in the 2006 season to determine if testis development and sperm characteristics were related to external morphology. Rio Grande strain male blue catfish were fed for six months either a non-enriched commercial catfish diet or an enriched diet where DHA and arachidonic acid was added. All males were described as to their weight gain, body proportions (length, head width and girth) and photographed. GSI, relative percentage of anterior testis, sperm count and motility time were recorded for each sacrificed male.

Testicular development (GSI) was not related to body weight, body length to head width ratio or body length to head girth ratio. Likewise, relative percent of anterior testis was not related to the above characteristics. There was no apparent relationship between body weight, body length to head width ratio or body length to head girth ratio to sperm count or sperm motility.

When 26 pairs of channel catfish female × blue catfish male were allowed to spawn naturally in pens in ponds two spawns were obtained (7.7%). When 13 pairs of channel catfish female × channel catfish male were allowed to spawn naturally three spawns were obtained (23.1%). The number of eggs/kg obtained and egg viability at 48 hours after fertilization was similar for channel male and blue male spawns, again indicating that there is no genetic incompatability between male blue catfish and female channel catfish. When low hatch rates are obtained for hybrid embryos, brood stock management, gamete quality and hatchery management are the problem, not species incompatabilities.

Objective 3b. *Determine the profile of estradiol hormone from serum plasma of 2-year-old females of channel catfish over a 12-month period, determine changes in oocyte maturation during vitellogenesis and identify the different cathepsins that are responsible for vitellogenin degradation and oocyte maturation in female catfish.*

Mississippi State University. The catfish industry is hampered by a chronic inefficiency resulting from the low spawning success of female brood stock for the annual production of fingerlings. Current estimates of spawning success of females range from 20 to 30%. An understanding of the relationship of annual changes in physiological indices during a reproductive cycle to oocyte maturation and successful spawning in channel catfish may contribute to an accurate prediction of successful spawns. The objective of this study was to evaluate the effects of plasma steriod concentrations (estradiol and testosterone), egg size and protein degredation by cathepsins D, L and B on in vivo egg maturation in four strains of channel catfish.

The first study of profiles of plasma estradiol and testosterone concentrations, size and protein content of eggs in 2- to 3-year-old channel catfish has been completed for four commercial strains of channel catfish, Gold Kist (2 strains), Thompson and NWAC103 for one year (age 2 to age 3). This study also included the first measurements of the activities of the proteolytic activities of cathepsins D, L, and B and their relationships to other processes involved in oocyte maturation. All of the parameters collectively evaluated may serve to assist in the selection of the best 2-year-old channel catfish female brood stock, and to determine the optimal timing of treatments of hormone injection to increase reproductive performance.

Groups of nine, 2-year-old female channel catfish brood stock obtained from each of four different strains/sources were tagged and stocked into four 0.1-acre earthen ponds (36 fish per pond, 9 fish per strain) in April. Blood and egg samples were collected from 12 fish in each pond (3 fish/strain) every month for 11 and 9 months, respectively, for blood and eggs. No individual fish within a strain was subject to sampling more than once every four months.

Great variation among individuals of the same strain precluded the identification of any significant, strain-specific differences for the variables under investigation. For all strains, mean plasma estradiol concentrations ranged from 0.02 to 0.29 ng/mL from June through December, and increased dramatically in January, peaking in February (3.4 to 3.7 ng/mL), and remained above 1.00 ng/mL through May.

Mean plasma testosterone concentrations increased from May through September (0.03 to 1.23 ng/mL), decreased in October, and then increased and remained at approximately 1 ng/mL through April. When variables from fish of all strains were collectively evaluated over time, concentrations of both plasma estradiol and testosterone significantly increased in July and again later from February to May. The increase in hormone concentration was accompanied by oocyte growth and increases in proteolytic activity of specific cathepsins, supporting the role of estradiol in regulating vitellogenesis.

During oocyte development, there were sequential relationships among hormone concentration, cathepsin activity, protein content, and predominant oocyte proteins. Plasma levels of vitellogenin gradually increased from February and peaked in May. Vitellogenin was enzymatically broken down into smaller protein units by cathepsins L, D, and B that individually predominated during different months when different stages of oocyte development occurred.

Mean activities of cathepsins D and L steadily increased beginning in October and were highest in March, whereas the activity of cathepsin B was variable from month to month. High levels of activity of cathepsin L occurred during February and March, suggesting its important role in protein degradation during that time, while peak activity of cathepsin B occurred during November to January. Activities of cathepsin D were the highest recorded, peaking in March, April, and May. Cathepsin B is more important in oogenesis or early vitellogenesis, cathepsin L assumes a principal role during middle vitellogenesis, and activity of cathepsin D peaks during late vitellogenesis.

Mean protein content of eggs was highest in October (3.08 to 3.795) when eggs appeared and decreased to levels of 0.54% to 2.14% for the remainder of the year (November through April) when eggs were present. From October to November the mean egg size increased by approximately 40%, to 1.0 to 1.4 mm, and remained at this size until May and June when size increased by approximately 75% to 100%.

Twenty hours subsequent to the injection of fish with either carp pituitary hormone or luteinizing hormone releasing hormone, plasma estradiol and testosterone concentration increased, activities of cathepsins L, D, and B increased, and egg size and protein content increased. These changes stimulated oocyte maturation. The percentages of spawning obtained were 18.8% of LHRH injected fish, 12.4% of CPE injected fish, 9.4% of fish not injected, and 0% of saline injected fish.

Injection of females with LHRHa can potentially serve as a tool to increase spawning success in appropriate commercial settings, particularly for improving 3-year-old catfish spawning success early in the spawning season. Low levels of plasma estradiol in all 3-year-old fish suggest that insufficient stimulation of vitellogenin production by estradiol may underlie the lack of vitellogenin incorporation

into developing oocytes. Sufficiently high peaks in estradiol concentration in July likely indicate a reproductively mature female. This information should serve as a foundation to apply in the evaluation of the relative effectiveness of exogenous hormone treatments in increasing the spawning success of channel catfish for producing both intraspecific and interspecific embryos.

Objective 3c. *Develop in vitro assays to evaluate sperm quality and evaluate their predictive ability in relation to fertilization and hatch.*

Louisiana State University. Channel catfish were seined from ponds during the months of April and May 2004. The males were killed and total lengths and weights were recorded. Testes were surgically removed and were suspended in calcium-free Hank's balanced salt solution (C-F HBSS) at 290 mOsmol/kg. The testes were cleaned by removing adherent blood and tissue, separated into visually estimated anterior and posterior sections, weighed, and crushed in C-F HBSS (1 g/20 mL) to release sperm. The sperm solutions were poured through a 100-µm filter into a 50-mL conical tube. Sperm motility was estimated after activation with deionized water and concentrations were calculated using duplicate hemacytometer counts. Optical density of the sperm solutions was measured using absorbance readings obtained by spectrophotometry (Spectronic 20 Genesys) at wavelengths of 400, 450, 500, 550 and 600 nm.

The most accurate absorbance readings for determining sperm concentrations from whole testis occurred at 500 nm ($y=2^9+1.99$, $R^2=0.531$). These results indicate that spectrophotometric assays can be used to determine sperm concentrations from crushed testis of channel catfish.

Flow cytometery is commonly used to evaluate cells from a wide variety of species. We have applied this technology to assess sperm quality in catfish. Several assays are being developed including evaluation of plasma membrane integrity, determination of the ratio of sperm cells to somatic cells, and evaluation of mitochondrial function. Studies were preformed this year using sperm subjected to a variety of treatments including refrigerated storage, cryoprotectant toxicity, freezing, and ultraviolet irradiation. The data from these studies are being analyzed and further studies are underway to develop practical methods to use flow cytometery as a tool for evaluation of catfish sperm.

Objective 4. *Develop economically viable standardized hatchery procedures and fertilization protocols to optimize hatching rate of hybrid embryos.*

Objective 4a. *Determine optimal sperm (fresh, frozen and refrigerated)-to-egg ratios for fertilization and hatch.*

Louisiana State University. The production of hybrid catfish fry is limited by factors including the inefficient use of sperm from the male blue catfish. The time, effort and expense involved in rearing a blue catfish male to maturity requires efficient use of the sperm obtained when the male is killed. The objectives of this study were to evaluate the effects of concentration on refrigerated and cryopreserved blue catfish sperm for: 1) sperm motility, 2) fertilization of channel catfish eggs, and 3) hatch of hybrid fry.

In 2004, only channel catfish males were available, and they were used for experimentation. Channel catfish were seined from ponds during the months of April and May 2004. The males were killed and total lengths and weights were recorded. Testes were surgically removed and were suspended in calcium-free Hank's balanced salt solution (C-F HBSS) at 290 mOsmol/kg. The testes were cleaned by removing adherent blood and tissue, separated into visually estimated anterior and posterior sections, weighed, and crushed in C-F HBSS (1g: 20 mL) to release sperm. The sperm solutions were poured through a 100-µm filter into a 50-mL conical tube. Sperm motility was estimated after activation with de-ionized water and concentrations were calculated using duplicate hemacytometer counts. The solutions were diluted to contain 1×10^8, 1×10^7 and 1×10^6 sperm cells/mL and were used for fertilization during artificial spawning with eggs from two females and sperm from three males (0.5 mL/400 eggs). The sperm concentration of 1×10^6 yielded 71 ± 16% fertilization for fresh sperm (3 ± 5% for thawed sperm); 1×10^7 yielded 88 ± 9% fertilization for fresh sperm (45 ± 37% for thawed), and 1×10^8 yielded 91 ± 10% fertilization for fresh sperm (48 ± 55% for thawed). The varied concentration of sperm used for artificial spawning yielded significant differences in fertilization and there is a correlation between sperm concentration and fertilization.

Six sexually mature male blue catfish were killed and their testes were surgically removed during the 2005 spawning season (May and June). The testes were cleaned of excess blood and tissue, weighed and placed in Ziploc bags containing 1:10 (testis weight:volume of extender) Hank's balanced salt solution prepared without calcium and magnesium. The testes were crushed and poured through a 100-µm filter. Sperm motility was estimated and concentrations of the samples were determined using hemacytometer counts. The initial sperm dilutions were divided into three groups. The first group was used as a control at the original concentration

Results at a glance...

☆ *Sperm concentrations can be reduced in currently used fertilization protocols by 100-fold with little reduction in subsequent hatch rate. This should result in much more efficient use of male broodfish when producing hybrid catfish.*

($3.6\times10^8 \pm 4.6\times10^7$ cells/mL). The remainder was diluted to final concentrations of 1×10^8, 1×10^7 and 1×10^6 cells/mL. The control and diluted samples were divided into two aliquots; one was cryopreserved at Genex Custom Collections, Inc., and the other was stored in a refrigerator (4°C) until used for fertilization. Eggs were stripped from gravid female channel catfish that had been injected with 100 µg/kg of synthetic LHRHa for artificial spawning. A monolayer of eggs was poured into 100-mL cups and fertilized using either one 0.5-mL straw of cryopreserved sperm or 0.5 mL of refrigerated sperm at the three concentrations. The fertilized eggs were placed into cups in a hatching trough for incubation. Neurulation was used as a conservative measurement of fertilization and was estimated in all cups at 24 hours after fertilization. The number of hatched fry was recorded at 120 hours after fertilization.

There was a significant difference in motility across the various concentrations and between refrigerated and cryopreserved sperm. In addition, there was a significant difference in neurulation, although there was no significant difference in hatch across the various concentrations (Table 37). Given that current hatchery practice is to use sperm dilutions prepared around 1:10 (weight:volume), this study suggests that refrigerated and cryopreserved blue catfish sperm can be diluted considerably (100 times greater) without reducing fertilization of channel catfish eggs.

Table 37. Mean neurulation and hatch rate for channel catfish × blue catfish hybrid embryos fertilized with either refrigerated or cryopreserved sperm at undiluted (approximately 4×10^8, 1×10^8, 1×10^7, or 1×10^6 sperm/400 eggs. Data for refrigerated and cryopreserved sperm treatments were pooled as no significant differences were found between these two sperm preparations. Means within a column sharing a letter were not significantly different.

	Mean ± SD	
Sperm/400 eggs	Neurulation	Hatch
Control	70 ± 14a	55 ± 17a
1×10^8	67 ± 18ab	58 ± 16a
1×10^7	64 ± 16ab	51 ± 21a
1×10^6	61 ± 11b	47 ± 15a

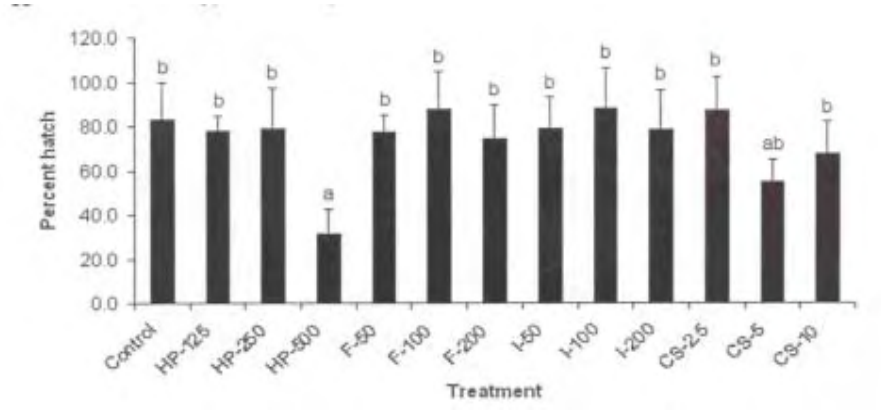
Objective 4b. *Determine the effects of commonly used therapeutics on hatching success.*

USDA-ARS. The chemotherapeutic and respective concentration yielding the greatest hybrid hatching success was identified. Four hybrid catfish egg masses were each divided into thirteen equal sub-masses. Each sub-mass was subjected to once daily chemotherapeutic treatment as a 15-minute static bath until eyed. The treatments were as follows: (1) Control (no treatment), (2) 125 ppm hydrogen peroxide, (3) 250 ppm hydrogen peroxide, (4) 500 ppm hydrogen peroxide, (5) 50 ppm formalin, (6) 100 ppm formalin, (7) 200 ppm formalin, (8) 50 ppm povidone iodine, (9) 100 ppm povidone iodine, (10) 200 ppm povidone iodine, (11) 2.5 ppm copper sulfate, (12) 5 ppm copper sulfate, and (13) 10 ppm copper sulfate. Egg masses were allowed to hatch to completion within individual containers. When hatching was complete, the fry were siphoned into a graduated cylinder and the volume of fry recorded. The total number of fry was calculated after determining the number of fry in 1 mL then multiplying times the total volume of fry collected. Hatching success was calculated as the percentage of eggs hatched.

Hatching success was high in the untreated controls (82.8%) and highly variable within treatments. Overall, hatching success was not significantly improved with chemo-therapeutic treatments; however, a tendency toward increased hatching success was observed among eggs treated with 100 ppm formalin (87.7%), 100 ppm iodine (88.1%), and 2.5 ppm copper sulfate (87.0%). A significant decrease in percent hatch was observed in eggs treated with 500 ppm hydrogen peroxide (Figure 13).

The optimal treatment frequency for maximizing hybrid hatching success was determined. Formalin is the most common therapeutant used to treat catfish egg diseases, and formalin yielded one of the highest hatching success rates in the first experiment. For these reasons, formalin was chosen as the therapeutant for this experiment. Four trials were conducted with four egg masses per trial to determine the optimal frequency of formalin application for maximizing hatching success. Formalin treatments were administered 0, 2, 3, or 4 times daily as a 100 ppm static bath. Egg masses were allowed to

Figure 13. Mean hatching success (%) of hybrid catfish eggs treated daily with increasing doses of hydrogen peroxide (HP), formalin (F), povidone iodine (I), or copper sulfate (CS). Numerals refer to dosage rate (ppm). Bars with common letters are not statistically different.



Results at a glance...

- ☆ *The frequency of formalin treatments should be three per day to maximize hatch rate of hybrid embryos. Four treatments per day is excessive. At 28°C, hybrid embryos are chemically sensitive to formalin between 42 to 46 hours post-fertilization, and formalin treatments should be avoided during this period.*

hatch to completion within individual containers. When hatching was complete, the fry were siphoned into a graduated cylinder and the volume of fry recorded. The total number of fry was calculated after determining the number of fry in 1 mL then multiplying times the total volume of fry collected. Hatching success was calculated as the percentage of eggs hatched. The optimal frequency of formalin treatments was determined to be three times daily (Table 38).

The effect of withholding formalin treatment during a putative sensitive developmental stage on hybrid hatching success was determined. A preliminary study was conducted to ascertain the developmental stage at which mortality most often occurs in hybrids. Briefly, hybrid eggs were collected throughout development, cleared in Stockard's solution and microscopically elevated for develop-mental differences indicative of egg mortality. At 28°C, mortality was observed between 42 and 46 hours post-fertilization. To determine the effect of withholding treatments during this potentially sensitive developmental period, formalin treatments (100 ppm) were administered three times daily such that treatments occurred at 42 hours post-fertilization (control) or were withheld from 42 to 44, 42 to 46, or 42 to 48 hours post-fertilization. Hatching success was calculated as previously described. Formalin treatments administered at 42 hours post-fertilization significantly reduced hatching success. Withholding treatments until 46 hours post-fertilization at 28°C yielded the greatest percent hatch (Table 39).

Table 38. Effect of daily formalin treatment frequency on hybrid hatching success.

	Frequency of daily formalin treatments			
	0x	2x	3x	4x
Percent hatch	12.7 ± 4.5a	31.4 ± 4.6b	51.6 ± 3.6c	33.7 ± 4.6b

Means followed by different letters are statistically different ($P<0.05$).

Table 39. Effect of formalin treatments administered at 42 hours post-fertilization (control) or withheld from 42 to 44, 42 to 46, or 42 to 48 hours post-fertilization on hybrid hatching success at 28ºC.

	Time of formalin treatment (hours post- fertilization)			
	42 h	44 h	46 h	48 h
Percent hatch	19.6 ± 5.3a	30.7 ± 11.0b	58.3 ± 3.9c	34.1 ± 8.5b

Means followed by different letters are statistically different ($P<0.05$).

WORK PLANNED

Louisiana State University reported and plans to continue to evaluate ultrasound as a means to evaluate female gonadal development. The University of Memphis reported and plans to continue to evaluate various hormones in vitro for stimulating oocyte maturation. Auburn University reported results for the effect of antibiotic treatment of refrigerated sperm. These experiments were not part of the original work plan, and are being conducted in addition to the original work planned.

IMPACT

At the beginning of this project, only about 4 to 5 million hybrid catfish fry were being hatched. Research results from this project have been important in increasing hybrid catfish production to more than 25 million fry hatched in 2006.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

Publications

Quintero, H. E., D. A. Davis and A. Abebe. 2006. Logit models for evaluation of spawning channel catfish (Ictalurus punctatus). Aquaculture Research (submitted).

Small, B.C. and N. Chatakondi. 2006. Efficacy of formalin as an egg disinfectant for improving hybrid catfish (Ictalurus punctatus × I. furcatus) hatching success. North American Journal of Aquaculture 68:9-13.

Doctoral Dissertations and Master of Science Theses

Ballenger, J. C. 2006. Genetic effects on the production of channel catfish (Ictalurus punctatus) female × blue catfish (Ictalurus furcatus) male hybrid fry. M.S. Thesis, Auburn University, AL.

Barrero-Monzon, M. 2005. Plasma steroid and vitellogenin concentrations and activity of cathepsins during oocyte maturation, and the influence of hormone injection in four commercial strains of channel catfish (Ictalurus punctatus) Ph.D. Dissertation. Mississippi State University, Starkville, MS.

Hutson, A.M. 2006. Evaluation of LHRHa implants and injections on the production of channel catfish (Ictalurus punctatus) female × blue catfish (Ictalurus furcatus) male fry. M.S. Thesis, Auburn University, AL.

Kristanto, A. H.2004. Evaluation of various factors to increase the efficiency of channel × blue hybrid catfish embryo production. Ph.D. Dissertation. Auburn University, AL.

Presentations

Ballenger, J., A. Hutson, D. Beam, G. Umali, A. Kristanto, M. Trask, M. Templeton, A. Davis, H. Quintero, F. Wang and R. A. Dunham. 2005. Effect of genetics on channel-blue hybrid catfish embryo production. Special Session-Hybrid Catfish Reproduction. World Aquaculture Society Meeting. New Orleans, LA. January 2005.

Barrero, M. L., L. D'Abramo, A. M. Kelly, L. A. Hanson, B. C. Small. 2005. Plasma steroid, cathpsin activity and egg size and protein content during in vivo oocyte maturation in four strains of channel catfish broodstock. Special Session-Hybrid Catfish Reproduction. World Aquaculture Society Meeting. New Orleans, LA. January 2005.

Baumgarner B.L. and T. R. Tiersch. 2006. Comparing reproductive performance of channel catfish females induced to spawn before and during the natural spawning season. WAS Aquaculture America 2006, February 14-16, 2006,Las Vegas, NV.

Beam, D. A. Hutson, A. Kristanto, J. Ballenger, M. Templeton, G. Umali, F. Wang and R. A. Dunham. 2005. Effects of confinement in bags or aquaria and exposure to the scent of conspecific males on the production of channel-blue hybrid catfish embryos by channel catfish females. Special Session-Hybrid Catfish Reproduction. World Aquaculture Society Meeting. New Orleans, LA. January 2005.

Bosworth, B. G. 2005. Comparison of carp pituitary extract, catfish pituitary extract, or LHRHa for induced spawning of channel catfish females to produce channel catfish × blue catfish hybrid fry. Special Session-Hybrid Catfish Reproduction. World Aquaculture Society Meeting. New Orleans, LA. January 2005.

Campbell, W. T., P. Pawiroredjo, A. M. Guitreau, and T. R. Tiersch. 2005. Evaluating sperm concentration in channel catfish. World Aquaculture Society Meeting. New Orleans, LA. January 2005.

Campbell, W. T. and T. R. Tiersch. 2006. Effects of blue catfish sperm concentration on fertilization of channel catfish eggs. WAS Aquaculture America 2006. Las Vegas, NV. February 2006.

Chatakondi, N., R. Yant and R. Dunham. 2005. Effects of age, strain and post-spawning protein levels in diets of female channel catfish and male blue catfish in hybrid catfish fry production. Special Session-Hybrid Catfish Reproduction. World Aquaculture Society Meeting. New Orleans, LA. January 2005.

Chatakondi, N., R. Yant, A. M. Kelly, B. C. Small and R.A. Dunham. 2006. Past , present and future of hybrid catfish in US aquaculture. WAS Aquaculture America 2006. Las Vegas, NV. February 2006.

Chatakondi, N., R. Yant, G. Umali, A. Kristanto and R. Dunham. 2005. Effective LHRHa dose based on varying maturity of channel catfish (Ictalurus punctatus) to optimize channel catfish (female) H blue catfish (male) hybrid fry production. Special Session-Hybrid Catfish Reproduction. World Aquaculture Society Meeting. New Orleans, LA. January 2005.

Dunham, R. 2006. Summary of highlights: improving reproductive efficiency to produce channel × blue hybrid catfish fry. WAS Aquaculture America 2006. Las Vegas, NV. February 2006.

Dunham, R., A.Hutson, N. Chatakondi, R. Beam, A. Kristanto, A. Chaimongkol, G. Umali and J. Ballenger 2006. Strain variation and the effect of LHRHa implantation for sperm production in blue catfish. WAS Aquaculture America 2006. Las Vegas, NV. February 2006.

Guitreau, A. M., B. E. Eilts, W. M. Burnside and T. R. Tiersch. 2006. Evaluation of ultrasonogaphy as a tool for assessing gonadal maturity in channel catfish females. WAS Aquaculture America 2006. Las Vegas, NV. February 2006.

Guitreau, A. M., B. E. Eilts, W. T. Campbell, P. Pawiroredjo, and T. R. Tiersch. 2005. Ultrasonography for evaluation of gonads, gonadal development, and oocytes in channel catfish. Special Session-Hybrid Catfish Reproduction. World Aquaculture Soc. Meeting. New Orleans, LA. January 2005.

Hughes, S. E., L. A. Littleton and R. P. Phelps. 2006. Selection criteria for channel catfish brood stock for use in induced spawning and hybrid production. WAS Aquaculture America 2006. Las Vegas, NV. February 2006.

Hutson A., Y. Zohar, E. Abraham, A. Kristanto, J. Ballenger, M. Templeton, G. Umali, D. Beam, F. Wang, C. Ligeon, P. Waters,Jr., Z. Liu and R. A. Dunham. 2005. Evaluation of LHRHa delivered via liquid injections or implants for the production of channel-blue hybrid catfish embryos. Special Session-Hybrid Catfish Reproduction. World Aquaculture Society Meeting. New Orleans, LA. January 2005.

Lessman, C.A., D. A. Perera, and B. Simco. 2006. Dynamic imaging of hybrid and channel catfish embryos: Schedule of normal development and the phenomenon of developmental arrest. WAS Aquaculture America 2006, February 14-16, 2006, Las Vegas, NV.

Littleton, L.A., S. E. Hughes and R. P. Phelps. 2006. Effect of timing of egg collection on ovulation and egg characteristics of channel catfish when used for hybrid production WAS Aquaculture America 2006. Las Vegas, NV. February 2006.

Pawiroredjo, P. A., N. Mandhani, S. G. Hall, and T. R. Tiersch. 2005. Quantifying the thermal requirements of catfish spawning. Special Session-Hybrid Catfish Reproduction. World Aquaculture Society Meeting. New Orleans, LA. January 2005.

Phelps, R. P., R. Hasty, A. Pendetar, L. Linley and N. Papanikos. 2005. Effects of temperature and body characteristics on the induced spawning of channel catfish and the production of channel H blue catfish hybrid fry. Special Session-Hybrid Catfish Reproduction. World Aquaculture Society Meeting. New Orleans, LA. January 2005.

Quintero, H. E., D. A. Davis and A. Abebe. 2006. Application of logistic regresion analysis in evaluation of channel catfish broodstock. WAS Aquaculture America 2006. Las Vegas, NV. February 2006.

Quintero, H. E., D. A. Davis, R. Dunham, R. P. Phelps, and A. Abebe. 2005. Evaluation of feeding regime and protein content on egg quality and fecundity from induced channel catfish females. Special Session-Hybrid Catfish Reproduction. World Aquaculture Society Meeting. New Orleans, LA. January 2005.

Quintero, H. E., D. A. Davis, R. Dunham, R. P. Phelps, A. Abebe and A.Hutson. 2006. Evaluation of fatty acid sources on egg quality and fecundity from induced channel catfish females. WAS Aquaculture America 2006, February 14-16, 2006. Las Vegas, NV.

Simco, B. and C. A. Lessman. 2005. Initial imaging of catfish oocytes and embryos by automated transparency scanners. Special Session-Hybrid Catfish Reproduction. World Aquaculture Society Meeting. New Orleans, LA. January 2005.

Small, B. C. and N. Chatakondi. 2005. Optimizing chemotherapeutic treatment of hybrid catfish eggs for maximal hatching success. Special session-Hybrid Catfish Reproduction. World Aquaculture Society Meeting. New Orleans, LA, January 2005.

Umali, G. M. and Rex A. Dunham. 2005. The economic significance of aquatic biotechnology in the production of channel × blue catfish embryo. Special session-Hybrid Catfish Reproduction. World Aquaculture Society Meeting. New Orleans, LA. January 2005.

◆ ◆ ◆

FEED FORMULATION AND FEEDING STRATEGIES FOR BAIT AND ORNAMENTAL FISH

Reporting Period
June 1, 2005 - August 31, 2006

Funding Level

Year 1	$103,118
Year 2	$139,603
Year 3	$130,525
Total	$373,246

Participants

University of Ark. At Pine Bluff (Lead Institution)	Rebecca Lochmann
University of Ark. At Pine Bluff (Ext.)	Nathan Stone
Texas A & M University	Delbert Gatlin
University of Florida	Craig Watson
University of Georgia	Gary Burtle

Administrative Advisor
Dr. Ron Lacewell
Texas A & M University
College Station, Texas

PROJECT OBJECTIVES

1. Manipulate diet composition and/or feeding strategy for economical production of "jumbo" golden shiners.

2. Manipulate diet composition and feeding strategy to increase immunocompetence and resistance to stress in bait and ornamental fish during:

   a. Production
   b. Transport and Live Display

3. Determine the relative contribution of natural foods and prepared diets to growth, response to low dissolved oxygen, and other health indices for bait and ornamental fish in different production systems.

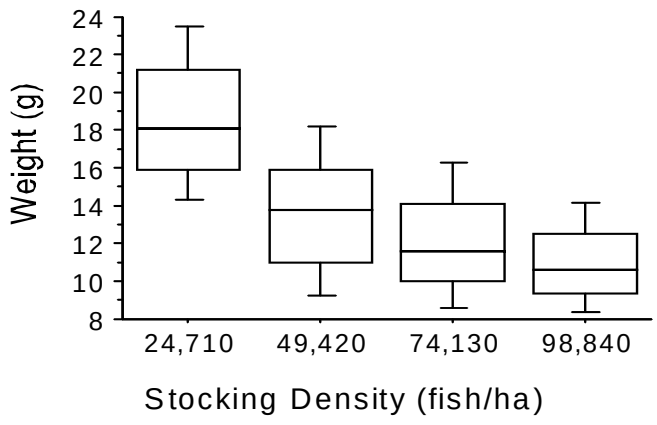
PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Objective 1. *Manipulate diet composition and/or feeding strategy for economical production of "jumbo" golden shiners*

University of Arkansas at Pine Bluff. The first objective was to determine an appropriate stocking density for juvenile golden shiners to maximize the production of jumbos (≥12g) within a single growing season. This density would then be used for a subsequent study evaluating feeding frequency and diet composition. Golden shiner juveniles (0.5 g) were stocked on July 25 into 12, 0.04-ha fenced and netted earthen ponds at four densities (24,710; 49,420; 74,130; and 98,840/ha) and cultured for 105 days. Fish were fed to satiation once daily with a commercial 42% protein extruded pellet. Ponds were aerated 10 hours nightly using 0.37-kW aerators. Secchi disk visibility was measured every 2 weeks, and total ammonia nitrogen, pH, chlorophyll *a*, dissolved oxygen and zooplankton were determined monthly. Recording thermographs were installed in two ponds and recorded water temperature every 6 hours. Fish were sampled monthly. Ponds were harvested November 7-8. Average fish weight and survival were estimated by weighing and counting five sub-samples of at least 25 fish. Weights (g) and lengths (mm) of a sample of at least 50 fish per pond were measured to determine condition and size variation. Remaining fish were bulk-weighed.

Average fish weight declined with increasing stocking density (Figure 1). At the lowest density, 98.4% of the weight at harvest was composed of jumbo fish. Survival ranged from 53 to 87% and was not significantly different among treatments. Gross yield increased with density from 366 to 753 kg/ha and was highly variable among ponds. Net yield of jumbos did not differ among the three higher density treatments. The 74,130/ha (30,000/acre) treatment

Figure 1. Box plots showing the distribution of fish weights at harvest.



resulted in an average gross yield of 639 kg/ha, of which 54% by weight was comprised of fish that weighed more than 12 g, and this density was selected for the next trial. Advanced fry were found in six ponds by August, documenting previously undescribed sexual maturity at 3 months of age in golden shiners. Juveniles stocked into study ponds had been raised from hatchery fry that were obtained on May 11, at 1 to 2 days of age.

Stocking juvenile golden shiners in late July, as was done in this study, results in lower single-season yields of jumbos when compared to direct stocking of hatchery fry at low densities. Previous work showed that direct stocking of fry in early May resulted in about 650 kg/ha of jumbos in a single season. However, the extra production of jumbos must be balanced against other uses for the ponds; juveniles used in this study were produced by stocking fry at 3.7 million/ha for 9 weeks, resulting in yields of about 900 kg/ha.

A second trial is underway to evaluate the effect of diet composition and feeding frequency on the growth and production of golden shiners. Juvenile golden shiners (average weight of 0.46 g) were stocked into 12, 0.04-ha earthen ponds at a rate of 74,100 fish/ha. Fish are being fed either once or twice daily with one of two diets (Table 1); a control diet (Diet 1) and an experimental diet (Diet 2), with the intent of matching the performance of fish fed the control diet but at a lower cost. The feed form is an extruded pellet (slow sink). Because the feed does not float, feeding to satiation is not possible. Fish are being fed at 3% body weight per feeding, adjusted weekly based on an assumed FCR of 1:1 and by sampling every 2 weeks. Other methods are the same as in trial 1. As of September 11, 2006, the increase in average weight for fish fed twice daily compared with that of fish fed once daily was approaching statistical significance ($P = 0.0503$), while diet effects were not statistically significant ($P = 0.32$).

Table 1. Composition of the diets[1] being tested for producing jumbo golden shiners in a single growing season.

	Amount (g/100g as fed)	
Ingredient	Diet 1 (control)	Diet 2 (No fish meal)
Menhaden fish meal (62%)	26.0	0.0
Poultry By-Product meal (60%)	15.0	34.0
Soybean meal (48%)	30.0	40.0
Corn	7.0	5.0
Wheat midds	13.8	12.8
Vitamin C (Stay-C)	0.146	0.146
Choline	0.58	0.58
Vitamin premix	0.4	0.4
Mineral premix	0.1	0.1
Poultry fat	7.0	7.0

[1] Diets contain approximately 42% total protein and 9-10% lipid by calculation.

Objective 2. *Manipulate diet composition and feeding strategy to increase immunocompetence and resistance to stress in bait and ornamental fish under simulated commercial conditions.*

Objective 2a. *Production*

Texas A&M University in collaboration with University of Arkansas at Pine Bluff. Three feeding trials were conducted at Texas A&M in recirculating systems with golden shiners to evaluate various potential immunostimulatory diet supplements. None of these attempts have resulted in a completely successful and conclusive feeding trial to date. The experimental diets prepared for these trials were based on the practical formulation originally proposed; however, it appears extrusion processing or a different method of particle size reduction may be needed to increase the utilization of these experimental diets by golden shiners.

We also supplemented the work originally planned by developing methodologies to quantitatively measure the immunocompetence and stress responses of baitfish under various conditions. A series of evaluations was conducted to define effective biological endpoints and/or physiological indicators of golden shiner health in response to various stimuli for rapid assessment of fish quality, and development of nutritional, pharmacological or husbandry strategies to enhance production efficiency. Some measurements of immunological and physiological responses have been developed for golden shiner including differential blood leucocyte counts, serum complement and cortisol assays. Our group (TAMU) recently has demonstrated that serum lysozyme activity of golden shiner and goldfish is very sensitive to the pH of the bacterial suspension (*Micrococcus lysodeikticus*), compared to hybrid striped bass and channel catfish. The optimal pH for lysozyme assay of golden shiner and goldfish was determined to be 5.9 and 6.0, respectively.

Our research team also found that neutrophil oxidative radical production could be analyzed according to a previously described procedure, but the isolation of head kidney from golden shiner is nearly impossible because that organ is almost invisible.

Results at a glance...

- *Prebiotics, immune stimulants and differences in protein or lipid content of diets had only limited impacts on general performance of golden shiner. However, the prebiotic GroBiotic-A® significantly improved survival of golden shiner exposed to the bacterium that causes columnaris disease.*

University of Arkansas at Pine Bluff in collaboration with Texas A&M University. A 14-week feeding trial was conducted at the University of Arkansas at Pine Bluff with golden shiner in aquaria to determine whether practical diets supplemented with Grobiotic-A®, extra lipid, or both could improve growth, survival, feed conversion, body composition, or survival upon exposure to low dissolved oxygen. Six diets similar to a commercial diet (30% protein and 9.6 kg energy/gram of protein) were formulated. Two diets contained the same protein components (primarily fish and poultry meals) and differed only in the amount of added lipid (4 and 10% poultry fat). The diet with 4% fat was the control. Two other diets were similar to diets 1 and 2 except they contained 2% Grobiotic-A®. Two additional diets contained poultry meal in place of fish meal on an estimated digestible protein basis. Twenty-five fish (1.2 ± 0.001g average weight) were stocked into each of four replicate 110-L tanks per

treatment in a flow-through system. Fish were fed twice daily to apparent satiation and group-weighed every 2 weeks to track growth. Weight gain, survival, and feed efficiency are shown in Table 1. Fish fed diets with Grobiotic-A® or no fish meal +4% poultry fat had slightly lower feed conversion ratio (feed offered/fish growth) than fish fed other diets. Statistical analysis of fish weight over time showed some transient differences, but final weight gain did not differ by diet. Posttrial fish were exposed to low dissolved oxygen for 24 hours with no mortality. Whole-body lipid was analyzed and there were no differences among treatments (Table 2). Because the golden shiners were not large enough to obtain blood for health assays at the end of the feeding trial, a subset of fish was maintained on their experimental diets for 12 more weeks. The pH optima for determining lysozyme activity was determined (5.9 – 6.0) and lysozyme assays are in progress.

Due to the lack of effect from the low-DO stress test, we performed a Columnaris challenge on a subset of golden shiners fed the control diet (basal – 4% poultry fat), the basal + 10% poultry fat diet, or the Grobiotic-A® + 10% poultry fat diet. Grobiotic-A® significantly enhanced survival of golden shiner relative to diets with 4 or 10% poultry fat and no Grobiotic-A®.

Table 2. Performance of juvenile golden shiners fed diets containing different concentrations of poultry fat (PF), Grobiotic-A® (GROB), or menhaden fish meal (FM) for 14 weeks[1]

Diet	Mean individual weight gain (g)	Feed conversion	Survival (%)	Whole-body lipid (%)
Basal - 4% PF	1.00±0.06	5.7±0.2b	80.0±2.8	4.1±0.7
GROB - 4% PF	1.15±0.04	5.2±0.2ab	78.0±1.2	4.8±0.7
No FM - 4% PF	1.06±0.05	5.0±0.2a	83.0±3.0	5.3±1.3
Basal - 10% PF	1.06±0.05	5.8±0.2b	88.0±2.3	3.7±0.6
GROB - 10% PF	1.18±0.03	5.0±0.1a	85.0±3.4	6.9±1.2
No FM - 10% PF	1.09±0.09	5.7±0.2b	84.0±2.8	5.7±0.6

[1]Means in columns with different letters are significantly different (P<0.10, Fisher's LSD).

Objective 2b. *Transport and Live Display*

University of Georgia. Whole-cooked soybeans are being compared to soybean meal in diets of golden shiners, feeder goldfish and fathead minnows. Golden shiners were stocked into aquaria in year 1 and year 2 but were subject to excessive mortality within a few days of stocking under a variety of culture conditions. Antibiotics applied to the golden shiners had no significant positive effect on survival. However, increasing salinity of the systems to 3 parts per thousand by addition of artificial sea salts improved survival of the golden shiners. Feeding trials are in progress that will utilize golden shiners held in aquaria at the elevated salinity. Goldfish and fathead minnow trials are in progress. Pond trials will begin after the aquarium trials have been completed.

Objective 3. *Determine the relative contribution of natural foods and prepared diets to growth, response to low dissolved oxygen, and other health indices of bait and ornamental fish in different production systems.*

Bait species

Texas A&M University. In three separate feeding trials in recirculating systems at Texas A&M with golden shiner, we have not been able to establish a reasonable amount of natural productivity in separate systems to assess the relative contribution of natural productivity. A modified culture system has been developed and is currently being tested for this purpose.

University of Arkansas at Pine Bluff. A feeding trial in outdoor pools is in progress at the University of Arkansas at Pine Bluff using the same diets described in Objective 2a. Methodological differences from the tank trial include less frequent fish sampling (monthly) to avoid mortalities due to handling stress, and monitoring natural food abundance through Secchi depth and chlorophyll *a* readings. At eight weeks, there were no differences in weight gain among treatments. Mortality has been low except for one pool where nearly all of the fish died suddenly from an undetermined cause (replicate removed from study). The feeding trial will last 2 to 4 more weeks, or as long as water temperature will support active daily feeding. All of the analyses described under Objective 2a will be conducted on these fish also, except that size may restrict the type of immunological tests that can be performed due to scant amounts of tissues such as kidney. In this case, alternative methods of assessing fish health will be used (see Work Planned).

University of Georgia. Pond studies are pending based on successful completion of feeding trials in tanks.

Ornamental species

University of Florida. During the first year of this project we submitted two species of fish, *Xiphophorus helleri* (swordtails) and *Brachydanio rerio* (zebra danios), to the treatments outlined in the original proposal.

There was a significant difference in the growth and perhaps survival of zebra danios produced in ponds receiving treatments of liquid fertilizer, cottonseed meal, an unprocessed meal diet, and a processed (pelleted and reground) diet. A significant difference in primary productivity (based on chlorophyll *a*) was also observed. Zebra danios fed an unprocessed and a processed diet in tanks also demonstrated a difference in growth. The low dissolved oxygen stress test was inconclusive, and perhaps inappropriate for demonstrating variations in ability of animals to endure stress based on the four pond treatments.

Detailed statistical analysis is in progress, but there appears to be a difference in growth, survival, and production (i.e. number of offspring produced) of swordtails based on pond trials with the four treatments. The data also has suggested the need for another study which would classify and quantify secondary productivity in these ponds (i.e. crawfish, tadpoles, insects, etc.) based on the different treatments. Studies for unprocessed and processed diets in tanks for swordtails are in progress.

WORK PLANNED

Texas A&M University (TAMU) is pursuing supplemental work to that originally planned. The University of Arkansas at Pine Bluff is collaborating on some aspects of this work:

1. Development of methodologies to quantitatively measure the immunocompetence and stress responses of baitfish under various conditions.

We are currently working on procedures to purify the lymphocytes of golden shiners from whole blood for a mitogen-induced proliferation assay. We also are exploring the potential application of methodology for cortisol extraction from beef cattle adrenal gland for assessment of whole-body cortisol in golden shiner. We also are evaluating the dynamics of whole-body minerals and vitamins such as selenium, zinc, and ascorbic acid in response to environmental stimuli such as harvesting, grading and shipping.

2. Investigation of dynamics of whole-body minerals and visceral vitamins and whole-body cortisol of golden shiners in response to handling and transportation stressors.

A study was recently conducted to monitor these responses at various stages of harvesting, handling and distribution of golden shiner. The samples were taken and preserved for analysis.

At UAPB, validation of a whole-body cortisol ELISA method for golden shiner is in progress.

IMPACTS

The overall goal of this project is to develop diets, feeding practices, and production strategies that enhance stress resistance and prolong survival of bait and ornamental fishes. For most participants it is too early to report impacts. However, the University of Florida reports that several producers have altered their stocking densities and feeding regimes for zebrafish and swordtails based on the preliminary findings of the pond studies in this project. The producers were enticed by the rapid growth and high survival rates demonstrated by feeding a processed diet, twice per day, and the stocking rates.

PUBLICATIONS

Publications

Lochmann, R. and N. Stone. 2006. Professors participating in multi-institutional research project. University of Arkansas at Pine Bluff, Pine Bluff, AR.

Presentations

A Special Session called "Diet and feeding strategies for bait and ornamental fish" will be held at the Aquaculture America 2007 meeting in San Antonio, TX, Feb 26-Mar 3, 2007. This session is based on results from the current project. The presentations included are:

Gatlin, D. Review of immune and stress responses of golden shiners *Notemigonus crysoleucas.*

Lochmann, R., T. Sink, H. Phillips, Li, P., and D.M. Gatlin, III. Effects of a feed additive, lipid concentration, and protein source on performance of golden shiner (*Notemigonus crysoleucas*).

Lochmann, R., N. Stone, and A. Kachowski. Stocking rates for juvenile golden shiners to maximize single-season production of jumbo (>12g) fish.

Sink, T., R. T. Lochmann, A. E. Goodwin, and E. Marecaux. Mortality rates in golden shiners *Notemigonus crysoleucas* fed diets high in fat and the prebiotic Grobiotic-A® prior to challenge with Flavobacterium columnare.

Watson, C. Growth and survival of ornamental fish based on pond fertilization and feeding.

Manuscripts in preparation

Li, P., Lochmann, R. T. & Gatlin, D. M. III. Determination of optimal pH for measurement of serum lysozyme activities of various warm water fishes. North American Journal of Aquaculture.

Lochmann, R., Sink, T., Phillips, H., Li, P. and Gatlin, D. M. III. Effects of a prebiotic (Grobiotic-A®), lipid concentration, and protein source on performance of golden shiner *Notemigonus crysoleucas* in tanks. Journal of the World Aquaculture Society.

Sink, T.D., R.T. Lochmann, A.E. Goodwin, and E. Marecaux. Mortality rates in golden shiner, *Notemigonus crysoleucas*, fed high-fat diets with or without the prebiotic Grobiotic-A® prior to challenge with Flavobacterium columnare. North American Journal of Aquaculture.

◆ ◆ ◆

SUPPORT OF CURRENT PROJECTS

Title	Yr	SRAC Funding	Other Support					Total SRAC+ Other Support
			University	Industry	Other Federal	Other	Total Other Support	
Publications, Videos and	1	50,000	43,950	-0-	-0-	-0-	43,950	93,950
Computer Software	2	60,948	30,737	-0-	-0-	-0-	30,737	91,685
	3	45,900	35,710	-0-	1,000	-0-	36,710	82,610
	4	60,500	41,000	-0-	-0-	-0-	41,000	101,500
	5	67,000	47,000	-0-	-0-	-0-	47,000	114,000
	6	77,358	52,975	-0-	-0-	-0-	52,975	130,333
	7	82,850	43,000	-0-	-0-	-0-	43,000	125,850
	8	77,507	47,000	-0-	-0-	-0-	47,000	124,507
	9	84,500	47,000	-0-	-0-	-0-	47,000	131,500
	10	78,700	30,000	-0-	-0-	-0-	30,000	108,700
	11	78,115	30,000	-0-	-0-	-0-	30,000	108,115
Total		**763,378**	**448,372**	**-0-**	**1,000**	**-0-**	**449,372**	**1,212,750**
Innovative Technologies and	1	314,409	193,931	-0-	-0-	-0-	193,931	508,340
Methodologies for Commercial-	2	287,451	217,676	-0-	-0-	-0-	217,676	505,127
Scale Pond Aquaculture	3	213,168	163,173	-0-	-0-	-0-	163,173	376,127
	4	170,096	106,405	-0-	-0-	-0-	106,405	276,501
Total		**985,124**	**681,185**	**-0-**	**-0-**	**-0-**	**681,185**	**1,666,309**
Improving Reproductive	1	118,390	86,891	-0-	-0-	-0-	86,891	205,281
Efficiency to Produce Channel ×	2	111,610	81,845	-0-	-0-	-0-	81,845	193,455
Blue Hybrid Catfish Fry	3	123,000	70,297	-0-	-0-	-0-	70,297	193,297
	4	123,000	72,121	-0-	-0-	-0-	72,121	195,121
Total		**476,000**	**311,154**	**-0-**	**-0-**	**-0-**	**311,154**	**787,154**
Feed Formulation and	1	103,118	39,363	-0-	-0-	-0-	39,363	142,481
Feeding Strategies for Bait	2	139,603	50,345	-0-	-0-	-0-	50,345	189,948
and Ornamental Fish	3	130,246	52,363	-0-	-0-	-0-	52,363	182,609
Total		**373,246**	**142,071**	**-0-**	**-0-**	**-0-**	**142,071**	**515,317**

SRAC RESEARCH AND EXTENSION PROJECTS

Project	Duration	Funding	Grant No.
*Analysis of Regional and National Markets for Aquacultural Products Produced for Food in the Southern Region. Dr. J. G. Dillard, Mississippi State University, Principal Investigator	04/01/88-06/30/90 **Project Total**	**$346,038**	87-CRSR-2-3218
*Preparation of Southern Regional Aquaculture Publications. Dr. J. T. Davis, Texas A&M University, Principal Investigator	01/01/88-12/31/90 **Project Total**	**$150,000**	87-CRSR-2-3218
*Performance of Aeration Systems for Channel Catfish, Crawfish, and Rainbow Trout Production. Dr. C. E. Boyd, Auburn University, Principal Investigator	03/01/88-10/31/90 **Project Total**	**$124,990**	87-CRSR-2-3218
*Develop a Statistical Data Collection System for Farm-Raised Catfish and Other Aquaculture Products in the Southern Region. Dr. J. E. Waldrop, Mississippi State University, Principal Investigator	06/01/89-11/30/90 **Project Total**	**$13,771**	88-38500-4028
*Immunization of Channel Catfish. Dr. J. A. Plumb, Auburn University, Principal Investigator	Yr. 1-05/02/89-04/30/90 Yr. 2-05/01/90-04/30/91 **Project Total**	$50,000 49,789 **$99,789**	88-38500-4028 89-38500-4516
*Enhancement of the Immune Response to *Edwardsiella ictaluri* in Channel Catfish. Dr. J. R. Tomasso, Clemson University, Principal Investigator	Yr. 1-05/02/89-04/30/90 Yr. 2-05/01/90-10/31/91 **Project Total**	$46,559 51,804 **$98,363**	88-38500-4028 89-38500-4516
*Effect of Nutrition on Body Composition and Subsequent Storage Quality of Farm-Raised Channel Catfish. Dr. R. T. Lovell, Auburn University, Principal Investigator	Yr. 1-05/02/89-04/30/90 Yr. 2-05/01/90-04/30/91 Yr. 3-05/01/91-12/31/92 **Project Total**	$274,651 274,720 273,472 **$822,843**	88-38500-4028 89-38500-4516 90-38500-5099

*Project Completed

Project	Duration	Funding	Grant No.
*Harvesting, Loading and Grading Systems for Cultured Freshwater Finfishes and Crustaceans. Dr. R. P. Romaire, Louisiana State University, Principal Investigator	Yr. 1-05/02/89-04/30/90 Yr. 2-05/01/90-04/30/91 Yr. 3-05/01/91-04/30/93 **Project Total**	$124,201 124,976 124,711 **$373,888**	88-38500-4028 89-38500-4516 90-38500-5099
*Preparation of Extension Publications on Avian Predator Control in Aquaculture Facilities. Dr. James T. Davis, Texas A&M University, Principal Investigator	05/01/90-12/31/92 **Project Total**	**$15,000**	89-38500-4516
*National Extension Aquaculture Workshop. Dr. Carole Engle, University of Arkansas at Pine Bluff, Principal Investigator	10/01/91-09/30/92 **Project Total**	**$3,005**	89-38500-4516
*Educational Materials for Aquaculturists and Consumers. Dr. J. T. Davis, Texas A&M University, Principal Investigator	Yr. 1-05/01/91-04/30/92 Total Yr. 1 Yr. 2-06/01/92-05/31/93 Yr. 3-06/01/93-12/31/94 **Project Total**	$3,971 35,671 $39,642 $58,584 34,500 **$132,726**	87-CRSR-2-3218 88-38500-4028 91-38500-5909 92-38500-7110
*Characterization of Finfish and Shellfish Aquacultural Effluents. Dr. J. V. Shireman, University of Florida, Principal Investigator	Yr. 1-05/01/91-04/30/92 Total Yr. 1 Yr. 2-06/01/92-05/31/93 Yr. 3-06/01/93-12/31/94 **Project Total**	$45,131 65,552 34,317 $145,000 $168,105 $128,937 **$442,042**	88-38500-4028 89-38500-4516 90-38500-5099 91-38500-5909 92-38500-7110
*Food Safety and Sanitation for Aquacultural Products: Microbial. Dr. J. L. Wilson, University of Tennessee, Principal Investigator	Yr. 1-04/01/92-03/30/93 Total Yr. 1 Yr. 2-06/01/93-05/31/94 Yr. 3-06/01/94-05/31/95 **Project Total**	$12,649 71,608 $84,257 $213,106 $237,975 **$535,338**	89-38500-4516 90-38500-5099 92-38500-7110 93-38500-8393

*Project Completed

Project	Duration	Funding	Grant No.
*Aquaculture Food Safety: Residues. Dr. George Lewis, University of Georgia, Principal Investigator	Yr. 1-09/11/92-09/30/93	$99,393	91-38500-5909
	Yr. 2-10/01/93-09/30/94	$44,631	90-38500-5099
		107,050	91-38500-5909
	Total Yr. 2	$151,681	
	Yr. 3-10/01/94-09/30/95	$89,463	93-38500-8393
	Yr. 4-10/01/95-09/30/96	$11,392	93-38500-8393
	Project Total	**$351,929**	
*National Coordination for Aquaculture Investigational New Animal Drug (INAD) Applications. (In cooperation with other Regional Aquaculture Centers and USDA)	Yr. 1-09/01/93-08/31/94		
	Project Total	**$2,000**	90-38500-5099
*Improving Production Efficiency of Warmwater Aquaculture Species Through Nutrition. Dr. Delbert Gatlin, Texas A&M University, Principal Investigator	Yr. 1-01/01/94-12/31/94	$28,148	90-38500-5099
		123,705	91-38500-5909
		128,444	92-38500-7110
	Total Yr. 1	$280,297	
	Yr. 2-01/01/95-12/31/95	$38,059	92-38500-7110
		175,450	93-38500-8393
		32,397	94-38500-0045
	Total Yr. 2	$245,906	
	Yr. 3-01/01/96-12/31/96	$23,907	93-38500-8393
		210,356	94-38500-0045
	Total Yr. 3	$234,263	
	Project Total	**$760,466**	
*Delineation and Evaluation of Catfish and Baitfish Pond Culture Practices. Dr. Michael Masser, Auburn University, Principal Investigator	Yr. 1-04/01/94-03/31/95	$75,530	92-38500-7110
		43,259	93-38500-8393
	Total Yr. 1	$118,789	
	Yr. 2-04/01/95-03/31/96	$113,406	94-38500-0045
	Yr. 3-04/01/96-03/31/97	$28,517	93-38500-8393
		72,281	94-38500-0045
	Total Yr. 3	$100,798	
	Project Total	**$332,993**	
*Optimizing Nutrient Utilization and Waste Control through Diet Composition and Feeding Strategies. Dr. Kenneth Davis, University of Memphis, Principal Investigator	Yr. 1-12/01/96-11/30/97	$241,476	95-38500-1411
	Yr. 2-12/01/97-11/30/98	$47,105	95-38500-1411
		210,047	96-38500-2630
	Total Yr. 2	$257,152	
	Yr. 3-12/1/98-11/30/99	$34,365	96-38500-2630
		199,811	97-38500-4124
	Total Yr. 3	$234,176	
	Project Total	**$732,804**	

*Project Completed

Project	Duration	Funding	Grant No.
*Management of Environmentally-Derived Off-Flavors in Warmwater Fish Ponds. Dr. Tom Hill, University of Tennessee, Principal Investigator	Yr.1-06/01/96-05/31/97	$29,349	93-38500-8393
		34,918	94-38500-0045
		186,560	95-38500-1411
	Total Yr. 1	$250,827	
	Yr. 2-06/01/97-05/31/98	$68,718	94-38500-0045
		97,393	95-38500-1411
		84,031	96-38500-2630
	Total Yr. 2	$250,142	
	Yr. 3-06/1/98-05/31/99	$154,621	96-38500-2630
		74,645	97-38500-4124
	Total Yr. 3	$229,266	
	Yr. 4-06/01/99-05/31/00	$80,900	98-38500-5865
	Yr. 5-06/01/00-05/31/01	$55,146	99-38500-7375
	Project Total	**$866,281**	
*National Aquaculture Extension Conference (In cooperation with other Regional Aquaculture Centers)	01/01/97-12/31/97	$3,392	93-38500-8393
		308	95-38500-1411
	Project Total	**$3,700**	
*Verification of Recommended Management Practices for Major Aquatic Species. Dr. Carole Engle, University of Arkansas at Pine Bluff, Principal Investigator	Yr. 1-01/01/97-12/31/97	$31,410	95-38500-1411
	Yr. 2-01/01/98-12/31/98	$7,186	95-38500-1411
		58,928	96-38500-2630
	Total Yr. 2	$66,114	
	Yr. 3-01/01/99-12/31/00	$62,781	99-38500-4124
	Project Total	**$160,305**	
Publications, Videos and Computer Software. Dr. Michael Masser, Texas A&M University, Principal Investigator (Continuing project)	Yr. 1-04/01/95-03/31/96	$50,000	94-38500-0045
	Yr. 2-04/01/96-03/31/97	$13,405	93-38500-8393
		47,543	94-38500-0045
	Total Yr. 2	$60,948	
	Yr. 3-04/01/97-03/31/98	$45,900	96-38500-2630
	Yr. 4-04/01/98-03/31/99	$60,500	97-38500-4124
	Yr. 5-04/01/99-03/31/00	$67,000	98-38500-5865
	Yr. 6-07/01/00-06/30/01	$77,358	00-38500-8992
	Yr.7-07/01/01-06/30/02	$82,205	2001-38500-10307
	Yr.8-01/01/03-12/31/03	$77,384	2002-38500-11805
	Yr.9-04/01/04-03/31/05	$916	2002-38500-11805
		83,196	2003-38500-12997
	Total Yr. 9	$84,112	
	Yr. 10-03/01/05-02/28/06	$78,700	2004-38500-14387
	Yr. 11-03/01/06-02/28/07	$78,115	2005-38500-15815
	Project Total	**$762,222**	

*Project Completed

Project	Duration	Funding	Grant No.
*Control of Blue-green Algae in Aquaculture Ponds. Dr. Larry Wilson, University of Tennessee, Principal Investigator	Yr. 1-01/01/99-12/31/99	$25,147	96-38500-2630
		105,167	97-38500-4124
		177,260	98-38500-5865
	Total Yr. 1	$307,574	
	Yr. 2-01/01/00-12/31/00	$975	96-38500-2630
		17,394	97-38500-4124
		158,608	98-38500-5865
		98,993	99-38500-7375
	Total Yr. 2	$275,970	
	Yr. 3-01/01/01-12/31/01	$26,186	97-38500-4124
		7,202	98-38500-5865
		188,550	99-38500-7375
		24,277	00-38500-8992
	Total Yr. 3	$246,215	
	Project Total	**$829,759**	
*Management of Aquacultural Effluents from Ponds. Dr. John Hargreaves, Mississippi State University, Principal Investigator	Yr. 1-04/01/99-03/31/00	$100,000	97-38500-4124
		127,597	98-38500-5865
	Total Yr. 1	$227,597	
	Yr. 2-04/01/00-03/31/01	$221,146	99-38500-7375
	Yr. 3-04/01/01-03/31/02	$106,610	2000-38500-8992
	Project Total	**$553,353**	
*****Development of Improved Harvesting, Grading and Transport Technology for Finfish Aquaculture. Dr. Ed Robinson, Mississippi State University, Principal Investigator	Yr. 1-01/01/01-12/31/01	$287,053	00-38500-8992
	Yr. 2-01/01/02-12/31/02	$14,259	98-38500-5865
		39,720	99-38500-5865
		14,757	00-38500-8992
		203,655	01-38500-10307
	Total Yr. 2	$272,391	
	Yr. 3-01/01/03-12/31/03	$15,000	00-38500-8992
		175,556	01-38500-10307
	Total Yr. 3	$190,556	2001-38500-10307
	Project Total	**$750,000**	
*****National Aquacultue Extension Conference-2007 (In cooperation with other Regional Aquaculture Centers)	11/01/05-10/31/06		
	Project Total	**$5,000**	2002-38500-11805

*Project Completed

Project	Duration	Funding	Grant No.
*Identification, Characterization, and Evaluation of Mechanisms of Control of *Bolbophorus*-like Trematodes and *Flavobacterium columnaris*-like Bacteria Causing Disease in Warm Water Fish. Dr. John Hawke, Louisiana State University, Principal Investigator	Yr. 1-03/01-03-02/28/04	$28,029	2000-38500-8992
		126,778	2001-38500-10307
		67,298	2002-38500-11307
	Total Yr. 1	$222,105	
	Yr. 2-03/01-04-02/28/2005	$27,126	2000-38500-8992
		47,498	2001-38500-10307
		151,614	2002-38500-11805
		1,000	2003-38500-12997
	Total Yr. 2	$227,238	
	Yr. 3-03/01/05-02/28/06	$24,074	2001-38500-10307
		15,417	2002-38500-11805
		107,279	2003-38500-12997
	Total Yr. 3	$146,770	
	Project Total	**$596,113**	
Improving Reproductive Efficiency to Produce Channel × Blue Hybrid Catfish Fry. Dr. Rex Dunham, Auburn University, Principal Investigator	Yr. 1-03/01/04-02/28/05	$1,000	2001-38500-10307
		114,934	2002-38500-11805
	Total Yr. 1	$115,934	
	Yr. 2 -03/01/05-02/28/06	$111,610	2003-38500-12997
	Yr. 3-03/01/06-02/28/07	$14,549	2002-38500-11805
		28	2003-38500-12997
		100,423	2004-38500-14387
	Total Yr. 3	$115,000	
	Yr. 4-Projected	$115,000	
	Project Total	**$457,544**	
Innovative Technologies and Methodologies for Commercial-Scale Pond Aquaculture. Dr. Claude Boyd, Auburn University, Principal Investigator	Yr.1-08/01/04-07/31/05	$1,053	2000-38500-8992
		167,433	2002-38500-11805
		145,923	2003-38500-12997
	Total Yr. 1	$314,409	
	Yr. 2-08/01/05-07/31/06	$39	2002-38500-11805
		116,359	2003-38500-12997
		171,053	2004-38500-14387
		$287,451	
	Yr.3-08/01/06-07/31/07	$120	2002-38500-11805
		8,529	2003-38500-12997
		101,220	2004-38500-14387
		103,299	2005-38500-15815
		$213,168	
	Yr.4-Projected	$170,096	
	Project Total	**$985,124**	
Feed Formulation and Feeding Strategies for Bait and Ornamental Fish. Dr. Rebecca Lochmann, University of Arkansas at Pine Bluff, Principal Investigator	Yr.1-05/01/05-04/30/06	$103,118	2003-38500-12997
	Yr.2-05/01/06-04/30/07	$139,603	2004-38500-14387
	Yr.3-Projected	$130,525	
	Project Total	**$373,246**	