SOUTHERN REGIONAL AQUACULTURE CENTER

EIGHTEENTH ANNUAL PROGRESS REPORT

For the Period Through August 31, 2005

December, 2005

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

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Dr. Craig S. Tucker, Director
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Stoneville, Mississippi 38776
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Fax: 662-686-3320
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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 acknowledges the contributions of the Project Leaders and Participating Scientists involved in the projects reported in this Eighteenth 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 our Board, IAC and TC, and 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

The farm-gate value of United States aquaculture exceeded $1 billion dollars in 2004, and nearly 70% of the crop was produced in the southeastern states. Aquaculture is an important part of southeastern agriculture, and its importance reaches far beyond the farm gate. Most of the support functions for the industry – such as feed manufacture and equipment fabrication – also take place in the region. The total economic impact of aquaculture is therefore many times the value of production alone.

The success of southeastern aquaculture has come with relatively little private sector support for research and development. The larger, more developed agricultural sector – such as poultry, cotton and soybeans – are supported by a vast infrastructure of agribusinesses that conduct 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 mechanism 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 18 years of operation, the Center has disbursed $12 million to fund 28 multi-state research and extension projects. More than 175 scientists from 30 institutions in the southeast have participated in Center projects.

In the past year, four research projects funded at $2.5 million were in progress. Work on those projects has been reported in 25 publications and 24 papers presented at meetings. The Center's "Publications" project is in its tenth year of funding and is under the editorial direction of faculty and staff at Texas A&M University. Eleven publications were printed this year, and eight more were in various stages of production. To date, SRAC "Publications" projects have generated more than 171 fact sheets with contributions from 158 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, a discovery in the "Disease" project will have a dramatic impact on catfish farming. Research conducted as part of that project led to discovery of a safe, inexpensive method to control the intermediate host of the trematode parasite *Bolbophorus damnificus*. Over the last 5 years, this

disease was discussed in doomsday language. In the near future, however, it may be considered no more than a manageable nuisance.

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 Group meetings 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 Eighteenth 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, 2005.

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:

Ivory Lyles, Arkansas Cooperative Extension System
Ken Roberts, Louisiana Cooperative Extension Service
Gaines Smith, Alabama Cooperative Extension System
David Morrison, Louisiana State University
Vance Watson, Mississippi State University, Chairman
Greg Weidemann, University of Arkansas
Harold R. Benson, Kentucky State University
W. S. Clarke, Virginia State University

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
Bill Cheek, LA
Jane Corbin, TN
Richard Eager, SC
Theop Inslee, OK
Austin Jones, MS
Shorty Jones, MS
Joey Lowery, AR
Robert Mayo, NC
Steve Minvielle, LA
Steve Price, KY
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
Allen Davis, AL
Lou D'Abramo, MS
Carole Engle, AR
Delbert Gatlin, TX
Conrad Kleinholz, OK
Ray McClain, LA
Steve Mims, KY
J. L. Wilson, TN

Members of the TC for Extension are:

Jimmy Avery, MS
Jesse Chappell, AL
Dennis DeLong, NC
David Heikes, AR
George Luker, OK
Greg Lutz, LA
Michael Masser, TX
Craig Watson, FL
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, 2005

Funding Level

Year 1	$ 50,000
Year 2	60,948
Year 3	45,900
Year 4	60,500
Year 5	67,000
Year 6	77,883
Year 7	83,850
Year 8	77,600
Year 9	84,500
Year 10	78,700
Total	$686,881

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.

Administrative Advisor
Dr. Joe McGilberry, Director
Mississippi State University Extension Service
Mississippi State, Mississippi

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 most 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 benefit of utilizing a region-wide pool of expertise to develop materials for distribution through the nationwide network of Extension Specialists and County Agents. This process makes efficient use of personnel at the State level, and results in high-quality educational materials that are readily available to scientists, educators, producers, and the general public.

RESULTS AT A GLANCE...

★ *158 authors from across the United States have contributed to SRAC's publication projects.*

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.

Producers. 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.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

During this current project year, eleven new fact sheets were completed and the Aquaplant web site updated. All have been distributed throughout the Southern Region and to interested Extension Specialists in other regions. Five fact sheets are currently in some stage of writing, production, or revision. Four fact sheets have currently not had drafts submitted.

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>.

RESULTS AT A GLANCE...

- ★ *Eleven fact sheets and a video were completed this year with four fact sheets in progress.*
- ★ *Twenty-seven scientists from across the Southern Region contributed to publications completed by SRAC this year.*
- ★ *SRAC has now published 171 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.*

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 1) Management of Environmentally Derived Off-Flavors in Warmwater Fish Ponds, and 2) Optimizing Nutrient Utilization and Waste Control through Diet Composition and Feeding Strategies will be developed.

A DVD on crawfish aquaculture will also be developed.

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 6 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 April through September of 2005, more than 39,000 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.

RESULTS AT A GLANCE...

- ★ *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>.*
- ★ *In the months from April through September 2005, more than 39,000 fact sheets were downloaded from the SRAC web site.*

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.

RESULTS AT A GLANCE...

Titles of some recent SRAC publications:

- ★ *Controlling Bird Predation at Aquaculture Facilities*
- ★ *Constructing a Simple and Inexpensive Recirculating Aquaculture System for Classroom Use*
- ★ *Anesthetics in Aquaculture*
- ★ *Production of Crawfish in Earthen Ponds without Planted Forage*
- ★ *Guidelines for Developing Aquaculture Research Verification Programs*
- ★ *Managing Ammonia in Fish Ponds*
- ★ *Cobia*
- ★ *Biology and Culture of the Hard Clam*
- ★ *Channel Catfish Fingerling Production*
- ★ *Koi and Goldfish*
- ★ *Liming Ponds for Aquaculture*

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.

PUBLICATIONS, MANUSCRIPTS OR PAPERS PRESENTED

Fact Sheets Completed (9/1/04 - 8/31/2005)

Barras, Scott C. and Kristina C. Godwin. Controlling bird predation at aquaculture facilities: Frightening techniques. SRAC Fact Sheet 401 (Revision).

Cline, David. Constructing a simple and inexpensive recirculating aquaculture system (RAS) for classroom use. SRAC Fact Sheet 4501.

Coyle, Shawn D., Robert M. Durborow and James H. Tidwell. Anesthetics in aquaculture. SRAC Fact Sheet 3900.

D'Abramo, Louis R., Cortney L. Ohs, Terrill R. Hanson and Jose L. Montanez. Semi-intensive production of red swamp crawfish in earthen ponds without planted forage. SRAC Fact Sheet 2401.

Engle, Carole, Jimmy Avery, Harry Daniels, David Heikes and Greg Lutz. Guidelines for developing aquaculture research verification programs. SRAC Fact Sheet 5000.

Hargreaves, John and Craig S. Tucker. Managing ammonia in fish ponds. SRAC Fact Sheet 4603.

Kaiser, Jeffrey B. and G. Joan Holt. Species profile: Cobia. SRAC Fact Sheet 7202.

Whetstone, Jack M., Leslie N. Strumer and Michael J. Oesterling revised from Lorio, Wendell J. and Sandra Malone. Biology and culture of the hard clam (*Mercenaria mercenaria*). SRAC Fact Sheet 433 (Revision).

Steeby, Jim and Jimmy Avery. Channel catfish fingerling production. SRAC Fact Sheet 1803.

Watson, Craig A. Jeffrey E. Hill, and Deborah B. Pouder. Species profile: Koi and goldfish. SRAC Fact Sheet 7201.

Wurts, William A. and Michael Masser. Liming ponds for aquaculture. SRAC Fact Sheet 4100.

Manuscripts in review

Dasgupta, Siddhartha. Economics of freshwater prawn farming in the United States.

Romaire, Robert and Ray McClain. Crawfish marketing.

Small, Brian C. Managing egg death and disease in catfish hatcheries.
Tucker, Craig S. Pond aeration (Revision).

Manuscripts in preparation

Hinshaw, Jeff and Anita Kelly. Species profile: Yellow perch.
Rakocy, Jim, Michael Masser and John Hargreaves. Aquaponics.

On-going project

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

♦ ♦ ♦

IDENTIFICATION, CHARACTERIZATION, AND EVALUATION OF MECHANISMS OF CONTROL OF *BOLBOPHORUS*-LIKE TREMATODES AND *FLAVOBACTERIUM COLUMNARE*-LIKE BACTERIA CAUSING DISEASE IN WARM WATER FISH

Reporting Period
March 1, 2003 - August 31, 2005

Funding Level

Year 1	$224,800
Year 2	$227,377
Year 3	$146,770
Total	$598,947

Participants

Louisiana State University (Lead Institution)	John Hawke (Project Leader), Richard Cooper
University of Tennessee	Andrew Mathew, Richard J. Strange
University of Arkansas at Pine Bluff	Andrew Goodwin
USDA/APHIS/WS (Starkville)	Brian Dorr, D. T. King
USDA/ARS (Stuttgart)	Andrew J. Mitchell
Mississippi State University College of Veterinary Medicine (Starkville)	Linda Pote, Larry Hanson, Mark Lawrence
Auburn University	John Grizzle, Joe Newton
Mississippi State University, Delta Research and Extension Center (Stoneville)	David Wise
Southern Illinois University	Anita Kelly
North Carolina State University	Michael Levy, James Flowers

Administrative Advisor

Dr. Jerald Ainsworth, Associate Dean
College of Veterinary Medicine
Mississippi State University
Mississippi State, Mississippi

PROJECT OBJECTIVES

1. Identify and characterize all of the life stages of the digenetic trematode (tentatively identified as *Bolbophorus* sp.) that infects channel catfish using both classical and molecular methods.

2. Evaluate integrated methods for snail control in catfish ponds.

    a. Monitor populations of catfish infected with *Bolbophorus* spp. to document the effect of parasite loads on growth and survival of the fish.
    b. Examine the efficacy of chemical control methods on snail populations.
    c. Examine the efficacy of biological control methods (snail-eating fish) on snail populations in ponds.

3. Develop and implement standardized methods for the isolation, culture, and antimicrobial susceptibility testing of strains of columnaris-like bacteria isolated from diseased fish.

4. Characterize archived strains of columnaris-like bacteria based on the following conventional and molecular techniques.

    a. Morphology
    b. Enzyme analysis
    c. Biochemical analysis
    d. Sequencing 16S ribosomal RNA and ribotyping

5. Develop challenge models for columnaris-like bacteria isolated from major warmwater aquaculture species in the southeast.

6. Using the challenge model for each species, correlate virulence with biotype and/or genotype of columnaris-like bacteria.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Objective 1. *Identify and characterize all of the life stages of the digenetic trematode(tentatively identified as* Bolbophorus *sp.) that infects channel catfish using both classical and molecular methods.*

Confirmation of *Bolbophorus* life cycle

Mississippi State University and USDA/ APHIS/WS. Two studies were conducted to confirm the life stages of *Bolbophorus damnificus* in American white pelicans and its snail host, *Planorbella trivolvis*. Three pelicans were pretreated with praziquantel, challenged

with *B. damnificus* metacercaria to establish patent infections, and were subsequently used to artificially infect *P. trivolvis*. Catfish were exposed to these infected snails, metacercaria from this challenge were fed to parasite free pelicans, and patent *B. damnificus* infections were established. Each life stage of this parasite was confirmed to be *B. damnificus* morphologically and molecularly. Data are being analyzed on cercaria and ova shedding.

A second study was conducted to determine potential snail hosts for *B. damnificus* and its life cycle in the snail. Ova from pelicans infected in Study 1 were used to artificially infect several snail species housed in aquaria at 80°F. Snails were checked weekly for cercaria shedding, and checked daily when they were positive. Time and number of cercaria shed was recorded and data are being analyzed.

Collections to Evaluate the Avian Host Range for *Bolbophorus*

USDA/ARS. A total of 106 aquatic birds have been collected and trematodes harvested from their alimentary canals for identification. Some of these trematodes have potential to be transmitted to cultured fish species. In 2003, 25 aquatic birds were collected including 5 pelicans, 10 cormorants, and 10 great egrets. In 2004, 54 birds were collected including 17 great egrets, 12 great blue herons, 11 snowy egrets, 6 cattle egrets, 6 green herons, 4 belted kingfishers, and 1 little blue heron. In 2005, 27 birds were collected including 6 belted kingfishers, 5 white pelicans, 2 great egrets, 9 black-crowned night herons, and 5 little blue herons. It appears that *Bolbophorus* spp. have been recovered only from white pelicans collected in 2003 and 2005. The trematode *Clinostomum* spp., one species of which is responsible for the yellow grub in fish, was found in great egrets, great blue herons, snowy egrets, black-crowned night herons, little blue herons, and cattle egrets. The gill trematode *Centrocestus formosanus* was recovered from green herons and great egrets. Identification of the trematodes is ongoing.

Confirmation of the Definitive Final Host of *Bolbophorus*

North Carolina State University. Adult *Bolbophorus damnificus* and immature *Bolbophorus* sp. type 2 have been recovered and identified from the American white pelican. Mature ovogenous *Bolbophorus* sp. type 2 have not been recovered from any avian species and identification of its definitive host remains a priority.

Mississippi State University. Birds (two each of American white pelicans, double-crested cormorants, great blue herons, great egrets) were live-captured in the Mississippi Delta. They were individually housed in pens with recirculating water tanks and fed catfish ad libitum daily until challenge. Birds were acclimated for at least 1 week. Fecal samples were collected daily starting at 48 hours prior to anti-helmintic treatment and continued until necropsy. At 7 days pre-challenge, birds were administered praziquantel at 34 mg/kg BW per os to eliminate all trematodes. At 7 days post-treatment birds were fed live fish naturally infected with *Bolbophorus damnificus* metacercariae (confirmed by a *B. damnificus*-specific polymerase chain reaction, PCR). Birds were necropsied 21 days post-challenge, intestinal contents of

each bird were examined; all parasites were removed, examined microscopically, identified and enumerated. A sub-sample of each parasite type was processed for electron microscopy and DNA analysis.

The only bird species that shed *B. damnificus* ova (confirmed by PCR) during the trial was the American white pelican. Adult *B. damnificus* were found in pelican 1 (one adult trematode) and pelican 2 (five adult trematodes). All other bird species were negative for *B. damnificus* and other trematodes.

This study confirms that the American white pelican is a host for *B. damnificus*. Results from this study demonstrate that artificial infections of *B. damnificus* could not be established in double-crested cormorants, great blue herons, and great egrets.

Confirmation of Intermediate Hosts of *Bolbophorus* spp.

North Carolina State University, USDA/ARS, Mississippi State University. *Planorbella trivolvis* snails collected from catfish ponds in Mississippi experiencing outbreaks of *Bolbophorus*-associated morbidity/mortality were screened for the shedding of forked-tailed cercariae in snails shipped to North Carolina. Two morphologically distinct types of bolbophorid cercariae were confirmed morphologically and genetically utilizing species-specific PCR. These were *1) Bolbophorus damnificus*, a serious pathogen of channel catfish, *Ictalurus punctatus*, and 2) *Bolbophorus* sp. type 2, a species not recovered from catfish but present in several other fish hosts. Interestingly, several snails were shown to be shedding both bolbophorid species simultaneously or sequentially. This indicated that both species were present in aquaculture ponds and they utilized the same molluscan host. A manuscript "Morphological description of the cercariae of *Bolbophorus damnificus* and *Bolbophorus* sp. with notes on North American Bolbophorids" by J. R. Flowers, M. F. Poore, L. M. Pote, R. W. Litaker and M. G. Levy was submitted to Comparative Parasitology in June 2004. Information in this manuscript will allow identification and speciation of bolbophorid cercariae based on light microscopic details.

RESULTS AT A GLANCE...

★ *Studies on the various life stages of* Bolbophorus damnificus *have revealed that the adult trematode resides in the gut of the American white pelican. The parasite has not been found in wild cormorants, great egrets, great blue herons, snowy egrets, cattle egrets, green herons, belted kingfishers and little blue herons. Attempts to artificially infect cormorants, great blue herons and great egrets failed whereas the white*

North Carolina *Planorbella duryi* snails were sent to Dr. L. Pote at Mississippi State University who was successful in infecting them with *B. damnificus*, indicating that the North Carolina snails are a permissive intermediate host. This indicates that in the presence of a suitable avian host, this infection is capable of further spread to the southeastern United States. Dr. Pote also provided several shipments of *P. trivolvis* positive for *B. damnificus* and *Bolbophorus*-

type 2 to Dr. Michael Levy at North Carolina State University, and has maintained the *P. trivolvis* snail colony which provided negative snails for other cooperators in this project.

The *Bolbophorus* trematode has been found in wild fish species including channel catfish and several centrarchids in Lake Chicot, Arkansas. Metacercarie recovered from a variety of fish demonstrated the following distribution: Only *B. damnificus* was recovered from catfish in aquaculture ponds. *Bolbophorus* species type 2 was recovered from white crappie and longear sunfish and largemouth bass. The fathead minnow was found to harbor both *B. damnificus* and species type 2. This is the first finding of a *B. damnificus* in a fish species other than catfish.

Both patent and pre-patent infections in infected snails were identified using PCR. Using PCR we also identified snails shedding either *B. damnificus* or type 2 exclusively. Cercariae were then fixed in hot 10% neutral buffered formalin. Ten cercariae of each type were examined for body length, body width, tail-stem length and width, furcae length and width, and oral sucker size. An additional large number of living cercariae were held under a cover slip and examined for the following characteristics: penetration glands, flame cells, organ primordial and tegumental spine arrangements. Differences between the two species strongly suggest that cercariae have distinguishing morphologic characteristics. Confirmation of these observations will be accomplished by examining additional cercariae during the coming season in order to rule out individual snail variation.

Fish Challenge Trials with *Bolbophorus* spp.

North Carolina State University. The potential pathogenic effect of both trematode species was investigated in a series of preliminary experiments. Hybrid striped bass (*Morone saxitalis* × *M. chrysops*), and channel catfish fingerlings were obtained from commercial farms in North Carolina where *Bolbophorus* is not known to be present. Snails were divided into two groups based on PCR identification of the *Bolbophorus* species that they shed. Infection rates were based on available numbers of cercariae less than 2 hours after emergence from the snails. Catfish were 2- to 3-inch fingerlings and hybrid striped bass were 1.5-inch fingerlings. An aliquot of cercariae was retained from each infection time and the challenge species reconfirmed using PCR. These results were not available until after challenge was completed due to the time involved in running the PCR assay.

Five groups of five bass were infected with 300, 500, or 550 *B. damnificus*, and two groups of bass were infected with either 40 or 285 *Bolbophorus* sp. type 2. Eight groups of five catfish were infected with 175, 350, 637, or 700 *B. damnificus* cercariae/fish. Three groups were infected with 300, 700, or 1,000 *Bolbophorus* species type 2. One group of fish was infected with 700 cercariae of a mixture of the two species due to a "switch" in the species shed by one or more snails in this group. All fish were necropsied and metacercariae removed and identified as to type using PCR.

All catfish infected with any dose of

B. damnificus developed the typical hemorrhagic lesions and most died beginning on day 4 post-infection. Several fish exposed to the lowest numbers of cercariae survived and were euthanized 6 weeks post-infection. Although catfish exposed to only *Bolbophorus* sp. type 2 failed to exhibit obvious signs of infection such as hemorrhagic lesions typical of a *B. damnificus* challenge, exposure to B. sp type 2 cercariae did result in these fish going 'off feed' for several weeks. A few degenerate metacercariae, none containing intact immature adult worms, were recovered. These were identified as type 2 by PCR.

Hybrid striped bass challenged with type 2 cercariae exhibited hemorrhagic lesions similar to those observed with *B. damnificus*-challenged catfish and mortality rates were similarly high. No morbidity or mortality was observed with hybrid striped bass challenged with *B. damnificus*. Only *Bolbophorus* species type 2 metacercariae were recovered from hybrid bass.

In Year 2 experimental infection of fish was continued with the two bolbophorid species. The potential pathogenic effect of both trematode species was investigated in a series of additional experiments complementing those performed in Year 1.

Laboratory-reared hybrid striped bass fingerlings and raceway-reared channel catfish fingerlings were purchased from commercial fisheries. Snails that had identified bolbophorid infections were placed in separate groups by species of cercariae as determined by species-specific PCR and cercariae were allowed to escape from their snail host into the water column. Samples of cercariae from each snail group were counted and cercarial yields were calculated. Within 4 hours of escape from the snails, cercariae were added to fish tanks containing experimental fish.

Groups of ten hybrid striped bass were separately exposed to *Bolbophorus damnificus* cercariae and *Bolbophorus* sp. cercariae at the following exposure rates: 500, 250, or 100 cercariae/fish. Groups of ten channel catfish were also separately exposed to *Bolbophorus damnificus* cercariae and *Bolbophorus* sp. cercariae at the following exposure rates: 250, 100, or 25 cercariae/fish.

At 5-days post-exposure, hemorrhagic lesions, lethargy, and decreased appetite were noted in the hybrid striped bass exposed to the *Bolbophorus* sp. cercariae at the rates of 500, 250, and 100 cercariae/fish. Mortality of the hybrid striped bass exposed to 500 cercariae/fish began at 6 days post-infection and all fish were dead by 11 days post-exposure. Hemorrhagic lesions in hybrid striped bass exposed to 250 and 100 cercariae per fish disappeared by 19 days post-exposure; however, large bumps under the skin were noted. None of the hybrid striped bass exposed to *Bolbophorus damnificus* developed lesions.

Conversely, channel catfish reacted to the *Bolbophorus damnificus* cercarial exposures. Hemorrhagic lesions and bumps were observed on catfish exposed to *B. damnificus* cercariae at the rates of 250 and 100 cercariae/fish in the morning of day 9 post-exposure. In the afternoon of day 9 post-exposure, three catfish exposed to 250

cercariae/fish and one catfish exposed to 100 cercariae/fish had died. Later, one catfish exposed to 250 cercariae/fish and one exposed to 100 cercariae/fish died at 14 and 23 days post-exposure, respectively. Some of the catfish exposed to 250 cercariae/fish developed exophthalmia and abdominal distension. None of the catfish exposed to the *Bolbophorus* sp. cercariae developed lesions. Also, lesions were not seen in the catfish exposed to *Bolbophorus damnificus* cercariae at the rate of 25 cercariae/fish.

The pathological consequences of *B. damnificus* infection in channel catfish were less severe compared with those seen in past experiments. This may be due to the larger size of fingerlings or the presence of fewer non-trematode pathogens. In previous challenges, fingerlings were collected from ponds and may have harbored other pathogens, whereas for this experiment we collected swim-up fry hatched in well water and laboratory-reared the fish.

These results demonstrate a high degree of specificity for the intermediate hosts for these two bolbophorid trematodes. *Bolbophorus damnificus* caused lesions only in catfish where *bolbophorus* sp. caused lesions only in hybrid bass.

Fish growth rates at the different parasite-exposure rates are currently being statistically analyzed. The number of encysed metacercariae for each fish (for each challenge parasite species and number) will also be determined. Development of methods for quantification and estimation of parasite loads in molluscan populations are also in progress.

Objective 2. *Evaluate integrated methods for snail control in catfish ponds.*

Objective 2a. *Monitor populations of catfish infected with* Bolbophorus *sp. to document the effect of parasite loads on growth and survival of the fish.*

Mississippi State University. Laboratory and field studies were conducted to evaluate the effects of sub-lethal trematode infections on growth, performance and disease resistance of channel catfish fingerlings. Trematode infections were established in populations of fish stocked in four, 0.1-acre ponds. A reservoir of trematode-infected snails was maintained in recirculating 300 gallon tanks located on the bank of each pond. Pond water was recirculated through each tank at a rate of 2 gallons per minute. The effluent (containing *Bolbophorus* cercariae) from the tank was directed back into the pond and served as the source of infection. Four additional ponds were used as control ponds. After 40 days, each population of fish was sampled to evaluate health status and 120 fish from each pond were transferred to 30-gallon aquaria to evaluate growth rates under controlled laboratory conditions using well water free of *Bolbophorus* cercariae. Only fish containing visible cysts (1 to 5 cysts per fish) were selected and used to evaluate growth potential. Fish were acclimated to laboratory conditions at 31 to 32°C for 3 weeks before

the start of the study. Following the conditioning period, fish were fed once daily for 9 weeks. Total weight gain, percent weight gain, specific growth rate, and feed efficiency were used to evaluate growth.

Evaluation of health and growth of channel catfish continually exposed to the cercarial stage of *Bolbophorus damnificus* throughout a production cycle

Mild trematode infections were established in pond populations of experimental fish by exposing fish to trematode cercaria. The percent of infected fish in each pond ranged between 20.4% and 1.6%. Mortalities directly related to trematode infections were not observed. *Edwardsiella ictaluri* and *Flavobacterium columnare* infections were diagnosed from all populations of fish and no differences in mortality were observed between trematode infected and non-infected fish. At the end of the production cycle, trematode infected fish consumed approximately 40% less feed compared to fish in control ponds.

Evaluation of health and growth of fish that have been infected with *Bolbophorus damnificus* cercariae by a single-pulse exposure

At the start of the acclimation period, trematode infected fish were significantly smaller compared to fish collected from control ponds. No differences in any of the measured parameters were observed between trematode-infected and non-infected fish at the end of 9 weeks. Although the final weight of trematode-infected fish was numerically smaller than control fish, percent weight gain and specific growth rate demonstrated a tendency towards compensatory growth of trematode-infected fish. Feed efficiency (0.86) was identical between treatment groups. Data indicates that once fish are removed from the source of infection, chronic trematode infections do not affect the growth potential of channel catfish.

Evaluation of health status and growth potential of channel catfish fingerlings infected with *Bolbophorus damnificus* under controlled laboratory conditions

Trematode infections were established under laboratory conditions by placing fingerlings in triplicate tanks containing *Planorbella trivolvis* snails shedding cercariae. Fish were left in the tank for 24 hours and snails were shedding cercariae at a rate of 770 ± 82 per 24 hours. Unexposed fish were maintained in three tanks under similar conditions. From each tank, trematode-infected or non-infected fish were transferred to six aquaria (30 fish/aquaria). Three aquaria from each replicate treatment tank received 7.5×10^5 CFU *E. ictaluri*/mL of water for 30 minutes (*Bolbophorus*-ESC and ESC-only groups). Fish in the remaining three aquaria were not exposed to *E. ictaluri* and served as *Bolbophorus*-only and negative control groups. The later two treatment groups were used in a second study and were challenged with *E. ictaluri* 28 days after exposure to *Bolbophorus* sp. cercariae.

No mortalities were observed in the *Bolbophorus*-only and negative control groups. Twenty-one days following exposure to *E. ictaluri*, the percent cumulative mortalities were 84.1 ± 16.2% in the *Bolbophorus*-ESC treatment and 45.9 ± 3.2% in the ESC-only

treatment. Mortalities were significantly different between the two groups. In the second study, when *E. ictaluri* exposure was delayed 28 days following *Bolbophorus* sp. infection, there was no difference in mortalities between the ESC-only (17.8 ± 4.0%) and combined *Bolbophorus*-ESC (21.5 ± 1.7%) exposed groups. Apparently, once fish are removed from the source of additional infections, chronic trematode infections do not increase the susceptibility of fish to ESC.

RESULTS AT A GLANCE...

★ *Mild, sub-lethal trematode infections can significantly reduce catfish growth by reducing feed consumption and increasing mortality associated with concurrent bacterial infections.*

Findings in these studies have significant implications for management strategies to control losses associated with trematode infections. Data collected from laboratory and field trials indicated that mild sub-lethal active trematode infections, commonly observed in channel catfish production systems, can significantly reduce production by reducing feed consumption and increasing losses associated with ESC. These studies also indicated that the presence of fully developed metacercariae does not compromise growth and health status of fish. These data support the contention that the deleterious effects of trematode infection are associated with penetration of the parasite and initial stages of encystment. Findings point to the need for increased surveillance for this disease and the benefit of initiating management protocols at the earliest stages of infection. In addition, breaking the trematode's life cycle by moving fish to non-infested water or by eradicating snails in the pond will eliminate the adverse effects associated with this disease.

RESULTS AT A GLANCE...

★ *The presence of fully developed* Bolbophorus *metacercariae does not affect growth or health of catfish. The deleterious effects of this infectious agent are therefore associated with penetration of the parasite and initial stages of encystment.*

Evaluation of the farm-wide economic impact of *Bolbophorus damnificus* infections of channel catfish

The economic impact of trematode infections was evaluated by conducting a disease-monitoring and production-efficiency study on a commercial catfish operation with ponds containing trematode-infected fish. Fish were sampled from each food fish production pond and examined for the presence of cysts that contain the metacercariae. Each pond was placed into one of four categories based on the percentage of infected fish in the sample. Ponds with trematode-infected catfish were placed into categories of light, moderate, or severe when the percentage of trematode-infected fish in the sample ranged from 0% to 33%, 33% to 66% or 66% to 100%, respectively. Ponds that did not contain trematode-infected fish were categorized as negative. Production records were grouped by infestation level for analysis. Of the 40 pond populations sampled, 17 were categorized as

negative, 6 as light, 6 as moderate, and 11 as severe. Fish from trematode-positive ponds consumed significantly less feed compared to fish from ponds that were categorized as trematode-negative. Fish from ponds in the trematode-negative category consumed on average 73.4 pounds/acre per day, and fish from ponds categorized as light, moderate and severe consumed 62.2, 47.5, and 47.2 pounds/acre per day, respectively. Similarly, production decreased as severity of infection increased. Compared to trematode-negative ponds, ponds in the light, moderate and severe categories produced 16.8%, 36.4%, and 44.5% fewer pounds of fish per acre, respectively. Net returns from ponds in the light category were reduced by 80.8% and production from ponds in the moderate and severe categories were not shown to cover variable costs of production. Ponds in the moderate category produced a net loss of $506 per acre and severe ponds produced a net loss of $631 per acre. These data from a commercial setting support results from experimental ponds and demonstrate that *Bolbophorus* infestations, regardless of severity, are a significant risk to commercial production of channel catfish.

Obective 2b. *Examine the efficacy of chemical control methods on snail populations.*

USDA/ARS. A total of seven trials were conducted from 2003 through 2005 to test the effectiveness of pond-shoreline treatments in controlling aquatic snails. Initially, four trials were conducted to compare a slurried-hydrated lime treatment with an established copper sulfate treatment. Copper sulfate and hydrated lime were applied at 4 pound and 80 pounds, respectively, per 100 feet of shoreline in a 6-foot swath. Trials were run under conditions of variable wind speed (0 to 16 mph) and treatment temperature (24 to 32°C). Both treatments effectively lowered the snail populations in the test cages. It appears that copper sulfate was more effective than lime in most trials, that hydrated lime treatments appeared to increase in effectiveness at higher temperatures (32°C vs 25°C), and that strong winds negatively impacted both treatments. Snail survival under all conditions in the four trials ranged from an average of 3.4% to 27.8% and 10.4% to 41.5% for copper sulfate and hydrated lime, respectively.

The goal of the second part of the study changed from comparing lime and copper sulfate to optimizing the hydrated lime treatment. Trials 5, 6, and 7 were conducted using only hydrated lime treatments at temperatures of 24 to 26°C and under low wind conditions. In Trial 5, the rate of hydrated lime was increased to 175 pounds per 100 feet of shoreline in a 6-foot swath. At that rate, effectiveness was increased and snail survival was less than 2%. Further optimization of the hydrated lime treatment was made by narrowing the treatment swath to 3 feet and reducing the amount of lime to 80 and 100 pounds per 100 feet of shoreline. Average snail survivals for the 80 and 100 pound treatments were 13.3% and 2.7%, respectively. The optimum pond shoreline treatment with slurried hydrated lime is

100 pounds of lime per 100 feet of shoreline applied in a 3-foot swath.

RESULTS AT A GLANCE...

★ *A shoreline treatment with slurried hydrated lime applied in a 3-foot swath at 100 pounds of lime per 100 feet of shoreline reduced snail populations by over 95%.*

Mississippi State University. The toxicity of copper sulfate to ram's horn snails was evaluated by establishing the 24-hour LC50 using Spearman-Karber analyses. Tests were conducted in 300-mL glass containers containing 200 mL of pond water (alkalinity = 235 mg/L as $CaCO_3$, hardness = 300 mg/L as $CaCO_3$, pH = 7.5, temperature = 23°C). Test concentrations of copper were arranged in a geometrically spaced dilution series. Each test concentration consisted of 4 replications with 5 snails per replication. Copper sulfate granules were dissolved in distilled water and delivered as a solution. After 24 hours, snails were removed from the test solution and placed in fresh, untreated water. End-points for the tests were death of the snails as determined by an additional 96-hours post-test observation period to confirm mortality. The effect of temperature (15, 20, 25, and 30°C) and alkalinity (0, 50, 100, and 200 mg/L $CaCO_3$) on the toxicity of copper to snails were also evaluated.

Laboratory tests showed copper sulfate crystals had a 24-hour LC50 of 0.6 mg/L Cu and, based on the alkalinity of the test water, was below the level considered toxic to fish. Alkalinity at the levels tested (0 to 200 mg/L $CaCO_3$) was not shown to effect to the toxicity of copper to snails. The LC50 concentration at an alkalinity of 0 mg/L $CaCO_3$ was 0.52 mg/L Cu versus 0.67 mg/L Cu at an alkalinity of 200 mg/L. Although there appeared to be a trend in the LC50 values toward decreasing toxicity with increasing alkalinity, these differences were not statistically significant. Analysis of data from this study showed a significant linear relationship between temperature and LC50 values for copper. As temperature increased from 15°C to 30°C, the LC50 values decreased from 1.1 mg/L to 0.18 mg/L Cu, representing a ten-fold increase in toxicity. This would be an important consideration when treating ponds with copper sulfate with respect to both snail and fish toxicity.

Three dose-titration trials were performed to determine the copper concentration required to kill snails under field conditions. Two trials were conducted in plastic tanks containing 200 gallons of pond water. Fish and snails were placed in three replicate tanks and dosed with a solution of copper sulfate at 0 (control) 1.25, 2.5, 5.0, and 10.0 mg/L Cu during the first trial and 0 (control), 0.375, 0.75, 1.25, and 2.50 mg/L Cu during the second trial. Each tank consisted of 20 snails confined in wire mesh cages and 10 channel catfish fingerlings to evaluate the toxicity of the treatment dose to fish.

The third trial was a non-replicated study conducted in 0.25-acre ponds containing approximately 1 acre-foot of water. In each pond, three sample sites were evenly distributed along the center long axis of the pond. At each sample site, 20 snails were confined in cages located at the surface and bottom of the pond. The toxicity of copper to

fish was evaluated in the 0.25-acre ponds by confining 10 channel catfish fingerlings in floating net-pens in close proximity to each snail sampling site. Each pond received a dose of 0 (control), 1.25, 2.5, 5.0, and 10.0 mg/L Cu by evenly applying the copper sulfate solution (200 gallons/pond) around the margins of the pond. Twenty-four hours after treatment, test snails were transferred to 1-L containers (containing the appropriate test water) and transported to the laboratory and observed for 72 hours. Twice daily, dead snails were removed and placed in a separate container containing untreated pond water. Dissolved oxygen concentrations in the test water and fish mortality were observed for 96 hours.

Titration trials in tanks and ponds were comparable, with laboratory toxicity trials and indicated the minimum effect dose (snail mortality >90%) of copper sulfate ranged between 0.75 and 1.25 mg/L Cu. Fish mortality was not observed at or below 1.25 mg/L Cu in the pond tank studies. Fish mortality (average mortality 5.3%), however, was observed at one of the three sample locations in one of the replicate ponds following treatment with 1.25 mg/L Cu.

Based on the results of the dose-titration trials, triplicate 0.25-acre and duplicate 10.0-acre experimental ponds were treated with 0.75 and 1.25 mg/L Cu to verify the minimum effective dose. Sample site configurations and treatment applications for tests conducted in the 0.25-acre ponds are described above. Sample site configurations for tests conducted in 10-acre ponds varied to accommodate pond size. Each 10-acre experimental pond contained 12 uniformly distributed sample sites consisting of 20 snails confined at the surface and bottom of the pond. An additional 20 snails were confined along the pond bank at equal intervals. Each 10.0-acre pond was managed as a commercial production pond and contained approximately 3,500 (average estimated size = 1 pound) fish per acre. No mortality or signs of disease were noted before any of the tests were conducted. Toxicity of the copper treatment to fish was evaluated by observing the pond stock for behavioral indicators of toxicosis and mortality. For each test conducted, a non-treated pond contained similar sample site configurations and served as a control.

Replicate single-dose pond treatments verified that treatment doses of 0.75 and 1.25 mg/L Cu were effective in killing snails. Average snail mortality in trials conducted in the 0.25-acre ponds ranged between 98.0% and 95.5% at the low treatment doses and was 100% at the high treatment dose. Fish mortality was observed in 1 of the 3 replicate ponds at each treatment dose. Similar results with respect to snail toxicity were observed in the single dose toxicity trials conducted in 10-acre ponds. Average snail mortality of the replicate trials at each sample location ranged between 92 and 98% following treatment, with 0.75 mg/L and between 98 and 100% following treatment with 1.25 g/L Cu. In contrast to the 0.25-acre pond trials, no fish mortality or behavioral signs of toxicosis were observed following treatment. In all pond trials, dissolved oxygen depletions were not observed for up to 168 hours after treatment. Fish mortality in the 0.25 acre ponds may be caused by exposure of confined fish to high concentrations of the applied chemical before it was completely mixed with the pond water.

Treatment efficacy was then evaluated in a 13.0-acre commercial channel catfish production pond. Moderate numbers of snails were

observed along the margins of the pond. Seven sample sites (surface and bottom cages containing 20 snails each) were placed a minimum of 50 yards from the pond bank and were distributed randomly across the pond. An equal number of cages containing 20 snails each were placed along the margins of the pond. In addition, natural snail populations along the margins of the test pond were sampled at five locations before and after treatment. A 20-foot section of pond bank consisting of uniform vegetation and levee slope was marked and divided into 2 equal sections. Snails were collected from a 10-foot section of the sample site before the pond was treated, and the remaining section was sampled 24 hours after the pond was treated. Snails collected from each section were placed in 4-L containers and transported to the laboratory for observation. Live snails were counted to estimate viable snail numbers and used to determine the number of snails per foot of pond bank in the sampled area. Production fish were monitored for behavioral signs of toxicosis and mortality. Snails confined in a similar configuration in an adjoining pond served as a control.

Results of the commercial field trial were comparable to tests conducted in the experimental ponds where application of copper sulfate at 1.25 mg/L was shown to be effective in killing snails. Average mortality of snails confined in cages ranged between 95.4 and 97.7%. The treatment was also shown to be effective against natural populations of snails along the margins of the pond. The average number of snails per foot of pond bank decreased from 21.5 snails to 0.18 snails 24 hours after treatment, representing a 99% reduction in viable snail populations in the habitat along the pond bank.

Treatment of the commercial pond resulted in changes in fish behavior and mortality that was likely related to the copper treatment. Within 4 hours of treatment application, an increase in the number of moribund fish were observed. Affected fish appeared lethargic or exhibited a spiraling swimming pattern. However, it is not thought that these observations were solely related to the chemical treatment. Prior to treatment, moribund and dead fish were present in the pond that was diagnosed with bacterial (*Edwardsiella ictaluri* and *Flavobacterium columnare*) and parasitic infections (*Bolbophorus* sp.). Moribund fish also exhibited clinical symptoms consistent with visceral toxicosis of catfish. Mortality rates in the pond were characterized as chronic and were estimated to be 150 to 200 fish per day. Farm management estimated total mortality in excess of 20,000 pounds. Following treatment, fish mortality increased within the first 24 hours. It was estimated that approximately 2,000 pounds of fish were lost following treatment, however, a majority of the fish had clinical signs of ESC. In addition to infectious disease, analysis of water quality 2 hours after treatment revealed low chloride to nitrite ratios and examination of fish gills and blood indicated acute nitrite toxicosis. On the

RESULTS AT A GLANCE...

★ *A low-dose, full-pond treatment with copper sulfate was developed that safely eradicates the trematode's intermediate host—the ram's horn snail.*

day of treatment, salt was added to the pond water and, after the first 24 hours, the daily mortality rate returned to pretreatment levels.

Objective 2c. *Examine the efficacy of biological control methods (snail-eating fish) on snail populations in ponds.*

Effect of diet conditioning on prey selection by blue catfish and redear sunfish

Mississippi State University, Southern Illinois University. Four aquaria were stocked with 300 juvenile blue catfish and four additional aquaria were stocked with 300 juvenile redear sunfish. The fish were given an initial "conditioning diet" which consisted of only one of the following: fish food – insect larvae, ram's horn snails or red-rimmed melania snails. After 2 weeks of feeding the "conditioning diets," 100 fish of each species were stocked into eight separate aquaria (16 aquaria total) and offered a known amount of their conditioning diet and a known amount of one of the other conditioning diets used above. Prey selection was determined by the frequency of selection of the various diets. A Chi-square contingency test was used to determine the influence of conditioning on food selection. A two-tailed test of binomial proportion was used to examine the significance of food preference in each conditioning experiment. Because fish that consume large amounts of food may bias results, the percentage of the total number of prey items the percentage of food for each fish was determined either by video or by examination of the stomach contents. For video analysis, a video camera was used to record a 2-hour segment of feeding. The data obtained was analyzed using the Observer (1997), a computer program specifically programmed for behavior analysis. Data obtained from this program was analyzed using a Chi-square contingency test.

Prey selection studies with the blue catfish revealed that regardless of the training or conditioning diet, blue catfish readily converted to catfish feed when it was offered. Redear sunfish preferentially consumed snails when available. Even redear sunfish trained to eat commercial fish food readily consumed snails and chironomids when available.

RESULTS AT A GLANCE...

★ *Ponds stocked with redear sunfish had significantly fewer snails than ponds without sunfish. Redear sunfish preferentially consumed snails and midge larvae when available, even when trained on pelleted diets prior to stocking ponds.*

Determination of the ability of redear sunfish to withstand conditions of commercial catfish culture

Southern Illinois University. In the spring, redear sunfish (small, medium, and large) were stocked at a rate of 300 fish/acre into four, 0.0-acre experimental ponds (12 ponds total) stocked with channel catfish at a production rate of 8,000 pounds/acre. Water quality variables such as dissolved oxygen,

temperature, alkalinity, carbon dioxide, ammonia, and pH were measured on a daily basis. The number of dead fish (catfish and sunfish) was recorded. In September, the ponds were seined and harvest size channel catfish removed. The number of surviving individuals of each species was recorded. The ponds were under stocked with 5-inch fingerlings to replace the fish removed for market. Survivability of redear sunfish was analyzed to determine if any correlation with water quality variables exist. The number of redear sunfish that died due to seining was recorded. In the spring of 2004, the ponds were seined and all redear were counted and weighed. Harvest size catfish were removed and 5-inch fingerlings were stocked to replace those removed.

In the fall of 2004, redear sunfish and channel catfish were removed from ponds. The number and type of snails within a one meter transect were counted and recorded. One hundred channel catfish from each pond were sampled and analyzed for the presence of trematodes on gills and in the flesh. This study was repeated in 2005.

In the 2004 study, ponds containing redear sunfish had significantly fewer snails than ponds without sunfish. The number and type of snails remaining in the ponds did not differ significantly when medium size or large sunfish were stocked. Redear sunfish trained to eat snails did not remove significantly higher numbers of snails than fish not trained or conditioned to snail diets. Survival of the redear sunfish was 100 percent in all ponds. The incidence of trematode infestation was still evident on channel catfish. Approximately, 25% of the catfish had trematodes on the gills and in some cases within the flesh.

In the 2005 study, the water temperatures in the ponds at Southern Illinois University averaged 5°C higher than in 2004. Catfish survival in all ponds was greater than 98%. Survival of redear sunfish was strongly correlated with the decreased dissolved oxygen and increased temperature in the ponds. Ponds that routinely had dissolved oxygen concentrations of 3 mg/L and temperatures of 32°C at sunrise had significantly higher mortality rates than ponds with higher morning dissolved oxygen concentrations. No significant correlations between alkalinity, carbon dioxide, ammonia, nitrite, or pH on survival of redear sunfish were observed. The incidence of trematode infestation was still evident on channel catfish. Approximately, 31% of the catfish had trematodes on the gills and in some cases within the flesh. The increase observed in the number of channel catfish with trematodes visible on the gills or in the flesh is likely due to the decrease in redear sunfish numbers in the ponds due to mortalities.

Objective 3. *Develop and implement standardized methods for the isolation, culture, and antimicrobial susceptibility testing of strains of columnaris-like bacteria isolated from diseased fish.*

Louisiana State University. Various agar media were evaluated for optimum primary isolation and maintenance of *Flavobacterium columnare*. Media under investigation included

both selective and non-selective cytophaga agar (CA), Hsu-Shotts (HS), Shieh (S), tryptone yeast extract (TYE), dilute Mueller Hinton (DMH) and Flavobacterium columnare growth medium (FCGM). Media were made selective by the addition of 5 µg/mL neomycin and 200 units/mL polymixin B. For primary isolation the media were prepared as agar plates and for maintenance the media were prepared as 20-mL slants in 50-mL tubes with 1 mL of saline added to preserve moisture. For the evaluation of primary isolation media, a standardized mixture of *F. columnare*, *Edwardsiella tarda*, *E. ictaluri*, *Aeromonas hydrophila*, and *Streptococcus difficilis* was prepared. This mixture was designed to mimic the mixture of aquatic bacteria that might be present in contaminated external sites such as the gills and skin of diseased fish. The mixture was inoculated onto the various test media to evaluate their ability to produce pure colonies of *F. columnare* while inhibiting contaminating bacteria.

Selective cytophaga agar (SCA) performed the best as a primary isolation medium for isolation of columnaris from a mixed inoculum of aquatic bacteria. The remainder of the media were ranked as follows: (2) SS (3) SHS, (4) DMH and (5) FCGM. Both DMH and FCGM produced no isolated *F. columnare* colonies.

For maintenance following isolation, TYE slants performed the best with some cultures maintaining viability as long as 84 days. The remaining media were ranked as follows: (2) CA, 52 days (3) DMH, 47days (4) HS, 34 days (5) S, 32 days and (6) FCGM, 23 days.

Some of the above-mentioned media were evaluated as broths for batch culture of *F. columnare*. A 40-mL volume of media was inoculated with 200 µL of a McFarland #5 standard inoculum and growth performance measured by colony forming units (cfu) per mL and absorbance at 600 nm following 24 hours of incubation at 28°C. *Flavobacterium columnare* growth medium (FCGM) out-performed other formulations tested producing mean absorbances of 0.2377 and colony counts of 2.2×10^9/mL. The clumping of cells, which is a problem in other broth media, was avoided in FCGM. The remaining broth media were ranked as follows: (2) Shieh (3) cytophaga and (4) DMH. For a summary of broth culture results see Table 1.

For disk-diffusion antimicrobial susceptibility testing, dilute Mueller Hinton (DMH) plates prepared with different levels of agar and nutrient were evaluated for clarity and consistency of zone size. To insure uniformity, one lot of each of five anti-microbial agents, one lot of Mueller Hinton medium, and one lot of equine serum were used in all disk diffusion test evaluations. The anti-microbial disks (BBL) chosen were Sulfamethoxazole : trimethoprim (SXT 25 μg), Sulfadimethoxine : ormetoprim

RESULTS AT A GLANCE...

★ *Selective cytophaga agar SCA has performed the best as a primary isolation medium in preliminary tests in isolation of Flavobacterium columnare from contaminated sites such as the gills and skin. For maintenance following isolation, tryptone yeast extract TYE medium as a moist slant, held cultures viable for as long as 84 days. For large batch broth culture, FCGM outperforms other formulations tested.*

Table 1. Growth of *F. columnare* in various broth media at 28°C for 24 hours. Data is presented as absorbance at 600nm and by colony forming units (cfu) per mL.

Test Medium	Mean Absorbance (600nm)	Mean Colony counts (CFU/mL)
DMH*	0.0858	1.3×10^7
Cytophaga*	0.1646	6.3×10^7
Shieh*	0.2078	3.1×10^8
FCGM*	0.2377	2.2×10^9

* Indicates significance at P = 0.05

(PRI-MOR 25 Fg), Oxolinic acid (OA 2 μg), Oxytetracycline (T 30 μg), and Florfenicol (FFC 30 μg). The basal MH broth used was (Difco) lot # 3126187, and the degranulated agar used was (Difco) lot #3265229. The concentrations of MH broth evaluated were 3 g, 3.5 g, 4 g, and 5 g/L. The agar concentrations that were evaluated were 9 g, 12 g, 15 g, and 17 g/L. Varying concentrations of MH broth were used to determine the optimum amount of nutrients for *F. columnare* growth. The varying agar levels were evaluated for their effects on zone appearance by limiting the gliding motility of the bacterium. Each medium was evaluated for thickness of growth, distinct zones, and uniformity of zone margins. Once the optimum concentration of broth to agar was determined, 5% equine serum lot #ANE18713 (Hyclone, Logan, Utah) was evaluated as a replacement for the more expensive fetal calf serum as a growth supplement for *F. columnare* cultures on MH agar plates.

The optimum media formulation for susceptibility testing was determined to contain 4 grams M-H broth and 17 grams of agar per liter with 5% equine serum. This medium gave the highest bacterial growth and allowed for better definition of zones of inhibition. Equine serum improved the growth of *F. columnare* cultures on dilute M-H agar, but not significantly different from fetal calf serum. Because of availability and cost of equine serum compared to fetal calf serum it was determined to be a suitable enrichment factor for the improved medium.

RESULTS AT A GLANCE...

★ *An improved medium has been developed for antimicrobial susceptibility testing of* Flavobacterium columnare.

Objective 4. *Characterize archived strains of columnaris-like bacteria based on conventional and molecular techniques.*

Morphologic and biochemical analysis of columnaris-like bacteria

Louisiana State University. Forty-nine strains of columnaris-like bacteria archived in the LADL and UAPB collections were analyzed using conventional biochemical testing in test tubes, the API 20E system, the API NE system

and the API ZYM system. These strains were obtained from a larger pool of yellow pigmented columnaris-like isolates that were subjected to screening procedures that involved a species-specific PCR (Bader et al. 2003), the five point characteristics of Griffin (1992) and the physiological characteristics of Bernardet and Grimont (1989). To conform to the Griffin screen, the bacterium must be shown to satisfy the following requirements: production of flat, spreading, yellow, and rhizoid colonies on cytophaga agar, growth in the presence of neomycin and polymixin B, production of gelatin degrading enzymes, binding of congo red dye to the colony, and production of chondroitin sulfate A degrading enzymes. Isolates were also tested for the presence of flexirubin-type pigments using the potassium hydroxide (KOH) method outlined in Bergey's Manual of Determinative Bacteriology 9th Edition (1994). The goal of this part of the project is to determine if *F. columnare* can be identified using conventional and commercially available biochemical testing schemes by labs that may not have molecular capabilities. Prior to the start of our study, 48 of 49 archived strains of columnaris-like bacteria conformed to the Griffin screen and were confirmed by PCR as *F. columnare.*

Results indicate that *F. columnare* should be shown morphologically to be a long, thin, gram negative rod (3 to10 micrometers length and 0.3 to 0.5 micrometers in width) with gliding and flexing motility, and form rhizoid, yellow-pigmented colonies on agar plates. Morphology of the cells and colony appearance is slightly variable among strains and is influenced by growth conditions and age of the culture. The isolate should be a strict aerobe, should not produce acid from carbohydrates, and should be cytochrome oxidase and catalase negative. Negative reactions were obtained in the GMD and TSI agar tests due to the lack of acid production from carbohydrates. The API 20E, API NE and API ZYM systems (bioMérieux-Vitek) were examined for usefulness in the identification of *F. columnare*. The API 20E and API ZYM systems were determined to be inadequate due to the lack of positive reactions on the strips. The API NE was very useful producing an adequate number of positive reactions. Isolates of *F. columnare* uniformly gave reactions resulting in the API NE code number 0441455 at 24 hours. The positive reactions in the API NE strip were as follows: esculin, D-glucose, L-arabinose, potassium gluconate, capric acid, malic acid, and citrate.

Adhesion to plastic, cultured cells, and isolated gills

Auburn University. Six types of plastic multiwell plates (BD Biosciences, Franklin Lakes, NJ) were compared for use in a bacterial adhesion assay. Two hours after washed *F. columnare* cells were added to the wells, there were significant differences among the plates. The same results were obtained with two isolates. Adhesiveness of *F. columnare* was greater for bacteria grown in Hsu-Shotts broth rather than in Shieh broth. The addition of calcium and magnesium to water used in the adhesion assay increased the adhesiveness of one isolate of *F. columnare* (PL-04-02) but had no effect on another isolate (PB-02-110). Other waters tested, which had high concentrations of NaCl, tended to reduce the adhesiveness of the isolates tested.

Isolates of *F. columnare* tested with the multiwell plate assay had a wide range of adhesiveness to plastic. As additional isolates are obtained for testing, these results will be compared to other types of results obtained by investigators at other institutions to determine if there is a relationship between adhesiveness and other characteristics. Attempts to quantify the adhesion of *F. columnare* to cultured cells was hindered by the adhesion of the bacteria to the glass or plastic substrate used for cell culture and by problems with accurate counting of bacteria stained by conventional methods. To overcome these problems, antibodies against *F. columnare* were made in rabbits as a first step in development of antibody-based methods for bacterial quantification.

Three types of assays for adhesiveness of *F. columnare* (isolated fish gills, larval fish, and cultured cells) were developed and then compared with a multi-well plastic plate assay. The assay with plastic plates was previously found to be useful for quantification of *F. columnare* adhesiveness.

For the gill assay, gills were dissected from channel catfish, bluegill, and common carp. For each fish, one section of gill was used as a control and two sections were exposed to *F. columnare*. After a 10-minute exposure to bacteria, gills were rinsed twice and homogenized. Plate counts of serial dilutions of the gill homogenate were used to quantify the *F. columnare* adhering to gills. There was a significant difference among *F. columnare* isolates adhering to for gills from bluegill (8 isolates) and common carp (3 isolates). Only two isolates were tested with normal channel catfish gills; however, there was a significant increase in the number of bacteria adherent to gills of channel catfish with proliferative gill disease or with *Aeromonas* infection.

A larval zebrafish assay for adhesiveness of *F. columnare* was developed. Whole fish were exposed to *F. columnare* for 1 hour, rinsed for 2 minutes, and then homogenized. The adherent *F. columnare* were enumerated by plate counts. There were significant differences in adhesiveness of the 11 isolates of *F. columnare* evaluated with this assay. The CFU/mg of fish varied over a 150-fold range for the isolates tested.

A cultured-cell assay was used to examine adhesiveness to EPC cells. The cell culture medium was removed from the cells and replaced by a suspension of *F. columnare* in either phosphate-buffered saline (PBS) or well water. After incubation at 30°C for 10 minutes, plate counts were used to determine the number of adherent bacteria. Seven isolates of *F. columnare* were tested for adhesiveness in hypotonic conditions (well water), and there was no significant differences among isolates. Four isolates were tested in PBS, and one isolate had significantly reduced adhesiveness. The assay with EPC cells was not satisfactory because the high concentration of sodium chloride in PBS reduces the adhesiveness of *F. columnare*. The use of fresh water during the incubation of EPC cells with *F. columnare* resulted in swelling of the EPC cells because of the hypotonic conditions. The variation in adhesiveness among the *F. columnare* isolates tested was small for the cultured-cell and gill assays.

Based on adhesion to plastic, the mean adhesion of 11 isolates of *F. columnare* isolated from external organs was higher than the mean for

11 isolates from internal organs. The virulence of 13 isolates (as reported by Thomas-Jinu and Goodwin in 2004) was not correlated with adhesion to plastic. Adhesion of 11 isolates of *F. columnare* to larval zebrafish was also not correlated with the virulence reported by Thomas-Jinu and Goodwin (2004). The lack of correlation between virulence and adhesion could be the result of additional passages in culture between the determination of virulence and the adhesion evaluations. There could also be differences in virulence or adhesion related to species of fish. Additional experiments will evaluate adhesion and virulence in simultaneous experiments.

Molecular identification of columnaris-like bacteria using rapid sequence analysis of a portion of the 16S ribosomal gene and the 16S-23S intergenic spacer region

Mississippi State University. Isolates of columnaris-like bacteria obtained from LSU and MSU were cultured, and DNA isolated using Purgene DNA isolation Kit (Gentra Systems, Inc., Minneapolis, Minnesota). A portion of the 16S and the entire 16S-23S intergenic spacer of one isolate was PCR-amplified using primers to regions of the 16S and 23S ribosomal sequences that are conserved among the gram negative bacteria. One predominant product was obtained and cloned into pPCR4 TOPO cloning vector (Invitrogen) and sequenced. This was an intergenic sequence containing the tRNA for alanine and the tRNA for isoleucine. Several products were expected, representing different ribosomal operons, but as of yet only this ITS product was found. Alignment of these sequences with the tRNA sequences from related organisms were used to identify conserved sequences, and primers were developed to allow direct PCR of the specific ITS and direct sequencing of the products. These PCR products have been produced and both strands of both products sequenced for all isolates.

The fragment of DNA between the 16S and 23S ribosomal RNA encoding (ITS) of a total of 50 *Flavobacterium columnare* case isolates were amplified by polymerase chain reaction using the common 16S-tRNA and tRNA-23S primer sets reported in year one of the project. The products were cloned and sequenced. The sequences consist of a total of 748 base pairs (bp) and include a 100 bp portion of the 16S fragment and 200 bp overlapping region. In sequence comparisons the 16S region was useful in identifying isolates that were not actually *F. columnare*. The ITS demonstrated substantial variation, however, at least 3 distinct clusters of similar sequences were identified. These clusters demonstrated 5-10% sequence differences in the ITS region but less than 2% divergence within a cluster. This suggests that the isolates represent at least 3 different strains. We are evaluating an additional 20 isolates and comparing sources to see if sequence data correlates with host or season. Also, the conserved sequences will be evaluated for differentiating diagnostic PCR. All sequencing data will be submitted to GenBank so that other diagnostic and research labs can use this information.

Ribotyping techniques to differentiate isotypes of columnaris-like bacteria

University of Tennessee. A number of *Flavobacterium columnare* isolates from fish

disease cases were acquired, as well as several *Flavobacterium columnare*-like bacteria, which share a close taxonomic relationship to the target organism, including *Flavobacterium hydatis, F. succinicans,* and *F. psychrophilum*. Those isolates are currently being typed using ribotyping methodologies, and assay components and procedural variations that provide the greatest fingerprint definition between the various isolates are being determined. Once established, the optimal methodology will be used to generate a fingerprint database of the above control isolates, which will then form the basis for comparison of wild type isolates obtained from other investigators involved with this project.

We have replicated and validated ribotyping methods developed to differentiate various isolates of *Flavobacterium columnare* (ATCC strains, wild type strains isolated from infected fish), *F. hydatis, F. resiovorum, F. aquatile, F. flevense,* and suspected *Flavobacterium spp.* obtained from various sources and other diverse species of bacteria that might inhabit aquatic environments (*Citrobacter freundii, Brochothrix thermosphacta, Aeromona veronii, Sphingomonas capsulate, Vibrio Cholerae, Pseudomonas stutzeri, Micrococcus luteus, Glaciecolia pallidula*). While the Qualicon database is quite extensive across many species of bacteria, the database for *Flavobacterium spp*. is rather limited, and thus as a part of this process, we are building a riboprint database, which will become available to other users of that system. In that analysis, we have noted good homology and yet acceptable separation of riboprints between various *Flavobacterium* species and good separation from and between most of the diverse isolates, including *Flavobacterium*-like bacteria.

We have also progressed in studies to determine the efficacy of pulsed-field gel electrophoresis (PFGE) analysis for identification and differentiation of a number of the above species, and including various strains of pathogenic *Flavobacterium columnare*. The first phase of this work has involved the development of culture methods and PFGE protocols, based on restriction digests, to obtain optimal fingerprints for identifying and separating various bacterial isolates. Because *F. colmnare* colonies cannot be readily separated from solid growth media, obtaining an appropriate mass of cells for PFGE assays via picks or loops, as is done with other bacterial species, was found to be difficult. Thus, isolates were grown in liquid culture, followed by centrifugation to obtain a cell pellet. Varying cell densities/concen-trations were used for DNA isolation procedures, following by restriction enzyme digests prior to running digested DNA through PFGE gels in conjunction with CHEF-mapper system (Bio-Rad Laboratories, Hercules, California). Test growth media have included ATCC Nutrient Broth 3, 1839 Harpo's HTYE, 1750 Anacker and Ordal Medium. Separate trials were conducted to test various restriction enzymes, including *Spe*I, *Xba*I, *Bam*HI, *Sma*I, *Apa*I. Additionally, varying densities of SeaKem Gold Agarose (Fisher Scientific, Far Lawn, New Jersey) were used for PFGE separation of bands.

For the PFGE analysis, *Spe*I, *Xba*I, *Bam*HI restriction enzymes produced small and numerous bands from DNA extracted from *Flavobacterium spp.* and some *Flavobacterium*-

like bacteria, and those patterns were found to be unsuitable for PFGE type analysis; whereas suitable fingerprints were generated for other gram-negative species, including *Salmonella choleraesuis* Braenderup control (ATCC BAA-664). We believe that restriction sites in *Flavobacterium* genome are too numerous for the three restriction enzymes above. *SmaI* and *ApaI* digests produced fewer but less distinct bands in *Flavobacterium spp*. and some Flavobacterium-like bacteria, whereas those enzymes again produced suitable fingerprints for other bacteria including control strains. Varying densities of PFGE gels did not provide better resolution of banding patterns.

Currently, we are implementing additional protocols and alternate restriction enzymes to develop more definitive PFGE typing patterns. Such protocols will likely require more lengthy DNA extraction procedures, which will in turn require longer turn-around times for sample analyses.

To date, our studies indicate that ribotyping may offer a more efficient and timesaving option for identification and differentiation of *Flavobacterium*, *Flavobacterium*-like, and non-*Flavobacterium* isolates.

RESULTS AT A GLANCE...

★ *Molecular (PCR) and conventional (API system) methods may be used for the identification of F. columnare. Molecular methods such as ribotyping, RAPD analysis, and sequencing of the intergenic spacer region between the 16S-23S ribosomal RNA genes can allow for discrimination between different genotypic strains.*

Determine the presence of unique outer membrane proteins of various strains of columnaris-like bacteria

Clemson University. Several outer membrane proteins (OMP) from *Flexibacter columnare* have been isolated and are consistently found in all *F. columnare*. Over the last two years we reported that a 30 kDa OMP isolated from *F. columnare* is a potent inducer of type II nitric oxide synthase (iNOS) and inducible prostaglandin H2 synthase (cyclooxygenase-2; COX-2) in isolated catfish phagocytes, and that these activities can be blocked using specific antibodies against the OMP.

The proteomics facility within the Clemson University Genomics Institute (CUGI) helped us to identify *F. columnare* OMPs, with an initial focus on the 30 kDa, 40 kDa, and larger OMPs. Protein fragments between 12 and 15 amino acid residues were isolated from each band, yet were not identified because of a lack of homology with any known proteins in current data banks. These fragments, how-ever, may be useful for generating PCR primer sets to identify larger coding regions of the OMP genes. Very recently, we constructed a cDNA library from a virulent Clemson University strain of *F. columnare* using a commercially available kit (Ambion) to remove 18S and 20S RNA, thereby enriching mRNA that was subsequently used to generate a long-PCR-based library (Clonetech). Our in-hand antibodies against OMPs recognized recombinant proteins expressed in the library, and those positive plasmids are being sequenced at the moment. Over the course of

the next 5 months recombinant OMP proteins will be expressed and purified, then screened for both *in vitro* and *in vivo* activity in channel catfish and tilapia phagocytes, and finally will be used as potential vaccines against our virulent strain of *F. columnare* The most important product of this study will be the availability of OMP-specific antibodies and recombinant OMPs for general use by the cooperators in this project.

Objective 5. *Develop challenge models for columnaris-like bacteria isolated from major warmwater aquaculture species in the southeastern United States.*

Internal genetic labeling of columnaris-like bacteria for use in the development of an effective challenge model

Mississippi State University and Auburn University. Our objectives for this project are to 1) ligate a *Bacteroides* consensus promoter sequence upstream of *gfp* mut3a to allow expression of green fluorescent protein in Flavobacterium *columnare,* 2) ligate the *gfp* gene and promoter into shuttle vector pCP11, 3) transfer the pCP11-gfp plasmid into *Flavobacterium columnare* for expression of green fluorescent protein, and 4) use the fluorescent-labeled *F. columnare* to develop an effective challenge model (in cooperation with Joe Newton at Auburn University).

Two 65 base-pair DNA oligonucleotides containing a consensus promoter sequence from *Bacteroides fragilis* were synthesized and hybridized. The double stranded DNA containing the promoter was then digested with *Eco*RI and *Sac*I and ligated upstream of *gfp* mut3a in plasmid pFPV25. Expression of the *gfp* gene from this plasmid (designated pFCgfp) in *E. coli* was confirmed using a fluorescence plate reader.

A *Spe*I-*Eco*RV fragment from pFCgfp was ligated into pCP11, and the resulting plasmid was designated pMWFCgfp. Expression of *gfp* from this plasmid in *E. coli* was also confirmed using a fluorescence plate reader. Another plasmid was also constructed by amplifying the *ermF* (erythromycin resistance) gene from pCP11 by PCR and cloning it into the *Eco*RI site of the broad host range plasmid pBBR1MCS4. The resulting plasmid, pBBRermF, will allow selection in *F. columnare* based on erythromycin resistance. We then transferred the *gfp* gene with the *Bacteroides* promoter from pMWFCgfp into pBBRermF on a *Sma*I/*Spe*I fragment to construct pBBRFCgfp.

Objective 3 has not been successfully completed despite numerous attempts to transfer pMWFCgfp and pBBRFCgfp into multiple *F. columnare* isolates. We have been utilizing a conjugation technique using *E. coli* SM10 l*pir* as a donor strain to attempt transfer of the plasmids into *F. columnare*. We have been using 25 columnaris strains that were collected from John Hawke (LSU-SVM) as well as an additional 20 isolates received from Joe Newton. This year, we have phenotypically characterized all the isolates to enable optimal growth conditions for *F. columnare* during the conjugation experi-

ments. We have also conducted experiments that resulted in adjustment of the erythromycin concentration used on the selection plates. In addition, we have obtained two additional plasmids, pCP23 and pCP29, from Mark McBride at University of Wisconsin, Milwaukee that will enable us to utilize other antibiotic selection markers. pCP23 carries a tetracycline resistance gene that is expressed in flavobacteria, and pCP29 carries a cefoxitin gene. Experiments are ongoing using our optimized growth/antibiotic conditions to transfer pMWFCgfp, pBBRFCgfp, pCP23, and pCP29 into the *F. columnare* isolates from our panel.

A nalidixic acid resistant *F. columnare* mutant (spontaneous–not recombinant) has been isolated that can be used as a tagged organism (if it is still virulent) until the *gfp* gene in *F. columnare* is successfully expressed. Several *F. columnare* isolates (including the nalidixic acid isolate) have been used in challenge experiments following the procedures of Andy Goodwin and S. Thomas-Jinu at the University of Arkansas at Pine Bluff. To date it has not been possible to cause columnaris disease in these experiments using their procedures.

Challenge models for channel catfish and golden shiners

University of Arkansas at Pine Bluff. In the first part of our study we compared the biochemistry, DNA sequence (by RAPD) and pathogenicity of a large group of columnaris strains. Channel catfish and golden shiners were subjected to temperature shock and then immersed in a bath of columnaris bacteria at a concentration sufficient to cause 60 to 70% mortality in 2 days using the more pathogenic of archived columnaris strains for the respective host. Each experiment was performed in triplicate with 20 fish per tank. Moribund fish were necropsied and the cause of death verified. Columnaris bacteria were re-isolated and identified by biochemical (tube tests) and molecular (randomly amplified polymorphic DNA, RAPD, Promega) techniques to verify that the fish died from infection by the challenge bacteria. In this work, we found that the catfish and cyprinid fish isolates fell in different clades, but that there was no correlation between these genetic and biochemical results, and any other measure including fish species of origin or pathogenicity to catfish and golden shiners.

Another way to look at differences between columnaris isolates is to challenge fish and then look for differences in response of the infections to practical disease treatments. Columnaris disease was produced in channel catfish, *Ictalurus punctatus* (Rafinesque) by bath exposure to 4 highly virulent isolates of *Flavo-bacterium columnare*. In untreated controls, mortality began 20 hours after exposure and was 100% by 48 hours after exposure. Mortality in channel catfish given antibiotic treatments with oxytetracycline (OTC) or a combination of sulfadimethoxine and ormetoprim (SOR) in feed prior to bacterial challenge was 0% with all four strains of *F. columnare*. Diquat was the most effective bath treatment; mortality with all four strains was 0%. With potassium permanganate, chloramine-T, hydrogen peroxide, and copper sulfate bath treatments, efficacy varied significantly among bacterial

strains and among treatments. Bath treatments with chloramine-T and potassium permanganate reduced mortality from 100% to 75% and 69%, respectively, but copper sulfate and hydrogen peroxide treatments were not effective.

Based on our results, oral antibiotics prevented columnaris disease but, of the bath treatments, only Diquat produced a dramatic reduction in the mortality of acutely infected fish. Diquat is labeled for aquatic use as an herbicide in the United States but in large ponds it is prohibitively expensive.

Challenge models for hybrid striped bass

Louisiana State University. Strains of *Flavobacterium columnare,* archived in the LSU Aquatic Diagnostic Laboratory repository, are being used in virulence studies in hybrid striped bass. Methods that produce uniform mortality rates of 75% or greater following exposure are classified as virulent strains of *F. columnare.* These criteria will be adopted for use to compare virulence of archived strains from various locations and various species outlined in Objective 6. Hybrid striped bass (20 g mean weight) were acclimated and held in the Aquatic Pathobiology Building at the LSU School of Veterinary Medicine. The strains (isolates) were evaluated for virulence in a standardized challenge procedure where scales were removed and the skin scarified in a 1 cm^2 area. Cultures of the *F. columnare* bacteria were swabbed on the scarified area rather than using the immersion method. This was done due to difficulties with clumping of the bacteria and difficulty enumerating bacteria in the challenge bath. Virulent strains of *F. columnare* colonized the scarified skin readily and caused infection and disease whereas avirulent isolates did not cause infection. Mortality was evident 96 hours after infection with virulent strains.

Objective 6. *Use challenge models for each fish species to correlate virulence with biotype and or genotype of columnaris-like bacteria.*

Channel catfish and golden shiners

University of Arkansas at Pine Bluff. Variability in *Flavobacterium columnare* pathogenicity makes disease treatment difficult because there is currently no way to easily recognize those strains that warrant aggressive treatments. In order to identify suitable markers, 17 isolates of *F. columnare* were cultured from six different fish species. The DNA from all isolates was analyzed using randomly amplified polymorphic DNA (RAPD). Bootstrap analysis of the RAPD data produced a tree with three major groups supported by scores of 80% to 100% similarity.

The remaining objective was to complete challenge assays to see if there were any stain differences in the virulence of columnaris isolates using the golden shiner as a host. Of the strains tested, two of catfish origin that were highly virulent in channel catfish (100% mortality) produced much lower mortality (30% to 40%) in golden shiners. For the other

isolates tested, pathogenicity in channel catfish and shiners was similar. There was no correlation between biochemical characteristics or RAPD genogroup and pathogenicity.

Hybrid striped bass

Strains identified by the RAPD grouping sysytem of Farmer 2004 were used in challenge studies. Representative isolates from each RAPD group and host species were obtained from our collection of archived strains at LSU. Strains used in the challenges were: LADL 04-046 isolated from channel catfish (RAPD Group I), LADL 04-066 isolated from large-mouth bass (RAPD Group I), PB-02-12 isolated from fathead minnow (RAPD Group II), and LADL 94-141 isolated from channel catfish (RAPD Group III). Strains LADL 04-066 largemouth bass (Group I) and PB-02-12 fathead minnow (Group II) were virulent in hybrid striped bass causing 100% mortality in 96 hours. Strains LADL 94-141 channel catfish (Group III) and LADL 04-046 channel catfish (Group I) were non-virulent in hybrid striped bass. Thus far virulence appears to be correlated more with host source than with RAPD group. Additional strains are currently being tested.

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IMPROVING REPRODUCTIVE EFFICIENCY TO PRODUCE CHANNEL × BLUE HYBRID CATFISH FRY

Reporting Period
March 1, 2004 - August 31, 2005

Funding Level	Year 1	$118,390
	Year 2	$111,610
	Year 3	$115,000
	Year 4	$115,000
	Total	$460,000
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 (°D) 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 °D-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.

Additionally, °D-values 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.

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 ($P < 0.05$).

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

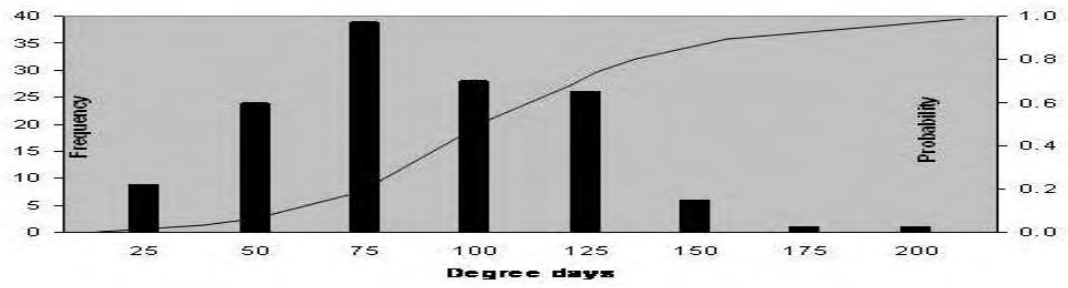

Figure 1. Spawning probabilities and spawning frequency at different degree-day values above the 21ºC threshold.

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. Brood fish 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 (2 mixed-sex and 1 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.

Brood fish 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.

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 supplemental 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 duet 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

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, *Ictalurus punctatus,* females fertilized with blue catfish, *I. furcatus,* 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.

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

ab Means followed by different letters are significantly different ($P < 0.05$) Duncan's multiple range test.

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 3 times per week with a liver supplement 2 times per week as well as fish offered the 42% protein diet fed six time per week. The treatment with the highest observed mean was 32% protein diet offered 6 times per week although there were no significant differences between fish offered the 32% protein diet 3 or 6 times per week or those offered the 42% protein diet 3 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 8 ponds, using two ponds per treatment. All females were 4-year-old Kansas strain. The fish 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 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 18:3n-3:18:2n-6 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, 3:2, respectively.

Dietary lipid treatments were evaluated using the following indicators: total number of eggs produced, fecundity (number of eggs per kilogram female), 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 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 18:3n-3:18:2n-6 (linseed oil, Diet 3) resulted in very poor fry production. The use of menhaden fish oil with DHA and ArA supplements and a ratio of 3:2 for n-3:n-6 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 (primarily18:3n-3 and 18:2n-6) producing n-3:n-6 ratio's 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.

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	% Fry survival
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

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

Auburn University. 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 4 consecutive years (Table 4). 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 three year period (Tables 5 and 6). 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. Additionally, strain of

male blue catfish affected hatching rate of hybrid embryos and sperm production. 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.

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

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

Table 5. 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 6. 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

Two strains of channel catfish were selected for increased body weight for six generations. The resulting females of the two select lines were compared to their corresponding randomly bred controls for hybrid fry production when they were 3 years old. Random control 1 produced more hybrid fry than random control 2. In both cases, select lines had reduced hybrid fry production compared to controls (Table 7). 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. 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.

Table 7. Fry/kg for two lines of channel catfish females selected for increased body weight for six generations and their randomly bred controls when injected with LHRHa and hybridized with blue catfish males when 3 years old.

Line	Fry/kg
Select 1	2,391
Random 1	3,105
Select 2	832
Random 2	1,265

The same two select lines were compared to one of their crossbreeds, select 2 female × select 1 male. The crossbreeding resulted in no heterosis, and the fry output of the crossbred females was the same as the maternal strain females (Table 8). This would be indicative of dominance for hybrid fry/kg or a genetic maternal effect. This can only be completely evaluated by examining the performance of both parental types and both reciprocal crossbreeds. The results also suggest that the lower fry output by the select lines compared to their random controls is not due to inbreeding unless there is a very strong maternal effect on reproduction as if inbreeding depression was the major explanation for the decreased fry output, the mean of the crossbreed should be at least higher than the lower performing parent.

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.

Table 8. Fry/kg for 2 lines of channel catfish females selected for increased body weight for 6 generations and their crossbreed (female×male) when injected with LHRHa and hybridized with blue catfish males when 3 years old. Means followed by different letters are significantly different (*P*=0.05).

Genotype	Fry/kg female body weight
Select 1 (S 1)	2,391a
Select 2 (S 2)	832b
S 2 × S 1	835b

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 brood fish 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. Brooders 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

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.*

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.

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 >70% success rate. To obtain the best induced spawning success brood fish

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.

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 > 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 9). 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 LHRH flowed much easier and more completely, but it seemed their time-frame for ovulation was wider. The LHRH 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 LHRH

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 microgram 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 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 10). 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 11). 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 11).

During the peak spawning season, the ovulation rates decreased (Tables 11 and 12). 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. During this peak period of the spawning season, little difference existed between fry output of individually bagged fish and those spawned in group tanks. Results were similar for the early and peak spawning (Tables 11, 12, and 13).

During the late spawning period, the injection regime with the highest ovulation rate was 10/50 at 100% using high line females (Table 14). The implant with the highest ovulation rate was 75 µg at 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 15-19).

Table 9. 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	6,482	55.5	1,348	1,788	239,100
Catfish PE	51	2.8	68	6,767	64.1	1,128	1,600	190,100
LHRHa	58	3	65	6,482	66.3	1,527	1,999	254,100
Standard Error		0.2	8.4	720	8.6	344	350	

Table 10. Ovulation percentage and hybrid fry/kg female body weight for channel catfish, *Ictalurus punctatus*, females fertilized with blue catfish, *I. furcatus*, 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	-
10/50	24	75	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	955

Table 11. Early season ovulation percentage and hybrid fry/kg female body weight for channel catfish, *Ictalurus punctatus*, females fertilized with blue catfish, *I. furcatus*, 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 12. Peak season ovulation percentage and hybrid fry/kg female body weight for channel catfish, *Ictalurus punctatus*, females fertilized with blue catfish, *I. furcatus*, 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 13. Comparison of early and peak season hybrid fry/kg female body weight for channel catfish, *Ictalurus punctatus*, females fertilized with blue catfish, *I. furcatus*, 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.

	Fry/kg	
LHRH Dose	Early	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 14. Late season ovulation percentage for channel catfish, *Ictalurus punctatus*, females fertilized with blue catfish, *I. furcatus*, 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 15. Egg quality and hybrid fry/kg female body weight during the early season for channel catfish, *Ictalurus punctatus*, females fertilized with blue catfish, *I. furcatus*, 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.

LHRH 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 16. Egg quality and hybrid fry/kg female body weight during the peak season for channel catfish, *Ictalurus punctatus*, females fertilized with blue catfish, *I. furcatus*, 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 17. Egg quality during the late season for channel catfish, *Ictalurus punctatus*, females fertilized with blue catfish, *I. furcatus*, 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 18. Percentage of eggs good or bloody during the early (E), peak (P) and late (L) season for channel catfish, *Ictalurus punctatus*, females fertilized with blue catfish, *I. furcatus*, 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 19. Percentage of eggs either "good" or "bloody" during the early (E), peak (P) and late (L) season for channel catfish, *Ictalurus punctatus*, females fertilized with blue catfish, *I. furcatus*, sperm. GMP grade LHRHa injections (priming dose/resolving dose, µg/kg) or research grade LHRHa implants (µg/kg) were used.

		% White			% Clumps		
Dose	Line	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	50
100 implant	high	-	-	0	-	-	25
125 implant	low	0	-	-	35	-	-

A second experiment was conducted during the late spawning period comparing 75-µg implants with10/50 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 20). In a third run, 28 out of 32 individual fish (87.5%) implanted with 75-µg ovulated, with 75.6% hatch.

Table 20. 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

Egg quality data was measured subjectively on a 5-point scale. 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 periods than implants. As the season progressed and the water warmed, these differences diminished and the overall latency period shortened (Table 21). By the late season, virtually no difference in latency existed among treatments, including no differences between injections and implants.

University of Memphis. Channel catfish ovarian follicles were treated in vitro with 17α, 20β-dihydroxyprogesterone 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 broodstock.

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 (Table 22, 23, and 24). 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 23 and 24).

Table 21. Mean latency period (hours ± standard deviation, SD) for female body weight for channel catfish, *Ictalurus punctatus*, females fertilized with blue catfish, *I. furcatus*, 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

Table 22. Mean eggs/kg female body weight (BW), hatching percentage, fry/kg female body weight and egg quality of channel catfish females (*Ictalurus punctatus*) exposed or not exposed to channel catfish male after injection with luteinizing hormone releasing hormone, LHRHa, when hybridized with blue catfish (*Ictalurus furcatus*) male (mean ± SD) in 2001.

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

Means followed by the same letter are not different (P>0.05) within each column.

Table 23. Mean spawning percentage, egg/kg female body weight (BW), hatching percentage, fry/kg female body weight and latency time at 29°C for channel catfish (*Ictalurus punctatus*) females injected with luteinizing hormone releasing hormone, LHRHa, with different exposures to channel catfish males (mean ± SD) in 2002.

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

Means followed by the same letter are not different (P>0.05) within each column.

Table 24. Ovulation % and fry/kg female body weight for channel catfish receiving 100-µg 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

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 (1g/20mL) to release sperm.

Sperm concentrations and motility estimates relative to gonad composition are summarized in Table 25. Sperm concentrations varied in relation to gonad composition.

Table 25. Summary of sperm concentrations and motility from whole testis and posterior and anterior sections.

	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

Means in a column with different letters were significantly different ($P<0.05$, n=21)

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 (i.e., 6 to 7 days versus 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, brood fish 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 2 and 3). However, upon closer examination, develop-ment 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 treat-ment 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 10mL/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 4). 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 5). GV identity was verified by presence of numerous nucleoli upon microscopic examination (Figure 6).

Additional images of catfish oocytes and embryos were made by automated transparency scanners. Daily use of 20-µL 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, MS, facility. The CAS system worked quite well in spite of the prolonged development time (i.e., 6-7 days).

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 antibodies (e.g., anti-cyclin B1) that may prove useful in our studies of oocyte maturation in catfish.

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., 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.

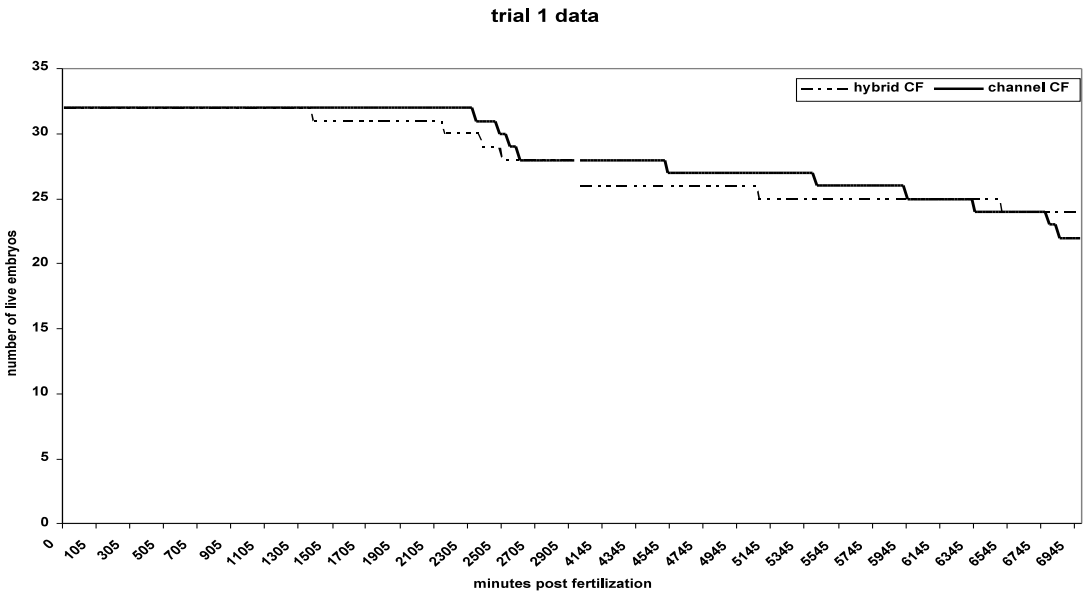

Figure 2. Viability of hybrid catfish and channel catfish embryos (trial 1) using CAS.

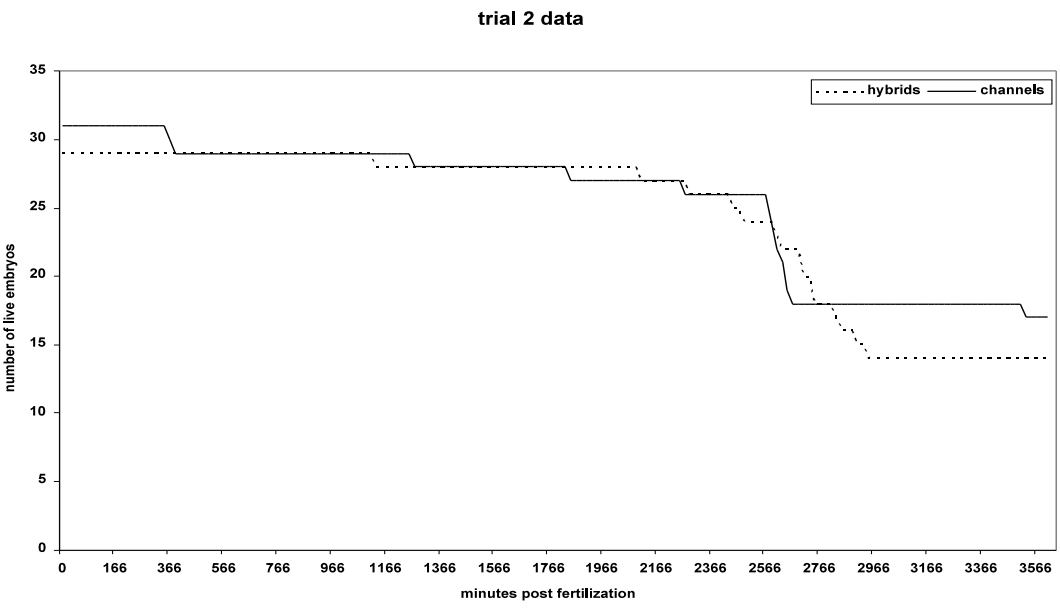

Figure 3. Viability of hybrid catfish and channel catfish embryos (trial 2) using CAS.

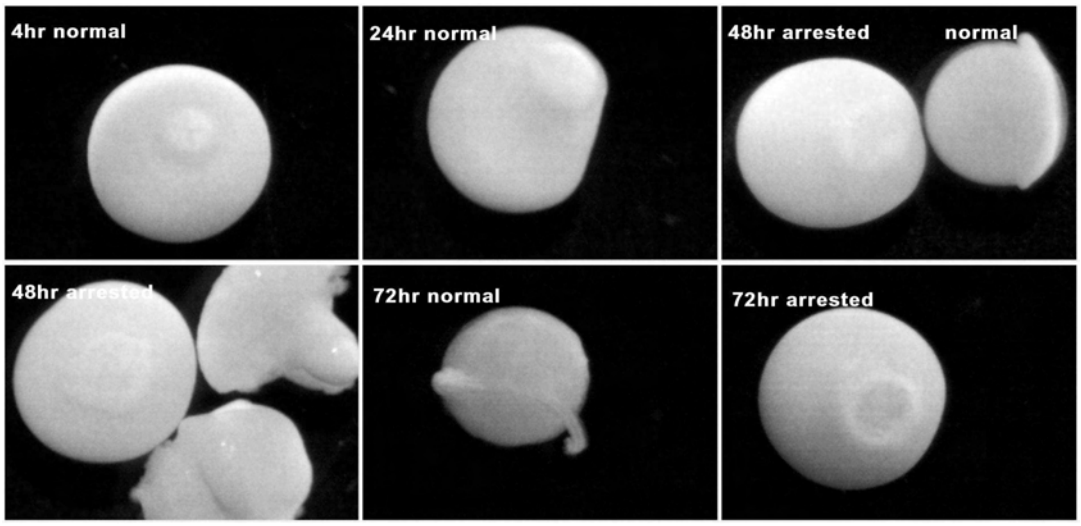

Figure 4. Digital photomicrographs of dechorionated, formalin-fixed hybrid catfish embryos. Embryos may arrest development in the cleavage stage but not immediately cytolyze.

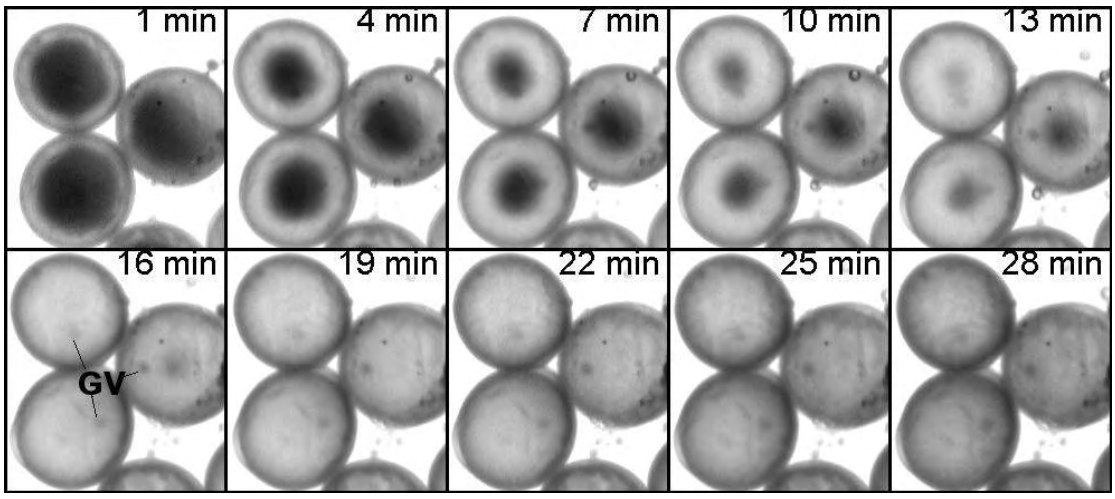

Figure 5. Channel catfish oocytes imaged by CAMMA and treated with 5% acetic acid. This treatment clears the yolky ooplasm and reveals the presence and location of the oocyte nucleus or germinal vesicle (GV).

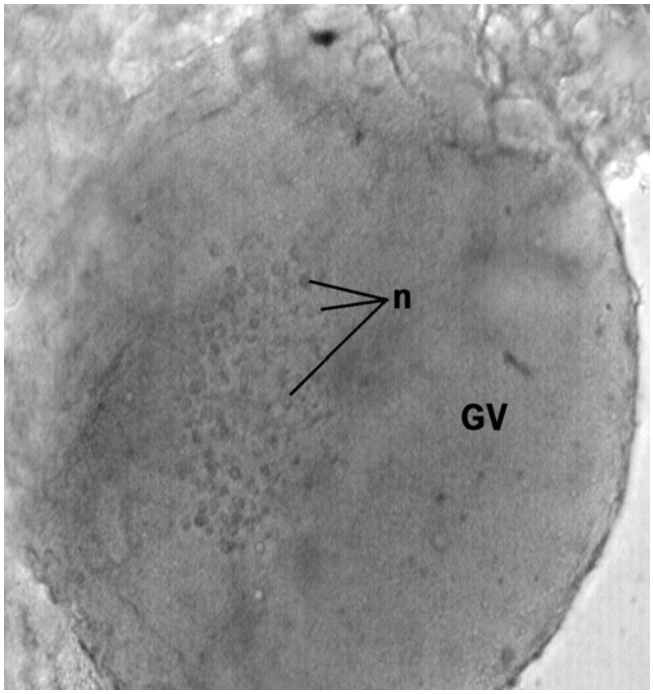

Figure 6. Isolated channel catfish oocyte germinal vesicle (GV) after 5% acetic acid fixation. Note the large number of characteristic nucleoli (n), a definitive set of landmarks for the GV.

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 artificiial 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 26). 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.

Table 26. 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

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 2-8 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 27). 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.

Table 27. Ultrasound and physical diameter measurements (mean ± SE) for channel catfish females (N=15). Means sharing a letter within each variable were not significantly different ($P \leq 0.05$).

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

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.

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 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.

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 twelve 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.

RESULTS AT A GLANCE...

★ *The effects and interactions of plasma steriod concentrations (estradiol and testosterone), egg size, protein content of eggs and protein degradation by cathepsins D, L and B on in vivo egg maturation channel catfish was determined for the first time.*

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 LHRH 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 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. 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.

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 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 (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 deionized 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 3 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 ($P<0.05$) 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) Hanks' 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 (3.6×10^8 ± 4.6×10^7 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 luteinizing hormone-releasing hormone (Peninsula Laboratories Inc., San Carlos) 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 28). Given that current hatchery practice is to use sperm

RESULTS AT A GLANCE...

★ *Sperm concentrations can be reduced in currently used fertilization protocols by 100-fold with little reduction in subsequent hatch rate.*

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 28. 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, Duncan's Multiple Range Test ($P \geq 0.05$).

Sperm/ 400 eggs	Mean ± SD	
	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 7).

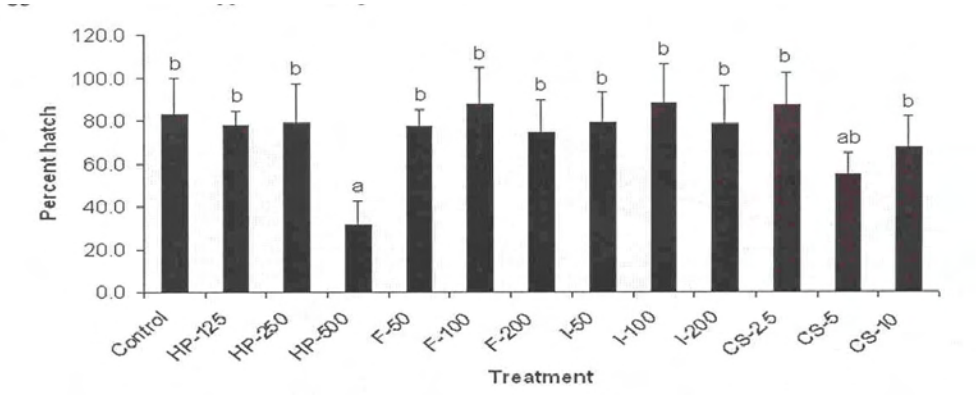

Figure 7. Mean hatching success (%) of hybrid catfish eggs treated daily with increasing doses of hydrogen peroxide (HP), formalin (F), povidone iodine (I), or copper sulfate (CS). Numerals refer to dosage rate (ppm). Bars with common letters are not statistically different.

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 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

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.*

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 29).

Table 29. 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).

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 developmental 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 30).

Table 30. 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 28°C.

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

IMPACTS

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. Feeding standard 32% protein floating catfish feed 6 times per week for 2 months prior to spawning gives equal or better fry production compared to high protein diets. Supplementation of brood fish diets with menhaden fish oil, DHA and ARA two months prior to spawning can double hybrid fry output. Utilization of the appropriate genetic line of channel catfish female can double and triple hybrid fry output. Injections of 30ug/kg female body weight of LHRHa followed by 150 µg or the utilization of 100 µg/kg implants generates the greatest number of fry/kg during the early and peak spawning periods. Hatch rate of hybrid embryos is improved if channel catfish females are stripped within 2 hours of first observation of egg release. 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 this tool should result in more efficient use of sperm, and more consistent fertilization rates. Sperm concentrations can be reduced 100-fold, from 2×10^8 to 2×10^6 sperm/800 eggs, and still obtain good hatch rates. The cost for the sperm savings is a reduction of 16% for relative hatch rate. The frequency of formalin treatments should be three times per day to maximize hatch rate of hybrid embryos and 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 to maximize hatch rate.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

Publications

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. In press.

Doctoral Dissertations

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.

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

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INNOVATIVE TECHNOLOGIES AND METHODOLOGIES FOR COMMERCIAL-SCALE POND AQUACULTURE

Reporting Period
August 1, 2004 - August 31, 2005

Funding Level

Year 1	$314,695
Year 2	$287,451
Year 3	$213,168
Year 4	$170,096
Total	$985,410

Participants

Auburn University (Lead Institution	Claude Boyd
Clemson University	David Brune, T. E. Schwedler
Louisiana State University	John Hargreaves
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 but farm-gate prices remain 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. *Continous 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, 6 feet × 10 feet × 4 feet deep, located within the 2-acre 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, 4) growth response

under raceway culture conditions as opposed to an "accelerated" fingerling culture pond.

Six, 1,800-gallon 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 (2.5 feet × 1.5 feet × 1 foot deep) with 1/16-inch mesh screens for one week and then transferred to bins with 1/8-inch mesh screens for an additional week. After having reached an average size of 1.2 to 1.4 g, fingerlings were released into the 1/4-inch mesh net-pens held within the 6 feet × 10 feet PAS cells. Each cell was supplied with water delivered by a 0.75-hp submerged aerator providing between 75 to 190 gpm 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).

Figure 1. Overview of 2-acre Clemson PAS unit with Fingerling Production Cells.

Figure 2. Six 60 ft^2 Fingerling Production Cells Containing Net-Pens and Aerator-Driven Water Flow

Figure 3. Individual Fingerling Production Bins Contained Within PAS Cells with Aerator-Driven Water Flow

Figure 4. Automatic Feeders Used to Feed Fingerlings During Initials Stages of Culture

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 0.33-acre PAS units (30 feet × 7 feet × 4 feet 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 (Figure 5).

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 prior to stocking and grow-out in conventional fingerlings ponds. A conventional, 0.5-acre fingerling culture pond as 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, 0.3-acre fingerling culture pond. To date, the fingerlings in both ponds are of similar size. This suggests the importance of converting fry or finger-lings 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. In 2006, fingerlings will be moved to floating feed on a faster schedule before pond stocking.

Mississippi State University. Work in Year 1 was devoted to retrofitting four, 1-acre ponds as modified PAS ponds. The four ponds have been surveyed and construction initiated. Preliminary excavation has been completed and gravel has been spread for the foundation. The next phases consist of pouring concrete foundations, laying concrete block walls, building and installing paddlewheels, completing backfill and finishing dirt work, and installing the hydraulic systems and electrical hook-ups. The remaining construction should be completed by spring of 2006.

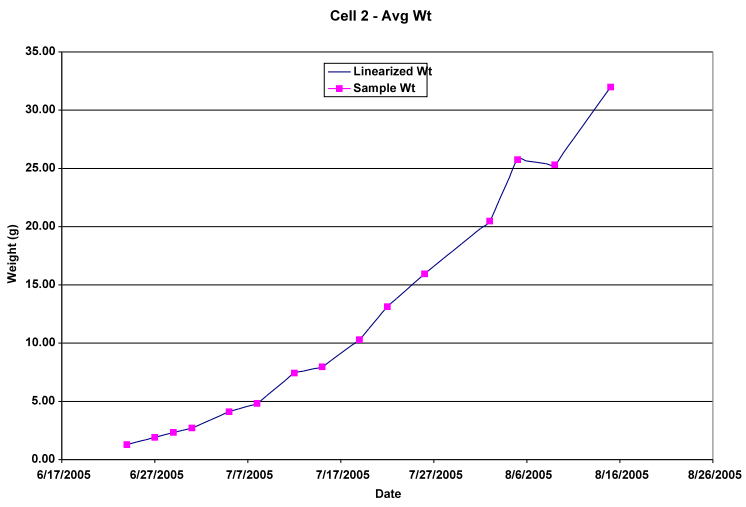

Figure 5. Growth of Catfish Fingerlings in PAS Cell 2 for First Eight Weeks of 2005 Season

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

University of Arkansas at Pine Bluff. 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 will result in improved yield, survival, feed conversion ratio (FCR) and growth compared to conventional multiple-batch pond culture. This will be accomplished by installing a

wire mesh barrier in existing earthen ponds. This study consists of ten, 0.25-acre ponds; five of these are control ponds and do not have barriers. The five treatment ponds have a 0.5 × 1.0-inch, PVC coated, wire mesh barrier that partitions off one-third of the pond. In the treatment ponds, fingerling catfish are being raised in 1/3 of the pond and larger carryover fish were stocked in the remaining 2/3 of the pond. The fish in the control ponds are allowed to co-mingle as in traditional multiple-batch culture. The ponds will be seined every 2 months during the growing season and average weights will be calculated. After harvest, survival and FCR will be calculated. Yield, survival, FCR, and growth will be compared among treatments and controls with t-tests. If warranted a partial budget analysis will be conducted. Preliminary results from the first sampling date are presented in the Table 1. Based on the results obtained at the completion of the current trials, a commercial-scale confinement system will be installed and evaluated on a commercial farm over the next year.

Table 1. Mean weights (g) of fingerlings 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	Confinement
Fingerlings		
Stocking (5/1/05)	25 ± 0a	25 ± 0a
Sampling (6/27/05)	53 ± 4.1a	56 ± 11.4a
Carryover		
Stocking (5/1/05)	408 ± 0a	408 ± 0a
Sampling (6/27/05)	696 ± 9.9a	708 ± 27.9a

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 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. This study should be conducted on a commercial farm, and a farm had been selected for the effort. Unfortunately, the producer was no longer able to continue in catfish farming. The LSU principal investigator currently is seeking the cooperation of other producers to conduct the research.

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

United States Department of Agriculture-Pine Bluff. Funding for work at this location does not begin until Year 2 of the main project (2005-2006). However, work was initiated in April 2005, despite the funding year not being synchronized with the catfish-growing season. Hybrid (channel × blue) catfish were stocked into nine, 38-m^3 raceways on 13 April 2005. Fish were stocked at 25, 50, 75, 100, 125, 150, 175, 200, or 225 fish/raceway. Mean individual fish weight was 0.085 kg at stocking. Salt was added to each raceway to ensure chloride concentration exceeded 100 mg/L. Fish were fed daily to apparent satiation with a 32% protein, floating feed. Dissolved oxygen, temperature and pH are measured daily, and total ammonia nitrogen, nitrite, nitrate, soluble reactive phosphorus, total nitrogen, total phosphorus, and chlorophyll *a* concentrations are measured weekly. Harvest is planned for October-November 2005.

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 1-acre pond at the National Warmwater Aquaculture Center, Stoneville, MS. The U-tube was fabricated from a 36-inch-diameter corrugated galvanized culvert (Figure 6) and buried to a depth of 20 feet below the bottom of a 1-acre pond. The unit was powered by a 240-volt, 3-phase, 5-hp, helical-geared Flender motor. The motor was vertically mounted on a 36-inch-diameter culvert elbow that was attached to the tube with a 12-inch band clamp. The motor turned a three-vane impeller attached to a 24-inch long × 2-inch diameter unsupported, steel shaft (Figure 7). 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 5.36 hp. The pump had an output of 8,300 gpm (Table 2).

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 5-hp, 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 5 feet below the water surface.

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

and configurations. 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 1.66 pounds O_2/hp-hr. These results were encouraging but less than desired for commercial application.

Two problems were noted during testing of the initial prototype. 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 supports near the end. This resulted in the impeller being located slightly above the bottom of the horizontal (discharge) end of the elbow (Figure 7). As the air-to-water ratio increased, back-flow from the pond was observed. This decreased water flow through the tube, and at higher ratios, flow through the tube ceased. Second, using this design, the water level is critical. If the water level dropped below the top of the discharge elbow, flow rate was affected (Figure 8). If the water level rose above the top of the elbow, the motor could be damaged. For commercial application, the unit should have at least a 2-foot "freeboard" to allow for normal variations in pond water level.

Figure 6. U-tube prior to installation. The tube was 35" I.D. x 20' tall and was made with 14 gauge galvanized steel.

Figure 7. View into the discharge end of the tube, showing the impeller, shaft, and motor (mounted on top).

Figure 8. As the water level dropped below the top of the culvert (as shown in this photograph) flow decreased; if water level rose above the top of the culvert the motor could be damaged.

Table 2. Performance of pump in prototype U-tube.

Impeller rpm	Motor amperage draw	Volts	kW	hp	Water Velocity (ft/sec)	Water output (gpm)	Pump efficiency (g/hp-hr)
150	13	230	4	5.36	2.56	8,109	90,772
125	11	205	2.55	3.41	1.98	6,278	110,460
100	8.1	148	1.43	1.92	1.5	4,770	149,040

In the second year of testing, the design will be modified in the following way: 1) The diameter of the "down" tube would be reduced to 24 inches. This would produce a faster "down" water velocity allowing for the entrainment of a greater air volume while retaining a relatively slow "up" velocity to maximize air-water contact time to enhance oxygen transfer; 2) A submersible motor would be used and it would be placed in the mouth of the "down" tube allowing larger water level fluctuations; 3) A venturi will be examined as a means of introducing air (or oxygen) 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 aerator failure.

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

Louisiana State University. The design of the low-speed paddlewheel mixer for crawfish pond has been completed. The device will be constructed and placed in crawfish ponds at the beginning of the crawfish production season in October.

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 3 × 5 inch 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. Issues to be addressed during the next year include: increasing the power output of the system, increasing the effective field strength of the system, further reducing the weight and size of the electrical components, and determining the most effective design for the electrodes.

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. Determination of energy use at the farm level has been initiated on four, channel catfish farms in west-central Alabama. Emphasis is being given to collection of data on electricity use for powering mechanical aerators, operating pumps, and feeding fish. The data collection will continue for two growing seasons.

Investigations of water use will 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 recharged 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 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. Rough drafts of cash flow budgets have been developed for five farm sizes. A survey instrument has been designed to gather data from lenders with portfolios in catfish, row crop, and livestock loans. Data to be 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 cash flow budgets to assess effects on cash flow and repayment capacity.

A basic mathematical programming economics model has been developed that incorporates grow-out and fingerling production activities. The model maximizes 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. To date, the model has been used to identify optimal fingerling size for under-stocking in grow-out production, with farm size treated both as an endogenous and exogenous variable. Sensitivity analyses developed include testing the relevant range of values for the following parameters: interest rates, feed conversion ratios, market prices of catfish, harvesting costs, levels of operating capital available, fingerling prices, fingerling production costs, survival rates, and feed prices.

IMPACTS

Work on most objectives has just started and it is too early to report impacts.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

Publications in print

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.

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

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

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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.

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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 Computer Software	1	50,000	43,950	-0-	-0-	-0-	43,950	93,950
	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
Total		**685,263**	**418,372**	**-0-**	**1,000**	**-0-**	**419,372**	**1,104,638**
Identification, Characterization, and Evaluation of Mechanisms of Control of *Bolbophorus*-like Trematodes and *Flavobacterium columnaris*-like Bacteria Causing Disease in Warm Water Fish	1	224,800	277,901	-0-	-0-	-0-	277,901	502,701
	2	227,377	285,420	-0-	-0-	-0-	285,420	512,797
	3	146,770	281,926	-0-	-0-	-0-	281,926	428,696
Total		**598,947**	**845,247**	**-0-**	**-0-**	**-0-**	**845,247**	**1,444,194**
Improving Reproductive Efficiency to Produce Channel × Blue Hybrid Catfish Fry	1	118,390	86,891	-0-	-0-	-0-	86,891	205,281
	2	111,610	81,845	-0-	-0-	-0-	81,845	193,455
	3	115,000	70,297	-0-	-0-	-0-	70,297	193,297
	4	115,000	72,121	-0-	-0-	-0-	72,121	195,121
Total		**460,000**	**311,154**	**-0-**	**-0-**	**-0-**	**311,154**	**787,154**
Innovative Technologies and Methodologies for Commercial-Scale Pond Aquaculture	1	314,695	193,931	-0-	-0-	-0-	193,931	508,626
	2	287,451	217,676	-0-	-0-	-0-	217,676	505,127
	3	213,168	163,173	-0-	-0-	-0-	163,173	376,341
	4	170,096	106,405	-0-	-0-	-0-	106,405	276,501
Total		**985,410**	**681,185**	**-0-**	**-0-**	**-0-**	**681,185**	**1,666,595**

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. 1-05/01/89-04/30/90 Yr. 2-05/01/90-04/30/91 Yr. 3-05/01/91-04/30/92 **Project Total**	$274,651 274,720 273,472 **$822,843**	88-38500-4028 89-38500-4516 90-38500-5099
*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. 3-05/01/91-12/31/92 **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.
*Preparation of Extension Publications on Avian Predator Control in Aqua-culture 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. 3-06/01/94-05/31/05 **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
*Aquaculture Food Safety: Residues. Dr. George Lewis, University of Georgia, Principal Investigator	Yr. 1-09/11/92-09/30/93 Yr. 2-10/01/93-09/30/94 Total Yr. 2 Yr. 3-10/01/94-09/30/95 Yr. 4-10/01/95-09/30/96 **Project Total**	$99,393 $44,631 107,050 $151,681 $89,463 $11,392 **$351,929**	91-38500-5909 90-38500-5099 91-38500-5909 93-38500-8393 93-38500-8393
*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

*Project Completed

Project	Duration	Funding	Grant No.
*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**	
*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**	

*Project Completed

Project	Duration	Funding	Grant No.
*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**	
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	2000-38500-8992
	Yr.7-07/01/01-06/30/02	$82,850	2001-38500-10307
	Yr.8-01/01/03-12/31/03	$77,507	2002-38500-11805
	Yr.9-04/01/04-03/31/05	$84,500	2003-38500-12997
	Yr.10-03/01/05-02/28/06	$78,700	2004-38500-14387
	Project Total	**$606,564**	
*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	
*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	2000-38500-8992
	Total Yr. 3	$246,215	
	Project Total	**$829,759**	
*Project Completed			

Project	Duration	Funding	Grant No.
*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	2000-38500-8992
	Yr. 2-01/01/02-12/31/02	$14,259	98-38500-5865
		39,720	99-38500-5865
		14,757	2000-38500-8992
		203,655	2001-38500-10307
	Total Yr. 2	$272,391	
	Yr. 3-01/01/03-12/31/03	$15,000	2000-38500-8992
		175,556	2001-38500-10307
	Total Yr. 3	$190,556	
	Project Total	**$750,000**	
*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**	
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	$29,931	2000-38500-8992
		126,836	2001-38500-10307
		68,033	2002-38500-11307
	Total Yr. 1	$224,800	
	Yr. 2-03/01/04-02/28/05	$58,160	2000-38500-8992
		9,378	2001-38500-10307
		159,839	2002-38500-11805
	Total Yr. 2	$227,377	
	Yr. 3-03/01/05-01/28/06	$24,074	
		15,417	2001-38500-10307
		107,279	2002-38500-11805
	Total Yr. 3	$146,770	2003-38500-12997
	Project Total	**$598,947**	
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
		146,209	2003-38500-12997
	Total Yr. 1	$314,695	
	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-Projected	$123,168	
	Yr.4-Projected	$170,096	
	Project Total	**$895,410**	

*Project Completed

Project	Duration	Funding	Grant No.
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
		117,390	2002-38500-11805
	Total Yr. 1	$118,390	
	Yr.2 -03/01/05-02/28/06	$111,610	2003-38500-12997
	Yr. 3-Projected	$115,000	
	Yr. 4-Projected	$115,000	
	Project Total	**$460,000**	