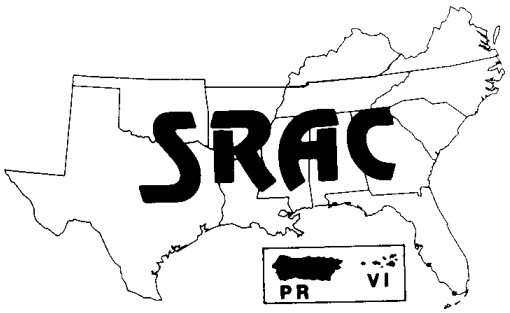
SOUTHERN REGIONAL AQUACULTURE CENTER



SEVENTEENTH ANNUAL PROGRESS REPORT

For the Period Through August 31, 2004

December, 2004

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

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SEVENTEENTH ANNUAL PROGRESS REPORT

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

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

INTRODUCTION

The farm-gate value of United States aquaculture exceeded $1 billion dollars in 2003, and nearly 70% of the crop was produced in the southeastern states. Aquaculture has become one of the cornerstones of southeastern agriculture and its importance to the region reaches far beyond the farm gate. Many 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. Further, if the overall economic value of aquaculture is viewed against a generally depressed agricultural economy, it is clear that aquaculture is a critical factor in the economy of the southeastern United States.

The success of southeastern aquaculture has come with relatively little private sector support for research and development. The larger, more developed agricultural sectors—such as poultry, cotton and soybeans—are supported by a vast infrastructure of agribusinesses that conducts most of the research needed to sustain commodity growth. Aquaculture, on the other hand, receives little private-sector R&D support, relying instead almost entirely on public-sector funds for technology development.

Although government agencies, particularly the United States Department of Agriculture, have provided significant support for aquaculture research and development, much of that funding is earmarked for specific use by specific institutions. The USDA-CSREES Regional Aquaculture Center program is the only funding 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 17 years of operation, the Center has disbursed $10 million to fund 27 multi-state research and extension projects. More than 175 scientists from 29 institutions in the southeast have participated in Center projects.

In the past year, four research projects funded at $1.9 million were in progress. Work on those projects has been reported in 20 publications and 19 papers presented at meetings. The Center's "Publications" project is in its ninth 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 eleven more were in various stages of production. To date, the "Publications" project has generated more than 170 fact sheets with contributions from 136 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, although the project is in only the second full year of funding, a discovery in the new "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.

Two results of the "Harvesting" project have already been adopted by the industry. A new seine developed at Mississippi State University allows catfish ponds to be harvested faster and with greater capture efficiency than

traditional seine designs. Seines based on the new design are already available from commercial netmakers. The second success is the floating platform grader developed at the University of Arkansas at Pine Bluff. The mechanical grader is so superior to conventional technologies at grading fish from mixed-sized populations that it may revolutionize catfish harvest technology, particularly for fingerling producers.

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 Seventeenth 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, 2004.

ORGANIZATIONAL STRUCTURE

The Agriculture Acts of 1980 and 1985 authorized the 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 southeastern region are identified each year by the Industry Advisory Council, which consists of fish farmers and allied industry representatives from across the southern region. The Technical Committee, consisting of research and extension scientists from all states within the region, works with the Industry Advisory Council to prioritize the problem areas. The two groups then work together to develop "Problem Statements" describing objectives of work to solve the problems with 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 of Directors. Regional aquaculture funds are allocated to participants in the SRAC projects approved by the Board of Directors and CSREES. Reviews of project proposals, progress reports, and recommendations for continuation, revision, or termination of projects are made jointly by the SRAC Technical Committee and Industry Advisory Council, and approved by the Board of Directors.

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 of Directors, Industry Advisory Council, Technical Committee, Steering Committees and project Work Groups are provided by the Administrative Center. This includes monitoring the 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 Office personnel as needed.

Operation and funding of the Center are approved by the Board of Directors for inclusion in the Grant Application submitted annually by the Administrative Center to USDA/CSREES. The Center staff also prepares and submits to USDA/CSREES for approval an Annual Plan of Work covering Center activities and projects to be funded. Following final approval, Letters of Agreement are prepared and executed by the Center 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 of Directors is the policy-making body for SRAC. Membership of the Board 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:

Harold R. Benson, Kentucky State University
W. S. Clarke, Virginia State University
Paul Coreil, Louisiana State University
David Morrison, Louisiana State University
Joe McGilberry, Mississippi State University Extension Service
Gaines Smith, Alabama Cooperative Extension System
Vance Watson, Mississippi State University, Chairman
Greg Weidemann, University of Arkansas

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 Technical Committee and Industry Advisory Council 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:

Steve Abernathy, LA
Neal Anderson, AR
Richard Eager, SC
Jack Finch, TN
J. B. Hanks, LA
R. C. Hunt, NC
Theop Inslee, OK
Austin Jones, MS
Shorty Jones, MS
Joey Lowery, AR
Michael Peirson, VA
Steve Price, KY
Brent Rowley, TX
Robert Schmid, TX
Dan Solano, FL
Brian Simmons, GA
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 Technical Committee to prioritize research and extension needs; (3) works with the Technical Committee 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 Technical Committee, 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
Gary Burtle, GA
Frank Chapman, FL
Harry Daniels, NC
Carole Engle, AR
Allen Davis, AL
Lou D'Abramo, MS
Delbert Gatlin, TX
Conrad Kleinholz, OK
Ray McClain, LA
Steve Mims, KY
Eric Thoman, VI
J. L. Wilson, TN

Members of the TC for Extension are:

Jimmy Avery, MS
Jesse Chappell, AL
Dennis DeLong, NC
Patricia Duncan, GA
David Heikes, AR
Jeff Hinshaw, NC
George Luker, OK
Michael Masser, TX
Mike Schwartz, VA
Mark Shirley, LA
Saul Wiscovich Terruel, PR
Eric Thoman, VI
Craig Watson, FL
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 of Directors. 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 of Directors 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 the Grant Application entering into funding agreement with USDA/CSREES for each fiscal year and Annual Plans of Work and Amendments to USDA/CSREES.
- 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 extension fact sheets, research publications and videos to research and extension contacts throughout the Southern Region, other RACs, USDA personnel, and the Aquaculture Information Center.
- Produce and distribute the "SRAC Annual Progress Report," which includes editing and proofreading the project reports, designing and, using desktop publishing, 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 Work Group announcements and Calls for Statements of Interest to research and extension directors and other interested parties throughout the Southern Region.
- Respond to numerous 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, 2003

Funding Level

Year 1	$50,000
Year 2	60,948
Year 3	45,900
Year 4	60,500
Year 5	67,000
Year 6	77,358
Year 7	82,850
Year 8	77,507
Year 9	84,500
Total	$606,563

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.

Results at a glance...

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

Extension Specialists. When this project was initiated, fewer than half the states had educational materials covering the major aquacultural species in their state. Now, educational materials are readily available to scientists, educators, producers, and the general public in all states. The benefit of using the SRAC program to produce timely, high-quality educational materials stems from the availability of a region-wide pool of scientists and specialists from which to select the most qualified authors. Reviewing, editing, and production are then centralized to develop uniformly high-quality materials for distribution through the nationwide network of Extension Specialists and County Agents. This process makes efficient use of personnel and resources at the State level while producing educational materials of the highest possible quality.

Educators. Several 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 is particularly helpful to people making decisions about entering the aquaculture business. Economic information is used by lending agencies and potential investors, as well as established producers who use the information to help make day-to-day decisions on farm management.

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

Results at a glance...

Titles of some recent SRAC publications:

- *Partitioned Aquaculture Systems*
- *Channel Catfish Virus Disease*
- *Acclimating Pacific White Shrimp,* Litopenaeus vannamei, *to Inland, Low-Salinity Waters*
- *Pond Mixing*
- *Hybrid Striped Bass Fingerling Production*
- *The HACCP Seafood Program and Aquaculture*
- *Growing Bull Minnows for Bait*

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

During this current project year, eleven new fact sheets and one video were completed. 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. Six 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 fact sheets are available at <http://www.msstate.edu/dept/srac> on the Internet.

WORK PLANNED

During the next project year six new fact sheets/species profiles, a project summary, and a DVD on water quality will be produced. The new fact sheets will address (1) economics of freshwater prawn production, (2) best management practices for pond aquaculture, (3) building a classroom recirculating system, (4) disease management of catfish hatcheries, (5) a species profile on cobia, and (6) species profile on queen couch.

Two fact sheets will be revised on the topics of: (1) production of freshwater prawns in ponds #484, and (2) avian predators #401.

A project summary from the Yield Verification project will be developed.

A DVD on water quality and testing will be developed.

IMPACTS

This is a highly productive project with significant regional, national, and international impact. Fact sheets and videos are regularly requested and used by clientele in all 50 states. Within the Southern Region, more than 80 fact sheets and six videos are distributed on request daily. Fact sheets generated within the Southern Region are also widely distributed by RACs and extension personnel in other regions. An average of 5 to 20 SRAC fact sheets and 3 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. Since the fact sheets are also accessible through numerous other university research and extension web sites, the total usage and

Results at a glance...

- ☆ *Eleven fact sheets and a video were completed this year with 11 fact sheets in progress.*
- ☆ *Twenty scientists from across the Southern Region contributed to publications completed by SRAC this year.*
- ☆ *SRAC has now published 171 fact sheets, 17 research publications, and 20 videos.*
- ☆ *Educators in schools and colleges use SRAC publications in classrooms throughout the U.S. and the world.*

impact is undoubtedly several times greater.

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

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>.*

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 9/1/2003-8/31/2004

Brune, David E., G. Schwartz, A. G. Eversole, J. A. Collier and T. E. Schwedler. Partitioned aquaculture systems. SRAC Fact Sheet 4500.

Camus, Alvin C. Channel catfish virus disease. SRAC Fact Sheet 4702.

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.

Davis, D. Allen. Acclimating Pacific white shrimp, *Litopenaeus vannamei,* to inland, low-salinity waters. SRAC Fact Sheet 2601.

Durborow, Robert. Protozoan parasites. SRAC Fact Sheet 4701.

Hargreaves, John. Pond mixing. SRAC Fact Sheet 4602.

Kelly, Anita M. Channel catfish broodfish management. SRAC Fact Sheet 1802.

Ludwig, Gerald. Hybrid striped bass fingerling production. SRAC Fact Sheet 302 (Revision).

Miget, Russell J. The HACCP seafood program and aquaculture. SRAC Fact Sheet 4900.

Romaire, Robert, W. Ray McClain and C. Greg Lutz. Crawfish aquaculture - harvesting. SRAC Fact Sheet 2400 (Revision).

Wallace, Rick. Growing bull minnows for bait. SRAC Fact Sheet 1200.

Fact Sheets in review

Steeby, Jim and Jimmy Avery. Channel catfish fingerling production.
Hargreaves, John A. and Craig S. Tucker. Management of ammonia in fish ponds.
Engle, Carole, Jimmy Avery, Harry Daniels, David Heikes, and Greg Lutz. Guidelines for the development of aquaculture research verification programs.
Watson, Craig A. Jeffrey E. Hill, and Deborah B. Pouder. Species profile: koi and goldfish.
Wurts, William and Michael Masser. Liming ponds for aquaculture.

Fact Sheets in preparation

Hargreaves, John and Craig Tucker. Copper use in aquaculture.
Hinshaw, Jeff and Anita Kelly. Species profile: yellow perch.
Rakocy, Jim, Michael Masser and John Hargreaves. Aquaponics.
Romaire, Robert and Ray McClain. Crawfish marketing.
Whetstone, Jack. Quahog clam.
Tucker, Craig S. Pond aeration (Revision).

Video completed

Durborow, Robert and Jim Tidwell. Culture of freshwater shrimp.

On-going project

Development of web site on aquatic weed management. Michael Masser.

DEVELOPMENT OF IMPROVED HARVESTING, GRADING AND TRANSPORT TECHNOLOGY FOR FINFISH AQUACULTURE

Reporting Period
January 1, 2001 - August 31, 2004

Funding Level

Year 1	$287,053
Year 2	$272,391
Year 3	$190,556
Total	$750,000

Participants

Mississippi State University (Lead Institution)	Edwin H. Robinson, Jason E. Yarbrough, Terry R. Hanson
University of Tennessee	Richard J. Strange
North Carolina State University	Harry V. Daniels, Thomas Losordo
University of Memphis	Bill A. Simco, Ken Davis (now at the Harry Dupree Stuttgart National Aquaculture Research Center)
University of Florida	Craig A. Watson, Roy P.E. Yanong
University of Arkansas at Pine Bluff	David Heikes, Carole R. Engle, Hugh W. Thomforde

Administrative Advisor

Dr. David Morrison
Assistant Director
Louisiana Agricultural Experiment Station
Baton Rouge, Louisiana

PROJECT OBJECTIVES

1. Develop and evaluate new gear and methods or modify existing methods to improve harvest (seining and trapping) efficiency and fish grading selectivity and reduce stress during these activities.

2. Evaluate methods relative to loading and transport of fish to reduce fish mortalities and the negative effects of stress on product quality.

3. Conduct comparative analyses of new technology and current technology for harvesting, grading, and loading fish.

ANTICIPATED BENEFITS

The primary benefit of this project will be to significantly improve profitability of the finfish aquaculture industry by improving harvesting efficiency, grading selectivity, and methods for loading and hauling fish, and by reducing the stress associated with these practices.

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PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Objective 1. *Develop and evaluate new gear and methods or modify existing methods to improve harvest (seining and trapping) efficiency and fish grading selectivity and reduce stress during these activities.*

Channel Catfish

Mississippi State University. Studies have shown that braided polyethylene (BPE) mesh is the choice for constructing seines and socks for harvest of catfish. Mesh sizes recommended for grading food sized catfish have been determined. Several prototype seines have been constructed and tested on commercial catfish farms. A prototype seine was used during the harvest of the USDA 103 strain of catfish at the National Warmwater Aquaculture Center (NWAC). Ten ponds (4 or 10 acre) were harvested with an average efficiency of catch of 93.5% in a single seine haul.

Full-sized commercial socks were built of BPE having a mesh size of 1-9/16 inch, 1-5/8 inch, and 1-11/16 inch. These socks were used to harvest commercial catfish ponds. The average population distribution for five size classes of fish is summarized in Table 1.

In addition to pond work, grading studies comparing mesh types and sizes were conducted in large tanks where the fish could be observed. The results are presented in Table 2. The knotted experimental, braided mesh hung in a square configuration, graded similarly to the next larger size of knotted conventional, twisted mesh hung in a diamond configuration.

A few catfish producers and commercial seining crews in Mississippi, Arkansas, and North Carolina are using the prototype seine and socks constructed using BPE. In some cases, the prototype seine has been modified by removing the mud rollers. Mud

Table 1. Average Percentage of Catfish Grading From Socks of Different Mesh Size After 6 Hours.

	Size Classes (pounds)				
Mesh size (inches)	<¾	¾-1	1-1¼	1¼ -1½	1½+
1-9/16	6	23	15	11	45
1-5/8	1	4	19	24	53
1-11/16	0	0	3	6	91

Table 2. Effect of mesh size and type on grading of catfish.

Mesh Type	Mesh size (inches or mm)	Size of fish held (pounds)
Conventional	1-5/8 inches	0.75 to 0.99
(knotted, twisted, diamond)	1-3/4 inches	1.00 to 1.24
	1- 7/8 inches	1.25 to 1.49
Experimental	1-9/16 inches	0.75 to 0.99
(knotted, braided, square)	1-5/8 inches	1.00 to 1.24
	1-11/16 inches	1.25 to 1.49
	1-3/4 inches	>1.50
Knotless	38.0 mm	0.75 to 0.99
(knotless, twisted, square)	39.5 mm	0.75 to 0.99

rollers work well in certain ponds but not in others. Removing the mud rollers reduces the cost of a typical seine by about $5,000. The relatively small amount of data that we have been able to collect on farms and anecdotal reports from farmers using the prototype seine confirm the data we have collected in controlled experiments.

To conclude our project, a "Cooperator Questionnaire" was sent to the three primary cooperators; two of the farmer cooperators responded. The questionnaire asked for an appraisal of the prototype seine and sock system as compared to conventional seining equipment. The responding cooperators typically used a conventional twisted-mesh seine, hung in a diamond configuration, mud lines of rolled rope or rolled mesh, and their sock attachment was a metal frame attachment. Only one of the cooperators had previously used a seine with mud rollers. Each of the responding cooperators evaluated various configurations of prototype seines.

When asked to compare characteristics of the prototype seines, one cooperator responded that the prototype seine without mud rollers, but with a closer float spacing, pulled similarly to a conventional seine. The other cooperator felt the prototype seine without mud rollers, but with a closer float spacing, pulled much better than a conventional seine. The primary difference being the amount of mud-dumping required with the push boat. This cooperator stated on many occasions he has had to use two push boats with a conventional seine and usually only had to push the prototype seine for approximately a quarter of the pond if at all. This response was typical of what we heard from most other farmers that used the seine.

Cooperators were asked to grade the various features of the prototype seine and sock system (Table 3). Both cooperators preferred the prototype seine with all the features except the mud rollers. They each felt that soil type/pond bottom firmness affected the efficiency of the seine with the large experimental mud rollers. One cooperator also stated fish were escaping between the mud roller and mud line attachment in areas of the aerator wash.

Cooperators were asked to tell what features they would request in a new seine. Each cooperator requested the braided polyethylene mesh hung on a square configuration, a large throat with the trap door-zippered sock attachment system, floats similar to those used on the experimental seine, closer

Table 3. Results of farmer-cooperator survey. A 1 to 5 grading scale was used, as follows: 1 = Liked it very much, 2 = Liked it, 3 = No difference, 4 = Disliked it, 5 = Disliked it very much.

Characteristic	Cooperator 1	Cooperator 2
Float type	1	1
Additional floats	1	1
Mesh material on seine and socks	1	1
Large throat entering sock	1	1
Trap door entering throat	1	1
Zippered sock attachment	1	1
Single rope mud line	1	3
Mud rollers	4	5

float spacing as used on the experimental seine, and a rolled rope mud line. The exceptions were that one cooperator wanted larger floats 15 feet either side of the large throat and the other cooperator wanted double throats (large throat on each end of the seine).

Cooperators also responded that they thought the primary advantages to the prototype seine system were: 1) less time to seine, 2) fish transitioned into sock much easier and appeared to be in better condition, 3) fish graded faster, 4) little if any aeration needed during socking, and 5) braided polyethylene appears to be much more durable.

Results at a glance...

☆ *A new seine technology that is currently being commercialized improves efficiency of catch, improves grading, and reduces seining time.*

Lastly, cooperators were asked if there were other features they would like to see tested or should further testing on the seine be conducted. One cooperator believed that further work was warranted on the mud roller system. Even though the mud roller system was the most disliked part of the prototype seine, the cooperators agreed that the rollers had merit but needed to be further refined.

Overall the prototype seine was well accepted and a modified version of the prototype will likely become an industry standard. A quote by one cooperator referring to the prototype seine was "I never knew seining could be so easy."

University of Arkansas at Pine Bluff. Two projects were conducted to develop in-pond fish grading technology for both market-size channel catfish and channel catfish fingerlings. Two horizontal floating platform graders with adjustable spacing were designed and fabricated. An eductor-style fish pump mechanism was also designed and fabricated. Design specifications for the fingerling grader were finalized during Year 2 and a demonstration grading system has been built. Public demonstrations were conducted in the spring of 2002 in Arkansas, Louisiana and Mississippi.

Design specifications for the foodfish grader were finalized in 2003. A controlled study at UAPB and commercial farms was successfully completed over this past year comparing the in-pond foodfish grading system to conventional live cars. An additional piece of equipment, a live car reel system, was developed to facilitate the process of crowding

fish while grading. This reel system can be added to any standard seine boat and allows one person to easily grade large quantities of foodfish. This live car reel system does not impede normal seining/ pushing operations and can also be used for crowding during load-out procedures. Public demonstrations were conducted in Arkansas, Louisiana and Mississippi.

Results at a glance...

- *An efficient in-pond fish grading system has been developed specifically for fingerlings, stockers, food-sized, and brooder-sized channel catfish.*

Over the course of this study, major advancements were made in the development of in-pond fish grading technology. The resulting design consists of three major components: 1) an adjustable horizontal bar grader, 2) a trailer with built in PTO-driven water pump, and 3) an eduction pump system that delivers fish to the 6-foot by 16-foot grading panel. This basic design has proven suitable for commercial fish farming use.

Various grading panel designs have been developed for fingerlings, stockers, foodfish, and broodfish. Additionally, an overlapping split-panel design was also developed to effectively sort fish into three groups with a single pass across the grading system. The basic eduction pump system was designed to integrate with standard sock frames used throughout the industry. Variations on the basic design include a quick-connect frame attachment for standard sock frames and a double-zippered tunnel system that integrates with the new zippered-sock designs developed at Stoneville.

University of Memphis. The stress response of channel catfish was evaluated in fish harvested by Mississippi State University personnel utilizing two types of seines. Approximately 34,500 food-sized fish were stocked into two, 8-acre ponds. The traditional seine utilized a rope mud line with a 4-foot live car opening. The experimental prototype seine utilized a mud line with rollers and an expanded opening for the live car. Stress response was evaluated in fish harvested on five different occasions at temperatures that varied from 12° to 30° C. Blood samples were taken from six fish at intervals during the harvest of the ponds using each of the seine types: 1) prior to the initiation of seining, 2) at the time fish were crowded into a live car, 3) midway through the harvesting process, and 4) from the last fish harvested from the live car. The seining process lasted for 2 to 4 hours. Plasma cortisol, glucose, and chloride were determined using accepted clinical assays. Responses (Table 4) associated with the two types of seines were evaluated utilizing a paired t-test for each sampling interval.

The stress responses evaluated were similar ($P > 0.05$) in fish collected utilizing the two types of seines. Cortisol concentrations were significantly lower ($P < 0.05$) in fish sampled with the experimental seine than with the traditional seine at 12EC. However, initial samples were lower in this pond, and the differences probably reflect pond differences rather than the effects of the two different harvesting techniques. Stress hormones of fish sampled prior to seining were relatively low, similar to those observed previously in unstressed fish. However, stress hormones increased significantly by the time fish were crowded into a live car. These stress hormones were near a maximum concentration and increased only slightly by the time the last fish were removed from the live car. The rate of secretion of stress hormones was such that these high concentrations were maintained for at least 1 hour.

Crowding fish into a live car using the traditional type of seine appeared to be more stressful compared to the experimental seine that utilizes a larger opening to the live car. Fish appeared to be more physically crowded and distressed as they were

Table 4. Blood characteristics of channel catfish at four intervals during harvest with traditional and experimental seines. Six fish were sampled per observation.

Temp	Sample	Cortisol (ng/mL)		Glucose (mg/100 mL)		Chloride (mEq/L)	
° C	Interval	Traditional	Experimental	Traditional	Experimental	Traditional	Experimental
12	1	24.7	9.4	27.8	23.4	121.7	126.4
	2	52.6	28.9	36.3	38.2	127.0	126.3
	3	68.1	56.5	53.2	47.3	123.2	126.2
	4	70.1	57.0	50.7	55.7	126.5	125.5
20	1	1.6	2.0	33.8	40.4	121.8	117.8
	2	33.3	34.7	78.1	100.1	117.0	120.3
	3	59.9	75.3	75.5	106.5	121.2	122.0
	4	140.1	93.7	109.7	143.2	115.7	119.2
29	1	25.5	25.7	54.5	47.6	117.5	122.2
	2	48.2	54.7	66.9	64.1	118.5	120.5
	3	79.6	77.5	103.1	95.0	115.0	111.5
	4	79.9	79.4	103.5	110.4	113.7	110.5
29	1	15.2		68.7		127.0	
	2	47.8	54.5	93.5	114.8	121.8	121.2
	3	65.3	68.1	115.6	161.7	122.0	113.5
	4	82.3	74.8	137.9	139.7	120.8	111.5
30	1	9.8	8.1	58.5	66.5	105.0	105.2
	2	55.8	26.1	112.1	96.3	102.0	110.7
	3	82.7	76.9	95.2	133.4	113.2	107.2
	4	79.0	46.5	110.9	100.3	108.2	104.3

forced through the more restrictive opening of the traditional seine type compared to the prototype seine. However, evaluation of hormonal responses did not provide a basis to distinguish differential stress associated with the types of seines.

Colder temperatures seemed to significantly slow and reduce the degree of the stress response. Fish sampled in December (12°C) developed lower cortisol concentrations than those sampled at other times of the year (temperatures of 29 to 30° C). The effects of lower temperature were even more dramatic in the increase of glucose in response to harvesting. Fish sampled at the end of the harvesting procedure at 12°C developed glucose concentrations less than half of that observed at 20° to 30°C. The glucose response was similar in fish harvested with the two types of seines. Plasma electrolytes in channel catfish remained stable throughout each sampling period and did not vary

with season. The ability to maintain osmoregulatory balance is probably an important component of the tolerance of channel catfish to aquaculture stressors.

University of Tennessee. The primary objective was to evaluate stress in fish species during aquacultural practices such as grading and transport using cortisol in the blood plasma as an indicator. Stress has often been associated with decreased disease resistance and suppressed immune system function. Therefore, a secondary objective was to conduct disease challenges to assess the increased susceptibility of channel catfish to *Edwardsiella ictaluri* after exposure to different degrees of confinement stress similar to that which would be experienced during grading. Progress toward the primary objective included the establishment and characterization of the cortisol, glucose and chloride assays that will be employed and the training of the project graduate student, in the techniques.

Three experiments were completed to evaluate the secondary objective. In the first experiment, small juvenile channel catfish were subjected to three levels of confinement stress and then challenged with a virulent strain of *E. ictaluri*. The degree of stress as measured by plasma cortisol was highly correlated with subsequent mortality to the disease challenge. Cortisol concentrations (mean ± standard deviation) for unstressed (23.6 ± 6.02 ng/mL), stressed 30 minutes (77.7 ± 19.16 ng/mL) and stressed 60 minutes (115.4 ± 26.65 ng/mL) were significantly different ($P < 0.05$) from each other. Significant increases in mortality occurred in conjunction with increases in cortisol (unstressed = 22.5 ± 4.2%; 30 minutes = 47.5 ± 5.2%; 60 minutes = 81.7 ± 6.8%). This provides a concrete example of a serious consequence of sublethal stress (immune suppression).

The second experiment was repeated as above, but used larger catfish fingerlings ready for stock-out to catfish ponds. The results were similar with plasma cortisol highly correlated to mortality, although both plasma cortisol and mortality were significantly higher for large fingerlings. Differences in cortisol levels were found between unstressed fish (31.4 ± 17.9 ng/mL) and stressed fish (30 minutes = 103.1 ± 20.7 ng/mL; 60 minutes = 109.6 ± 24.9 ng/mL). Although cortisol did not differ ($P > 0.05$) between the 30 and 60 minutes stressed groups, mortalities in the large fingerlings increased significantly with level of stress (unstressed = 25.8 ± 7.4%; 30 minutes = 62.5 ± 10.4%; 60 minutes = 100 ± 0.0%). This study demonstrates the relationship between cortisol and increased susceptibility of channel catfish to enteric septicemia of catfish (ESC). This indicates that large fingerlings become stressed much more easily and the resulting immunosuppression from stress is greater. Implications are that cortisol is an effective measure of stress as well as highly correlated with infection rates. Also, fish farmers must consider the length or amount of stress to place on channel catfish fingerlings in order to reduce infection rate from *E. ictaluri*. Size of channel catfish fingerlings must also be considered during stress events, as size affects stress increases and corresponding infection rates with ESC.

The third experiment also used a series of disease challenges where fish were subjected to three levels of stress and exposed to *E. ictaluri*. In each series the dose of *E. ictaluri* was lowered by half beginning at the established LD-30. For the high dose challenge, cortisol concentrations differed ($P < 0.05$) among stressor treatments (unstressed = 23.6 ± 2.5; 30 minutes = 77.7 ± 7.8; 60 minutes = 115.4 ± 10.9 ng/mL). Additionally, increases in mortality occurred in conjunction with increases in cortisol (unstressed = 22.5 ± 4.2; 30 minutes = 47.5 ± 5.2; 60 minutes = 81.7 ± 6.8%). The medium dose challenge exhibited similar results for cortisol concentrations (unstressed = 10.5 ± 3.5; 30 minutes = 88.5 ± 10.6; 60 minutes = 129.5 ± 31.5 ng/mL). Mortality rates were similar to the high dose challenge (unstressed = 7.5 ± 4.2; 30 minutes = 60.8 ± 13.9;

60 minutes = 94.2 ± 5.8%). While cortisol concentrations were similar (unstressed = 6.7 ± 3.4; 30 minutes = 80.5 ± 10.0; 60 minutes = 21.3 ± 39.7 ng/mL) for the low dose challenge, mortality rates were lower (unstressed= 4.2 ± 3.8; 30 minutes = 18.3 ± 8.2; 60 minutes = 27.5 ± 5.2%) but still significantly different from each other. This study demonstrates 1) both confinement stress and bacterial pathogen concentration affect disease susceptibility of channel catfish, and 2) stress and the concurrent physiologically high concentrations of cortisol are more highly correlated with disease susceptibility than bacterial pathogen load.

In summary, the level of *E. ictaluri* in the water was not important. When catfish fingerlings become stressed they are more susceptible to ESC at any dose. At each dose, cortisol readings indicated that the more stressed the fingerlings became, the greater the mortality rate at that dose. This may help to eliminate the *E. ictaluri* "bloom" theory during the ESC window of 20E to 28EC. When fish become stressed, they also become more susceptible to ESC anytime *E. ictaluri* is present at any concentration during the window.

Striped Bass

North Carolina State University. An 18-foot portable in-pond horizontal floating grader was manufactured based on the design developed at the University of Arkansas at Pine Bluff (UAPB). Initial trials using large phase-II hybrid striped bass (HSB) fingerlings were not successful in transferring the hybrid striped bass from the holding net to the floating grader. The majority of fish did not enter the inductor box even after extreme crowding. Those that did enter the box were buffeted around before being deposited on the grader with obvious signs of trauma (redness and bleeding on different parts of the body). Based on the discouraging results of these trials, a series of modifications were made to the inductor box design. These modifications were equally ineffective in improving the flow of fish from the holding net to the horizontal grader section and caused 15 to 20% of the fish to have scale loss and hemorrhaging. In addition, this design caused from 5 to 7% mortality of the graded fish. A second series of modifications were made to the inductor box and the front of the grader. These modifications were successful in effectively moving the fish from the net holding pens to the grader section without external signs of trauma.

Results at a glance...

☆ *Modifications of the catfish grader improved grading of both fingerling and adult hybrid striped bass.*

The final modifications to the grader include the following: 1) Elevation of the pump hose from the bottom of the inductor box to the top half; 2) Increase of outlet hose from 12 to 18 inches diameter; 3) Installation of vertical sleeves on the outside of the box to allow adjustment of water depth; 4) Decrease the angle of the entire grader front panel from 90E to 60E to reduce the height at which fish enter the grading area.

Seven separate trials were done on Phase-II fingerlings (average weight = 50 to 100 g) in 0.25-acre experimental ponds. Fish were harvested by seining then transferred while still in the water to a large rectangular holding net (live car). The holding net was attached to the grader inductor box with a section of net material fitted with a large nylon zipper. This allowed the fish to be crowded from the holding car toward the inductor box for eventual grading. The purpose of these trials was to calibrate the fingerling grader panel, to allow estimates of settings required to select different sizes of fish, and to develop operating protocols for use of the grader (pump speed, bar spacing and grading rate at different water temperatures).

Individual lengths, widths and weights were made on 100 fish from randomly selected samples of

fish prior to grading (= Pregrade), those that passed through the grader bars (= Passed), and those that were retained on the bars (= Retained). To estimate mortality or the presence of external trauma caused by the grader, a sample of 20 fish from the group that passed the grader and from the group that were retained on the grader were placed into individual holding nets and observed for 48 hours. Dead fish were removed at 24 and 48 hours. All the fish were removed after 48 hours and inspected for external condition (scale loss, coloration, and hemorrhaging).

The fingerling grader panel successfully separated different sizes of fingerlings (Figure 1). The results shown in this figure are representative of the results obtained from the seven trials. Variation in the Retained group (coefficient of variation, CV, = 5.1%) and Passed group (CV = 9.6%) was reduced compared to the Pregrade group (CV = 11.3%) by grading. No mortality was observed in any of the seven trials even after 48 hours in the holding nets. Less than 1% of the fish exhibited signs of external trauma. Trauma was limited to minor scale loss without associated redness or hemorrhaging. The presence of grass carp (*Ctenopharyngodon idella*) along with the fingerlings may have contributed to some of the observed trauma. The carp were large (over 7 pounds) and thrashed about vigorously on the top of the horizontal grader while among the fingerlings. Some trauma to the HSB was likely caused by the actions of these larger fish.

Once the grader and nets were set up in the pond, a process that took approximately 15 to 20 minutes, the grader could process approximately 10,000 fingerlings in 30 minutes. At temperatures below 10 EC fish activity was so slow that grading was less effective as the fish would not pass through the bars.

Three trials were conducted on a commercial HSB farm (White Rock Fish Farm, Vanceboro, NC) in 3-acre ponds used for foodfish production. A total of approximately 13,000 pounds of fish (average weight = 150 to 350 g) were graded. The grader was set up and operated using the same procedures as were used during the fingerling trials, except a foodfish grader panel was installed. Individual measurements on fish and observations of mortality and external trauma were made in a similar manner to the methods used on fingerlings. Variation in the Pregrade group (CV = 11.3%) was reduced by grading for the Retained group (CV = 6.6%) and Passed group (CV = 8.0%) (Figure 2). Total fish mortality for the three trials was less than 1%. Approximately 5% of the fish showed external trauma similar to that seen in the fingerling trials (minor scale loss). The presence of very large (over 20 pounds) black carp likely contributed to some of the trauma experienced by the HSB. Despite the large size of the carp, the inductor box would successfully move them through on to the grader panel where they would thrash around in their attempt to escape. Any HSB in close proximity to the carp would be battered by the carp. The Asian black carp was stocked into these particular ponds to control the ram's horn snail, one of the hosts of the white grub. Some means of excluding large fish from entering the grader and mixing with smaller fish is needed to reduce trauma to the HSB.

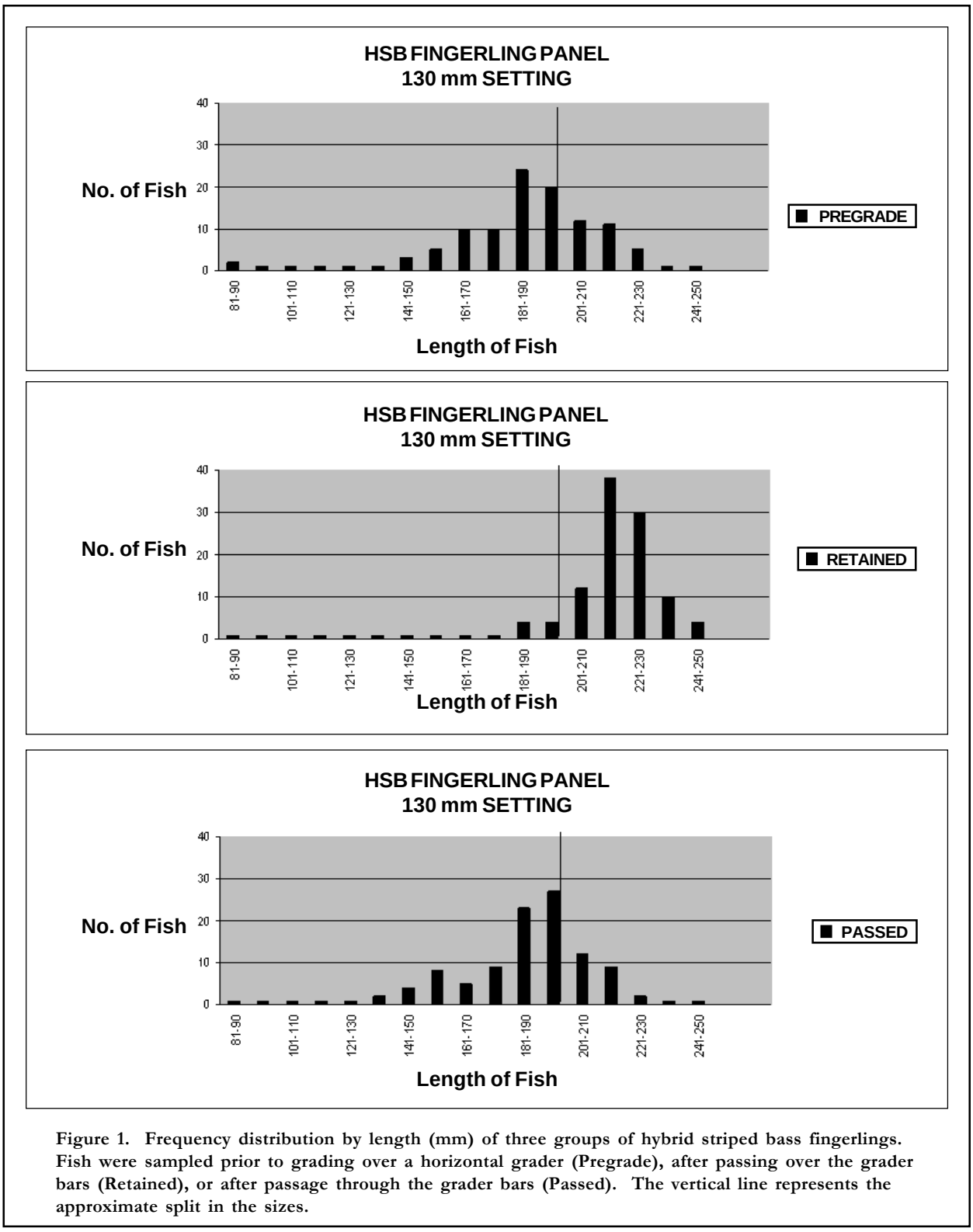

Figure 1. Frequency distribution by length (mm) of three groups of hybrid striped bass fingerlings. Fish were sampled prior to grading over a horizontal grader (Pregrade), after passing over the grader bars (Retained), or after passage through the grader bars (Passed). The vertical line represents the approximate split in the sizes.

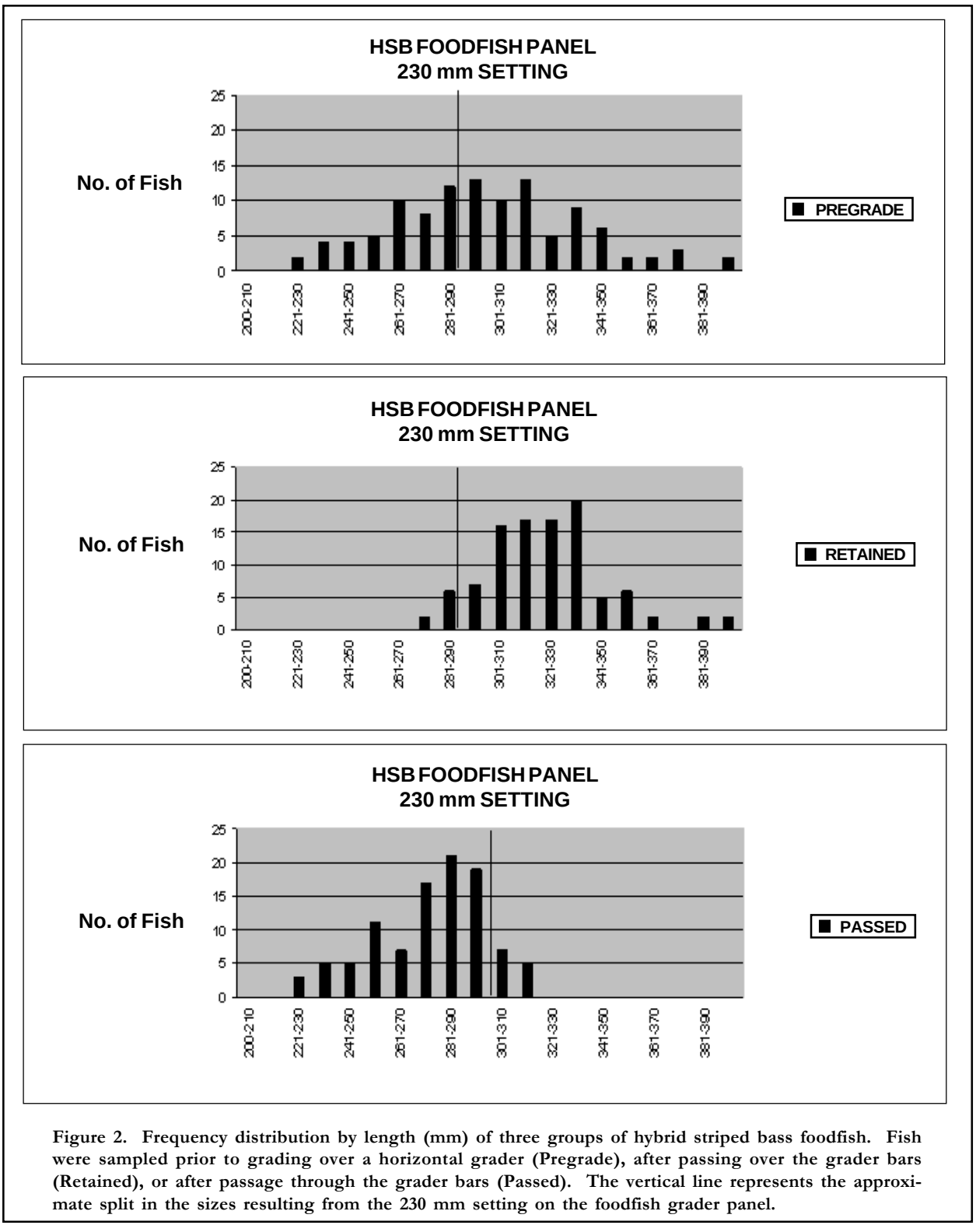

Figure 2. Frequency distribution by length (mm) of three groups of hybrid striped bass foodfish. Fish were sampled prior to grading over a horizontal grader (Pregrade), after passing over the grader bars (Retained), or after passage through the grader bars (Passed). The vertical line represents the approximate split in the sizes resulting from the 230 mm setting on the foodfish grader panel.

Ornamental Fish

University of Florida. Survey and analysis of existing practices (including man-hours) provide producers with accurate information of the economic cost of their existing practices. In 1999 tropical fish comprised $43,184,000 or 50.4% of total aquaculture sales in Florida (Florida Agricultural Statistics Service). A total of 183 surveys were mailed out with 75 being returned and 24 of those returned agreeing to work with University of Florida Tropical Aquaculture Laboratory (UF/TAL) more extensively. Table 5 shows the taxonomic groups of fish represented by farms responding to the survey.

Table 5. Taxonomic groups of aquacultured fish reported in survey.

Common Name	Order	Family
Barbs	Cypriniformes	Cyprinidae
Cichlids	Perciformes	Cichlidae
Corycats	Siluriformes	Callichthyidae
Danio	Cypriniformes	Cyprinidae
Gourami	Perciformes	Osphronemidae Trichogastrinae)Helostamotidae
Guppies	Cyprinodontiformes	Poeciliidae
Halfbeak	Beloniformes	Hemiramphidae
Koi	Cypriniformes	Cyprinidae
Mollies	Cypinodontiformes	Poeciliidae
Pacu	Characiformes	Characidae (Serrasalminae)
Platys	Cyprinodontiformes	Poeciliidae
Plecostomus	Siluriformes	Loricariidae
Rainbow Fish	Atheriniformes	Melanotaeniidae
Rasbora	Cypriniformes	Cyprinidae
Sharks	Cypriniformes	Cyprinidae
Swordtails	Cyprinodontiformes	Poeciliidae
Tetra	Characiformes	Characidae
Tilapia	Perciformes	Cichlidae
Shrimp	Phylum Anthropoda	Order Decapoda
Snails	Phylum Gastropoda	

Man-hours were directly observed and timed per activity with a stopwatch on four different ornamental farms. Two were egg layer farms and the other two had a combination of egg layer and live bearer ponds. Table 6 shows the time in minutes and the number of people required per activity.

Trapping and grading modifications were studied. Newly designed traps with larger ingress sizes were more successful at trapping higher numbers and larger fish (i.e. cichlids) based on farmer's evaluations. The most commonly used mechanical grader is a floating box design with several inserts of varying bar width. Each insert must be purchased separately and can be expensive if several inserts are needed. A one-piece adjustable grader box that can quickly

Table 6. Time (minutes) for each activity from observation.

Activity	Minimum Time	Mean	Maximum Time	Median # People/Activity
Seining	6.01	10.01	20	2
Deploy Traps	0.33	7.28	20.27	1
Collect Traps	0.83	3.76	6.88	1
Transport Pond to Bldg	3	6.12	12	2
Grading	5.92	17.22	47.17	2
Count/Pack Orders	45	167.5	420	2
Transport Bldg to Buyer	0.5	135.87	300	1

be adjusted with a moving knob to accommodate many different settings was loaned out for evaluation of cost and time effectiveness to local ornamental farmers. The adjustable grader ranges in grader spacing sizes of 6.4 to 29 mm (0.25 to 1.125 inches). Figure 3 shows both types of graders. The grader was loaned to three different fish farms. One farm was an egg-layer farm, one had both egg layers and live bearers, and the third was a cichlid farm. The initial response to the grader was not positive because the farmers were not used to working the grader box in the water for separation of the fish. In the insert design, the slotted inserts are along the bottom and sides of the insert, allowing fish to swim horizontally out of the grading box. However, in the adjustable grader, only the bottom of the grader box is slotted and fish must therefore swim down to leave the box. Fish generally do not exit through the bottom slots and the box must be maneuvered in the water. All three farmers felt that this slowed down the grading process and would not use this type of grader over the box with inserts.

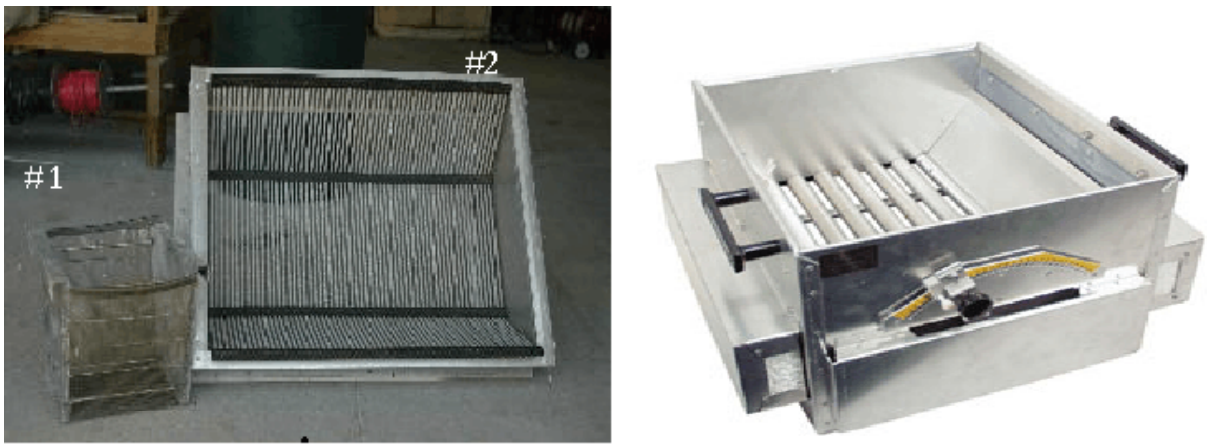

Figure 3. Mechanical graders evaluated in this project. The box on the left is a floating box with separate inserts and the box on the right is an adjustable grader box.

Objective 2. *Evaluate methods relative to loading and transport of fish to reduce fish mortalities and the negative effects of stress on product quality.*

Baitfish

University of Arkansas at Pine Bluff. Many of the expenses involved in hauling fish by truck are costs associated with moving enough water to keep the fish alive. As fish are crowded to reduce costs, the first limiting water quality problem is dissolved oxygen. The development of pure oxygen diffuser systems solved this problem and made it possible to haul fish at much higher densities. After oxygen, the next density-limiting factor is ammonia secreted by the fish. Current loading rates are limited by the necessity to haul enough water to prevent ammonia from reaching hazardous levels. The objective of our study was to find a better way to handle ammonia accumulation so that fish densities could be increased and hauling costs reduced.

Studies were conducted to determine the effect of simulated fish-hauling conditions on ammonia excretion rates of the golden shiner, *Notemegonus crysoleucas*. Studies were conducted at 15, 20, and 25EC in three recirculating systems for 24 hours using freshly harvested fish that were fasted and acclimated for 2 days. Each recirculating system consisted of a reservoir, three stocking tanks, air pump, floating bead filters and 1-micrometer bag filter. Total ammonia nitrogen, pH, temperature and DO were measured at the beginning of the experiment by stopping water flow. After 30 minutes, water flow was restarted to flush for three and a half hours. The procedures were repeated six times in 24 hours. The experiment was repeated four times using different batches of shiners. Average hourly ammonia excretion rates under three temperatures were obtained (Table 7).

Maximum average daily ammonia excretion rates at three temperatures were applied to calculate the size of floating biological filters (FBFs) and zeolite (clinoptilolite) for one vat (100 gallons water, 140 pounds fish) in hauling trucks. Considering the limitations of temperature, salinity and the operational feasibility, zeolite filters are more promising than FBFs for live fish transport. Ammonia secretion by golden shiners ranges from 3 to 16 g NH_3-N/kg fish per hour depending on fish size and water temperature. Detailed calculation of the volume of biofilter, weight of zeolite, or amount of ammonia-absorbing chemical required to control ammonia were made and tested. Removal of the ammonia produced by 2,200 pounds of shiners hauled on a truck for 24 hours would require 660 pounds zeolite, 42 cubic feet of floating bead biofilter, or $300 to $500 of commercial ammonia removal chemicals.

Table 7. Hourly ammonia excretion (mg NH_3-N/kg fish per hour) by golden shiners

Exp. No.	Unit fish size (g)	Hourly Ammonia Excretion Rates (mg NH_3-N/kg per hour)		
		Treatment I	Treatment II	Treatment III
		25°C	20°C	15°C
1	2.7	13.8 ± 4.0	10.1 ± 2.2	4.8 ± 1.2
2	2.7	15.0 ± 6.1	11.2 ± 3.1	7.4 ± 2.4
3	3.6	6.2 ± 2.7	5.8 ± 2.4	4.1 ± 2.1
4	3.6	11.1 ± 2.9	5.2 ± 1.7	2.9 ± 1.6

Laboratory testing of pH buffer systems to mimic the beneficial effects of carbon dioxide accumulation was conducted. Attempts to design chemical buffer systems sufficient to lower the pH below 7.0 and maintain it at a suitable level for 24 hours were very difficult to design due to variable water chemistry on farms, accumulation of carbon acid in the water, and chemical toxicity. It is probably possible to develop chemical pH control strategies on a farm-by-farm basis. A carbon dioxide/oxygen mixing system designed to mimic the beneficial effects of carbon dioxide accumulation was tested in hauling vats. Carbon dioxide and oxygen mixtures do provide beneficial pH lowering and tranquilization effects in fish haulers, but the solubility of carbon dioxide is so high that it is extremely difficult to prevent the accumulation of excessive levels over time. A successful system would require continuous pH monitoring and metering of carbon dioxide.

The University of Memphis. Small fish, larvae and eggs are often shipped in plastic bags filled with a small amount of water and an oxygen atmosphere. During shipment they are subjected to various stressors associated with the transportation of fish. Comparisons were made between traditional plastic bags and "breathing" bags made of a material that permits exchange of oxygen between the environment and the water in the bag. Initial observations indicated that the traditional bags provide a suitable amount of dissolved oxygen for a longer period of time than the "breathing" bags. The slow rate of diffusion of oxygen from the surface of the breathing bag to the central water mass limits the volume of water that can be used. Oxygen quickly limits survival in breathing bags that contain a large volume of water because the ratio of surface area for diffusion to water volume is low. However, small "breathing" bags that contained a small amount of water and no oxygen atmosphere supported individual fish for several days. Ammonia and carbon dioxide typically increased and pH decreased with increased transport time. Controlling these variables could increase the time fish can be shipped in bags.

Further studies were conducted comparing traditional plastic bags to Kordon "breathing" bags (Novalek, Inc., Hayward, CA). These bags are made of a plastic that permits exchange of gases between the environment and the water in the bag. Complete filling of the "breathing" bags with water and the lack of an air space may result in less mechanical movement that could reduce stress in the fish. Four liters of water were added to both types of bags and they were stocked with *Pimephales promelas* (average weight 2 grams) in triplicate at different densities. Survival and water quality characteristics were determined after 48 hours (Table 8).

Survival was good in both types of bags at densities of 30 or fewer fish/bag. Oxygen limited survival in breathing bags that contained 40 to 60 fish/bag. Ammonia and carbon dioxide typically increased and pH decreased with increased transport time. Controlling of these variables could increase the time fish can be shipped in bags. In other studies, small breathing bags that contained a small amount of water and no oxygen atmosphere supported individual crappie fish (six fish, total weight = 120 grams) for 4 days.

Accumulation of ammonia is a major factor that limits density and time of transport of minnows. The rate of excretion of ammonia by fathead minnows was evaluated under various conditions. Reduced temperatures have reduced ammonia excretion rates in a variety of fish. The use of anesthetics or ammonia absorbing materials could also enhance currently used techniques. The influence of temperature, size, and the anesthetic MS 222 on the rate of ammonia excretion by fathead minnows was evaluated (Table 9).

In general, ammonia excretion rates increased with increased temperature. Smaller fish were expected to have a higher excretion rate than larger fish, but

Table 8. Water characteristics and survival of *Pimephales promelas* stocked at different densities in triplicate in two types of shipping bags for 48 hours.

Bag Type	Fish per bag	Survival%	Temp. °C	Oxygen mg/L	CO_2 mg/L	pH
Traditional	10	90	17.9	5.7	35	6.6
Kordon	10	100	16	4.6	20	6
Traditional	20	100	19.2	4.3	28	6.6
Kordon	20	95	20.9	3.4	66	6.6
Traditional	30	97	18.2	2.7	43	6.3
Kordon	30	97	20	1.5	50	6.6
Traditional	40	82	20.8	7.4	88	6.4
Kordon	40	0	22.7	0.5	265	6.6
Traditional	60	53	21.7	6.6	95	6.4
Kordon	60	0	22.7	0.5	265	6.6

Table 9. Influence of temperature, size and MS222 on ammonia excretion by flathead minnows.

Temp (°C)	Ave. Wt. (grams)	MS 222 (g/L)	NH_3 excretion (mg/kg/hr)
16	1.65	0	8.5
17	0.70	0	9.0
	0.70	0.4	11.1
20	1.65	0	8.2
	0.71	0	14.0
	0.71	0.4	13.0
23	1.37	0	14.7
	0.70	0	12.6
	0.43	0	13.6
25	1.65	0	18.2
	1.65	0.2	15.9
	1.65	0.4	17.0

this pattern did not seem to hold for the size ranges evaluated (0.43 to 1.65 g per fish). The anesthetic MS 222 resulted in a slight reduction of excretion rates in three of the four times evaluated. Quantification of factors that affect the accumulation of ammonia during transport may permit more efficient shipment protocols.

Ornamental Fish

University of Florida. Studies of stress reduction and blood cortisol response were conducted. Dosage rates to reach light transport sedation for the blue gourami *Trichogaster trichopterus* were determined and should have applications to other species (Table 10). Blood cortisol levels were measured from untreated controls and from stressed individuals treated with one of five different compounds designed to alleviate stress. Compounds tested were metomidate, quinaldine, tricaine methanesulfonate (TMS), salt, and Hypno® (Jungle Laboratories Corp., Cibolo, TX). Anesthetics (but not salt) resulted in reduced blood cortisol levels relative to controls following a 1-week post-treatment period.

Table 10. Concentrations of commonly used anesthetics needed to reach light sedation for blue gourami.

Treatment	Recommended for ornamentals	Actual for blue gouramis
Hypno®	0.10 mL/L	0.14mL/L
Quinaldine	10-25 mg/L	5 mg/L
Metomidate	0.2-1.0 mg/L	0.8 mg/L
TMS	10-70 mg/L	60 mg/L

Harvesting, grading, and transport methods were studied. Harvest method studies showed that trapping fish yields better scores for appearance and behavior than does seining fish. During the experiment, no trapped fish died after a 1-week holding period following harvest whereas 24% of seined fish died. When seining, making a box in the water with the seine and netting fish into the transport container is better than dumping all of the fish at once.

Grading can occur either with or without oxygen available at pond side or in a building. Experiments using both treatments yielded no significant difference in survival, appearance, or behavior. Moreover, even with no oxygen added, measured dissolved oxygen did not fall below 6.37 mg/L in any of the treatments.

Transportation water used in harvesting fish from a pond to a holding facility was evaluated. Trials showed using aerated well water or a 50-50 mixture of pond and aerated well water (or system water) had improved behavior over using pond water exclusively. When using additives (oxygen, acriflavine neutral, methylene blue, quinaldine, TMS, eugenol, or salt) for transportation from a pond to a building, acriflavine neutral had significantly lower survivorship than other treatments used. Also, the fish treated with acriflavine were inferior in appearance to control fish.

Various 24-hour bath treatments were evaluated to include tetracycline, acriflavine neutral, salt, potassium permanganate, nitrofurazone, Quick Cure® (Aquarium Products, Glen Burnie, MD), formalin, and methylene blue. Use of acriflavine neutral resulted in the lowest (i.e. worst) appearance and behavior scores. Overall, tetracycline yielded the best appearance and behavior at the final evaluation; nevertheless, tetracycline gave relatively low scores for behavior in the initial evaluation. In addition, the fish treated with potassium permanganate, Quick Cure, formalin, and nitrofurazone also had significantly higher appearance and behavior scores than controls. This indicates a benefit to using one of these additives when prophylactically treating fish coming in from a pond.

Results at a glance...

☆ *Anesthetics help alleviate stress during transport of ornamental fish and should have application to transport of other species.*

When transporting fish to a distributor, additives can improve the quality of the fish shipped. We tested methylene blue, acriflavine neutral, quinaldine, salt, TMS, eugenol, Biogard® Feed, and Biogard® Bath. Salt was the most consistently beneficial additive for appearance and behavior. Acriflavine neutral yielded lower appearance scores than other treatments, including the control.

University of Tennessee. The University of Florida conducted experiments on anesthetic effects on ornamental fish during transport and the stress effects during these experiments were measured at the University of Tennessee using cortisol as a

biological indicator. Bioassay tests for cortisol had to be revised for the University of Florida due to small amounts of blood plasma obtained from gouramis. Two sets of cortisol bioassays were run for the University of Florida. Data are reported in the University of Florida report, above.

Objective 3. *Conduct comparative analyses of new technology and current technology for harvesting, grading, and loading fish.*

Channel Catfish

Mississippi State University. Studies to compare harvest efficiency of two types of seines were initiated in Year 2 at the National Warmwater Aquaculture Center (NWAC), Stoneville, Mississippi. A conventional twisted polyethylene mesh seine (CTPE) with a standard frame and CTPE sock and a braided polyethylene seine with mudrollers, large funnel, and zipper attached BPE sock have been tested. Year 2 data are shown in Table 11. Third year data indicate that the catch efficiency is improved by about 15% using the prototype seine (63 vs 55%, for prototype and conventional seines). Catch efficiency was determined by comparing the weight of fish caught during seine hauls with the estimated weight of fish in the pond. Seining time was reduced by 50% using the prototype seine (152 minutes for the conventional seine vs 76 minutes for the prototype seine). Also about 50% less time and manpower are needed to attach the sock to the prototype seine. Additionally, the prototype seine typically did not "mud down" as often as the conventional seine. Although the actual numbers differ between years for seining time and efficiency, the trends were the same. On farm demonstrations conducted during the last year of the project further demonstrated that the prototype seine significantly reduced seining time.

Table 11. Harvest efficiency data. CTPE = conventional twisted polyethylene; BPE = braided polyethylene.

Parameters	Seine Type			
	Year 2		Year 3	
	CTPE	BPE	CTPE	BPE
Mean Seining Time (min.)	90	60	152	76
Mean Stock Attachment Time (min.)	11	5	10	6
Labor to Attach Sock	2	1	2	1
Mean Efficiency (%)	69	83	63	55

University of Arkansas at Pine Bluff. Formal testing began in Year 2 to test the modifications made to the grader and to gather data for economic analysis. The UAPB grader took from 2 to 6 minutes to grade 10,000 pounds of catfish. There was no difference due to size proportions of catfish. Dissolved oxygen levels were not significantly different in the control live car after 14 hours of grading than in the pond. Little direct mortality was observed due to either grading technology, but all the mortality (seven fish in all) occurred in the control live car. Both the UAPB and control live car grading technologies significantly reduced the number and weight of sub-harvestable fish. However, the UAPB grader retained only 27 to 47 pounds of sub-harvestable fish while the control live car retained approximately 3 to 4 times the weight of sub-harvestable fish (69 to 159 pounds). Overall the UAPB grader graded out from 46 to 112 pounds (5 to 11% of the total weight graded) more sub-harvestable size fish than the control live car. This resulted in a 12.5% increase in average weight of fish available for transport to a processing plant. Both these differences were statistically significant. There was no difference in weight of harvestable-size fish retained by the graders.

A series of grading trials were conducted on commercial catfish ponds in project Years 2 and 3 to determine if a new in-pond horizontal floating bar grader is more efficient and outperforms current live car-grading techniques. Three replicate trials were conducted at UAPB at three different temperature ranges (> 26EC, 12.8 to 26EC, < 12.8EC) with three fish size distributions: (75:25, 50:50, 25:75 sub-harvestable to harvestable fish) in 2002 and 2003. Data summarized in Figures 3 and 4. Commercial trials were replicated three times during each temperature range with the size distribution of fish in the pond at harvest time. Grading accuracy was measured by determining the proportion and weight of sub-harvestable fish (<1.25 pounds) and harvestable fish (>1.25 pounds) retained by the grader as well as those returned to the pond. Mechanical injury and mortality were measured by visual inspection, and stress was measured by mean glucose and cortisol levels. Grading accuracy was measured by determining the proportion and weight of sub-harvestable fish (<1.25 pounds) and harvestable fish (>1.25 pounds) retained by the grader as well as those returned to the pond. Mechanical injury and mortality were measured by visual inspection, and stress was measured by mean glucose and cortisol levels.

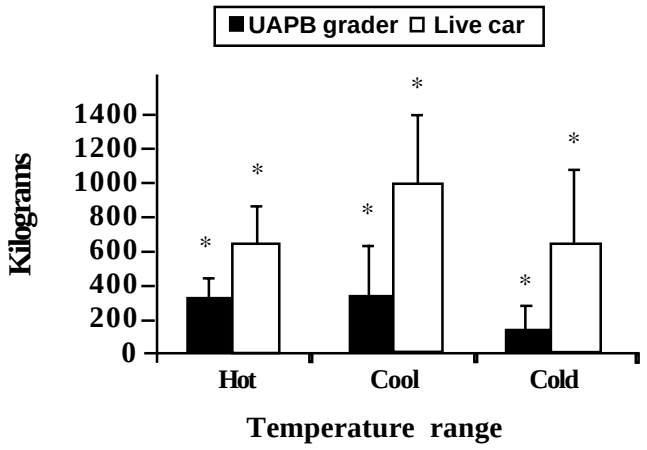

Figure 1. Mean kilograms of sub-harvestable fish left after grading by the UAPB grader (black columns) and the live car (white columns) after grading at three different temperature ranges on commercial farms. Columns with an "*" denotes a significant difference ($P < 0.05$) as determined by a paired t-test.

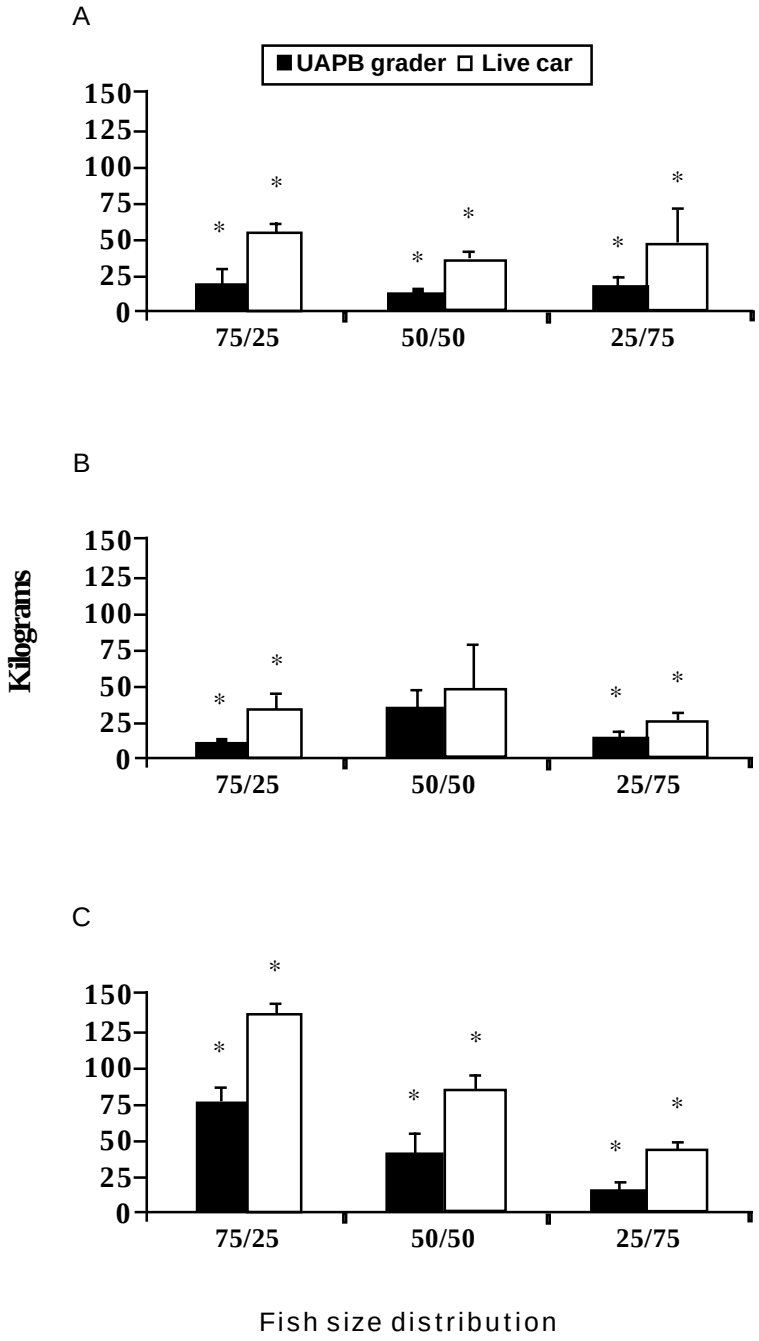

Figure 4. Mean kilograms of sub-harvestable fish left after grading by the UAPB grader (black columns) and the live car (white columns) after grading in hot (A), cool (B), and cold (C) temperature ranges with three different size distributions of sub-harvestable to harvestable fish. Columns with an "*" denote a significant difference ($P < 0.05$) as determined by a paired t-test.

Grading speed was significantly greater with the UAPB grader (230 to 480 pounds/minute) than the traditional live car (1 to 1.5 pounds/minute). The UAPB grader significantly decreased the proportion of sub-harvestable fish during all temperature ranges and for all size distributions of fish whereas the traditional live car did not significantly reduce the population of sub-harvestable fish in the 25:75 population during either the hot or cold trials. The mean percentage of sub-harvestable fish was 17% before grading in the 25:75 trial and was reduced to 4% after grading with the UAPB grader. Similar results were observed for the 50:50 size populations with the UAPB grader reducing the population from a mean of 31 to 8% sub-harvestable fish while the traditional live car reduced the population to 16% sub-harvestable fish. For the 75:25 size distribution the UAPB grader reduced the population from a mean 63 to 15% sub-harvestable fish while the traditional live car reduced the population only to 29% sub-harvestable fish. The live car grader did significantly grade fish during the cool trial (even at the 25:75 ratio), reducing the percentage of sub-harvestable fish from 16 to 6%.

In the commercial farm trials, only the UAPB grader significantly reduced the proportion of sub-harvestable fish. This was true for all temperature ranges. Traditional live car grading did not grade out enough sub-harvestable fish to cause a significant change in the proportion of sub-harvestable fish. The UAPB grader reduced the population from 16 to 8% during the hot trials, from 26 to 11% during the cool temperature trials, and from 17 to 4% during the cold trials.

Mean weight of sub-harvestable fish retained by each grader varied widely among trials, but was significantly less in the UAPB grader as compared to the traditional live car except during the cool trial with the 50:50 size distribution. Across all UAPB trials, two to three times more sub-harvestable fish were returned to the pond with the UAPB grader based on retained weight of sub-harvestable fish. The UAPB grader retained 22 to 42 pounds of sub-harvestable fish during the hot temperature trials, 24 to 71 pounds during the cool water trials, and 35 to 160 pounds in cold water. By comparison, the traditional live car retained 79 to 120 pounds of sub-harvestable fish in hot water, 53 to 106 pounds in cool water, and 90 to 294 pounds in cold water. Compared across temperature ranges, the weights of sub-harvestable fish retained by the UAPB grader were: 24 to 86 pounds with the 50:50 ratio; 24 to 160 with the 75:25 ratio, and 26 to 37 pounds with the 25:75 ratio. By comparison, the following weights were retained by the live car grader: 79 to 181 pounds with the 50:50 ratio, 81 to 295 pounds with the 75:25 ratio, and 53 to 106 pounds with the 25:75 ratio. The UAPB grader retained an average of 70 ± 31 pounds of sub-harvestable fish whereas the traditional live car retained 105 ± 66 pounds (a third more) during the cool trial.

In the commercial trials, the UAPB grader had significantly fewer kilograms of sub-harvestable fish remaining after grading than the traditional live car. The UAPB grader retained 657 pounds of sub-harvestable fish during the hot trials, 700 pounds during the cool trials, and 306 pounds of sub-harvestable fish during the cold trials. By comparison, the traditional live car retained 1340 pounds during the hot trials, 2070 pounds during the cool trials, and 1340 pounds during the cold trials. Thus, the UAPB grader returned from two to four times more weight of sub-harvestable fish than the traditional live car.

Grading performance of the traditional live car was significantly influenced by both size distribution and temperature. Grading performance was lowest at the 75:25 ratio during the cold trials, and best during the 50:50 ratio during the hot trials. There was also a significant interaction effect between size distribution and temperature. However, temperature did not affect grading performance for the UAPB

grader. Size distribution did significantly ($P< 0.05$) affect performance of the UAPB grader, and the interaction term for temperature and size distribution was also significant. Grading performance was worst during the 75:25 ratio and best during the 25:75 ratio. Glucose and cortisol levels in fish graded with the two grading technologies were not significantly different.

An economic analysis was performed using data from previously reported field trials to determine whether farmer adoption of this grader is economically feasible. Scenarios for four farm sizes were evaluated. Analyses conducted included a partial budget, payback period, net present value, internal rate of return, and Taguchi quality-loss function analysis to quantify and compare economic losses due to deviation from the target fish size.

Partial budget results indicated positive net benefits for all farm sizes (160, 320, 640, and 1180 acres). Net benefits increased with farm size, market price, and increased dockage penalties. Larger farms had the greatest benefit because they had more fish across which to spread the fixed cost of grading. As market price increased, the value of grading increased because returning more fish to the pond for additional growth generates a greater return from the greater overall weight sold. Dockage penalties affected the value of grading. Lower tolerance levels for sub-marketable fish and lower prices paid (higher dockage penalties) for small fish increased the value of grading. Payback periods ranged from 0.1 to 2.0 years depending on the scenario. Net present values were positive and increased with increasing farm size. Estimated internal rates of return were higher than the current opportunity cost of capital and increased with increasing farm size. The UAPB grader saved from $770 to $5,575/year, depending on farm size, by reducing the variation in individual fish size of loads of fish sent to processing plants. Results indicated that the UAPB grader is a profitable short- and long-term investment for small and large farms. This increase in profitability results from reducing the inefficiency of the live car grader by returning sub-marketable fish to the pond. Annual yields and weight of fish sold will generate greater returns to the investment in fingerlings and feed by not selling sub-marketable fish prematurely. These results indicate that producer adoption of the UAPB grader is economically feasible for the scenarios analyzed.

Baitfish

University of Arkansas at Pine Bluff. Existing enterprise budgets for baitfish are over 5 years old. These budgets have been updated to reflect current costs of production. Data acquisition forms have been developed to evaluate the current costs of transporting baitfish. These forms were developed with input from industry cooperators and are currently on review by others. These will be used to develop a database of cost information related to fish transportation. This database will serve as the basis of comparison for comparing and evaluating the new technologies to be developed.

IMPACTS

Channel Catfish

Braided polyethylene (BPE) mesh is recommended for construction of catfish seines and socks based on performance characteristics determined in research and commercial catfish ponds. Seines have been commercialized and this design will likely serve for the standard for the industry. The development of new seine technology should increase profits because the new technology improves the efficiency of catch by 15 to 20%, improves grading, and reduces seining time by 45 to 50%.

The fish grading work could potentially impact both producers and processors of channel catfish food fish. The adjustable nature of the fish grader allows

more control over the size of fish retained. This could lead to more harvesting flexibility and more marketing options for producers. Another advantage is that fish can be graded immediately after seining, allowing more accurate inventory estimates to be related to the plant. Data suggest the in-pond mechanical grader removes more sub-marketable fish as compared to conventional socks. This technology will let foodfish producers retain more sub-marketable fish in the production pond while improving efficiency at the processing plant. Fingerling producers marketing graded channel catfish fingerlings can benefit greatly from in-pond grading as it eliminates the need for costly vat grading facilities, drastically reduces the time and labor requirement of other grading methods and can eliminate costly haul-backs. To date, eight catfish fingerling facilities, three commercial catfish foodfish facilities and one hybrid striped bass facility have adopted this technology.

Results at a glance...

- *The in-pond, platform fish grader is a profitable short- and long-term investment for all sizes of farms. The major economic benefit results from returning sub-marketable fish to the pond for additional growth.*

Although live car grading has been the industry standard for over 40 years, there are problems and uncertainties associated with this technique. This study has shown that the UAPB grader can reliably grade fish more accurately and consistently at a wider range of temperatures than the traditional live car. Moreover, the traditional live car was shown to not grade significant proportions of small fish at the mesh size selected for this study.

When catfish fingerlings become stressed they are more susceptible to ESC at any concentration of bacteria. Cortisol concentrations showed that the more stressed the fingerlings became, the greater the mortality rate proving that cortisol is a good biological indicator of stress and associated mortality to ESC.

Crowding fish into a live car using the traditional type of seine appeared to be more severe compared to the prototype seine that utilizes a larger opening to the live car. Fish were seemingly more physically crowded and distressed as they were forced through the more restrictive opening of the traditional seine type compared to the prototype seine. Thus using the new seine design could help alleviate stress to fish during seining and grading.

Striped Bass

An 18-foot portable in-pond horizontal floating grader was manufactured based on the design developed at the University of Arkansas at Pine Bluff (UAPB). A series of modifications were made to the inductor box and the front of the grader. These modifications were successful in effectively moving the fish from the net holding pens to the grader section without external signs of trauma. In-pond graders have the potential to significantly reduce the labor and costs associated with harvesting and minimize mortality caused by excessive handling.

Ornamental Fish

Survey and analysis of current practices provides producers with information on the economic cost of existing practices, which can be used to improve management. New trap design has increased effectiveness of harvest yield for larger size fish such as cichlids. From data collected in experiments, acriflavine neutral, which is a commonly used chemical, had no distinct benefits and can actually lower the overall quality of the fish. The use of tetracycline, potassium permanganate, Quick Cure®, formalin, and nitrofurazone can be recommended for use prophylactically in treating fish directly from the

pond. For shipping fish, salt can be recommended as the best treatment tested.

Extension publications that are in press will detail specific methods and technologies that reduced time and produced higher levels of survival, reduced levels of stress, and improved behavior and appearance, at each point of operations involved in harvesting, grading, and transportation. These results will also be summarized in a 20-minute extension video.

Baitfish

Using ammonia production data, it is now possible to predict the final ammonia concentration in tanks used to haul baitfish. While field trials of potentially practical systems were unsuccessful (carbon dioxide) or not conducted (biofilters or zeolite), innovative farmers may find ways to develop these ammonia reduction ideas for practical applications.

The anesthetic MS-222 resulted in a slight reduction of ammonia excretion rates. Decrease in the accumulation of ammonia during transport may permit more efficient shipment protocols. The complete filling of the breathing bags with water and the lack of an air space may result in less mechanical movement that could reduce stress.

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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, 2004

Funding Level

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

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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 80EF. 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. Collections of aquatic birds now include 5 pelicans, 10 cormorants, and 10 great egrets collected in 2003. In 2004, 54 more aquatic 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. Trematodes were harvested from the alimentary canal of collected birds. *Clinostomum spp*. (one species of which is responsible for the yellow grub in fish) were found in great egrets, great blue herons, snowy egrets, and cattle egrets. Specimens of the gill trematode *Centrocestus formosanus*, may have been recovered from green heron and great egrets (definitive identifications not yet made). It appears that *Bolbophorus* spp. has only been recovered from white

pelicans previously collected in 2003. 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.

Results at a glance...

☆ *Three distinct types of cercariae were confirmed in snails collected from ponds experiencing mortality from trematode infestations:* Clinostomum marginatum, *the causative agent of "yellow grub disease";* Bolbophorus damnificus, *a serious pathogen of channel catfish; and* Bolbophorus *species type 2, a species not recovered from catfish but present in several other fish hosts and pathogenic for hybrid striped bass. The ram's horn snail,* Planorbella trivolvis, *is the primary intermediate host and has been shown to harbor both types of* Bolbophorus.

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 anthelmintic 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* metacercaria (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.

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.*

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.

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 co-operators 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. Hybrid-striped bass and channel catfish fingerlings were obtained from commercial farms in North Carolina, where *Bolbophorus* spp. are 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.

Groups of 10 catfish fingerlings were maintained in aquaria at 26EC and were infected with 500 or 100 *B. damnificus*/fish; 500 *Bolbophorus* sp. type 2. Both groups of *B. damnificus* catfish experienced typical hemorrhagic lesions, loss of appetite and 100% mortality. Catfish infected with *Bolbophorus* sp. type 2 experienced no obvious clinical signs.

Groups of 10 hybrid bass fingerlings were maintained in aquaria at 26EC and infected with 100, 250 or 500 *Bolbophorus sp.* type 2 or 500 *B. damnificus*. All HSB experienced hemorrhagic lesions typical of bolbophorid infection. None of the fish in the lower challenge dose groups experienced changes in appetence and none died. HSB infected with 500 *Bolbophorus* sp. type 2 experienced severe hemorrhagic lesions and 9 of 10 fish died. However, most fish continued to feed until near death. HSB infected with 500 *B. damnificus* remained lesion-free without obvious clinical signs. Weight data and metacercaria recovery rates are in the process of being analyzed. Fish host species specificity was therefore confirmed, as seen during year 1. However, an outbreak of columnaris in the catfish may complicate interpretation of our results and necessitate repetition of this experiment during Year 3.

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 were 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* cercaria) 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 32EC 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. Other production parameters and histopathology are not available at this time.

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

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.*

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 x 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. Data indicates that once fish are removed from the source of additional infections, that chronic trematode infections do not appear to 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 are significant in terms of management strategies aimed at controlling losses associated with trematode infections. Preliminary 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 mortality associated with ESC. These studies also indicated that the presence of fully developed metacercariae does not appear to compromise the growth performance and health status of fish. This data supports the idea that the deleterious effects of this infectious agent are associated with penetration of the parasite and initial stages of encystment. Findings point to the need for increased surveillance for this disease and that management strategies are initiated at the onset of infection. In addition, breaking the trematode life cycle by moving fish to non-infested water or by eliminating snail populations in the pond will eliminate the adverse effects associated with this disease.

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

USDA/ARS. Four trials were conducted comparing pond shoreline treatments of copper sulfate and hydrated lime against snails. Copper sulfate and hydrated lime were applied at 1 pound and

20 pounds respectively per 25 feet of shoreline in a 6-foot swath width. Both treatments effectively lowered the snail populations in the test cages. Preliminary information indicates that the copper sulfate was more effective than lime in most trials, that hydrated lime treatments appear to be more effective than other treatments at higher temperatures (31E C vs 25EC), and that strong winds negatively impact the copper sulfate treatments more than the hydrated lime treatments. Survival under all conditions in the four trials ranged from 3 to 27% and 10 to 41% for copper sulfate and hydrated lime respectively. A fifth trial was run with hydrated lime alone. The rate of hydrated lime was doubled and the effectiveness was increased so that the rate of snail survival dropped to less than 2%.

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.*

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 = 23EC). Test concentrations of copper were arranged in a geometrically spaced dilution series. Each test concentration consisted of four 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, un-treated water. Endpoints 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 30EC) 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 15EC to 30EC, 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 were 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 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.*

Mississippi State University

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

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 two-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 will be 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.

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

Redear sunfish (small, medium and large) were stocked in spring at a rate of 50 fish/acre into four, 0.05-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) were recorded. In September, the ponds were seined and harvest size channel catfish removed. The number of surviving individuals of each species were recorded. The ponds were "under-stocked" with 5-inch fingerlings to replace the fish removed for market. Survivability of redear sunfish will be 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 following spring, the ponds will be seined and all redear will be counted and weighed. Harvest size catfish will be removed and 5-inch fingerlings will be stocked to replace those removed.

Results at a glance...

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

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 the gills and in the flesh. 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. All other variables are still being analyzed.

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 Fg/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.

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.*

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.

Results at a glance...

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

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 Fl 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 28EC. *Flavobacterium columnare* growth medium (FCGM) outperformed 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.

Table 1. Growth of *F. columnare* in various broth media at 28ºC 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 $P = 0.05$

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 antimicrobial agents, one lot of Mueller Hinton medium, and one lot of equine serum were used in all disk diffusion test evaluations. The antimicrobial disks (BBL) chosen were Sulfamethoxazole : trimethoprim (SXT 25 Fg), Sulfadimethoxine : ormetoprim (PRI-MOR 25 Fg), Oxolinic acid (OA 2 Fg), Oxytetracycline (T 30 Fg), and Florfenicol (FFC 30 Fg). 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 3g, 3.5g, 4g, and 5g/L. The agar concentrations that were evaluated were 9g, 12g, 15g, and 17g/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.

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 the species-specific PCR of 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 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 30EC 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. The plastic plate assay and the larval fish assay appear most promising for future studies and will be used for comparison with virulence assays.

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

Results at a glance...

☆ *Both 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.*

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 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 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.

Ribotyping has been performed on 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 riboprints between those species are being analyzed along with those from 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, a riboprint database is being build that will become available to other users of that system. In the analysis, good homology and yet acceptable separation of riboprints was noted between various Flavobacterium species, with good separation from and between most of the diverse isolates, including Flavobacterium-like bacteria. Isolate Flavocolum23463, which was one of the cultures provided from ATCC as a control strain of *Flavobacterium columnare*, typed out as non-Flavobacterium (closest match to *Bacillus megaterium)*. ATCC later determined that there were problems with those original cultures, which ATCC re-isolated and reshipped. While this problem occurred with 2 isolates causing some delay, it does point out the power of the ribotyping analysis to identify and delineate organisms.

The above species and subtypes/strains are being subjected to a pulsed field gel electrophoresis PFGE analysis, to compare the ability of the two analyses to separate organisms. PFGE analysis, through modification of PCR primers and/or restriction enzymes may provide more opportunity to adjust the procedure to produce optimal fingerprints for identifying and separating various bacterial isolates.

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

Clemson University. Several outer membrane proteins (OMP) from *Flavobacterium columnare* that are consistently found in all *F. columnare* isolates tested thus far have been isolated. As reported in the 2003 SRAC Annual Progress Report, a 30 kDa OMP was isolated from *F. columnare* that 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 these activities can be blocked using specific antibodies against the OMP. In 2004, iNOS and COX-2 were found that are induced in phagocytes from several species of fish, including striped bass, *Morone saxitilis*, mummichog, *Fundulus heteroclitus*, and tilapia, *Oreochromis niloticus*, and as seen with channel catfish phagocytes, iNOS and COX-2 expression can be blocked using specific antibodies against the 30 kDa OMP. These collective data are being assimilated in a manuscript to be submitted for publication in early 2005.

Several continuing projects are underway and planned. In addition to the 30 kDa and 40 kDa proteins previously mentioned, larger OMPs were recently isolated using SDS-PAGE that show *in vitro* biological activity in fish phagocytes (and a mouse macrophage cell line). As part of the Clemson University Genomics Institute (CUGI), a new proteomics facility is now in operation, and this facility has agreed to help identify *F. columnare* OMPs, with an initial focus on the 30 kDa OMP. Our ultimate goal over the course of the next year is to construct a cDNA library from a virulent Clemson University strain of *F. columnare* and screen this library with our antibodies. This approach, and that of using PCR probes that may be designed from proteomics data (protein sequences of identified proteins), will lead to the expression of recombinant OMP proteins. These recombinant OMP proteins will then be 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.

Results at a glance...

- *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 these activities can be blocked using specific antibodies against the OMP.*

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. The 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).

Objective 1 has been completed. Two 65 bp 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.

Objective 2 has been completed. 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.

This year, another plasmid was constructed to attempt expression of *gfp* in *F. columnare*. First, the *ermF* (erythromycin resistance) gene from pCP11 was amplified by PCR, cloned it into pCR2.1 (Invitrogen), and subcloned 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. The *gfp* gene with the *Bacteroides* promoter was transferred 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. A conjugation technique using *E. coli* SM10 l*pir* as a donor strain has been used to attempt transfer of the plasmids into *F. columnare*. Twenty-five columnaris strains that were collected from John Hawke (LSU-SVM) in the previous year has been used, as well as an additional 20 isolates received from Joe Newton this year. Currently, attempts have been made to transfer pMWFCgfp and pBBRFCgfp into *F. columnare* by electroporation. In addition, a *Flavobacterium johnsoniae* isolate has been obtained from David Hunnicut at Penn State, Erie to use as a control for the conjugation and electroporation experiments.

Objective 4 is still in progress. 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 S. Thomas-Jinu and Andy Goodwin 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. 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.

Another way to look at differences between columnaris isolates is to challenge fish and then look for differences in the response of the infections to practical disease treatments. Columnaris disease was produced in channel catfish by bath exposure to 4 highly virulent isolates of *Flavobacterium columnare*. In untreated controls, mortality began 20 hours after exposure and was 100% by 48h. 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 4 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 strains and among treatments. Bath treatments with chloramine-T and potassium permanganate significantly reduced mortality from 100% to 75% and 69%, respectively, but copper sulfate and hydrogen peroxide treatments were not effective. Based on these 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 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 Louisiana Aquatic Diagnostic Laboratory repository will be initially screened for high virulence isolates using protocols developed by R. Cooper. Methods that produce uniform mortality rates of 70-80% with exposure to virulent strains of *F. columnare* will be adopted for use to compare virulence of archived strains from various locations and species in Objective 6. The hybrid striped bass (15 to 20 g mean weight) are currently being acclimated in the Aquatic Pathobiology Building at the LSU School of Veterinary Medicine.

Strains identified by RAPD profiling and will be used in challenge studies. Representative isolates from each RAPD group and host species will be obtained from our collection of archived strains at LSU. The isolates will be evaluated for virulence in an immersion challenge model described by Andy Goodwin for cyprinids and catfish.

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, seventeen 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 80-100% similarity. Virulence of the isolates will be determined by bath exposure of channel catfish and golden shiners to broth cultures of *F. columnare* using the methodology developed in Objective 5.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

Publications in Print

Thomas-Jinu, S. and A. E.Goodwin. 2004. Morphologic and genetic characteristics of *Flavobacterium Columnare* isolates: Correlations with Virulence in Fish. Journal of Fish Diseases 27:29-35.

Thomas-Jinu, S. and A. E. Goodwin. 2004. Acute columnaris infection in channel catfish: Efficacy of treatments practical in warmwater aquaculture ponds. Journal of Fish Diseases 27:23-28.

Presentations

Farmer, B. and J. Hawke. 2004. Improved methods for the diagnosis and characterization of *Flavobacterium columnare*. Annual Meeting of the Fish Health Section, American Fisheries Society, July 25-29, Shepherdstown, WV.

Hanson, L. A., L. M. Ford, B. Scheffler and J. P. Hawke. 2004. Sequence analysis of the 16S-23S intergenic spacer of *Flavobacterium columnare* to identify potential strains. Annual Meeting of the South Central Branch of the American Society for Microbiology, Nov. 5-6. Mississippi State, MS.

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IMPROVING REPRODUCTIVE EFFICIENCY TO PRODUCE CHANNEL × BLUE HYBRID CATFISH FRY

Reporting Period
March 1, 2004 - August 31, 2004

Funding Level

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

Participants

Auburn University (Lead Institution)	Rex Dunham, Allen Davis, Ron Phelps
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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, *Ictalurus punctatus*. Spawning begins when water temperatures consistently remain above 21EC 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 (36EC). This study attempted to use degree-days (ED) 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 21EC threshold yielded a constant value of 98 ± 4 ED for the heat requirement of channel catfish to initiate spawning.

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

Additionally, ED values above 21EC 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 row followed by the same letter do not differ significantly (*P*<0.05).

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

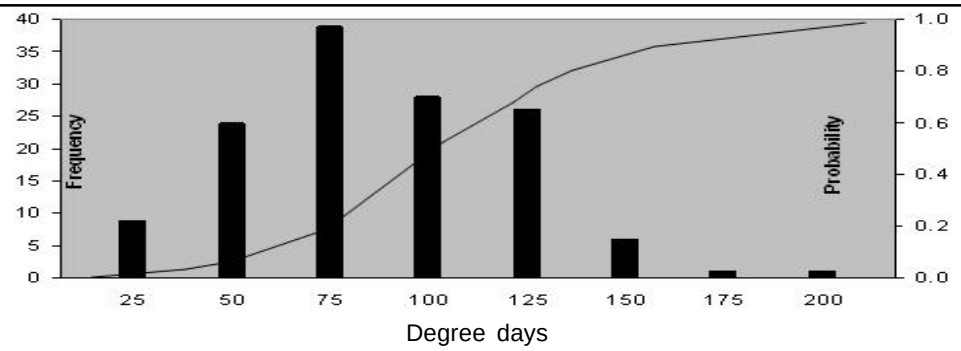

Figure 1. Spawning probabilities and spawning frequency at different degree day values above the 21EC threshold.

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. Female brood stock were maintained in 0.1-acre ponds at a density of 1,300 pounds/acre. Fish were offered a commercial floating feed (32% protein) 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. Floating vertical pump aerators were used when oxygen concentrations dropped below 2 mg/L. Ammonia and nitrite concentrations were estimated twice weekly. Hardness and alkalinity were measured at the beginning and at the end of the experiment. Three months prior to the onset of spawning, four dietary treatments were assigned to four replicate ponds each containing three strains of catfish. A factorial 2 × 2 design was used to evaluate feed quality and feeding rate. The two test diets were: 32% typical practical catfish feeds, and 42% high fish meal practical catfish feeds, and the feed was offered either three or six times a week, to apparent satiation. Females were spawned in three periods (early, middle and late spawning periods), and egg mass, egg diameter, fertilization rate at 48 hours, and biochemical analyses, were recorded as indicators of egg quality. Fish strains used were selected based on previous spawning productions, such as differences in performance related to feed quality and feeding rate.

Statistical analyses of the spawning data is currently under way to determine the influence of the various dietary treatments as well as the influence of strain, age, sex and spawning period. All statistical analyses were performed using SAS version 8.2 software. The effects of feeding rate and feed quality on fish performance, fecundity and egg quality, related to strain and spawning period are being evaluated. Preliminary analysis indicates nutrition affected hybrid embryo production. Definitive recommendations cannot be made because of apparent genotype × nutrition, age, egg mass position and tank size effects on hatch rate and fry/kg, and analysis is ongoing to account for these factors. Once the spawning data is analyzed selected egg samples representing good and bad quality spawns will be selected and further analyzed for biochemical composition.

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 channel catfish strains 1 through 5 consistently produced greater numbers of hybrid fry than AU strains 6 through 10 for three consecutive years (Table 2). Additionally, AU-1 produced greater numbers of fry than AU-7 in a fourth year. Nutrition × genotype interactions were observed for hybrid fry produced/kg female body weight. 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 2. 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	1857
AU-2	75	7,133	2154
AU-3	100	11,997	1283
AU-4	82	6,545	1005
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

Mississippi State University. Groups of nine, 2-year old female channel catfish brood stock obtained from each of 4 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 for blood and eggs, respectively. No individual fish within a strain was subject to sampling more than once every four months. Plasma estradiol, plasma testosterone, cathepsins, protein content of eggs and egg size were measured. No noteworthy differences in the mean values of the physiological indices monitored were observed among the four strains during each month.

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

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

Auburn University. Hybrid channel × blue catfish can be obtained by induced spawning and artificial fertilization but with variable results. A threshold degree of maturity must be reached before 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 brood fish (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 28EC in 60-gallon aquaria and injected with LHRHa at 20 Fg/kg as a preparatory injection followed 12 hours later with 100 Fg/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 EC × time in hours to first egg release) was 1,156 ± 275. The mean degree hour response time was 1,416 ± 107 at 24EC , 1,228 ± 211 at 26EC and 981 ± 278 at 28EC. The percentage of females that ovulated were 58, 62.5 and 87.5% at 24, 26, and 28EC, respectively. The majority of females which did ovulate did so between 58 to 64 hours at 24EC, 48 to 52 hours at 26EC and 24 to 40 hours at 28EC 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 24EC an average of 70 ± 60 g were obtained/kg, at 26EC 126 ± 41, and at 28EC 154 ± 34. The number of eggs/g of eggs also varied by temperature, 71 ± 11, 53 ± 6, and 48 ± 10 at 24, 26 and 28EC respectively. Egg quality varied with how soon eggs were taken after the first egg was released. For females at 28EC, 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 ± 2120, respectively).

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, IA and Syndel International, Inc., Vancouver, BC, 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 Fg/kg female BW initial injection followed by 80 Fg/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. Treatment means were compared by mixed model ANOVA.

There were no differences among treatments for any of the variables measured (Table 3). 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.

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 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.

Table 3. 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	6482	55.5	1348	1788	239,100
Catfish PE	51	2.8	68	6767	64.1	1128	1600	190,100
LHRHa	58	3.0	65	6482	66.3	1527	1999	254,100
Standard Error		0.2	8.4	720	8.6	344	350	

Auburn University. Luteinizing hormone releasing hormone analogue, LHRHa, injections were more effective than carp pituitary extract, gonadotropin hormone releasing hormone, GnRH, salmon GnRH and ovaprim injections for producing channel catfish female × blue catfish hybrid catfish embryos. LHRHa injections (one or two priming injections and a resolving injection) ranging from 10/50 to 30/150 Fg/kg female body weight were compared. In general, the higher dosages of 20/100 and 30/150 were the most effective, but the dosage must be decreased as the spawning season progresses to maintain maximum effectiveness. At the end of the spawning season 10/50 is an

effective dosage. LHRHa implants were more effective than injections for producing channel catfish female × blue catfish hybrid catfish embryos. 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. Fry/kg produced ranged from 200 to 3,000 for the various treatments.

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 brood stock.

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 sometimes increased and sometimes decreased hybrid fry production (Table 4). Method of exposure appears to have an effect. If water is introduced from separate tanks containing males is introduced, positive effects on hybrid fry production, whereas visual or actual contact appears to have negative effects (Table 5).

Table 4. 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 agonist, 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	90[a] ± 30	6,822[a] ± 2,268	31.1[a] ± 6.7	2,246[a] ± 652	31[a] ± 5	3.3[a]± 0.15
Exposed	100[a] ± 0	7,358[a] ± 1,756	40.5[b] ± 1.6	3,031[b] ± 1,028	30[a] ± 5	3.7[b]± 0.11

[a,b] means followed by the same letter are not different (P>0.05) within each column.

Table 5. Mean spawning percentage, egg/kg female body weight (BW), hatching percentage, fry/kg female body weight and latency time at 29EC for channel catfish (*Ictalurus punctatus*) females injected with luteinizing hormone releasing hormone agonist, 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	80[a] ± 42	9,368[a] ± 1,519	14.4[a] ± 0.64	1,351[a] ± 219	31[a] ± 0.10
- 150 no male	80[a] ± 42	8,288[a] ± 2,671	52.9[b] ± 0.45	4,384[b] ± 1413	31[a] ± 0.10
30 + 150 high male	90[a] ± 31	8,211[a] ± 3,882	23.2[c] ± 0.11	1,901[a] ± 899	32[b] ± 0.52

[a,b] means followed by the same letter are not different (P>0.05) within each column.

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 6. Sperm concentrations vary in relation to gonad composition.

Table 6. 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 ± 4.5[a]
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 ± 4.6[a, 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 ± 4.6[b]

[a,b] 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. Factors that require additional study include requirement for pathogen control of scanned embryos, density of scanned embryos in relation to fluid volume and peristaltic pump flow during the 6 days of development. Initial imaging of catfish oocytes suggested the feasibility of adapting this technique developed originally for zebrafish and called Computer-Aided Meiotic Maturation-Assay (CAMMA). Although fully-grown oocytes of catfish are significantly larger than those of zebrafish, the preliminary results indicate that CAMMA may be successfully used to follow oocyte maturation in the catfish system. Factors that require additional study include 1) best saline/medium formulation to support oocyte viability and maturation in vitro, 2) relationship of oocyte clearing to cell cycle stages of the oocyte and 3) optimal hormone milieu to elicit oocyte maturation in vitro.

Currently, data is being extracted from stacks of scanned images from catfish oocytes treated with various media and hormones, and embryonic development is being analyzed. Catfish oocytes and ovary extracts will be screened for reaction with cell-cycle control protein antibodies (e.g., anti-cyclin B1) that may prove useful in studies of oocyte maturation in catfish.

Louisiana State University. Ultrasound is a non-invasive technique that 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, and oocyte diameter by use of ultrasound in channel catfish.

During February through June, 2004, channel catfish gonads were evaluated at three different life stages: fingerlings (under 1 pound), market-sized foodfish (1 to 2 pounds), and brood stock (more than 3 pounds). Fish were scanned using a linear ultrasound probe (3 to 10 mHz), and gonadal sex was verified by dissection. To evaluate ovarian development, twelve females were given injections of artificial luteinizing hormone-releasing hormone. Of these, five were strip spawned. Fish were scanned

daily to monitor gonadal development.

Gonads were correctly identified as testis or ovary for fingerlings (57%), food fish (90%), and brood stock (86%). Immature gonads were difficult to distinguish from surrounding tissues. Mature testes were partially visible, but we could not quantify their development due to lack of contrast with surrounding tissues. Unlike testes, mature ovaries were easily distinguished and their development quantified by measuring ovarian diameter, calculating the ratio of ovarian diameter to body wall diameter (OD:BD), and measuring oocyte diameter. There were no significant differences ($P < 0.05$) in ovarian diameter or in OD:BD between strip-spawned and non-spawning females (Table 7). Strip-spawned females had significantly larger ($P < 0.01$) 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 7. 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 ± 5.8[a]	54.2 ± 7.4[a]	0.87 ± 0.03[a]	0.84 ± 0.05[a]	1.8 ± 0.4[a]	1.8 ± 0.5[a]
2	65.8 ± 5.1[a]	50.4 ± 9.1[a]	0.89 ± 0.03[a]	0.85 ± 0.04[a]	1.9 ± 0.4[a]	1.9 ± 0.5[a]
3	70.6 ± 6.6[a]	63.9 ± 7.4[a]	0.89 ± 0.03[a]	0.88 ± 0.05[a]	2.2 ± 0.5[a]	1.8 ± 0.4[b]
4	60.6 ± 0.0[a]	64.3 ± 11.3[a]	0.90 ± 0.00[a]	0.87 ± 0.06[a]	2.0 ± 0.4[a]	1.8 ± 0.5[b]

*Ovarian diameter : Body wall diameter

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.

Groups of nine, 2-year-old female channel catfish broodstock obtained from each of 4 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.

For all strains, mean plasma estradiol concentrations ranged from 0.02 to 0.29 ng/mL from June through December, and increased dramatically in January, peaking in February (3.4 to 3.7 ng/mL), and remained above 1.00 ng/mL through May. Mean plasma testosterone concentrations increased from May through September (0.03 to 1.23 ng/mL), decreased in October, and then increased and remained at approximately 1 ng/mL through April. 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. 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 1.0-1.4 mm, and remained at this size until May and June when size increased by approximately 75 to 100%.

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 3d. *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-ì m 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 ($y = 2^{-9}x + 1.199$, $R^2 = 0.531$). 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. 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. The sperm solutions were poured through a 100-Fm 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 x 10^8, 1 x 10^7 and 1 x 10^6 sperm cells / mL and were used for fertilization of during artificial spawning with eggs from two females and sperm from 3 males (0.5 mL / 400 eggs). The sperm concentration of 1 x 10^6 yielded 71 ± 16% fertilization for fresh sperm (3 ± 5% for thawed sperm); 1 x 10^7 yielded 88 ± 9% fertilization for fresh sperm (45 ± 37% for thawed), and 1 x 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.

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 submasses. 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 ($P > 0.05$) improved with chemotherapeutic 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 ($P<0.05$) decrease in percent hatch was observed in eggs treated with 500 ppm hydrogen peroxide (Figure 2).

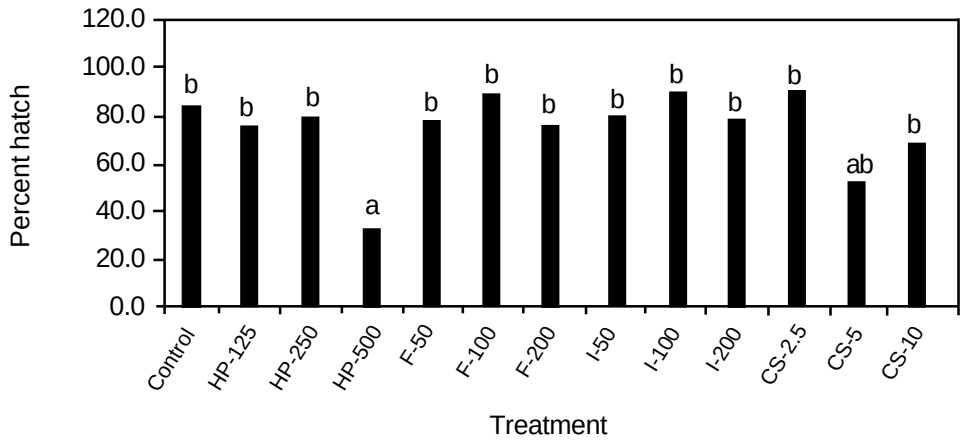

Figure 2. Mean hatching success (%) of hybrid catfish eggs treated daily with increasing doses of hydrogen peroxide (HP), formalin (F), povidone iodine (I), or copper sulfate (CS). Numerals refer to dosage rate (ppm). Bars with common letters are not statistically different.

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 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 8).

Table 8. Effect of daily formalin treatment frequency on hybrid hatching success.

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

[abc]Means having different superscript 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 28EC, 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 ($P<0.05$) hatching success. Withholding treatments until 46 hours post-fertilization at 28EC yielded the greatest ($P< 0.05$) percent hatch (Table 9).

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 28EC, 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.*

Table 9. 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 28EC.

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

[abc]Means having different superscript 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. 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

Hatch rate of hybrid embryos is improved if channel catfish females are stripped within 2 hours of first observation of egg release. 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.

The frequency of formalin treatments should be 3X per day to maximize hatch rate of hybrid embryos and 4 treatments per day is excessive. At 28EC, hybrid embryos are chemically sensitive to formalin between 42-46 hours post-fertilization, and formalin treatments should be avoided during this period to maximize hatch rate.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

Doctoral Dissertations

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

Presentations

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

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

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

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

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

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

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

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

Hutson A., Y. Zohar, E. Abraham, A. Kristanto, J. Ballenger, M. Templeton, G. Umali, D. Beam, F. Wang, C. Ligeon, P. Waters,Jr., Z. Liu and R. A. Dunham. 2005. Evaluation of LHRHa delivered via liquid injections or implants for

the production of channel-blue hybrid catfish embryos. Special Session-Hybrid Catfish Reproduction. World Aquaculture Society Meeting. New Orleans, LA. January 2005.

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

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

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

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

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

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

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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	1	50,000	43,950	-0-	-0-	-0-	43,950	93,950
Computer Software	2	60,948	30,737	-0-	-0-	-0-	30,737	91,685
	3	45,900	35,710	-0-	1,000	-0-	36,710	82,610
	4	60,500	41,000	-0-	-0-	-0-	41,000	101,500
	5	67,000	47,000	-0-	-0-	-0-	47,000	114,000
	6	77,358	52,975	-0-	-0-	-0-	52,975	130,333
	7	82,850	43,000	-0-	-0-	-0-	43,000	125,850
	8	77,507	47,000	-0-	-0-	-0-	47,000	124,507
	9	84,500	47,000	-0-	-0-	-0-	47,000	131,500
Total		**606,563**	**388,372**	**-0-**	**1,000**	**-0-**	**389,372**	**995,936**
Development of Improved	1	287,053	218,353	-0-	-0-	-0-	218,353	505,406
Harvesting, Grading and	2	272,391	227,188	-0-	-0-	-0-	227,188	499,579
Transport Technology for	3	190,556	232,823	-0-	-0-	-0-	232,823	423,379
Finfish Aquaculture								
Total		**750,000**	**678,364**	**-0-**	**-0-**	**-0-**	**678,364**	**1,428,364**
Identification, Characterization,	1	224,800	277,901	-0-	-0-	-0-	277,901	502,701
and Evaluation of Mechanisms	2	227,377	285,420	-0-	-0-	-0-	285,420	512,797
of Control of *Bolbophorus*-like	3	146,770	281,926	-0-	-0-	-0-	281,926	428,696
Trematodes and *Flavobacterium*								
columnaris-like Bacteria Causing								
Disease in Warm Water Fish								
Total		**598,947**	**845,247**	**-0-**	**-0-**	**-0-**	**845,247**	**1,444,194**
Improving Reproductive	1	118,390	86,891	-0-	-0-	-0-	86,891	205,281
Efficiency to Produce Channel ×	2	111,610	81,845	-0-	-0-	-0-	81,845	193,455
Blue Hybrid Catfish Fry	3	123,000	70,297	-0-	-0-	-0-	70,297	193,297
	4	123,000	72,121	-0-	-0-	-0-	72,121	195,121
Total		**476,000**	**311,154**	**-0-**	**-0-**	**-0-**	**311,154**	**787,154**

SRAC RESEARCH AND EXTENSION PROJECTS

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

*Project Completed

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

*Project Completed

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

Project	Duration	Funding	Grant No.
*Management of Environmentally-Derived Off-Flavors in Warmwater Fish Ponds. Dr. Tom Hill, University of Tennessee, Principal Investigator	Yr.1-06/01/96-05/31/97	$29,349	93-38500-8393
		34,918	94-38500-0045
		186,560	95-38500-1411
	Total Yr. 1	$250,827	
	Yr. 2-06/01/97-05/31/98	$68,718	94-38500-0045
		97,393	95-38500-1411
		84,031	96-38500-2630
	Total Yr. 2	$250,142	
	Yr. 3-06/1/98-05/31/99	$154,621	96-38500-2630
		74,645	97-38500-4124
	Total Yr. 3	$229,266	
	Yr. 4-06/01/99-05/31/00	$80,900	98-38500-5865
	Yr. 5-06/01/00-05/31/01	$55,146	99-38500-7375
	Project Total	**$866,281**	
*National Aquaculture Extension Conference (In cooperation with other Regional Aquaculture Centers)	01/01/97-12/31/97	$3,392	93-38500-8393
		308	95-38500-1411
	Project Total	**$3,700**	
*Verification of Recommended Management Practices for Major Aquatic Species. Dr. Carole Engle, University of Arkansas at Pine Bluff, Principal Investigator	Yr. 1-01/01/97-12/31/97	$31,410	95-38500-1411
	Yr. 2-01/01/98-12/31/98	$7,186	95-38500-1411
		58,928	96-38500-2630
	Total Yr. 2	$66,114	
	Yr. 3-01/01/99-12/31/00	$62,781	99-38500-4124
	Project Total	**$160,305**	
Publications, Videos and Computer Software. Dr. Michael Masser, Texas A&M University, Principal Investigator (Continuing project)	Yr. 1-04/01/95-03/31/96	$50,000	94-38500-0045
	Yr. 2-04/01/96-03/31/97	$13,405	93-38500-8393
		47,543	94-38500-0045
	Total Yr. 2	$60,948	
	Yr. 3-04/01/97-03/31/98	$45,900	96-38500-2630
	Yr. 4-04/01/98-03/31/99	$60,500	97-38500-4124
	Yr. 5-04/01/99-03/31/00	$67,000	98-38500-5865
	Yr. 6-07/01/00-06/30/01	$77,358	00-38500-8992
	Yr.7-07/01/01-06/30/02	$82,850	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
	Project Total	**$606,564**	

*Project Completed

Project	Duration	Funding	Grant No.
*Control of Blue-green Algae in Aquaculture Ponds. Dr. Larry Wilson, University of Tennessee, Principal Investigator	Yr. 1-01/01/99-12/31/99	$25,147	96-38500-2630
		105,167	97-38500-4124
		177,260	98-38500-5865
	Total Yr. 1	$307,574	
	Yr. 2-01/01/00-12/31/00	$975	96-38500-2630
		17,394	97-38500-4124
		158,608	98-38500-5865
		98,993	99-38500-7375
	Total Yr. 2	$275,970	
	Yr. 3-01/01/01-12/31/01	$26,186	97-38500-4124
		7,202	98-38500-5865
		188,550	99-38500-7375
		24,277	00-38500-8992
	Total Yr. 3	$246,215	
	Project Total	**$829,759**	
*Management of Aquacultural Effluents from Ponds. Dr. John Hargreaves, Mississippi State University, Principal Investigator	Yr. 1-04/01/99-03/31/00	$100,000	97-38500-4124
		127,597	98-38500-5865
	Total Yr. 1	$227,597	
	Yr. 2-04/01/00-03/31/01	$221,146	99-38500-7375
	Yr. 3-04/01/01-03/31/02	$106,610	2000-38500-8992
	Project Total	**$553,353**	
Development of Improved Harvesting, Grading and Transport Technology for Finfish Aquaculture. Dr. Ed Robinson, Mississippi State University, Principal Investigator	Yr. 1-01/01/01-12/31/01	$287,053	00-38500-8992
	Yr. 2-01/01/02-12/31/02	$14,259	98-38500-5865
		39,720	99-38500-5865
		14,757	00-38500-8992
		203,655	01-38500-10307
	Total Yr. 2	$272,391	
	Yr. 3-01/01/03-12/31/03	$15,000	00-38500-8992
		175,556	01-38500-10307
	Total Yr. 3	$190,556	2001-38500-10307
	Project Total	**$750,000**	
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. 2-03/01-04-02/28/2005	$58,160	2000-38500-8992
		9,378	2001-38500-10307
		159,839	2002-38500-11805
	Total Yr. 2	$227,377	
	Yr. 3-Projected	$146,770	
	Project Total	**$598,947**	

*Project Completed

Project	Duration	Funding	Grant No.
Improving Reproductive Efficiency to	Yr. 1-03/01/04-02/28/05	$1,000	2001-38500-10307
Produce Channel × Blue Hybrid Catfish Fry.		117,390	2002-38500-11805
Dr. Rex Dunham, Auburn University,	Total Yr. 1	$118,390	
Principal Investigator	Yr. 2 -Projected	$111,610	
	Yr. 3-Projected	$123,000	
	Yr. 4-Projected	$123,000	
	Project Total	**$476,000**	
Innovative Technologies and Methodologies	Yr.1-08/01/04-07/31/05	$1,053	2000-38500-8992
for Commercial-Scale Pond Aquaculture.		167,433	2002-38500-11805
Dr. Claude Boyd, Auburn University,		146,209	2003-38500-12997
Principal Investigator	Total Yr. 1	$314,695	
	Yr. 2-Projected	$287,451	
	Yr.3-Projected	$123,168	
	Yr.4-Projected	$170,096	
	Project Total	**$895,410**	