SOUTHERN REGIONAL AQUACULTURE CENTER

SIXTEENTH ANNUAL PROGRESS REPORT

For the Period Through August 31, 2003

December, 2003

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

Work summarized in this report was supported in part by Grant Nos. 98-38500-5865, 99-38500-7375, 00-38500-8992, 2001-38500-10307, 2002-38500-11805 and 2003-38500-12997 from the United States Department of Agriculture sponsored by the Cooperative State Research, Education, and Extension Service.

SIXTEENTH 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 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, 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 Sixteenth 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 in 2002 exceeded $1 billion dollars, with nearly 70% of the total production occurring in the southeastern states. Aquaculture has become one of the stars 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, so the total economic impact of aquaculture is 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.

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

Although government agencies, particularly the United States Department of Agriculture, have provided significant support for aquaculture research and development, much of that funding is earmarked for specific uses 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.

One measure of the productivity of the Regional Aquaculture Center program is the hundreds of high-quality, peer-reviewed scientific articles, graduate theses, and technical papers that have been generated since program inception in 1987. We are particularly proud of the work conducted by scientists in the southern region, as exemplified by the work summarized in this Annual Report.

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 not complete, two products 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.

Research to address the impact of aquaculture on the environment—including work in the "Effluents" project reported here—has been critical in recent regulatory activities. In September 2002, the United States Environmental Protection Agency published proposed regulations for aquaculture effluents. The proposal excludes pond culture systems from regulation, which means that most aquaculture producers in the south will not be saddled with costly or ineffective regulations. This good news is, in large part, the result of EPA's analysis of data derived from projects organized and supported by the Southern Regional Aquaculture Center. The scientists and farmers who developed the Center's aquacultural effluents projects in 1991, 1996, and 1999 showed remarkable foresight by anticipating the need for credible scientific information to support reasonable decisions on environmental regulation.

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 Sixteenth 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 the Southern Regional Aquaculture Center during this reporting period cover the life of the projects from their initiation date through August 31, 2003.

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
Ivory Lyles, Arkansas Cooperative Extension Service
David Morrison, Louisiana State University
Joe McGilberry, Mississippi State University Extension Service
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
J. Neal Anderson, AR
James Bardsley, GA
Richard Eager, SC
James P. Ekstrom, TX
J. B. Hanks, LA
R. C. Hunt, NC
Austin Jones, MS
Joey Lowery, AR
Robert Mayo, NC
Bryan P. Plemmons, VA
Steve Price, KY
Marty Tanner, FL
Rafe Taylor, 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
Allen Davis, AL
Delbert Gatlin, TX
Andrew Goodwin, AR
John Hargreaves, LA
Rebecca Lochmann, AR
Ray McClain, LA
Steve Mims, KY
Craig Watson, FL
J. L. Wilson, TN

Members of the TC for Extension are:

Jimmy Avery, MS
Jerry Crews, AL
Dennis DeLong, NC
David Heikes, AR
Tom Hill, TN
Jeff Hinshaw, NC
Greg Lutz, LA
Mike Masser, TX
Brian Nerrie, VA
Nathan Stone, AR
Jack Whetstone, SC
Forrest Wynne, KY

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

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

PROJECT CRITERIA

Projects developed within SRAC should meet the following criteria:

- Addresses a problem of fundamental importance to aquaculture in the Southern Region;
- Involves participation by two or more states in the Southern Region;
- Requires more scientific manpower, equipment, and facilities than generally available at one location;
- Approach is adaptable and particularly suitable for inter-institutional cooperation, resulting in better use of limited resources and a saving of funds;
- Will complement and enhance ongoing extension and research activities by participants, as well as offer potential for expanding these programs;
- Is likely to attract additional support for the work which is not likely to occur through other programs and mechanisms;
- Is sufficiently specific to promise significant accomplishments in a reasonable period of time (usually up to 3 years);

PROJECT DEVELOPMENT PROCEDURES

The IAC initiates the project development process by identifying critical problems facing aquaculture in the region. The TC and IAC then jointly prioritize problem areas and recommend the most important research and extension needs to the Board 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 .... Page 10

Management of Aquacultural Effluents from Ponds .... Page 15

Development of Improved Harvesting, Grading and
Transport Technology for Finfish Aquaculture .... Page 33

Identification, Characterization, and Evaluation of Mechanisms
of Control of *Bolbophorus*-like Trematodes and *Flavobacterium*
columnaris-like Bacteria Causing Disease in Warm Water Fish .... Page 47

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	77,883
	Year 7	83,850
	Year 8	77,600
	Total	$523,681

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

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

PROJECT OBJECTIVES

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

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

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

ANTICIPATED BENEFITS

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

Extension Specialists. When this project was initiated, fewer than half the states had educational materials covering the major aquacultural species in their state. The concept of using the SRAC program to produce timely, high-quality educational materials is based upon the benefit of utilizing a region-wide pool of expertise to develop materials for distribution through the nationwide network of Extension Specialists and County Agents. This process makes efficient use of personnel at the State level, and results in high-quality educational materials that are readily available to scientists, educators, producers, and the general public.

Educators. Several colleges and universities in the United States use SRAC technical fact sheets as reference material in aquaculture and fisheries courses.

> **Results at a glance...**
>
> ☆ *123 authors from across the United States have contributed to SRAC's publication projects.*

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.

> **Results at a glance...**
>
> *Titles of some recent SRAC publications:*
>
> ☆ *Aquatic Weed Management – Herbicide Technology and Application Techniques*
> ☆ *Saprolegniasis (Winter Fungus) and Branchiomycosis of Commercially Cultured Channel Catfish*
> ☆ *Cost Economics of Smallscale Catfish Production*
> ☆ *Species Profile: Sturgeon*
> ☆ *Aquaculture Food Safety – Residues*

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.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

During this current project year, six new fact sheets, one species profile and one comprehensive CD were written. All have been distributed throughout the Southern Region and to interested Extension Specialists in other regions. Approximately seventeen fact sheets and a video are currently in some stage of writing, production, or revision.

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, five fact sheets will be revised and seven new fact sheets, including one species profile, will be produced. The new fact sheets will address (1) economics of freshwater prawn production, (2) catfish yield verification, (3) control of blue-green algae, (4) trout diseases (particularly or only viral diseases), (5) best management practices for pond aquaculture, (6) disease management in catfish hatcheries, and (7) a species profile for cobia.

Five fact sheets will be revised on the following topics: (1) production of freshwater prawns in ponds, (2) avian predators #401, (3) forage species #140, (4) forage species #141, and (5) forage species #142.

IMPACTS

This is a highly productive project with significant regional and national impact. Fact sheets and videos are requested and used by clientele in all 50 states on a regular basis. Within the Southern Region, more than 80 fact sheets and six videos are distributed on request daily. Fact sheets generated within the Southern Region are also widely distributed by RACs and extension personnel in other regions. An average of 5 to 20 SRAC fact sheets and 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 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

Results at a glance...

- ☆ *Seven fact sheets and a comprehensive CD were completed this year with17 fact sheets in progress.*
- ☆ *Fourteen scientists from across the Southern Region contributed to publications produced by SRAC this year.*
- ☆ *SRAC has now published 161 fact sheets, 17 research publications, and 19 videos.*
- ☆ *Educators in schools and colleges use SRAC publications in classrooms throughout the U.S. and the world.*

several colleges and universities also use SRAC technical fact sheets as part of the reference material used in the course.

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

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

Results at a glance...

☆ *All fact sheets completed by this project to date are available on the Internet at <http://www.msstate.edu/dept/srac>.*

PUBLICATIONS, MANUSCRIPTS OR PAPERS PRESENTED

Fact Sheets Completed (7/1/02 - 8/31/2003)

Avery, Jimmy. Aquatic Weed Management – Herbicide Technology and Application Techniques. SRAC Fact Sheet 3601.
Durborow, Robert and David Wise. Saprolegniasis (Winter Fungus) and Branchiomycosis of Commercially Cultured Channel Catfish. SRAC Fact Sheet 4700.
Engle, Carole and Nathan Stone. Cost Economics of Smallscale Catfish Production. SRAC Fact Sheet 1800.
Hargreaves, John. Pond Mixing. SRAC Fact Sheet 4700.
Mims, Steven and Andy Lazur. Species Profile: Sturgeon. SRAC Species Profile 7200.
Santerre, Charles R. and George W. Lewis. Aquaculture Food Safety – Residues. SRAC Final Project Report 6001.
Terhune, Jeffery S., David J. Wise, Jimmy L. Avery and Lester H. Khoo. Infestations of the Trematode *Balbophorus* sp. in Channel Catfish. SRAC Fact Sheet 1801.

Computer Software

Masser, Michael P. CD of all SRAC Fact Sheets and Species Profiles.

Manuscripts in review

Brune, David. Partitioned Aquaculture Systems.
Camus, Alvin. Channel Catfish Virus Disease.
Durborow, Robert and David Wise. Common Catfish Parasites.
Kelly, Anita M. Channel Catfish Broodfish Management.
Ludwig, Gerald. Hybrid Striped Bass Fingerling Production. SRAC Fact Sheet 302 (Revision).
Romaire, Robert, W. Ray McClain and C. Greg Lutz. Crawfish Aquaculture – Harvesting. SRAC Fact Sheet 242 (Revision).
Wallace, Rick. Growing Bull Minnows.

Manuscripts in preparation

Avery, Jimmy and Jim Steeby. Channel Catfish Fingerling Production.
D'Abramo, Louis. Intensive Culture of Crawfish.
Durborow, Robert. Fish Anesthetics.
Hargreaves, John and Craig Tucker. Copper Use in Aquaculture.
Hargreaves, John. Ammonia in Fish Ponds (Revision).
Hinshaw, Jeff and Anita Kelly. Species Profile: Yellow Perch.
Rakocy, Jim, Michael Masser and John Hargreaves. Aquaponics.
Romaire, Robert and Ray McClain. Crawfish Marketing.
Rosati, Ronald. Building Simple Recirculating Systems for the Classroom.
Tucker, Craig S. Pond Aeration (Revision).

Videos in preparation

Durborow, Robert and Jim Tidwell. Culture of Freshwater Shrimp.

On-going project

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

◆ ◆ ◆

MANAGEMENT OF AQUACULTURAL EFFLUENTS FROM PONDS

Reporting Period
April 1, 1999 to August 31, 2003

Funding Level	Year 1	$227,597
	Year 2	222,146
	Year 3	106,610
	Total	$555,353

Participants	Mississippi State University (Lead Institution)	John Hargreaves, Thomas Cathcart
	Auburn University	Claude Boyd
	University of Arkansas at Pine Bluff	Carole Engle, Nathan Stone
	Louisiana State University	Robert Romaire, Ray McClain
	North Carolina State University	Harry Daniels
	Virginia Polytechnic Institute and State University	Greg Boardman
	Waddell Mariculture Center	Craig Browdy

Administrative Advisor
Dr. Marty Fuller, Associate Director
Mississippi Agricultural and Forestry Experiment Station
Mississippi State University
Mississippi State, Mississippi 39762

PROJECT OBJECTIVES

1. Develop additional information to characterize the components of aquaculture effluents that represent the greatest potential risk of deleterious environmental impact (e.g. suspended solids, total phosphorus).

2. Evaluate the impact of aquaculture pond effluent discharge on receiving stream water quality.

3. Evaluate a range of water management techniques appropriate for ponds as a means of reducing the quantity and improving the quality of discharged water.

4. Develop and evaluate models for predicting risks to the environment and the costs and benefits of implementing Best Management Practices (BMPs).

5. Based upon existing information, supplemented by project findings, develop a comprehensive set of BMPs that can be implemented to reduce the environmental impacts

of pond aquaculture in general. Develop supplemental BMPs particular to the various pond cultured species in the region that will complement the generic, pond-system BMPs. These BMPs would include best culture practices, waste handling and management, and water quality management and reuse.

6. Convene a series of workshops to educate and inform producers and regulators on the characteristics and management of aquaculture effluents from ponds, including BMPs, based on the best available information and that minimize environmental impact and satisfy regulatory compliance requirements.

ANTICIPATED BENEFITS

Results of this project will provide simple management alternatives to reduce the volume and improve the quality of effluents, possibilities for water reuse, and inexpensive treatment methods based on sedimentation. This project will provide beneficial effluent management practices to producers of channel catfish, striped bass, baitfish, crawfish, and marine shrimp. Development of practical, environmentally sound management practices that minimize the effect of pond effluents on receiving streams will reduce the environmental impact and contribute to the sustainability of the regional aquaculture industry. Information generated by this project can be used by regulators and permit writers to provide effective and coherent regulation of aquaculture effluents.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Objective 1. *Develop additional information to characterize the components of aquaculture effluents that represent the greatest potential risk of deleterious environmental impact (e.g. suspended solids, total phosphorus).*

Auburn University. About 53% of total suspended solids, total phosphorus, total nitrogen, and biochemical oxygen demand are associated with particles less than 5 micrometers in diameter. A water retention time in settling basins of 8 hours will improve effluent quality significantly and a settling time of 2 to 4 hours is sufficient to reduce total suspended solids in effluents to 75 to 90% of original concentrations. Solids removal is associated with declines in the mineral fraction with little change in organic solids concentrations in effluents. Application of aluminum sulfate at 25 to 50 ppm does not improve the efficiency of solids.

Estimates of runoff from watersheds suggest that settling basins to treat storm runoff from watershed-type catfish ponds require volumes of 30 to 40% of pond volume in order to provide a retention time of 8 hours. Thus, because of the large volume required, settling basins do not appear to be feasible for treating storm runoff. Settling basins for treating intentional discharge from partial drawdown or complete draining would need to be only 10 to 20% of the volume of the largest pond on the farm because the quality of catfish pond effluents is relatively high except for the final 20 to 25% of water released when ponds are drained completely.

Most existing catfish farms in Alabama extend to property lines or streams, and there seldom is space for installing settling basins. However, settling basins could be considered an essential component in the design of new farms. On farms without settling basins, it is possible to use the pond being harvested as its own settling basin. Water levels should be lowered to 20 to 25% of full volume, drains should be closed and fish harvested by seining. Once fish have been removed, the water should be allowed to stand until most of the suspended solids have settled, usually within 2 to 3 days. The water should then be released slowly to prevent resuspension of solids. It is recommended that the valve only be opened to 25% of its maximum capacity during final draining. The valve should be closed at the beginning of rainfall and not reopened until water has cleared.

Results at a glance...

- ✰ *Treatment of the final 20% of water discharged when ponds are drained can be accomplished with sedimentation basins designed with a hydraulic retention time of 8 hours. However, a settling time of 2 to 4 hours is sufficient to reduce total suspended solids in effluents to 75 to 90% of original concentrations.*

Alabama channel catfish farmers try to maintain chloride concentrations of 50 to 100 mg/L in ponds by annual salt applications. The average and standard deviation for chloride concentration in salt-treated ponds was 87.2 ± 37.5 mg/L. The maximum chloride concentration was 189 mg/L. The maximum limit for chloride concentration in Alabama streams allowed by the Alabama Department of Environmental Management is 230 mg/L. It is unlikely that effluents from salt-treated catfish ponds would violate the in-stream chloride standard of 230 mg/L or harm aquatic life in streams. Nevertheless, chloride concentrations in ponds should be measured before salt application as a safeguard against excessive salt application and chloride concentrations above the in-stream chloride standard.

Mississippi State University. In commercial channel catfish ponds dominated by a dense bloom of the blue-green alga *Oscillatoria agardhii*, about 51% of total suspended solids have a diameter of less than 5 micrometers. Similar results are obtained from fractionation of solids in water collected from ponds dominated by inorganic turbidity (55% of the total suspended solids are less than 5 micrometers). Treatment of water collected from a pond dominated by a dense bloom of *O. agardhii* with alum between 0 and 50 ppm results in a solids reduction rate of 1.11 grams of solids per gram of alum. Size fractionation of pond water following alum treatment indicates that the proportional removal of solids less than 5 micrometers was greatest, although the greatest absolute solids reduction rate occurs in whole (not fractionated) pond water. The proportion of solids in the smallest size fractions increases with alum dose, suggesting that larger solids are selectively settled by alum treatment.

North Carolina State University. Fractionation of solids in overflow water from hybrid striped bass ponds shows that approximately 95% of total solids and 65% of total suspended solids are less than 5 micrometers in diameter. More than 80% of biochemical oxygen demand, total phosphorus and total nitrogen in pond water is associated with particles less than 5 micrometers. More than 90% of the solids in the pond water overflow are dissolved and about 25% of total solids are volatile. Suspended solids, which account for 10% of total solids, are approximately 63% volatile, with solids in the smaller particle fractions more volatile than in the larger fractions. The amount of settleable solids in hybrid striped bass pond water overflow is negligible, suggesting that sedimentation alone, without the use of chemical amendments to coagulate and precipitate solids, would not be an effective treatment of this fraction of pond water.

Effluents from the initial and final stages of pond draining collected and characterized during pond harvest indicate that total suspended solids concentrations are 40% higher in the final 10% of water drained from the ponds compared to the first 10%. This increase in total suspended solids is primarily from small particles of the mineral fraction of the pond sediment. Where almost all of the suspended solids in the first 10% of water drained are volatile, only 60% of the suspended solids in the final water are volatile. Because total suspended solids account for only approximately 10% of total solids in these waters, the concentration of total solids is only slightly higher (less than 8%) in effluent from the final stages of pond draining than in the initial stages. Concentrations of total phosphorus and total nitrogen in the effluent do not increase significantly from the beginning of pond draining to the end.

University of Arkansas at Pine Bluff. A field study characterized suspended solids and 5-day biochemical oxygen demand (BOD_5) in baitfish pond effluents by serial fractionation, and evaluated exiting drainage ditches as possible solids settling systems. Total suspended solids (TSS) concentration in effluents at the point of discharge from ten ponds on five farms averaged 36 ppm during the draining of the first 10% of pond volume and increase by approximately 70% during the last 10% of the effluent volume. Volatile suspended solids (VSS) concentration is more variable but average about 50% of TSS. Organic matter (BOD_5) concentration averages 9.0 ppm and does not increase significantly in the last 10% of effluent volume. Screening through a 10-micrometer mesh removes more mineral than organic solids, and reductions in TSS, although small, are statistically significant. Volatile suspended solids and BOD_5 generally do not change with reductions in TSS, except small but significant reductions in the first 10% of effluent after screening through a 41-micrometer mesh. Based on averages, 75 to 82% of suspended particles are less than 5-micrometers and smaller particles are more organic. The last 10% of effluent is characterized by smaller particles than the first 10% of effluent volume. Volatile suspended solids concentration in the last 10% of effluent had a stronger correlation ($r = 0.91$) with BOD_5 than the first 10% ($r = 0.71$). Total suspended solids concentration is weakly correlated with BOD_5 ($r = 0.55$ in the first 10% and 0.71 in the last 10%).

Louisiana State University. Water budgets calculated over three production seasons in experimental crawfish ponds indicate that the average annual water requirement is 7.5 feet. More than 71% of intentional water inflow over the three production seasons replaces losses from evaporation, evapotranspiration by rice planted as crawfish forage, and seepage, with intentional and unintentional effluent release accounting for the remainder. Precipitation is a significant water inflow in these shallow-water ecosystems, contributing nearly 40% of the total over three production seasons. Intentional water discharge during summer drawdown (an established management practice) is consistent, averaging 10.8 inches. Unintentional discharge is highly correlated with the magnitude of precipitation events. Precipitation from October to June in year one (25.1 inches) and year three (27.2 inches) resulted in very low unscheduled water release (3.6 and 2.5 inches, respectively). Unintentional water release in the second production season, representative of normal precipitation patterns in southern Louisiana (53.6 inches) was 17.7 inches (24% of annual water budget).

A water budget model was developed to determine seasonal effluent quantity and mass loading for solids and nutrients representative of crawfish production systems in south-central and southwest Louisiana. Precipitation, evaporation, evapotranspiration, and infiltration data for southern Louisiana was obtained from the National Climatic Data Center and published research. Model output closely agreed with annual water budgets calculated from the experimental crawfish ponds over three crawfish production seasons, with an average deviation of 11% (range: 3 to 25%). Unintentional and intentional effluent discharge and seasonal mass loading for TSS, TP, TN, and $CBOD_5$ were determined under various simulation scenarios which include type of crawfish production system (permanent, rice-crawfish double-cropping, and rice-crawfish rotational system), annual precipitation (average, wet, and dry years), added pond storage capacity (0, 2, 4, and 6 inches), and intentional flushing to improve water quality (1, 2, 3 exchanges per year). Most water additions from pumping replace losses from evaporation, evapotranspiration, and seepage (usually 60% or higher). Unintentional discharge, largely from precipitation, usually ranged from 13% in an average year to as much as 30% in a high rainfall year, with most of the discharge occurring in winter. Seasonal mass loading (pounds/acre) for single-crop crawfish production systems were as follows: TSS, 848; TP, 1.99; TN, 10.6; and $CBOD_5$, 18.5. Eighty-nine percent of TSS and 70% of TP, TN, and $CBOD_5$ released from experimental crawfish ponds during summer drawdown occur with the first 5% and last 20% of water released.

Waddell Mariculture Center. Settling of the last 20 to 30% of shrimp pond drainage often, but not always, reduces total nitrogen and total phosphorus. Reductions are sometimes as great as 80% of initial levels, and are typically only significant during the first hour of sedimentation. Proportional reductions in total nitrogen and total phosphorus are not as great as proportional reductions in suspended solids, turbidity, and BOD_5. Reductions in turbidity and suspended solids are similar. Reductions in suspended solids during the first hour of settling are greater than reductions that occur during the subsequent 19 hours. In high-density ponds (i.e., stocked with 100 postlarvae/m^2), settling during the first 30 minutes reduces turbidity by 45 to 92%. In lower-density ponds (i.e., stocked with 10 to 50 postlarvae/m^2), settling during the first 30 minutes reduces turbidity by 5 to 49%. Planktonic algae is the primary component of turbidity in effluent and is only modestly affected by sedimentation. Although there is a tendency for high-density ponds to have greater initial levels of suspended solids, turbidity, and BOD_5, and greater reductions (both in magnitude and proportion) in these parameters during relatively short sedimentation periods than lower-density ponds, there is substantial overlap between these groups. The findings preclude firm statements regarding the benefit of sedimentation based solely upon shrimp stocking density.

Initial levels and the effect of sedimentation on suspended solids, turbidity, and BOD_5 vary greatly among ponds, regardless of pond location or stocking density. Factors that contribute to this high variability include those that affect sediment resuspension during harvest, in particular, sludge pile size and location with respect to drainage flow, slope of the pond bottom, operation of aerators during harvest, and shrimp activity. Sedimentation prior to water release may be particularly beneficial when pond harvest strategies that encourage the flushing of sludge are employed.

Objective 2. *Evaluate the impact of aquaculture pond effluent discharge on receiving stream water quality.*

Mississippi State University. Seventeen stream sample locations were selected and geo-referenced within four sub-watersheds with variable development of the landscape to aquaculture ponds. Stream sampling occurred during base flow conditions and during the only significant runoff event that occurred during spring 2000. Despite elevated stream flow during this event, few ponds were observed to be discharging water. No changes in stream water quality could be attributed to aquaculture ponds. In some stream reaches, the magnitude of in-stream variation in water quality over short distances was large.

A study was conducted to evaluate the duration and settling characteristics of initial pond effluent from levee ponds. Settling characteristics of effluent samples collected at the discharge point were determined. In addition, spatial and temporal variation of water quality in channels receiving the effluent was measured. When draining was initiated, shear forces generated by water moving into the drain pipe caused scouring in a zone around the entrance to the drain pipe. The initial flush of discharged water consisted of pond water and a slurry of sediment that had accumulated over the screen inside the pond. The initial discharge was very high in total suspended solids (43,860 mg/L), volatile suspended solids (3,770 mg/L), BOD_5 (118 mg/L), and nutrients. Within 2.5 minutes of the initial discharge, total suspended solids decreased to 1,790 mg/L, volatile suspended solids to 205 mg/L, and BOD_5 to 29 mg/L. By 30 minutes, the poorly consolidated sediment from around the drain structure inside the pond had been discharged, and the effluent quality was identical to the bulk pond water for the remainder of the draining period (approximately 5 days). For the effluent, 15.2% of the total solids were discharged with the first 1.7% of the total volume discharged. The initial solids concentration was 900 times higher than the solids concentration in the bulk pond water. In contrast, the BOD_5 of the initial flush of water discharged was only about nine times higher than the BOD_5 of the bulk pond water and concentrations decreased more rapidly than total suspended solids. Therefore, cumulative discharge of BOD_5 as a function of water volume discharged did not have an initial spike of similar magnitude as for total suspended solids; rather, cumulative discharge of BOD_5 was roughly proportional to the volume of water discharged. These results suggest that the ratio of mineral solids to organic matter of the initial flush of water was relatively higher than that of subsequently discharged water.

The median settling velocity decreased from 0.3 cm/second in the initial effluent to 0.06 cm/second after 30 minutes. Total suspended solids in a ditch 100 meters from a catfish pond outfall increased to a maximum 30 minutes following the initiation of pond discharge, but then decreased to concentrations that were less than or equal to initial ditch concentrations. The duration of poor water quality of initial effluent from catfish ponds with internal drains is brief (less than 30 minutes) and discharged solids settle rapidly within receiving ditches.

University of Arkansas at Pine Bluff. A field study evaluated exiting drainage ditches for solids settling. Surveyed ditches ranged from 1,050 to 2,300 feet in length and from 12 and 39 feet in width. Study ditches included vegetated and non-vegetated types. Volatile suspended solids (VSS) decreased by 14.1% over the first 100 meters (110 yards) of ditch. Total suspended solids (TSS) remained unchanged because of increases in fixed suspended solids (FSS). Current velocity in the ditch explained up to 65% of the variability in change in solids. There was a net increase in TSS when average velocity exceeded 2 feet/second. Fractionation may have affected the character of organic particles by breaking large particles into fine particles.

Additional work was conducted to characterize nutrient concentrations in baitfish pond effluents and receiving streams. Ten baitfish ponds were sampled and characterized from December 2000 through June 2001 in the central Arkansas Delta ecoregion. Effluent samples taken during the first and last 10% of pond drainage volume were analyzed for total nitrogen, total phosphorus, BOD, and TSS concentrations. Pond drainage ditches were sampled along the ditch length to determine overall reduction of TSS, and ditch water quality was sampled and analyzed prior to stream discharge. Upstream samples were collected concurrently with pond and ditch samples and were analyzed for similar nutrient concentration and physical characteristics. There were no significant differences in effluent quality between the first 10% and the last 10% of effluent volume, except the last 10% had significantly higher TSS concentrations than the first 10%. There was no significant difference in nutrient concentrations in effluents sampled at the standpipe and effluents sampled at the end of drainage ditches. Filtering effluents through a 5-micron mesh screen did not significantly reduce nutrient concentrations. There was also no significant difference in standpipe effluent concentrations versus those found upstream of the discharge point. In general, concentrations of measured parameters in commercial baitfish pond effluents were lower than or similar to those reported for commercial catfish ponds during the same seasons.

Louisiana State University. Drainage ditches were evaluated for sedimentation of solids, nutrients, and organic matter discharged during final summer drawdown (maximum mass loading) from experimental crawfish ponds. In an initial study, the TSS were reduced over a distance of 800 feet from 28% in a wide, shallow, non-vegetated ditch to 80% in a narrow, deep heavily vegetated ditch, prior to entering a receiving stream. In contrast, TSS increased 15% over a distance of 800 feet when effluent was discharged into a shallow, narrow, non-vegetated ditch. In a subsequent study, the TSS were reduced 79% over a distance of 800 feet, and 93% at 2,400 feet prior to entering the receiving stream when effluent was discharged through a heavily vegetated drainage canal (filter strip). Concomitant reductions in TP, TN, and $CBOD_5$ ranged from 26% to 77%.

Waddell Mariculture Center. Water quality in pond drainage after passage through a discharge canal indicates that total suspended solids, turbidity, and BOD_5 at the farm discharge are at levels similar to pond effluent after 20 hours of sedimentation. This suggests that significant water quality improvements occur during passage through the drainage canal from the pond to the facility outfall.

Objective 3. *Evaluate a range of water management techniques appropriate for ponds as means of reducing the quantity and improving the quality of discharged water.*

Mississippi State University. A study was conducted over 3 years to evaluate the accuracy of a mathematical model used to predict performance of a management strategy to reduce pond effluent volume and groundwater use requirements. The strategy consisted of increasing the water storage capacity (depth) of one pond by 1 foot in an interconnected 2-pond or 4-pond module. Tested pond system configurations included three conventional production ponds linked to one production-storage pond; one conventional production pond linked to one production-storage pond; and one conventional (control) pond. During the study, effluent release, groundwater use, precipitation, evaporation, and infiltration were monitored to validate the model. Additionally, ponds were stocked at commercial rates and water quality (dissolved oxygen, temperature, total ammonia, nitrate, chlorophyll *a*, conductivity, alkalinity, and hardness) and occurrences of disease (proliferative gill disease, enteric septicemia of catfish) were monitored to determine whether the management strategy had unintended consequences.

Results at a glance...

☆ *Effluent volume can be reduced by increasing pond depth to increase rainwater storage capacity and linking the combined production-storage pond to one or three adjacent conventional ponds. Effluent volume was reduced by more than 50% and groundwater consumption was reduced by more than 40% compared to conventionally managed ponds. Linking ponds and reusing stored water has not affected fish growth, occurrence of diseases, or water quality.*

The study period encompassed years that were both drier and wetter than normal. There was good agreement between the model and actual pond performance, indicating that the model has utility for a broad range of precipitation/evaporation regimes. Significant reductions in effluent release and groundwater use can be achieved using this approach. Harvest size, water quality, and occurrence of disease indicate no negative consequences of linking ponds.

During 2000, groundwater use in the 1:1 system was about 26% less than in the control pond. In the 3:1 system, groundwater use was about 29% less than in the control pond. This represented a water savings of approximately 24 cm and 27 cm, respectively. During 2001, groundwater use in the 1:1 system was about 58% less than in the control pond. In the 3:1 system, groundwater use was about 45% less than in the control pond. This represented a water savings of approximately 34 cm and 27 cm, respectively.

The groundwater use data appear consistent with site precipitation. Although both 2000 and 2001 had total precipitation values somewhat greater than the 30-year site average, the critical period (May-October) showed marked differences in the 2 years. The 30-year average precipitation for May through October in Stoneville, MS is approximately 55 cm. Values for 2000 and 2001 were 42.8 and 70.6 cm, respectively. The differences were more marked in the critical months of July-August, during which only 1.5 cm of precipitation fell in 2000. This value is only 10% of the 30-year July-August mean for Stoneville (15 cm). During 2001, July-August precipitation was nearly 30 cm, which is about twice the 30-year mean. Although 2000 and 2001 were both somewhat wetter years than normal over 12 months, the warm months of 2001 were much wetter than those of 2000. This difference appeared to largely account for the ground water use differences observed in these years.

During 2000, groundwater use was roughly equivalent in the six treatment ponds. The decreased groundwater requirement of the treatment ponds was probably due to the storage capacity of the production-storage ponds. Once stored water was exhausted (as it was during the dry summer of 2000), groundwater requirements of the six ponds were similar.

During the wetter summer of 2001, the production-storage ponds required no groundwater. In the four treatment production ponds, use of stored rain water accounted for a substantial decrease in groundwater requirements relative to the control pond. In the production-storage ponds, residual stored water, perhaps coupled with other as yet unexplained effects, eliminated groundwater requirements entirely for the year.

The largest task remaining in this project is analysis of the continuous measurements of pond depth and discharge. This task is continuing.

North Carolina State University

Water budgets. A water budget describing water use on a commercial hybrid striped bass farm in North Carolina had been calculated from data collected over a two-year period. The farm has 78 water acres and consists of 18 ponds of approximately 4.5 acres each. All water on the farm is groundwater and no surface water is used. Water is used to fill ponds after draining, to add water to make up for evaporation and seepage, and to flush ponds as needed for water quality reasons. Ponds are usually drained and dried after all fish are harvested, although sometimes scheduling demands require that ponds are immediately restocked after harvest, in which case ponds are not drained between crops. Discharge volume was calculated with a basic water budget equation.

Much of the discharge from the farm occurs during the summer and fall. Twenty-four major discharge events (not due to rainfall) were measured during 2001. There were no large discharges of water during winter (December-March) and most water was discharged between April and August.

On average, the farm discharges 48 inches of water annually.

Fixed-film Filter Media. Two synthetic filter materials were evaluated for their ability to remove suspended solids and nutrients from hybrid striped bass pond effluents. BioStrata is made of black corrugated PVC sheets layered and glued into a honeycomb block form and has a surface area to volume ratio of 110 ft^2/ft^3. A total of 7 ft^3 was used in a tank. Koi brushes are cylindrical brushes 24 inches in length by 4 inches wide. Twelve rows of six brushes each were suspended vertically in the center compartment of a tank. A tank with no baffles and no filter media served as a control. Tanks were rectangular fiberglass (96 inches by 24 inches by 24 inches) and fitted with baffles to contain the filter material and prevent laminar flow. Water was pumped into the tanks at one end, flowed over/through the filter media, and drained from the other end. Standpipes at the drain end of each tank maintained water depth at 24 inches. Three tanks were used in each trial: one containing BioStrata media, one with Pond (or Koi) brushes, and one control tank.

Water was pumped from a 0.25-acre hybrid striped bass grow-out pond stocked at 6,000 fish/acre. Total suspended solids concentration in the pond water during these trials ranged from 63 to 170 mg/L. Most of this turbidity was from suspended clay particles; the pond had very little phytoplankton and chlorophyll *a* concentrations were very low. Water quality was measured at the inflow and outflow of each tank approximately every 3 days during each trial, which lasted between 15 and 22 days. Four hydraulic loading rates ranging from 1.4 to 16.6 gallons per minute were tested during the study, resulting in hydraulic retention times in the filter units between 2.5 hours and 12 minutes, respectively.

Neither filter media was consistently effective in removing solids and nutrients from pond water. The honeycomb BioStrata media reduced concentrations of chlorophyll *a* by 20-40% but usually resulted in increased concentrations of nitrogen, phosphorus, and suspended solids. The brush media removed between 10-30% of nitrogen and less than 5% of solids, but was ineffective in removing phosphorus and chlorophyll *a* from pond water. Because of the very low abundance of phytoplankton in the pond water being filtered, changes in chlorophyll *a* concentrations as result of filtration through media were very small, although they appear large expressed as a proportion of influent concentration. Nutrient and solid removal among sampling dates and within and among trials varied widely. Many times there were no consistent differences in water quality between inflowing and outflowing water. Thus, mean removal rates should be interpreted cautiously.

Solids that accumulated in the tanks during filter-media trials were collected, dried and weighed. The solids were primarily inorganic and were approximately 15% volatile solids. In two trials, brush media removed 3-4 times the weight of solids removed in the control tank, and the honeycomb BioStrata media removed the most solids, 9 to 15 times the amount removed by the control tank. In another trial, the effectiveness of the two media was reversed and the honeycomb BioStrata media removed 4 times the solids as the control, and the brush media removed 15 times the amount removed by the control. The most efficient removal of solids required the filtration of 115 L of pond water to remove 1 g of dried solids (this was using the honeycomb BioStrata media at flow rates of 1.4 gallons per minute); the least efficient required 20 times that volume (brush media at 8.4 gallons per minute).

Water quality. Fish production and water quality in annually drained hybrid striped bass ponds was compared to that in ponds managed with no discharge. Twelve, 0.25-acre ponds were managed according to common commercial practices for hybrid striped bass production. After 3 years, fish production was not significantly different between the two treatments. Although total suspended solids concentration was greater in undrained ponds, there were no other differences in water quality between the two water management regimes.

Turbidity and total suspended solids concentration in pond water were greater and feed consumption and mean weight of fish were lower in ponds that were not dried between crops compared to ponds that were dried and received soil or water amendments. The relative effectiveness of different amendments suggest that applications of anionic polyacrylamide are more effective in reducing turbidity and improving water quality than alum or gypsum when applied to pond water in soil-water mesocosms.

University of Arkansas at Pine Bluff. As a water conservation technique in response to declining aquifer levels, re-use of pond water is growing popular in the Arkansas baitfish industry. Predation of fry by cyclopoid copepods present in re-used water is the greatest challenge to widespread adoption of this practice. A study was conducted to evaluate treatments affecting zooplankton populations so that water can be re-used and the volume of effluent reduced. The aim of this study was to evaluate methods of restarting the zooplankton bloom in pond water held from previous production operations. The abundance and evolution of rotifer and copepod populations in ponds containing old water, old water treated with 0.25 ppm Dylox, and mechanically filtered old water were compared to ponds filled with ground water. Zooplankton were sampled and water quality was monitored daily for 6 weeks. Rotifer abundance increased in ponds in all treatments during the first 8 days. Average rotifer density over 8 days did not differ between treatments. However, average copepod abundance was affected by treatments. New water had significantly fewer copepods than Dylox-treated or old water, but did not have fewer copepods than mechanically filtered water. Mechanical filtration compared more favorably to ponds filled with ground water than to ponds treated with Dylox or not treated. Filtration minimized adult copepods, while maintaining sufficient rotifer density for baitfish culture. Mechanically filtered old water has good potential of providing sufficient food (rotifers and nauplii) for newly stocked fry while minimizing the risk of copepod predation on fry.

Cyclopoid copepod predation has been established as an important factor causing low and variable survival rates during sunshine bass fingerling production. A concentration of 500 copepods/L can result in no survival of 5-day-old fry stocked at 20/L for 24 hours. Concentrations of 50 copepods/L and below resulted in survival rates not significantly different from controls with no copepods. Farmers will encounter cyclopoid copepod concentrations between 50 and 500 copepods/L in pond water fertilized to enhance rotifer blooms or in water held from previous operations. This study investigated the effect of cyclopoid copepod concentration on survival rates of sunshine bass, golden shiner, fathead minnow, and goldfish fry. Survival of golden shiner, fathead minnow, and goldfish was 95-100% at all copepod concentrations (0, 100, 200, 300, 400, and 500/L). Survival rates of sunshine bass (75.0-93.1%) were not significantly different among copepod concentrations ranging from 0 to 300/L. Sunshine bass fry survival at 400 copepods/L (62.5%) was significantly higher than at 500 copepods/L (39.4%), and significantly lower than at 0 and 100 copepods/L. On the basis of these results, stocking sunshine bass fry into ponds with fewer than 300 cyclopoid copepods/L probably represents a low risk of predation. Concentrations of cyclopoids between 300 and 500/L represent a higher risk of predation. Concentrations of cyclopoids exceeding 500/L will probably result in extremely low survival and poor yields. Five-day-old golden shiner, fathead minnow, and goldfish stocked in old pond water with 500 cyclopoid copepods/L or less could have acceptable survival rates.

Louisiana State University. Several water management techniques were evaluated alone and in combination in empirical field trials to reduce mass loading of solids and nutrients in crawfish ponds during the production season and final summer drawdown. The BMPs included cessation of crawfish harvesting activities 2 weeks prior to draining, rapid draining versus slow draining, draining from the surface as opposed to the bottom; retaining the last 20% of pond water followed by slow draining, and allowing the last 20% of pond volume to evaporate. The most effective means for reducing quantity and improving the quality of discharged water was the addition of 6 inches of water storage capacity and eliminating the last 20% of the pond volume when possible.

Objective 4. *Develop and evaluate models for predicting risks to the environment and the costs and benefits of implementing Best Management Practices (BMPs).*

University of Arkansas at Pine Bluff. Partial enterprise budgets are being developed for the various effluent management strategies evaluated in this project. Budget analyses were completed for sedimentation basin management options for commercial catfish ponds. In all, 108 different scenarios were analyzed for sedimentation basins on catfish farms. Budgeting work on the integrated production-storage pond strategies for catfish culture was also completed. Seventy-two different scenarios were analyzed for production-storage ponds. Preliminary cost data have been collected on the fixed-film filter options under study in this project.

Sedimentation Basins. Costs associated with settling basins are dependent on the size and number of basins, and whether sufficient land is available for basin construction or if existing production ponds must be retrofitted and taken out of production. Sizing of settling basins is controlled by factors such as the type of effluent to be treated (draining or storm overflow), layout of ponds, size of the largest foodfish pond, the number of drainage canals, and the scope of regulations governing the release of aquacultural effluents. The number of settling basins is affected by the hydraulic residence time (HRT) which is calculated from Stoke's Law. The HRT, in turn, is affected by the size distribution of suspended particles.

The number of settling basins is also affected by the number of drainage outlets on a farm. Some farms may drain in four to five different directions. Furthermore, farms that have ponds that are not contiguous would need a greater number of basins. Three farm size scenarios were considered in an analysis of settling basin costs: a 160-acre farm with approximately 140 acres of water, a 320-acre farm with 280 acres of water and a 640-acre farm with 560 acres of water. Average sizes of foodfish ponds in this analysis were assumed to be 10 and 15 acres, while fingerling ponds were 5 acres each.

Larger farm sizes result in higher and more variable costs. Investment costs included excavation of settling basins and the installation of stationary re-lift pumps to drain effluents from excavated basins. Annual operating costs consisted of copper sulfate applications (to promote sedimentation of phytoplankton cells), the annual cost of pumping, and levee mowing and maintenance, whereas annual fixed costs refer to depreciation of basins and pumps, interest on investment, and the opportunity costs associated with land taken out of production for the settling basin. Estimates of lost revenue due to production foregone from retrofitted production ponds were $300, $346, and $480/acre for the 160-, 320-, and 640-acre farms, respectively. Larger farm sizes will result in higher and more variable costs. Investment costs included excavation of settling basins and the installation of stationary re-lift pumps to drain effluents from excavated basins.

Large investments are needed for the construction of settling basins. This investment cost depends heavily on the drainage layout of the farm and the scope of regulations governing the release of effluents. Regulations that require settling basins on catfish farms would increase total investment cost on catfish farms by $126 to $2,990/ha and total annual costs by $19 to $367/ha.

Utilization of existing foodfish ponds for settling basins represents a more economical approach for the treatment of harvest/draining effluents than construction of settling basins. This is particularly true for those scenarios where all effluent volume must be treated. This difference was a consequence of the extremely high cost associated with excavating a sedimentation basin sufficiently deep to collect all farm effluents by gravity flow. Finally, compliance costs for the treatment of overflow effluents were moderate to high and strongly influenced by farm size.

For farms on which existing fish ponds would have to be converted to settling basins, over half of the cost was due to the production foregone and annual fixed costs of the pond. Requiring catfish farmers to construct settling basins would impose a disproportionately greater financial burden on smaller farms. The magnitude of the increased costs associated with settling basins was too high relative to market prices of catfish for this technology to be economically feasible.

Production-Storage Ponds. Two configurations (1:1 and 1:3), based on the number of production ponds served by each production-storage pond, were assumed. Increased depth of the combined production-storage pond increases the storage capacity of the system but incurs higher earthmoving costs. Three additional depths were considered: 12, 24, and 36 cm and seepage values of 0.0 and 1.0 mm/d were assumed. Cost estimates were developed for farms with average foodfish pond sizes of 10 and 15 acres. Fingerling ponds were not linked.

In total, 24 scenarios were defined for each of three farm sizes (total = 72 scenarios). Depth of storage pond and pond configuration were the two most important factors affecting implementation costs of this technology. For instance, estimated total investment costs for a 160-acre farm with 10-acre foodfish ponds, 1:1 configuration, and 0 mm/d infiltration rate, ranged from $76,123 to $215,088 as the additional depth of storage ponds was increased from 12 to 36 cm. However, if the configuration is 3:1, investment costs decreased (ranging from $44,782 to $115,947).

Fixed-film Filtering Systems. Investment and operating costs were estimated for the fixed-film honeycomb and brush filtering systems. Based on the assumptions used and on the experimental data available, costs of this filtering system ranged from $0.03 to $0.07/kg of fish produced with this treatment option.

Farm-Level Economic Effects of Imposing Effluent Treatments on Hybrid Striped Bass Farms. A Mixed-Integer Programming (MIP) model was developed to evaluate the farm-level effect of imposing effluent treatment options on hybrid striped bass farms. Settling basins and constructed wetlands entail high cost for farmers with high reduction in effluents. Filtering treatments incur high cost without much reduction in nutrient concentration in effluents. Not flushing water from the pond or not draining the pond annually reduces effluent volume. Reduction in the amount of water flushed or drained from the pond also decreases operating costs without any additional investment cost associated with adoption of the treatments. It is estimated that various effluent treatment options would increase production costs on average by $0.001 to 6.79/kg. Based on the MIP model, no annual draining and not flushing pond water are the best operational treatments. When discharge standards are imposed, the model selected the no-draining treatment. By not draining ponds, farms would minimize treatment cost by reducing effluent volume. Additional work is needed on the long-term risks associated with not flushing or not draining hybrid striped bass production ponds.

Objective 5. *Based upon existing information, supplemented by project findings, develop a comprehensive set of BMPs that can be implemented to reduce the environmental impacts of pond aquaculture in general. Develop supplemental BMPs particular to the various pond cultured species in the region that will complement the generic, pond-system BMPs. These BMPs would include best culture practices, waste handling and management, and water quality management and reuse.*

Auburn University. Funding from this SRAC effort was used to supplement additional funds from the Alabama Catfish Producers in developing BMPs for Alabama catfish farming. An environmental audit form for assessing the status of environmental management on catfish farms was developed. This instrument was used to identify potential problems that can possibly be solved with BMPs. The audit form was used by project participants to conduct environmental audits of aquaculture production facilities. A document containing best management practices to reduce the volume and improve the quality of channel catfish farm effluents was prepared through the cooperation of Auburn University, Alabama Catfish Producers Association, Natural Resources Conservation Service (NRCS), and the Alabama Department of Environmental Management. These BMPs were reviewed by all agencies involved and were presented to the farmers for comment. Farmer meetings were held to assure that farmers were aware of the BMPs and had opportunity for input. The BMPs are maintained by NRCS at <http://www.al.nrcs.usda.gov/about/so_sect/eng/ aq_bmp.html> in the form of Guide Sheets, and they also have been published by Auburn University. Guide sheets were developed for the following topics:

- Reducing Storm Runoff into Ponds
- Managing Ponds to Reduce Effluent Volume
- Erosion Control on Watersheds and Embankments
- Pond Management to Minimize Erosion
- Control of Erosion by Effluents
- Settling Basins and Wetlands
- Feed Management
- Fertilization of Catfish Ponds
- Water Quality Protection to Improve Effluents
- Water Quality Enhancers
- Therapeutic Agents
- Fish Carcasses
- General Operations and Worker Safety
- Emergency Response and Management

These BMPs will be referred to in the Alabama Department of Environmental Management regulations that will be made for aquaculture effluents.

Louisiana State University. Current NRCS Conservation Practices were reviewed to assess their applicability to development of a set of BMP effluent and watershed management guidelines for the crawfish aquaculture industry. Specific attributes evaluated for each conservation practice included applicability, need for clarification or modification, economic feasibility, environmental effectiveness, need for additional research, and need for educational programs for users. A set of BMPs for aquaculture production in Louisiana was drafted and reviewed by various commodity, state, and federal agencies. The revised document "Louisiana Aquaculture Best Management Practices" was completed and published in spring 2003, and includes individual reviews of BMPs for crawfish pond production, catfish pond production and recirculating system production (for alligators, finfish, or shedding crustaceans). Crawfish pond production BMPs focus on reducing pumping costs, improving flushing efficiency, and minimizing sediment loading during draining.

Objective 6. *Convene a series of workshops to educate and inform producers and regulators on the characteristics and management of aquaculture effluents from ponds, including BMPs, based on the best available information and that minimize environmental impact and satisfy regulatory compliance requirements.*

A workshop was convened on 6-7 November 2000 in Roanoke, VA. The objectives of the workshop were to (1) develop a prioritized list of practices that will minimize environmental impacts of aquaculture and be economically acceptable to producers; and (2) familiarize state regulators and consultants with the aquaculture effluents issue and provide the information necessary to develop effective and reasonable regulations.

In Arkansas, information on effluents and BMPs for pond aquaculture has been extended to producers. Two Extension newsletter articles were published. Results of the water re-use zooplankton study were presented to baitfish producers at the UAPB Aquaculture Field Day (attendance was approximately 300 people). A poster presentation on effluents and BMPs was exhibited at the Aquaculture Field Day and a presentation was made on the same topic at the annual convention of catfish and baitfish producers (150 people). Extension faculty assisted the Arkansas Bait and Ornamental Fish Growers Association in adapting proposed BMPs for catfish production to baitfish, and in developing the association's BMP document.

Most scientists in this SRAC project are active participants in activities coordinated by the Joint Subcommittee on Aquaculture's Aquaculture Effluent Task Force, including the various subgroups representing the species and areas of specialization of project scientists.

WORK PLANNED

The research supported by this project has been completed. A Final Project Report of findings will be prepared and published.

IMPACTS

The technical guidelines for several straightforward options for effluent treatment or volume reduction have been evaluated and are now available for consideration and implementation by producers of fish in ponds. Project personnel have been involved with the development and dissemination of information on best management practices to fish producers and to federal regulatory authorities considering regulation of aquaculture pond effluents. The comprehensive cost estimates and the best management practices developed in this study could potentially provide valuable information for both the Environmental Protection Agency (EPA) and the aquaculture industry as EPA proceeds with their rule-making effort to develop Effluent Limitation Guidelines.

Generalized results of the comprehensive cost estimates related to sedimentation basins have been discussed by members of the Economics Subgroup of the JSA Aquaculture Effluents Task Force with officials of the Environmental Protection Agency and the aquaculture industry. It is likely that the results of these studies will play a role in decisions made as EPA proceeds with their rule-making effort to develop Effluent Limitation Guidelines.

Best Management Practices developed as part of this project are being implemented in Alabama, Arkansas, and Louisiana. In Alabama, BMPs are the basis for regulation by the Alabama Department of Environmental Management (ADEM). The Arkansas Bait and Ornamental Fish Growers Association used SRAC information in developing a set of Best Management Practices for bait and ornamental fish farms. Association members have committed to following these recommended practices in order to minimize any possible environmental impacts from their farms. In Louisiana, BMPs appropriate for the state's diverse aquaculture industries have been collated in a manual for distribution to fish farmers.

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

Reporting Period
January 1, 2001 - August 31, 2003

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
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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.

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.

Some catfish producers and commercial seining crews in Mississippi, Arkansas, and North Carolina are continuing to use the prototype seine and socks constructed using BPE. In some cases, the prototype seine has been modified by removing the mud rollers. Mud rollers work well in some 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, agree with the data we have collected in controlled experiments.

University of Arkansas at Pine Bluff. Two projects are currently underway to develop in-pond fish grading technology for both market-size

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	1-1 ¼	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

channel catfish and channel catfish fingerlings. Two horizontal floating platform graders with adjustable spacing have been designed and fabricated. An eductor-style fish pump mechanism has also been 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 food-fish grader were finalized in 2003. A controlled study at UAPB and commercial farms was successfully completed over this past year comparing the in-pond food-fish 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 food-fish. This live car reel system does not impede normal seining/pushing operations and can also be used for crowding during load-out procedures.

University of Memphis. Preliminary data has been obtained on the stress response of channel catfish harvested by Mississippi State University personnel. Two types of seines were evaluated: one was the traditional seine utilizing a rope mud line with a 4-foot live car opening; the other was a prototype seine utilizing a mud line with rollers and an expanded opening for the live car. Blood samples were collected from 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. Samples have been obtained from fish harvested on six different occasions, at temperatures that varied from 12° to 29°C. Preliminary analyses from four sampling periods indicate that stress hormones of fish sampled prior to seining are 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 was removed from the live car. The rate of secretion of stress hormones was such that these high concentrations were maintained for at least one hour. 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. However, preliminary evaluation of cortisol hormone 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 23° to 29°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 of less that half of that observed at 23° to 29°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. This 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 and the fourth is underway to evaluate the secondary objective. In the first completed 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. 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. 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. Findings revealed that the dose or 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 20° to 28°C. When fish become stressed, they also become more susceptible to ESC anytime *E. ictaluri* is present at any concentration during the window.

A fourth experiment is underway which will examine the effects of various anesthetics on the stress response of channel catfish. Cortisol levels will be taken from the fingerlings to determine the stress level for each anesthetic type after the stress treatment. The fingerlings will then be challenged with *E. ictaluri* to see if reduced stress effects from anesthetizing will lower mortality rates due to ESC. These results suggest that mortalities could be reduced during stress events produced by grading or transport of catfish fingerlings.

Striped Bass

North Carolina State University. An in-pond portable grader has been designed and constructed for use on hybrid striped bass farms. The grader was modified from the original design that was developed for channel catfish. On-farm demonstrations/trials have been conducted on commercial hybrid striped bass farms. Based on these trials, the transfer box has been modified to improve the passage of fish from the holding net onto the grader panels. Significant changes to the intake end of the portable in-pond grader have been made. The initial design was not successful in transferring the hybrid striped bass from the holding net to the floating grader. The current design has been modified after numerous field tests but is still causing unacceptable levels of scale loss and lesions that lead to mortality of the fish. Up to 35% of the fish had noticeable cuts or lost scales. The floating grader portion of the design can effectively grade a large number of different sized fish. To date, we still do not have a good working design to proceed with larger-scale grading trials. Removal of fish from the holding nets is still problematic and our cooperating farmer is no longer able to continue working with us as a result of this. Modifications to the grader should be completed by fall 2003 and testing of the grader will continue.

University of Tennessee. Progress toward evaluation of stress in striped bass during grading includes the establishment and characterization of the cortisol, glucose and chloride assays that will be employed and the training of the graduate student responsible for the work. Blood samples will be taken from striped bass used in harvest technology projects by researchers at North Carolina State University to evaluate stress indicators. To date no blood samples have been received.

Ornamental Fish

University of Florida. A survey was distributed to all the tropical fish farms in the state of Florida seeking information on existing technologies and practices, and suggestions for new technologies and/or practices they would recommend investigating. Results of year one observations and direct farm input to questionnaires and site visits have been analyzed and evaluated in regards to objectives. An extension program to summarize these findings is being developed for industry participants. Collaboration with other project participants at Memphis State University has been initiated to develop a base-line for measuring stress in selected ornamental fish, using blood cortisol levels. Use of new sedatives and stress reduction compounds (Metomodate, Aqua Vitae) are being tested for impact on fish stress and condition during and after harvesting, grading, and transportation.

Results at a glance...

☆ *New trap designs for harvest of ornamental fish decrease personnel requirements and increase the quality of larger species such as cichlids.*

Year 2 addressed modifications to existing technology (i.e. traps, graders, etc.) and a comparison of modified technology. Baseline cortisol levels were measured for the blue gourami, *Trichogaster trichopterus*, including those treated with five different compounds designed to alleviate stress (Metomodate, Quinaldine, MS-222, Salt, and Hypno).

In Year 3, we continued to modify trap design and evaluation of traps by farmers. A more detailed observation of man-hours was conducted. A study was performed to refine practices of harvesting and transportation to reduce mortalities. Newly designed traps are more successful at catching larger fish (such as cichlids) based on farmers' initial evaluation. A principal study was performed on anesthetic usage to inhibit cortisol increase due to acute stress (i.e. harvesting, grading, transport, handling, etc.). Dosage rates to reach light (transport) sedation in the blue gourami, *Trichogaster trichopterus*, were studied and should have application for other species.

University of Tennessee. The University of Florida is conducting experiments on anesthetic effects on ornamental fish during transport. We are helping them examine stress effects during these experiments 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. We have run two sets of cortisol bioassays for the University of Florida and have been helping them to design experiments, interpret results, and refine experiments to eliminate compound stress effects for future studies. We are currently waiting for the third set of bloods plasma samples from their next experiment.

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. 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 25°C 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-micron 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 2).

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 (378.5 L water, 63.5 kg 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. This work was continued during Year 3 but has not been completed, but will be completed during the next 9 months.

The University of Memphis. Facilities have been constructed for use in this project to evaluate the effect of handling on the stress response of fish. An analytical laboratory has been developed that will enable us to monitor changes in water conditions when fish are subjected to various stressors associated with the transportation of fish. Small fish, larvae and eggs are often shipped in plastic bags filled with a small amount of water and an oxygen atmosphere. Preliminary comparisons have been made of traditional plastic bags with "breathing" bags made of a material that permits exchange of oxygen between the environment and the water in the bag. Initial observations indicate that the traditional bags provide a suitable amount of dissolved oxygen for a longer period of time than the "breathing" bags. The slow rates 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 that

Table 2. 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 25°C	Treatment II 20°C	Treatment III 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

requires oxygen to diffuse for a considerable distance. 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 of these variables could increase the time fish can be shipped in bags.

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 3. 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

Results at a glance...

☆ *A new seine design improved harvest efficiency for channel catfish by 15 to 20% and reduced seining time by 45 to 50%.*

Table 3. Harvest Efficiency Data

Parameters	Seine Type: CTPE (Year 2)	BPE (Year 2)	CTPE (Year 3)	BPE (Year 3)
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

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.

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 found (7 fish in all) was 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 (> 26°C, 12.8 to 26°C, < 12.8°C) with three fish size distributions: (75:25, 50:50, 25:75 sub-harvestable to harvestable fish) in 2002 and 2003. Data is summarized in Figures 1 and 2. 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

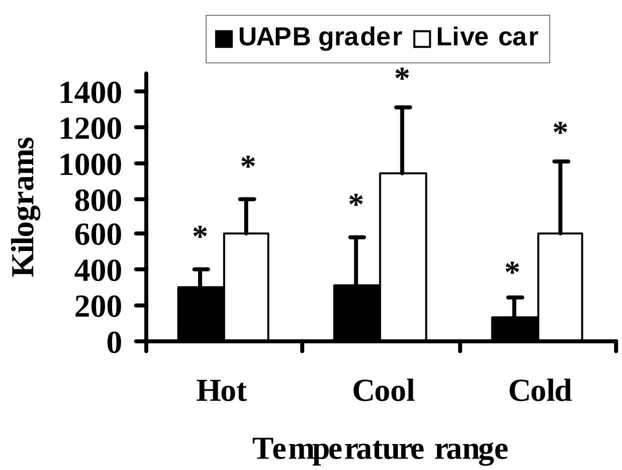

Figure 1. Mean kilograms of sub-harvestable fish left after grading by the UAPB grader (black columns) and the live car (white columns) after grading at three different temperature ranges on commercial farms. Columns with an "*" denotes a significant difference (P < 0.05) as determined by a paired t-test.

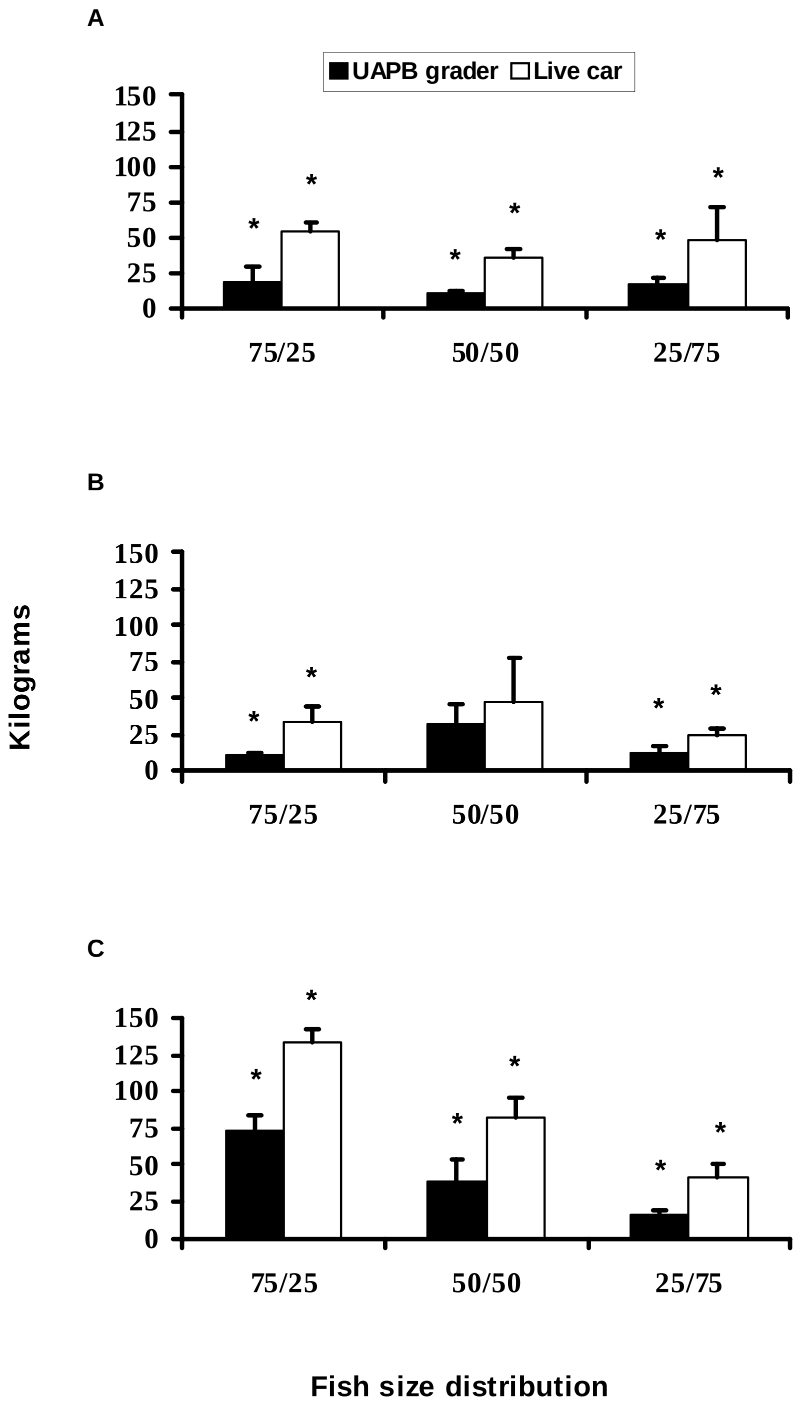

Figure 2. Mean kilograms of sub-harvestable fish left after grading by the UAPB grader (black columns) and the live car (white columns) after grading in hot (A), cool (B), and cold (C) temperature ranges with three different size distributions of sub-harvestable to harvestable fish. Columns with an "*" denote a significant difference ($P < 0.05$) as determined by a paired t-test.

of sub-harvestable fish (<0.57 kg) and harvestable fish (>0.57 kg) 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 speed was significantly greater with the UAPB grader (105 to 219 kg/minute) than the traditional live car (0.5 to 0.6 kg/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%.

Results at a glance...

☆ *Use of in-pond grading equipment is superior to traditional live-car nets for grading channel catfish at all temperatures. The addition of a new live-car reel system has greatly enhanced fish crowding capabilities and can improve efficiency of grading and load-out operations.*

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 10 to 19 kg of sub-harvestable fish during the hot temperature trials, 11 to 32 kg during the cool water trials, and 16 to 73 kg in cold water. By comparison, the traditional live car retained 36 to 55 kg of sub-harvestable fish in hot water, 24 to 48 kg in cool water, and 41 to 134 kg in cold water. Compared across temperature ranges, the weights of sub-harvestable fish retained by the UAPB grader were: 11 to 39 kg with the 50:50 ratio; 11 to 73 with the 75:25 ratio, and 12 to 17 kg with the 25:75 ratio. By comparison, the following weights were retained by the live car grader: 36 to 82 kg with the 50:50 ratio, 37 to 134 kg with the 75:25 ratio, and 24 to 48 kg with the 25:75 ratio. The UAPB grader retained an average of 32 ± 14 kg of sub-harvestable fish whereas the traditional live car retained 48 ± 30 kg (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 298 kg of sub-harvestable fish during the hot trials, 317 kg during the cool trials, and 139 kg of sub-harvestable fish during the cold trials. By comparison, the traditional live car retained 607 kg during the hot trials, 938 kg during the cool trials, and 609 kg during the cold trials. Thus, the UAPB grader returned from two to four times more kg 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.

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.

WORK PLANNED

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. Work will continue on evaluating prototype seines on commercial catfish farms as well as by controlled studies in NWAC ponds. Trials will be conducted under warm and cool water temperatures to account for seasonal variation in fish behavior.

University of Arkansas at Pine Bluff. Additional grader performance data will continue to be collected through 2004. Grading comparisons will be made to conventional live cars with larger mesh sizes than the original study. Several manuscripts are in preparation and will be completed. Food-fish grading demonstrations will also be conducted in 2004.

University of Memphis. Analysis of blood samples will be completed to determine the stress responses in fish harvested by two different types of seines. This information will be helpful to determine which type of seine enables harvesting of fish with the least adverse effects.

University of Tennessee. Work planned to meet the secondary objective includes continuing our challenge experiments as described above for experiment four and possibly an antibiotic treatment study with *E. ictaluri*. Further work includes finishing publications for the third and fourth experiment in our research.

Striped Bass

University of North Carolina. We anticipate having a functional grader in the early fall 2003. As soon as the grader is found to satisfactorily grade fish without causing external damage, we will be able to conduct trials on a weekly basis to collect the data as we had originally proposed.

Ornamental Fish

University of Florida. Additional studies will be performed on anesthetics for inhibition of cortisol increase. A feed and immersion product will be tested for an increase in post-stress survival rates. Traps will be modified based on farmer input of effectiveness and a new grader will be tested. Any modified equipment will be lent out for evaluation and adoption of these techniques and equipment by industry will be measured.

University of Tennessee. Work planned to meet the primary objective includes finishing bioassays for the University of Florida once plasma samples are received. We also plan to continue assisting with experimental design and discussion of results.

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. Work will begin on the evaluation of varying loading rates and using various commercial water quality stabilizers on closed system long-hauls on fish survival and profitability of baitfish aquaculture.

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

Channel Catfish

Mississippi State University. Studies will continue under controlled conditions at the NWAC to compare new seining technology to conventional seining technology. Benefits and costs for the harvest technologies developed for catfish will be estimated.

University of Arkansas at Pine Bluff. The economic analysis is underway. A partial budget analysis will be used to determine the net change in profit resulting from adoption of the in-pond grading technology on various sized catfish farms. This change is expected to be affected by farm size, dockage rates, and market price. Thus, sensitivity analyses of these parameters will be conducted. An investment analysis and quality loss function analysis are planned to complete the economic analysis.

Baitfish

University of Arkansas at Pine Bluff. Economic evaluation of new transport technologies will be collected and summarized and data on mortalities associated with the new technologies collected. Additional costs and benefits associated with new transport technologies will be compared to existing technologies within a partial budget framework. This analysis will be extended to evaluate the effect on farm profits of adoption of new technologies.

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. Even though work is continuing on prototype seines, the seines have been commercialized. 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 food-fish 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 food-fish facilities and one hybrid striped bass facility have adopted this technology.

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.

Striped Bass

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

The new trap design has impacted the effectiveness of harvest yield for larger size fish such as cichlids. Anesthetic impacts appear promising in inhibiting cortisol increase and perhaps reducing stress to ornamental fish.

Baitfish

Studies on ammonia excretion rates of golden shiners provide a basis for calculating the type of filter that is best for transporting live fish. Based on the data collected thus far, it appears that zeolite filters are more promising than floating-bead filters for live fish transport.

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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, 2003

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

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	North Carolina State University	Michael Levy, James Flowers
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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 80^{o}F. Snails were checked weekly for cercaria shedding, and checked daily when they were positive. Time and number of cercaria shed was recorded and data are being analyzed.

Collections to Evaluate the Avian Host Range for *Bolbophorus.*

USDA/ARS. Bird collections have begun and five pelicans, 10 cormorants, and 10 great egrets were collected in 2003. Parasites from these hosts have not been processed.

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 for the coming 2 years.

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.*

Confirmation of Intermediate Hosts of *Bolbophorus* spp.

North Carolina State University, USDA/ARS, Mississippi State University. *Planorbella trivolvus* 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. Three distinct types of cercariae were confirmed morphologically and genetically utilizing species-specific PCR. These were (1) *Clinostomum marginatum,* the causative agent of 'yellow grub disease', (2) *Bolbophorus damnificus*, a serious pathogen of channel catfish, *Ictalurus punctatus*, and (3) *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 *Bolbophorus* species simultaneously or sequentially. This indicated that both species were present in aquaculture ponds and they utilized the same molluscan host.

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 fat head 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

Results at a glance...

☆ *The* Bolbophorus *trematode has been found in wild fish species including channel catfish and several species of centrarchids. Only* Bolbophorus damnificus *was recovered from catfish in aquaculture ponds.* Bolbophorus *species type 2 was recovered from white crappie, longear sunfish and largemouth bass. The fat head minnow, was found to harbor both species, and this is the first finding of* Bolbophrus damnificus *in a fish species other than catfish.*

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.*

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.

Further experimental infections utilizing greater numbers of fish are planned in order to confirm these preliminary results.

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 120-L aquaria to evaluate growth rates under controlled laboratory conditions using well water free of *Bolbophorus* cercariae. Only fish containing visible cysts (1 to 5 cysts per fish) were selected and used to evaluate growth potential. Fish were acclimated to laboratory conditions at 31 to 32°C for 3 weeks before the start of the study. Following the conditioning period, fish were fed once daily for 9 weeks. Total weight gain, percent weight gain, specific growth rate, and feed efficiency were used to evaluate growth.

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

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

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.*

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.

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.*

Mississippi State University and USDA/ARS. Four trials were completed comparing pond shoreline treatments of copper sulfate and hydrated lime against snails. Copper sulfate and hydrated lime were applied at 589 g and 11,897 g respectively per 10 linear meters of shoreline in a 2-m swath or 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 (31°C vs 25°C), 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...

☆ *Copper sulfate or hydrated lime applied along pond margins reduces populations of the trematode's intermediate host–the ram's horn snail. This is the most effective approach to managing the disease.*

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

Mississippi State University. Juvenile redear sunfish measuring 2 to 2.5 inches were obtained from Logan Hollow Fish Farm, Murphysboro, Illinois, and juvenile blue catfish measuring 4 inches were obtained from

Little Grassy Fish Hatchery, Giant City, Illinois, for use in this study. Blue catfish have been trained to take insect larvae and small ram's horn snails, however, the redear sunfish are just converting from insect larvae to snails. The redear sunfish should be trained to the snails by the end of the year. The red rimmed melania snails were not available in sufficient quantities and have been removed from the conditioning diets. We are currently conducting the prey selection studies with the blue catfish.

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 plate media are being evaluated for optimum primary isolation and maintenance of *Flavobacterium columnare.* Media under investigation include; cytophaga agar (CA), selective cytophaga agar (SCA), Hsu-Shotts (HS), Shieh (S), Selective Shieh (SS), Tryptone Yeast Extract (TYES) and *Flavobacterium columnare* growth medium (FCGM). SCA has performed the best as a primary isolation medium in preliminary tests in isolation of columnaris from contaminated sites such as the gills and skin. Additional work is planned using *Aeromonas hydrophila* and *Edwardsiella ictaluri* as competitive organisms. For maintenance following isolation, FCGM or CA prepared as wet slants perform well. For broth culture, FCGM broth outperforms other formulations tested.

> **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, flavobacterium growth medium FCGM or cytophaga agar CA slants perform well. For large batch broth culture, FCGM outperforms other formulations tested.*

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. Zone sizes around sensitivity disks have been most consistent and clearly readable on plates prepared with 15 g/L of agar in 20 g/L Mueller-Hinton medium. Future research will examine the addition of fetal calf serum to the Mueller Hinton medium to enhance growth and zone clarity. Variability in susceptibility among strains has been great with oxytetracycline susceptibility ranging from 24 mm to 47 mm and Romet susceptibility ranging from 0 to 38 mm. Ultimately proposed methods will be submitted to the NCCLS for adoption into the M42 R document.

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-three strains of columnaris-like bacteria archived in the LADL and UAPB collections are being analyzed using conventional biochemical testing in tube tests, 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 procedure 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). 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.

Preliminary results indicate that *F. columnare* should be shown 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 forming rhizoid, yellow-pigmented colonies on solid agar plates. 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 systems (bioMérieux) have proven useful giving consistent results in preliminary tests. Morphology of the cells and colony appearance is variable among strains and is influenced by growth conditions and age of the culture.

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.

The isolated gill assay for bacterial adhesion was found to be too labor intensive for a routine technique. This problem can be overcome by using a whole-fish adhesion assay. This method involves exposing live mosquitofish *Gambusia affinis* to washed cultures of *F. columnare* for 1 hour, washing and macerating the fish, and then quantifying the number of bacteria by plate count. Preliminary experiments with this method had a large range (10 fold) of results for replicated trials, so future work will be related to improving the precision of the assay.

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. Twenty-eight isolates of columnaris-like bacteria were obtained from LSU and four case isolates from MSU were cultured and DNA isolated using Purgene DNA isolation Kit (Gentra Systems, Inc., Minneapolis, Minnesota). A portion of the 16s and the entire 16s–23s intergenic spacer of one isolate was PCR-amplified using primers to regions of the 16s and 23s ribosomal sequences that are conserved among the gram negative bacteria. One predominant product was obtained and cloned into pPCR4 TOPO cloning vector (Invitrogen) and sequenced. This was an intergenic sequence containing the tRNA for alanine and the tRNA for isoleucine. Several products were expected, representing different ribosomal operons, but as of yet only this ITS product was found. Alignment of these sequences with the tRNA sequences from related organisms were used to identify conserved sequences, and primers were developed to allow direct PCR of the specific ITS and direct sequencing of the products. These PCR products have been produced and both strands of both products sequenced for all 32 isolates. The sequence information is being edited and compiled. We will then perform sequence comparisons between isolates to determine the extent to which strains can be defined by ribotyping. 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.

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

Clemson University. A 40 kDa outer membrane protein (OMP) from *Flavorbacterium columnare* has been isolated and purified that is consistently found in all *F. columnare* isolates tested thus far, including a reference strain from ATCC. Both polyclonal and monoclonal antibodies were generated against this 40 kDa OMP for use as a pan–*F. columnare* probe. In addition to the 40 kDa OMP, a 30 kDa OMP was isolated and purified from a Clemson isolate and found to be expressed only in channel catfish *F. columnare* isolates. The purified Clemson 30 kDa OMP was used to generate both polyclonal and monoclonal antibodies for identifying channel catfish isolates of *F. columnare*.

Recent studies show that the 30 kDa OMP is a potent inducer of type II nitric oxide synthase (iNOS) and inducible prostaglandin H2 synthase (cyclooxygenase-2; COX-2) in isolated catfish phagocytes. reover, antibodies specific for the

Results at a glance...

☆ *A portion of the 16s ribosomal RNA gene and the entire 16s–23s intergenic spacer region of the ribosomal RNA genes was PCR amplified using conserved primers. One predominant product was an intergenic sequence containing the tRNA for alanine and the tRNA for isoleucine. Sequencing this region should allow for discrimination among different strains of* Flavobacterium columnare*.*

Results at a glance...

☆ *A 40 kDa outer membrane protein (OMP) from* Flavobacterium columnare *has been isolated and purified that is consistently found in all* F. columnare *isolates tested thus far. A 30 kDa OMP was isolated and purified from a Clemson isolate and found to be expressed only in channel catfish* F. columnare *isolates.*

30 kDa protein block iNOS and COX-2 expression, as well as chemotaxis of catfish phagocytes towards the 30 kDa protein. Additional studies demonstrated that the 30 kDa OMP also induces iNOS and COX-2 in the mouse macrophage cell line (RAW.274.1), and that this effect can be blocked by our specific antibodies. Current studies are designed to determine if the 30 kDa and 40 kDa OMPs are biologically active in tilapia. Moreover, the potential of this 30 kDa OMP as a biological response modifier in adjuvant treatments of channel catfish and tilapia columnaris is currently under examination. Other studies are focused on the role of the 30 kDa and 40 kDa OMPs in the pathogenesis of columnaris in channel catfish and tilapia.

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. Objectives for the first year of 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, and (3) transfer the pCP11–gfp plasmid into *Flavobacterium columnare* for expression of green fluorescent protein.

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.

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.

A conjugation technique is being developed using *E. coli* SM10 l*pir* as a donor strain to allow transfer of pMWFCgfp into *F. columnare*. Over 25 columnaris strains have been collected from LSU–SVM and from clinical cases at MSU–CVM to test as recipient strains for the conjugation.

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.

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. Methods being explored for strain differentiation are random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) fingerprinting.

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

Channel catfish and golden shiners.

University of Arkansas at Pine Bluff. Variability in *Flavobacterium columnare* pathogenicity makes disease treatment difficult because there is currently no way to easily recognize those strains that warrant aggressive treatments. In order to identify suitable markers,17 isolates of *F. columnare* were cultured from six different fish species. The DNA from all isolates was analyzed using randomly amplified polymorphic DNA (RAPD). Bootstrap analysis of the RAPD data produced a tree with three major groups supported by scores of 80-100% similarity. Virulence of the isolates will be determined by bath exposure of channel catfish, *Ictalurus punctatus* (Rafinesque), and golden shiners, *Notemigonus crysoleucas* (Mitchill), to broth cultures of *F. columnare* using the methodology developed in Objective 5.

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SUPPORT OF CURRENT PROJECTS

Title	Yr	SRAC Funding	Other Support: University	Industry	Other Federal	Other	Total Other Support	Total SRAC+ Other Support
Publications, Videos and Computer Software	1	50,000	43,950	-0-	-0-	-0-	43,950	93,950
	2	60,948	30,737	-0-	-0-	-0-	30,737	91,685
	3	45,900	35,710	-0-	1,000	-0-	36,710	82,610
	4	60,500	41,000	-0-	-0-	-0-	41,000	101,500
	5	67,000	47,000	-0-	-0-	-0-	47,000	114,000
	6	80,546	52,975	-0-	-0-	-0-	52,975	133,525
	7	83,850	43,000	-0-	-0-	-0-	43,000	126,850
	8	77,600	47,000	-0-	-0-	-0-	47,000	124,600
Total		**526,344**	**341,372**	**-0-**	**1,000**	**-0-**	**342,372**	**868,716**
Management of Aquacultural Effluents fromPonds	1	227,597	105,319	-0-	-0-	-0-	105,319	332,922
	2	222,289	117,051	-0-	-0-	-0-	117,051	353,970
	3	150,740	109,516	-0-	-0-	-0-	109,516	260,256
Total		**600,626**	**331,886**	**-0-**	**-0-**	**-0-**	**331,886**	**947,148**
Development of Improved Harvesting, Grading and Transport Technology for Finfish Aquaculture	1	287,053	218,353	-0-	-0-	-0-	218,353	505,406
	2	272,391	227,188	-0-	-0-	-0-	227,188	499,579
	3	190,556	232,823	-0-	-0-	-0-	232,823	423,379
Total		**750,000**	**678,364**	**-0-**	**-0-**	**-0-**	**678,364**	**1,428,364**
Identification, Characterization, and Evaluation of Mechanisms of Control of *Bolbophorus*-like Trematodes and *Flavobacterium columnaris*-like Bacteria Causing Disease in Warm Water Fish	1	224,800	277,901	-0-	-0-	-0-	277,901	502,701
	2	227,377	285,420	-0-	-0-	-0-	285,420	512,797
	3	145,770	281,926	-0-	-0-	-0-	281,926	428,696
Total		**598,947**	**845,247**	**-0-**	**-0-**	**-0-**	**845,247**	**1,444,194**

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	$124,201	88-38500-4028
	Yr. 2-05/01/90-04/30/91	124,976	89-38500-4516
	Yr. 3-05/01/91-04/30/93	124,775	90-38500-5099
	Project Total	**$373,952**	
*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	$3,971	87-CRSR-2-3218
		35,671	88-38500-4028
	Total Yr. 1	$39,642	
	Yr. 2-06/01/92-05/31/93	$59,000	91-38500-5909
	Yr. 3-06/01/93-12/31/94	34,500	92-38500-7110
	Project Total	**$133,142**	
*Characterization of Finfish and Shellfish Aquacultural Effluents. Dr. J. V. Shireman, University of Florida, Principal Investigator	Yr. 1-05/01/91-04/30/92	$13,081	88-38500-4028
		82,747	89-38500-4516
		49,172	90-38500-5099
	Total Yr. 1	$145,000	
	Yr. 2-06/01/92-05/31/93	$168,105	91-38500-5909
	Yr. 3-06/01/93-12/31/94	$128,936	92-38500-7110
	Project Total	**$442,041**	
*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	$12,649	89-38500-4516
		71,608	90-38500-5099
	Total Yr. 1	$84,257	
	Yr. 2-06/01/93-05/31/94	$213,106	92-38500-7110
	Yr. 3-06/01/94-05/31/95	$237,975	93-38500-8393
	Project Total	**$535,338**	
*Project Completed			

Project	Duration	Funding	Grant No.
*Aquaculture Food Safety: Residues.	Yr. 1-09/11/92-09/30/93	$99,393	91-38500-5909
Dr. George Lewis, University of	Yr. 2-10/01/93-09/30/94	$44,631	90-38500-5099
Georgia, Principal Investigator		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	Yr. 1-09/01/93-08/31/94		
Centers and USDA)	**Project Total**	**$2,000**	90-38500-5099
*Improving Production Efficiency of	Yr. 1-01/01/94-12/31/94	$28,148	90-38500-5099
Warmwater Aquaculture Species		123,705	91-38500-5909
Through Nutrition. Dr. Delbert		128,444	92-38500-7110
Gatlin, Texas A&M University,	Total Yr. 1	$280,297	
Principal Investigator	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	Yr. 1-04/01/94-03/31/95	$75,530	92-38500-7110
Catfish and Baitfish Pond Culture		43,259	93-38500-8393
Practices. Dr. Michael Masser,	Total Yr. 1	$118,789	
Auburn University, Principal	Yr. 2-04/01/95-03/31/96	$113,406	94-38500-0045
Investigator	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	Yr. 1-12/01/96-11/30/97	$241,476	95-38500-1411
Composition and Feeding Strategies.	Yr. 2-12/01/97-11/30/98	$47,105	95-38500-1411
Dr. Kenneth Davis, University of		210,047	96-38500-2630
Memphis, Principal Investigator	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	$80,546	00-38500-8992
	Yr.7-07/01/01-06/30/02	$83,850	2001-38500-10307
	Yr.8-01/01/03-12/31/03	$77,600	2002-38500-11805
	Project Total	**$476,344**	
*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
		188,639	99-38500-7375
		24,277	00-38500-8992
	Total Yr. 3	$246,304	
	Project Total	**$829,848**	
*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
		151	99-38500-5865
		14,757	00-38500-8992
		243,224	01-38500-10307
	Total Yr. 2	$272,391	
	Yr. 3-01/01/03-12/31/03	$34,088	99-38500-5865
		15,000	00-38500-8992
		141,468	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-Projected	$227,377	
	Yr. 3-Projected	$146,770	
	Project Total	**$598,947**	
*Project Completed			