SOUTHERN REGIONAL AQUACULTURE

THIRTEENTH ANNUAL PROGRESS REPORT

For the Period Through August 31, 2000

December, 2000

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

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PREFACE

In 1980, Congress recognized the opportunity for making significant progress in aquacultural development, and included in Title XIV of the Agriculture and Food Act of 1980 and later, in the Food Security Act of 1985, the authority to establish aquacultural research, development, and demonstration centers in the United States (Subtitle L, Sec. 1475[d]) to enhance viable and profitable aquaculture production for the benefit of consumers, producers, service industries, and the American economy. It was envisioned that the centers would be used in a national program of cooperative research and extension activities in association with colleges and universities, state Departments of Agriculture, federal facilities, and nonprofit private institutions with demonstrated expertise in aquaculture research and development. Eventually, five such centers were established, one each in the northeastern, southern, north-central, western, and tropical Pacific regions of the country. The 1990 Farm Bill (Food, Agriculture Conservation, and Trade Act of 1990; P.L. 101-624) reauthorized funding for the Regional Aquaculture Center program.

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.

ACKNOWLEDGEMENTS

The Southern Regional Aquaculture Center (SRAC) would like to acknowledge the contributions of the Project Leaders and Participating Scientists involved in the projects reported in this Thirteenth 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

Beginning with the first projects funded by SRAC, interest among aquaculture research and extension scientists in SRAC 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 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.

The Regional Aquaculture Center program is acknowledged to be an unusually efficient and productive use of Federal funds. The efficiency of the program lies in the unique approach to research, in which problems are identified at the local level and then solved using a coordinated, team approach. The team approach to problem-solving, which makes use of the best scientific talent in each region, results in a highly productive research and extension program. One measure of that productivity is the hundreds of high-quality, peer-reviewed scientific articles, graduate theses, and technical papers that have been generated since inception of the RAC program. As a further illustration of the productivity and impact of the Centers on American aquaculture, nearly 40% of the scientific articles contributed by U.S. scientists to one of the leading international aquaculture journals in a recent year were funded wholly or in part by the RACs. On a more local scale, some feeling for the productivity of the SRAC program can be gained by taking a glance at the number of extension publications produced through our Publications project (pages 17-18) or the number of peer-reviewed scientific publications generated as part of research projects (see, for example, the publications associated with the "Off-Flavor Management" project listed on pages 29-31).

A more important measure of success is the extent to which the results of projects have influenced or improved domestic aquaculture. As examples, results of recent or ongoing SRAC projects are being widely adopted by the industry to reduce feed costs, to improve the shelf-life of aquaculture products, and to reduce the incidence of environment-derived off-flavors in pond-raised fish.

Research to address the impact of aquaculture on the environment has proven to be particularly valuable. In August 2000, the United States Environmental Protection Agency announced its intention to develop effluent regulations for the aquaculture industry. Most of the technical information submitted to the USEPA to aid in development of sensible, yet effective, rules for warmwater pond aquaculture was derived from past SRAC projects. Further, the scientists and farmers who developed the most recent SRAC aquacultural effluents project in 1998 and 1999 showed remarkable foresight in anticipating the announcement of rulemaking by USEPA. Included in that project are research objectives that may provide key information leading to reasonable approaches to environmental management.

This Thirteenth Annual Progress Report of the Southern Regional Aquaculture Center covers the activities of the Administrative Center during the past year. Progress reports on the six multi-year research and extension projects supported by SRAC during this reporting period cover the life of the projects from their initiation date through August 31, 2000.

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.

The Board of Directors, the policy-making body for SRAC, utilizes recommendations from an Industry Advisory Council (IAC) and a Technical Committee (TC) to determine priorities for new and continuing aquaculture research and extension projects for the Southern Region. IAC membership represents different segments of the aquaculture industry throughout the region and provides valuable inputs for identifying priorities from an industry perspective. The TC is composed of research and extension scientists from essentially all states within the region and identifies priorities from a technical perspective. These groups provide valuable inputs into the SRAC program by identifying and developing priority research and extension needs in aquaculture. Using recommendations from these two groups, the SRAC Board of Directors selects priority categories for project development and funding.

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 of Agriculture Section (AHS) of the Board of Agriculture of the National Association of State Universities and Land Grant Colleges.

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
David E. Foster, Arkansas Cooperative Extension Service
Stephen Jones, Alabama Cooperative Extension Service
J. Charles Lee, Mississippi State University
David Morrison, Louisiana State University
Daniel Smith, Clemson University Cooperative Extension Service
Vance Watson, Mississippi State University
Greg Weidemann, University of Arkansas

Ex-officio Board members are:

Lester Myers, Chairman, Industry Advisory Council
Michael Masser, Co-chairman, Technical Committee
J. Larry Wilson, Co-chairman, Technical Committee
Craig S. Tucker, 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 on-going 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
Randy Deshotel, LA
R. C. Hunt, NC
Austin Jones, MS
Robert Mayo, NC
John Morrison, AL
Lester Myers, MS (Chairman)
Marty Tanner, FL
R. R. Waldrop, TX
Jerry Williamson, AR
Gary Youmans, SC

IAC members serve up to three-year appointments having staggered terms with options for re-appointment.

The IAC (1) recommends to the Board research and extension needs and priorities from an industry perspective; (2) reviews project proposals and accomplishment and termination reports; and (3) recommends to the Board, jointly with the Technical Committee, actions regarding new and continuing proposals, proposal modifications and terminations.

TECHNICAL COMMITTEE

The TC is composed of representatives from participating research institutions and state extension services, other state or territorial public agencies as appropriate, and non-profit 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:

- Gary Burtle, GA
- Wallis Clark, FL
- J. A. Collier, SC
- Harry Daniels, NC
- Allen Davis, AL
- Carole Engle, AR
- Delbert Gatlin, TX
- Andrew Goodwin, AR
- John Hargreaves, MS
- Ray McClain, LA
- Stephen Smith, VA
- Jim Tidwell, KY
- J. L. Wilson, TN (Co-chair)

Members of the TC for Extension are:

- Jimmy Avery, MS
- David Cline, AL
- Dennis DeLong, NC
- Robert Durborow, KY
- G. J. Flick, Jr., VA
- David Heikes, AR
- Tom Hill, TN
- Jeff Hinshaw, NC
- Andy Lazur, FL
- Greg Lutz, LA
- Mike Masser, TX (Co-chair)
- Nathan Stone, AR
- Jack Whetstone, SC

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

The TC (1) recommends to the Board research and extension needs and priorities from a scientific perspective; (2) develops problem statements for research and extension areas under consideration; (3) plans, develops, and implements regional proposals; (4) reviews proposals and accomplishment and termination reports; and (5) 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:

- 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);
- can provide the solution to a problem of fundamental importance or fill an information gap.

PROJECT DEVELOPMENT PROCEDURES

Research and extension priorities and statements of problems defining priority areas are jointly developed and recommended to the Board by the Industry Advisory Council and the Technical Committee. Using their recommendations as guidelines, the Board selects specific problem areas to be funded and appoints a Steering Committee (comprised of research, extension and industry representatives from the IAC, TC and other agencies) and an Administrative Advisor. The Steering Committee has full responsibility for developing a definitive research and extension Problem Statement, recommending levels of funding for each year of the proposed work, and preparation of the subsequent project proposal.

An Administrative Advisor is appointed by the Board for each active project area, and serves as the coordinator for activities related to the project, providing continuous linkage between the Work Group, Steering Committee and SRAC. Responsibilities of Administrative Advisors are outlined in the SRAC Operations Manual.

Following review of the Problem Statement by the IAC and TC, and review and approval by the Board, announcements to convene an *ad hoc* Work Group are made regionally to (1) institutions and individuals identified by the Steering Committee; (2) extension and research directors of 1862 and 1890 Land Grant Universities within the Southern Region; and (3) other institutions, agencies and organizations within the Southern Region having demonstrated capabilities in the area under consideration.

All *ad hoc* Work Group participants desiring to participate in a proposed research and extension activity must submit a "Commitment to Participate" form. Participants will also have an opportunity to make appropriate comments and suggestions relative to the development of the proposal and their interest and capability in participating. This information is used by the Steering Committee to draft a proposal, recommending the best qualified participants, as well as tentative funding allocations, to address objectives outlined in the Problem Statement.

Project proposals are reviewed by the Steering Committee, IAC, TC, all proposed 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.

Final selection of projects and levels of funding are determined by the Board. Most projects have an expected duration of three years. Following final approval by the Board of Directors and CSREES, work described in the research and extension project is implemented. Participating scientists, along with the Steering Committee, comprise the permanent Work Group for the research and extension effort and are responsible for implementation and conduct of the proposed work.

Separate allocations are made for research and extension to ensure strong programs in each of these areas. All funds allocated for extension activities are administered through the respective State Cooperative Extension Services.

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 submision 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 instituions 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 Requests for Proposals 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 are cumulative reports detailing the progress of research and extension work accomplished for the duration of the project through August 31 of the current year. These reports are prepared by the respective Project Leaders in conjunction with the institutional Principal Investigators.

PUBLICATIONS, VIDEOS AND COMPUTER SOFTWARE

Reporting Period
April 1, 1995 - August 31, 2000

Funding Level

Year 1	$50,000
Year 2	60,948
Year 3	45,900
Year 4	60,500
Year 5	74,000
Total	$291,348

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. Daniel Smith, Director
South Carolina Cooperative Extension Service
Clemson University
Clemson, South Carolina

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 courses in aquaculture and fisheries. Educational institutions at the elementary and secondary level use SRAC extension materials in the classroom to make students aware of aquaculture production and associated trades as a possible vocation.

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

Producers. Information on the use of therapeutants, pesticides, methods of calculating treatment rates, and possible alternative crops and marketing strategies is in constant demand by aquaculturists. Videos that demonstrate 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 project year, eight new fact sheets were written and one was revised. All have been distributed throughout the Southern Region and to interested Extension Specialists in other regions. Approximately 15 fact sheets are currently in some stage of writing or revision.

The fourth publication in the "RAC Results" series was completed this year. These publications highlight the impacts of SRAC projects in a brief, popular format. The most recent RAC Results publication, "Analysis of Regional and National Markets for Aquacultural Food Products Produced in the Southern Region" summarizes the 1988-1990 SRAC project of the same name.

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, another publication in the "Species Profile" series will be prepared for sturgeon, in addition to nine new fact sheets. The new fact sheets will address 1) the economics of small-scale catfish production, 2) marine shrimp culture, 3) application of aquatic herbicides, 4) use of copper-based algicides, 5) pond mixing and circulation, 6) operation of oxygen meters, 7) toxicity of aquaculture chemicals, 8) the economics of hybrid striped bass culture, and 9) grass carp for weed control. Three fact sheets will be revised on the following topics: 1) pond construction, 2) oyster culture, and 3) aquatic herbicides. In addition there will be one research summary written by research personnel from the completed SRAC project "Aquaculture Food Safety: Residues".

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 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

Results at a glance...

- *Nine fact sheets were completed this year with 15 more in progress.*
- *Twenty-nine scientists from across the Southern Region contributed to publications produced by SRAC in this project year.*
- *SRAC has now published 157 fact sheets and 20 videos.*
- *Educators in schools and colleges use SRAC publications in classrooms throughout the U.S. and the world.*

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.

> ***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*

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

PUBLICATIONS, MANUSCRIPTS OR PAPERS PRESENTED

Fact Sheets Completed (6/1/99 - 8/31/2000)

Brunson, Martin W. and Joe E. Morris. Species Profile: Sunfish. SRAC Fact Sheet 724.
Hinshaw, Jeffrey M. Trout Farming, Carrying Capacity and Inventory Management. SRAC Fact Sheet 222.
Kleinholz, Conrad W. Species Profile: Bigmouth Buffalo. SRAC Fact Sheet 723.
Lutz, C. Greg. Pet Turtle Production. SRAC Fact Sheet 439.
Mims, Steven D., William L. Shelton and Forest Wynne. Production of Paddlefish. SRAC Fact Sheet 437.
Stone, Nathan, Carole Engle, David Heikes and Donald Freeman. Bighead Carp. SRAC Fact Sheet 438.
Tidwell, James H., Shawn D. Coyle and Timothy A. Woods. Species Profile: Largemouth Bass SRAC Fact Sheet 722.
Tucker, Craig S. and Martine van der Ploeg. Managing Off-Flavor Problems in Pond-Raised Catfish. SRAC Fact Sheet 192.
Tucker, John W., Jr. Species Profile: Grouper Aquaculture. SRAC Fact Sheet 721.

Manuscripts in review

Daniels, H. V. Species Profile: Southern Flounder. SRAC Fact Sheet 726.
Faulkner, Greg. Maintenance and Repair of Seines.
Hinshaw, Jeffrey M. Trout Production: Handling Eggs and Fry. SRAC Fact Sheet 220 (Revision).
Silva, Juan L., Gale R. Ammerman, and Stuart Dean. Processing Channel Catfish. SRAC Fact Sheet 183 (Revision).
Silva, Juan L. and Stuart Dean. Processed Catfish: Product Forms, Packaging, Yields and Product Mix. SRAC Fact Sheet 184 (Revision).

Manuscripts in preparation

Gatlin, Delbert M., III. Improving Production Efficiency of Warmwater Aquaculture Species Through Nutrition. SRAC Project Summary.

Hodson, Ron. Hybrid Striped Bass: Pond Production of Food Fish. SRAC Fact Sheet 303 (Revision).

Rakocy, James. Use of Aquaculture Effluents.

Rakocy, James. Recirculating Aquaculture Tank Production Systems: Integrating Fish and Plant Culture. SRAC Fact Sheet 454 (Revision).

Silva, Juan L. Food Safety Techniques for Aquacultural Products.

Treece, Granvil. *Artemia* Production for Marine Larval Fish Culture. SRAC Fact Sheet 702.

Treece, Granvil and D. Allen Davis. Culture of Small Zooplankters for the Feeding of Larval Fish. SRAC Fact Sheet 701.

Treece, Granvil. Algae Production for Marine Larval Fish Culture.

Watanabe, Wade O. Species Profile: Mutton Snapper (*Lutjanus analis*).

RAC Results

Engle, Carole R. Analysis of Regional and National Markets for Aquacultural Food Products Produced in the Southern Region.

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MANAGEMENT OF ENVIRONMENTALLY-DERIVED OFF-FLAVORS IN WARMWATER FISH PONDS

Reporting Period
June 1, 1996 - August 31, 2000

Funding Level	Year 1	$250,827
	Year 2	250,142
	Year 3	229,266
	Year 4	80,900
	Year 5	56,100
	Total	$867,235

Participants	University of Tennessee, Lead Institution	Thomas K. Hill
	University of Arkansas at Pine Bluff	Peter W. Perschbacher
	Auburn University	Claude E. Boyd
	Auburn University	R. Thomas Lovell
	Louisiana State University	Leslie C. Plhak
	Louisiana Tech University	H. Lynn Walker
	University of Memphis	King-Thom Chung
	University of Mississippi	Daniel K. Schlenk
	Mississippi State University	David J. Wise
	Texas A & M University	Delbert M. Gatlin

Administrative Advisor
Dr. Don O. Richardson, Dean
Agricultural Experiment Station
University of Tennessee
Knoxville, Tennessee

PROJECT OBJECTIVES

1. Evaluate the feasibility of decreasing the incidence of fish off-flavors by reducing the amount of phosphorus available to support phytoplankton growth.

    a. Evaluate methods of reducing phosphorus input by diet modification by determining the minimum phosphorus requirement for food-sized channel catfish and quantifying the reduction in waste phosphorus generation by food-sized catfish fed "low-phosphorus" feeds relative to presently available feeds.

b. Evaluate methods of removing phosphorus from pond waters by studying methods of enhancing rates of phosphorus removal from pond waters by pond bottom soils and determining the feasibility of precipitating phosphorus from pond waters as sparingly soluble aluminum or calcium salts.

2. Evaluate the feasibility of reducing the incidence of fish off-flavors by manipulating pond phytoplankton biomass and taxonomic composition using biological and chemical control measures.

   a. Evaluate the effect of filter-feeding fishes on water quality and reduction or elimination of off-flavor in pond-raised channel catfish.

   b. Develop microbial pathogens to control blue-green algal abundance.

   c Determine whether plant phenolics (tannins) can control growth of microorganisms that produce odorous compounds in warmwater fish ponds.

   d. Evaluate the effect of routine, low-level treatments of ponds with copper sulfate on phytoplankton communities, off-flavor incidence, and water quality in channel catfish ponds.

3. Determine the feasibility of managing fish off-flavors by reducing rates of 2-methylisoborneol (MIB) uptake by fish and/or enhancing rates of MIB elimination from fish.

4. Develop statistical models describing the within-pond variation in the degree of off-flavor in fish populations under various conditions.

5. Develop analytical techniques for assessing flavor qualities in fish.

6. Develop publications to educate producers and processors on the ecology of environmentally-derived off-flavors, off-flavor management, and the results of this project.

ANTICIPATED BENEFITS

The overall goal of this project is to reduce the incidence of unacceptable flavor quality in pond-cultured fish. If this goal is accomplished, the aquaculture industry will benefit from increased farm profits and market expansion resulting from improved consumer attitude toward aquaculture products.

Use of the revised phosphorus allowance in commercial catfish feeds should reduce the phosphorus input to catfish ponds and thus reduce nutrients available to support algae growth. Similarly, use of alternative phosphorus supplements or phytase enzymes to increase utilization of phytate phosphorus in the feed may be beneficial in reducing phytoplankton growth and thus reduce occurrence of off-flavor. Regardless of the impact on algal communities, these studies will lead to more efficient use of phosphorus from feeds.

The use of chemical substances for reducing phosphorus levels in pond water could provide a simple procedure for channel catfish farmers and other aquaculturists to use in reducing the amount of phosphorus in waters of ponds to which large amounts of feed are applied. The benefits of the compounds (aluminum sulfate, calcium oxide, and calcium sulfate) chosen for use in this research are that they are common compounds, they are relatively inexpensive, they are environmentally safe and would not pose a food safety risk, and they would be easy to apply. If one or more of these compounds can reduce phytoplankton blooms, and especially blooms of blue-green algae, there does not appear to be any reason that farmers would not accept them readily.

The two biological control measures under investigation (use of filter-feeding animals and use of natural algal pathogens) are particularly attractive because they avoid the use of chemical control measures. In the case of control measures using filter-feeding fish or clams, economic returns from harvest of the animals stocked for algae control may be an added benefit. Other improvements in water quality may also occur. For example, a state fish hatchery is participating in the large-scale evaluation of planktivorous fish with the hopes of addressing chronic problems with low dissolved oxygen levels.

Several chemical control measures are being investigated, including the use of copper sulfate and natural compounds such as plant phenolics. These studies should also lead to the development of one or more novel chemical treatments that can be used to control noxious phytoplankton blooms.

Additional studies focus on enhancing the elimination of MIB from channel catfish, so that off-flavor fish may be purged more effectively prior to processing. These approaches may be of significant economic value to the aquacultural industry. Also, improved methods of analysis for geosmin and MIB that are comparable or better than sensory methods with regards to sensitivity and comparable or better than GC analysis in terms of objectivity will be developed. Immunoassay methods have these benefits and can also be formatted into rapid and simple test kits for industry. These methods will provide the industry with a better tool for quality control and fish grading as well as the research community with a better tool to study off-flavor development and control.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Objective 1A. *Evaluate methods of reducing phosphorus input by diet modification by determining the minimum phosphorus requirement for food-sized channel catfish and quantifying the reduction in waste phosphorus generation by food-sized catfish fed "low-phosphorus" feeds relative to presently available feeds.*

The minimum dietary available phosphorus requirement for food-size channel catfish fed commercial type diets was determined in a 7-month pond study. The available phosphorus requirement based on subclinical measurements (bone breaking strength and alkaline phosphatase activity) was found to be 0.3% of the diet, although the requirement for maximum growth was less than this. These data indicate that the available phosphorus requirement for commercial catfish feeds should be 0.3% of the diet, which is approximately 25% lower than the present National Research Council recommendation.

The availability of different forms of phosphorus in practical feed ingredients was determined for channel catfish. A reference diet and test diets containing either menhaden fish meal, fish meal analog, meat and bone meal, soybean meal, cottonseed meal, corn, rice bran, wheat, and wheat middlings were fed to channel catfish after which fecal samples were collected and analyzed to determine the availability of different forms of phosphorus from the ingredients. The various feed ingredients varied considerably in terms of their phosphorus composition and availability to the fish. Wheat, sorghum, and cottonseed meal had the greatest phosphorus availability of the plant feedstuffs while fish meal analog had the greatest availability of the animal feedstuffs. Also in this study, the uptake and mineralization of different forms of phosphorus and nitrogen in feces from channel catfish fed the various ingredients were determined.

Digestibility trials were also conducted with channel catfish to evaluate several different feedstuffs which have been genetically modified to contain low concentrations of phytic acid. The phytic acid which is typically found in reasonably high concentrations in plant feedstuffs is indigestible and thus excreted by fish. The low-phytate varieties of barley, corn, and soybean meal which have been evaluated have elevated phosphorus availability to channel catfish. Thus, as these feedstuffs become more readily available, they may provide a dietary means of reducing phosphorus excretion by this species.

Another study was conducted to evaluate diet formulations and feeding strategies to marginally meet the phosphorus requirement of channel catfish while minimizing dietary phosphorus input. Fingerling channel catfish were fed one of three practical diets with either no phosphorus supplementation (approximately 0.2% available phosphorus), minimal phosphorus supplementation from dicalcium phosphate (0.3% available phosphorus), or standard phosphorus supplementation from dicalcium phosphate (0.5% available phosphorus) for 8 weeks in aquaria. Two additional treatments included feeding the diet containing 0.2% available phosphorus with intermittent feeding (two days every other week or every fourth week) of the diet containing 0.5% available phosphorus. Samples of pectoral spines and whole-body tissues indicated adequate phosphorus status of channel catfish could be maintained with minimal phosphorus input by feeding the unsupplemented diet in conjunction with the phosphorus-supplemented diet for two days every fourth week.

> ## *Results at a glance...*
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> ✰ *Several studies conducted in this project showed that dietary phosphorus intake by catfish can be reduced substantially without affecting fish growth or health. Although the effect of reducing dietary phosphorus on the incidence of off-flavor is not clear, these findings should allow for more efficient feed use by fish and formulation of less expensive feeds.*

A pond study was conducted to quantify the reduction in waste phosphorus generation by food-size channel catfish fed experimental diets formulated to contain 28% protein and 0.4% available phosphorus from either dicalcium phosphate (water-soluble) or defluorinated phosphate (water-insoluble) phosphate. No statistical differences were observed in weight gain, feed conversion, survival, bone ash, and bone phosphorus between fish fed the two diets. There were no significant differences in total phosphorus, soluble phosphorus, and chlorophyll *a* concentrations in pond water between the two dietary treatments.

A pond study was conducted in Mississippi to quantify the reduction in waste phosphorus generation by food-sized channel catfish fed low phosphorus diets. A basel diet was formulated to contain 32% protein without supplemental phosphorus (0.2% available phosphorus). Supplemental phosphorus was added to the basal diet to provide available phosphorus of 0.3 and 0.4%, respectively, using dicalcium phosphate. There were no significant differences in total phosphorus, soluble phosphorus, and chlorophyll *a* concentrations in pond water between the two dietary treatments. However, in a similar study conducted in Alabama where phosphorus in catfish feed was increased from 0.6% (0.2% bioavailable) to 1.0% (0.6% bio-available), there were significant increases in total phosphorus and phytoplankton production.

Objective 1B. *Evaluate methods of removing phosphorus from pond waters by studying methods of enhancing rates of phosphorus removal from pond waters by pond bottom soils and determining the feasibility of precipitating phosphorus from pond waters as sparingly soluble aluminum or calcium salts.*

In 1996, laboratory and pond studies were conducted to determine the feasibility of precipitating phosphorus from waters as sparingly soluble aluminum or calcium salts through the application of aluminum sulfate (alum), calcium oxide (lime), or calcium sulfate (gypsum). The gypsum application had the greatest effect, and the treatment was repeated in 1997 at a higher application rate. At the higher rate (increasing total hardness to 200 ppm), gypsum significantly reduced total phosphorus and chlorophyll *a* levels. More frequent applications of alum to pond water (1998) showed distinct short-term effects but little long-term change in the pond water quality. Repeated applications of 7 pounds/acre of agricultural limestone at 2-day intervals also reduced soluble phosphorus concentrations but had little effect on phytoplankton. A 120-day pond trial was completed which evaluated the effects of periodic additions of alum on the availability of phosphorus in the pond environment. Addition of alum at 50 ppm every other week did not affect total production or feed conversion of channel catfish in 0.1-acre ponds. In addition, waterborne phosphorus concentration, primary productivity, algal species composition and sediment oxygen demand generally were not affected by alum addition; however, there was an obvious reduction in the amount of filamentous algae and other vegetation in ponds treated with alum.

> ## *Results at a glance...*
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> ☆ *In areas where ponds are filled with soft water, the combination of periodically adding gypsum to the water and tilling empty pond bottoms is an effective method of reducing waterborne phosphorus levels during the production season.*

In 1997 and 1998, drying and tilling empty pond bottoms before filling the ponds resulted in lower phosphorus levels in the water during the production season. Incorporation of alum, agricultural limestone, or sodium nitrate in the tilled soil did not reduce phosphorus levels or improve water quality above drying and tilling alone.

In 1999, the treatments were as follows: control, dry tilling of pond bottoms, and dry tilling of pond bottoms followed by periodic gypsum applications to maintain 200 ppm total hardness. The 1999 study showed that the dry-till with gypsum treatment was superior to dry-till alone in lessening phosphorus availability and phytoplankton abundance.

The 2000 experiments involve a comparison of the dry-till with gypsum treatment with a water circulation treatment in comparison to control ponds. Preliminary findings suggest that water circulation is not as effective as the dry-till with gypsum treatment, but the study will not conclude until October 2000.

Objective 2A. *Evaluate the effect of filter-feeding fishes on water quality and reduction or elimination of off-flavor in pond-raised channel catfish.*

Six species of filter-feeding macroorganisms were identified as candidates for biological control and tested in mesocosms in 1997 for their ability to filter off-flavor algae. Nile tilapia, blue tilapia, and silver carp significantly reduced numbers of *Oscillatoria perornata* and *Anabaena circinalis* (major producers of MIB and geosmin, respectively). Threadfin shad significantly reduced numbers of *A. circinalis*. Two species of local, unionid clams did not produce measurable effects. Based on these findings, a silver carp system was evaluated in 0.25-acre ponds in 1998. Abundance of *O. perornata* was reduced, but the alga was not eliminated. In addition, difficulty was encountered in adapting a silver carp system to channel catfish production ponds.

Evaluations of threadfin shad and of Nile tilapia (in cages) indicated that best algae control was obtained with Nile tilapia. Tilapia were evaluated in cages at 500 and 1000/acre, which is lower than previously reported to provide control of *O. perornata*. Best control was obtained at the "full" Nile tilapia stocking rate of 2000/acre. A polyculture system based on the "full" Nile tilapia stocking rate is in place at a state fish hatchery in two, 2.5-acre fingerling ponds stocked with 10,000 catfish fingerlings/acre, with another equivalent pond system as a control. During the 4 weeks with Nile tilapia, reductions were seen in fluctuations in algal abundance, numbers of odor-producing blue-green algae, and the incidence and intensity of off-flavor in catfish. Complete control of off-flavor may require a longer period than the 4 weeks found necessary in the relatively small experimental ponds. Improvements in ammonia, nitrite, and oxygen levels have also been observed in ponds with tilapia.

Objective 2B. *Develop microbial pathogens to control blue-green algal abundance.*

Fungal and bacterial pathogens of *Anabaena* and *Oscillatoria* were isolated from commercial catfish ponds. In laboratory studies these agents lysed cells of *Anabaena* and *Oscillatoria*, and selectively removed these species from mixed cultures containing beneficial algae and blue-green algae.

A fungal pathogen was selected for evaluation in replicated tests that were conducted in

210-gallon tanks to study control of *Oscillatoria perornata*. The tanks were filled with water from a commercial catfish pond, stocked with catfish fingerlings, and treated with preparations of a fungus. The fungus controlled *O. perornata*, but high oxygen demands were observed. Preparations of the fungus are being developed that will minimize the oxygen demand.

A bacterium that is pathogenic to species of *Anabaena* and *Oscillatoria* was isolated from pond water. When comparisons were made using a number of databases, no definitive match for the DNA sequence of the 16S rRNA gene for the bacterium could be established at the genus or species level. Therefore, the bacterium could represent a genus that has not been described. When the bacterium was evaluated as a biological control agent in laboratory studies, the average reductions in chlorophyll *a* were 94 to 98% for *Oscillatoria* spp. and 13 to 98% for *Anabaena* spp. No significant reductions in chlorophyll *a* were noted for *Chlorella vulgaris*, *Scenedesmus subspicatus, Selenastrum capricornutum*, *Microcystis aeruginosa*, or *Plectonema boryanum*. Shake flask cultures of the bacterium produced approximately 3 billion plaque-forming units/milliliter of culture broth. Therefore, 1 liter of culture broth when uniformly distributed in one acre-foot of pond water, would result in an initial concentration of approximately 2,400 plaque-forming units/milliliter of pond water. In replicated tests conducted in 210-gallon tanks containing water from commercial catfish ponds, the bacterium selectively removed species of *Anabaena* and *Oscillatoria.* When the bacterium was inoculated to pond water, *Oscillatoria perornata* was reduced from an initial density of 2,700 filaments/milliliter to 0 filaments/milliliter after 48 hours. The colonial blue-green alga *Microcystis* became dominant as the species composition of the phytoplankton changed in the treated pond water. Results of laboratory and tank tests indicated that the bacterium did not adversely affect channel catfish fingerlings.

> ## *Results at a glance...*
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> ✰ *A bacterium isolated from pond water selectively attacks odor-producing blue-green algae while having no effect on beneficial algae or catfish. The bacterium shows promise as a biological control agent for the algae that cause off-flavors.*

The bacterium was tested in ponds. Analyses of pond water following inoculation indicated that the bacterium was present in the water for up to 5 days after inoculation. While the results of preliminary pond experiments were encouraging, future tests in ponds need to be conducted under more uniform environmental conditions to facilitate interpretation of results.

Objective 2C. *Determine whether plant phenolics (tannins) can control growth of microorganisms that produce odorous compounds in warmwater fish ponds.*

The bacterium *Streptomyces tendae* is known to synthesize geosmin, an earthy off-flavor contaminant of aquatic products. Experiments were conducted to determine the antimicrobial effects of tannic acid and related compounds such as propyl gallate, methyl gallate, and gallic acid on the growth of *Streptomyces tendae.* Well-diffusion assays and biomass determinations were performed. The biomass determination method is more sensitive than the well-diffusion assay. The results of these experiments indicate that tannic acid is inhibitory to *S. tendae* at levels as low as 0.3 ppt. Propyl gallate is inhibitory at higher concentrations, but methyl gallate and gallic acid have no inhibitory effects at concentrations up to 1 ppt. Olfactory evidence suggests that tannic acid may inhibit geosmin synthesis.

It was also demonstrated that tannic acid and related compounds are inhibitory to the growth and pigment synthesis of off-flavor producing *Nostoc* sp. strain MAC. The minimum inhibitory concentrations of tannic acid, propyl gallate, and gallic acid in augmented pond water were 320, 240, and 500 micrograms/disk, respectively. Tannic acid, propyl gallate, and gallic acid also exhibited inhibitory activity to *Cytophaga columnaris*, a ubiquitous, gliding fish pathogen, at 150, 300, and 300 ppm. Methyl gallate was effective at 500 ppm. The protein precipitation and polysaccharide binding capacities, lipophilicity and other physico-chemical properties of these compounds were measured in order to understand possible mechanisms for their antibacterial action. Tannic acid, a polymeric compound with multiple hydroxyl groups, had at least a nine times greater capacity for binding protein and glycogen than the other test compounds. These results suggest that the hydroxyl group availability of tannic acid is essential for antibacterial activity. Therefore, it is likely that these compounds may have some beneficial effect in controlling the microbial population in ponds and may have impact on the phytoplankton biomass.

Objective 2D. *Evaluate the effect of routine, low-level treatments of ponds with copper sulfate on phytoplankton communities, off-flavor incidence, and water quality in channel catfish ponds.*

Eighteen, 0.4-ha earthen ponds in northwest Mississippi were used in a 3-year study to evaluate the effect of weekly copper sulfate applications on the incidence and economic impact of environment-derived off-flavors in channel catfish. Each spring when water temperatures increased above 70°F, nine of the ponds were treated weekly with 5 pounds/acre copper sulfate by placing the required amount of copper sulfate crystals in a burlap bag which was then placed in the current produced by a paddlewheel aerator. Copper treatments were discontinued each fall when water temperatures fell below 70°F. Overall prevalence of off-flavor was reduced by 80% for ponds treated with copper sulfate relative to control ponds, and episodes of off-flavor were of shorter duration in treated ponds. Off-flavors never delayed fish harvest from treated ponds, whereas off-flavors delayed fish harvest on ten occasions in control ponds. Average annual fish harvest was 5,250 pounds/acre from ponds treated with copper sulfate and 4,760 pounds/acre from control ponds. The 9% reduction in fish harvest from control ponds was due to infectious disease outbreaks in one or two ponds each year where harvest was delayed due to off-flavor. Enterprise budgets showed that average net

Results at a glance...

- ✰ *Weekly, low-level treatments of catfish ponds with copper sulfate reduced the incidence of off-flavor by 80% and increased net revenues by over 40% compared to untreated ponds.*

returns above variable costs were $770/acre for control ponds and $1,100/acre for ponds treated with copper sulfate. Variation in net returns was twice as great for control ponds as for treated ponds, indicating increased stability in production and economic returns when off-flavors were managed using copper sulfate. High variation in annual economic performance on control ponds resulted from one or more ponds having high net returns while one or more ponds had extremely poor returns due to protracted episodes of off-flavor. Stability in production and costs is a means of reducing risk and is a positive factor farmers can use to better plan their cash flow needs throughout the production season and in the longer term.

Objective 3. *Determine the feasibility of managing fish off-flavors by reducing rates of 2-methylisoborneol (MIB) uptake by fish and/or enhancing rates of MIB elimination from fish.*

Three compounds were initially identified as potential enhancers of MIB elimination based on their ability to increase the activity of cytochrome P450, the enzyme system thought to be involved in metabolizing the off-flavor compound, 2-methylisoborneol (MIB). One of the three compounds (3-methylcholanthrene) significantly increased the residence time of MIB in channel catfish. After initial success with clofibric acid, further investigation only showed a trend toward enhanced elimination. The last compound, ethanol, provided the best results observed in enhancing MIB elimination, but only following MIB exposure. Pretreatment with each chemical did not affect MIB uptake or elimination. Treatment with ethanol following MIB uptake nearly doubled the rate of MIB elimination. Although a direct correlation was observed between temperature and MIB elimination, temperature failed to have any synergistic effect on the enhanced elimination by any of the three compounds.

Analysis of MIB elimination in the Uvalde strain of channel catfish indicated tremendous variation in the response of fish and their ability to eliminate MIB. Variation between individuals is nearly 35%. The factors controlling the variation in response are unclear. No relationship has been observed between individual isoforms of cytochrome P450 and MIB elimination in this particular strain. Examination of MIB metabolism in Uvalde strain channel catfish with induced enzymes indicated that MIB is not metabolized.

To determine whether extrahepatic (tissues other than the liver) biotransformation of MIB may be occurring, the metabolism and disposition of radio-labeled MIB was examined in Uvalde channel catfish as well as another strain of channel catfish (USDA 103) and the channel catfish x blue catfish hybrid. No metabolites were observed in plasma from animals treated with an intra-arterial dose of radio-labeled MIB. Elimination of MIB from the two strains and hybrid was accurately predicted using a three compartment pharmacokinetic model. There was no significant difference in terminal half-lives between strains, but significant differences in other predicted pharmacokinetic parameters, such as total clearance, were observed with the hybrid strain, which had a 10-fold greater clearance.

Objective 4. *Develop statistical models describing the within-pond variation in the degree of off-flavor in fish populations under various conditions.*

Work on this objective is not complete.

Objective 5. *Develop analytical techniques for assessing flavor qualities in fish.*

Monoclonal antibodies have been produced that bind to 2-methylisoborneol (MIB). This led to the development of immunochemical methods (ELISA) to detect MIB down to levels of 0.01 ppb, low enough to be comparable to the human sensory threshold for MIB. Zeolite was tested as a material for MIB absorption and concentration. Using ELISA, zeolite was shown to absorb small molecules (glycoalkaloids) but not antibodies. Zeolite, however, was shown to be less efficient for MIB absorption than activated carbons, when compared using a purge and trap apparatus.

An eight-member sensory panel was trained using the Sensory Spectrum Method. A preliminary study was conducted to evaluate the effectiveness of various processing procedures in reducing off-flavor in catfish. Fillets of each flavor rating were either dipped or vacuum tumbled in water, dairy whey or 3% lemon juice. The panel gave significantly higher scores for the geosmin note and lower scores for the chicken-like note for off-flavor level 5 compared to level 1, regardless of the treatment. Lemon juice significantly increased the geosmin note, whereas dairy whey reduced it. Vacuum tumbling with lemon juice reduced the green/corn note (considered a desirable note) compared to dipping in lemon juice.

Objective 6. *Develop publications to educate producers and processors on the ecology of environmentally-derived off-flavors, off-flavor management, and the results of this project.*

See list of publications on pages 29-31.

WORK PLANNED

Work on all objectives is proceeding on schedule and no changes in the project have occurred this year.

IMPACTS

Much of the work in this project has not been completed, so it is difficult to determine impacts. However, information generated in two of the project objectives is already being used in the aquaculture industry. First, work to investigate phosphorus availability of various feedstuffs has been used by feed manufacturers to refine commercial diet formulations, with a cost savings to the farmer. Second, the use of routine, low-level copper sulfate treatments developed for preventing algae-related off-flavors has been widely adopted by many commercial producers in Mississippi and Arkansas.

Although other results of this project are too preliminary to have an impact on the aquaculture industry, several of the treatments and practices being investigated show promise. For example, phosphorus levels in ponds can be reduced by precipitating phosphorus as aluminum or calcium salts, or by treating the pond bottom to reduce phosphorus flux from soils to water. These practices could be an important management procedure for improving quality of pond water and effluents and in combating off-flavor. Another example of a potentially effective practice is the use of filter-feeding fishes, which has been shown to be effective in controlling odor-producing algae in small-scale systems. Perhaps the most intriguing result is the success achieved using bacterial pathogens of odor-producing blue-green algae. If these results can be transferred to pond-scale eco-systems, the work may lead to a novel, safe, and effective method of controlling flavor problems in fish.

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OPTIMIZING NUTRIENT UTILIZATION AND REDUCING WASTE THROUGH DIET COMPOSITION AND FEEDING STRATEGIES

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PROJECT OBJECTIVES

1. Determine the effects of diet composition on fish production, nutrient utilization, and excretion of organic and nitrogenous wastes.

    a. Evaluate the effects of minimizing protein concentrations via amino acid supplementation of diets for channel catfish. The proposed research should be based on, and augment, available information concerning protein and amino acid nutrition of this species.

b. Evaluate manipulations of dietary protein concentration and energy density as well as inclusion of specific diet additives to improve growth efficiency and nitrogen retention while limiting excretion of wastes by channel catfish and hybrid striped bass (sunshine bass).

2. Assess the effects of various feeding strategies and techniques on fish production, nutrient utilization, and waste reduction.

   a. Optimize feeding strategies in relation to water temperature for channel catfish production. Of particular interest is delineation of more precise feeding strategies when water temperatures are cool (spring, late fall) and extremely hot (late summer, early fall).

   b. Evaluate alternative feeding strategies including manipulation of diet composition in relation to such variables as water temperature and fish size for channel catfish, baitfish, and hybrid striped bass (sunshine bass).

   c. Develop and refine feeding strategies for crawfish that effectively enhance production by augmenting the forage-based system.

3. Develop publications to effectively extend information derived from this project to feed manufacturers and fish producers.

ANTICIPATED BENEFITS

The overall goal of this project is to improve the efficiency of nutrient utilization in aquaculture feeds and forage, which has two important implications. First, efficient use of feeds and forage should make farming more profitable because feed costs represent a large fraction of the total cost of aquaculture production. Second, optimizing nutrient retention may improve culture system water quality and reduce the impact of aquaculture on the environment by decreasing waste production.

Objective 1. Work on this objective will provide information that will increase the efficiency of commercial diet assimilation by channel catfish and hybrid striped bass, with a concomitant reduction in waste generation. These improvements should increase the cost-effectiveness of producing these fish in aquaculture and limit potential negative environmental impacts from waste production.

Protein is the most expensive component of channel catfish diets and a primary source of waste nitrogen in production ponds. Commercial catfish feeds contain proteins of animal and plant origin that enter the production pond in uneaten feed and fish feces. Nitrogen is released from feed and feces by bacterial decomposition which contributes to poor water quality. Thus, reducing the protein content of catfish diets could help to reduce feed cost and might improve water quality in catfish ponds.

Research with other aquatic species suggests that effective reduced-nitrogen diets can be made by balancing dietary protein to match the amino acid composition of a species-specific “ideal protein." An ideal-protein diet for channel catfish should possess an amino acid composition similar to that in the whole body of catfish, an indispensable amino acid content that meets all minimum dietary requirements, and a quantity of dispensable amino acids sufficient to meet the demands of protein synthesis during rapid growth. Such a diet might be utilized more efficiently than diets in which the amino acid composition is less strictly controlled.

Work under this objective includes evaluating the efficacy of several reduced-nitrogen, ideal-protein diets for channel catfish. It is hoped that these diets will increase dietary nitrogen utilization, improve fish growth, and reduce the amount of nitrogenous waste entering catfish ponds under typical production conditions.

The total cost of catfish diet ingredients can be reduced approximately 5% by using all-plant protein ingredients and balancing acids in the diet with crystalline amino acids compared to a similar diet containing fish meal to balance amino acids. Nitrogen and phosphorus utilization may improve when channel catfish are fed diets with balanced amino acid content, leading to better overall dietary efficiency and improved profitability.

This objective also includes work on dietary enhancement for the culture of reciprocal cross hybrid striped bass (sunshine bass). Increasing the lipid composition and supplementation of specific feed additives in hybrid striped bass diets may result in growth enhancement and increase nutrient utilization.

Objective 2. Precise feeding regimens for use when water temperatures are cool (spring, late fall) and extremely hot (late summer and early fall) may improve production efficiency and nutrient utilization in channel catfish farming. Improved feed management strategies that utilize daily feeding frequency and timing to optimize fish growth would significantly reduce feed costs by lowering labor requirements, reduce wear on machinery, and lead to greater production efficiency. Other feeding strategies, such as reducing daily feed allowance by feeding more concentrated feeds (high protein percentage), will allow the farmer to feed more conservatively and thereby waste less feed.

Formulating better baitfish and crawfish diets is expected to improve overall performance of golden shiners and crawfish in commercial production systems and improve profitability. Results from this objective will provide producers with viable, cost-effective feeding strategies that can enhance production by augmenting the forage-based food system. Identification of effective, low-cost feeds and practical feeding strategies will facilitate efficient supplemental feeding practices that minimize waste while maintaining optimal production in forage deficient ponds.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Objective 1a. *Evaluate the effects of minimizing protein concentrations via amino acid supplementation of diets for channel catfish. The proposed research should be based on, and augment, available information concerning protein and amino acid nutrition of this species.*

Texas A & M University. This project was initiated in March of 1997 with a 2-year pond feeding trial to evaluate the use of lysine supplementation to reduce total dietary protein and limit nitrogenous waste excretion in channel catfish production. Two experimental diets containing 25% crude protein and a standardized reference diet containing 30% crude protein from practical ingredients were fed to mixed sizes of channel catfish in earthen ponds. One of the experimental diets did not contain supplemental lysine, whereas the other was supplemented with 0.5% of lysine HCl to provide the same level of lysine as the reference diet. Fish in each pond were selectively harvested by grader seine in October 1997, May 1998, and October 1998 after which fingerling fish were added back to each pond. The final harvest took place in May 1999. Yield of marketable fish at each intermediate harvest was not affected by diet, and total yield data indicated that fish production was not negatively affected by reducing dietary protein from 30 to 25% of diet. Further, lysine supplementation of the diet with 25% protein did not confer any added benefits, and no effects on water quality could be attributed to the dietary manipulations. Body composition of fish fed the various diets was determined and indicated that reducing dietary protein increased nitrogen retention.

Louisiana State University. The project was initiated in the spring of 1997. Objectives were to determine the effects of reduced-protein diets on production yield, feed conversion efficiency, dressing percentage, and body composition of pond-raised channel catfish during a continuous, three-year production period, as well as effects of lowered-nitrogen diets on pond water quality. Fingerling fish were stocked in 16, 0.2-acre ponds, at 10,000 fish/acre in late spring. Fish were fed one of four, isocaloric, extruded (floating) catfish feeds formulated to contain 26-30% crude protein. Each diet was assigned to four, randomly selected ponds and fish were fed daily as much as they would consume in 30 minutes.

Results at a glance...

☆ *The crude protein level in catfish feeds can be reduced to at least 28% without affecting fish production. Reduced protein levels in feeds will reduce feed costs and improve protein utilization.*

Diets being tested were 30%, 28%, and 26% crude protein, plus a control diet. Reductions in dietary crude protein from 30 to 26% were achieved by decreasing the dispensable (dietary non-essential) amino acid content of the diets by 10 to 20% (28 and 26% CP diets, respectively), while maintaining concentrations of all indispensable (dietary essential) amino acids at minimum required levels. The ratio of each dispensable amino acid to lysine was held constant in all diets except the control. Diets were manufactured at a commercial feed mill.

Four partial harvests have been conducted to date, in the fall of 1997, the spring and fall of 1998, and the spring of 1999. After each harvest, fingerlings were restocked to maintain 10,000 fish/acre. At each harvest, 100 fish were taken from each pond for determination of body composition and dressing percentage. Water quality parameters and chlorophyll *a* concentrations were monitored twice weekly to determine the effects of dietary treatments on pond water quality.

Production data from the third and final year of the project, 1999, were invalidated because aeration was lost during a power outage, resulting in a massive summer fish kill. The final harvest of the project will be held in October 2000. Although the 1999 production data were invalidated by the summer fish kill, 100 fish were collected from each pond in the fall for determination of body composition and dressing percentage, as was done in previous years. Those data have been included in the body composition and dressing percentages in Table B below.

Results through spring 1999 harvest are shown in Tables A and B:

Table A. Production

Treatment	Total Diet Fed (kg)	Total Weight of Fish Harvested (kg)	Feed Conversion Ratio
Control	21,723	7,124	3.0
30% CP	19,461	7,181	2.7
28% CP	19,220	6,450	3.0
26% CP	18,800	6,467	2.9

Table B. Body Weights / Fat Content / Dressing Percentages (standard error in parenthesis)

Average Treatment	Visceral Fish Weight (g)	Dressed Fat (%)	Yield (%)
Control	531 (82)	2.5 (<0.1)	58.6 (0.2)
30% CP	577 (81)	2.7 (0.1)	59.2 (0.2)
28% CP	597 (53)	2.4 (0.1)	58.3 (0.2)
26% CP	539 (60)	2.2 (0.1)	58.6 (0.2)

The University of Georgia. Channel catfish stocked in earthen ponds at the rate of 10,000 fingerlings per acre were fed soybean meal-corn-wheat middling diets that were formulated to contain 0.65% or 0.94% as methionine by the addition of DL-methionine or 0.94% as methionine by addition of menhaden fish meal. In the first year of a two-year trial, channel catfish yields were similar between all-plant ingredient diets and a diet that contained menhaden fish meal. The all-plant ingredient diet cost 5% less than the fish meal diet but had similar production. Water quality parameters, including ammonia, nitrite, and total phosphorus, were similar in all treatments during the first year. After five partial harvests in the first year, a residual of smaller fish were carried over until the second year when stocking density was brought to 10,000/acre. The second

Results at a glance...

☆ *Catfish feeds with all-plant protein can be used without affecting fish production. The cost of feeds with all-plant ingredients is about 5% less than traditional feeds.*

year production cycle ended with similar gross catfish yield among treatments over the two year period (10,461, 10,789, and 10,270 pounds/acre, respectively). The three diet formulas did not appear to affect proximate body composition. However, when feed intake was considered, the net protein utilization was 8.7% higher when 0.94% methionine was added to soybean meal-corn-wheat middling diets versus the addition of 0.65% methionine. Although the implications for water quality improvement were not shown in this short trial, the prospects for recovery of significantly more protein nitrogen by the channel catfish should encourage the practice of adequate methionine supplementation to all-plant protein diets.

Objective 1b. *Evaluate manipulations of dietary protein concentration and energy density as well as inclusion of specific diet additives to improve growth efficiency and nitrogen retention while limiting excretion of wastes by channel catfish and hybrid striped bass (sunshine bass).*

Auburn University. Feeding 12.5% less of a 32% protein feed to catfish in production ponds resulted in the same yield of fish as feeding a 28% protein feed to satiation. Feed efficiency and economics were improved by feeding the 32% protein feed at the reduced rate. Feeding 22.5% less of a 36% protein feed resulted in significantly less fish production than with 28% and 32% protein diets.

A follow-up study was conducted to further examine the relationship between feeding rate and dietary protein. In that study, feeding 12.5% less of a 32% protein feed to channel catfish in production ponds resulted in the same fish yield as feeding a 28% protein feed to satiation, whether fish populations were uniform-sized fingerlings or mixed fingerling and market-size fish (9:1 ratio of fingerlings to large fish). However, in mixed-size populations, the higher protein diet increased profitability and reduced feeding dominance of larger fish over smaller fish. The percentage of total fish yield provided by the fish stocked as fingerlings was 73 and 66% of total yield when fed the higher and lower protein diets, respectively.

An all-plant, commercial type of diet with no phosphorus supplement, containing 0.20% available phosphorus, was found sufficient for maximum weight gain by channel catfish grown to marketable size in ponds. However, 0.30% available phosphorus is recommended for production diets for catfish growth in ponds. Increasing the dietary phosphorus to higher concentrations reduced muscle and visceral fat composition of the carcass. Further, dietary phosphorus levels of 0.40 to 0.42% were required for maximal survival after challenge to the fish bacterial pathogen, *Edwardsiella ictaluri*.

Three diets with different concentrations of crude protein were tested in channel catfish ponds. Fish were fed to satiation with 28% crude protein feed, and the other two diets (32 and 36% crude protein) were applied in amounts calculated to provide the same crude protein input as the 28% crude protein diet. Using this practice, feed application was smaller as feed crude protein increased, and phosphorus and organic matter loads to the ponds decreased accordingly. The feeding practice and diets used in this study had no measurable effects on nitrogen concentrations in pond waters and effluents or on fish production. In spite of smaller phosphorus inputs with 32 and 36% protein feed, only a small fraction of the applied phosphorus remained in the water column, and the differences in phosphorus input in feeds among treatments did not affect phytoplankton production or pond water or effluent phosphorus concentrations. When fish ponds were drained for harvest, the quality of effluent did not change until about 75% of the water had been released. Water quality in effluents then deteriorated because the pond bottom was disturbed by outflowing water, fish activity, and harvest. By holding the last 25% of water in ponds for 12 to 24 hours after fish removal, much of the suspended matter was removed by sedimentation. The water can then be released slowly to prevent resuspension of sediment, and a better quality effluent obtained.

Phosphorus budgets were prepared for channel catfish ponds that received one of five diets ranging from 0.60 to 1.03% phosphorus. Fish production did not differ among diets. There were few differences among treatments with respect to soluble reactive phosphorus, total phosphorus, and chlorophyll *a* concentrations or gross primary productivity. Phosphorus loss in effluents when ponds were drained for harvest did not differ among treatments. Phosphorus removed from ponds in fish at harvest and the amounts of phosphorus adsorbed by bottom soils increased as dietary phosphorus concentration increased. Low-phosphorus diets did not decrease phytoplankton productivity or improve effluent quality. Uptake of phosphorus by bottom soils is a major factor controlling phosphorus concentrations in pond water. Low-phosphorus diets can be beneficial in catfish pond management by reducing the phosphorus load to bottom soils and conserving their ability to adsorb phosphorus.

A study of the biochemical oxygen demand (BOD) of waters from ten channel catfish ponds at Auburn, Alabama, revealed that the 5-day BOD seldom exceeded 8 ppm and that the ultimate BOD was usually less than 30 ppm. Water samples from catfish ponds usually needed to be diluted only 2 or 3 times to permit 5-day BOD measurements, and nitrification occurred even during a 5-day incubation period. Catfish pond waters were not extremely high in ammonia nitrogen concentration, and ammonia nitrogen introduced in the ammonium chloride-enriched dilution water caused an appreciable increase in BOD of some samples. Plankton respiration is a major component of carbonaceous BOD in catfish pond waters. Thus, the BOD is not expressed as rapidly during 5-day incubations as in typical wastewater. The ultimate BOD would be a good measurement of oxygen demand for catfish pond effluents, but it is difficult to measure. Data from this study suggest that ultimate BOD can be estimated from the 5-day BOD, but the correlation is not strong. An alternative is to develop a short-term BOD measurement specifically for effluents from channel catfish and other aquaculture ponds. This study suggests that a 10-day BOD conducted without nitrification inhibition or addition of ammonia nitrogen in dilution water might be a better alternative to standard 5-day BOD or ultimate BOD measurements normally used in wastewater evaluation.

A study was also conducted to determine rates of gaseous ammonia loss (volatilization) from ponds. Daily rates of volatilization ranged between 9 and 71 milligrams of nitrogen per square meter, and averaged 4% of total ammonia nitrogen in channel catfish ponds receiving high feed levels. Abundant N and the high N:P ratio in pond waters prevented appreciable biological nitrogen fixation. There were four main N losses from ponds: fish harvest (32%), denitrification (17%), ammonia volatilization (12%), and accumulation in pond soils (23%).

Mississippi State University, Starkville. Two experiments were conducted in flow-thru aquaria with sunshine bass at two water temperatures

(80 and 90°F). Six semipurified diets were prepared which contained three protein levels (45, 40, and 35%) and two lipid levels (5 and 15%) to yield varying dietary energy/protein ratios of 6, 7, 8, 9, 10, and 11 kcal/g protein. Fingerling sunshine bass (about 3-4 g/fish) were randomly distributed at a rate of 25 fish/tank. Triplicate groups of fish were randomly assigned to each diet and fed to satiation daily for 8 weeks. Overall growth and nutrient utilization values were significantly higher for fish maintained at 80°F compared to fish kept at 90°F. Feed consumption decreased with increasing dietary E/P ratio. All of the responses except hepatosomatic index (HSI) had the same pattern at both temperatures. Feed efficiency, protein efficiency ratio and protein conversion efficiency were highest at a dietary E/P ratio of 9 kcal/g protein. Whole body lipid deposition and intraperitoneal fat (IPF ratio) accumulation were increased with increasing dietary lipid levels. At these two temperatures HSI changed differently, but HSI correlated with liver glycogen levels at both temperatures. At 90°F, liver glycogen deposition was positively correlated with dietary carbohydrate levels. The lower energy conversion efficiency of fish held at 90°F indicates an increased energy requirement for maintenance and/or activity in these fish. We are unable to explain the reduced growth and nutrient utilization by the fish maintained at the elevated temperatures. Perhaps stress effects of high temperatures, associated with release of heat-shock protein and/or metabolic changes mediated through isoenzyme shifts may be responsible.

A study was conducted to investigate HSP70 (heat-shock protein) synthesis in sunshine bass exposed to stressful water temperatures. Fish were acclimated at 80 and 90°F for 4 weeks. They were subjected to heat shock by exposure to water temperatures 3.5, 7, and 10.5°F above their respective acclimation temperatures for 2 hours. Increased thermotolerance was observed in fish acclimated at 90°F as they survived up to 97°F compared to fish acclimated at 80°F which died at 93°F. Fish pre-conditioned at 80°F did not exhibit a change in HSP70 synthesis when heat shocked. Increased levels of HSP70 synthesis were observed in fish acclimated at 90°F and exposed to elevated temperatures. Accumulation of HSP70 was correlated with increasing temperature in liver, gut and gill tissues. This was evident in brain tissue only when other stress factors were reduced or eliminated from the experiment.

An experiment was conducted to identify changes in certain enzyme or isoenzyme activities at elevated temperatures in sunshine bass. Liver esterase was found to be present in two isoenzyme forms at 77°F compared to only a single form at the higher (90°F) temperature.

Texas A & M University. Three studies have been conducted with hybrid striped bass to investigate a variety of dietary manipulations on growth and nutrient utilization. Two different feeding trials have been completed with hybrid striped bass in which the effects of dietary lipid level and carnitine supplementation were evaluated. Providing dietary lipid at 10% or 15% rather than 5% or 20% significantly enhanced weight gain of hybrid striped bass but dietary carnitine supplementation did not influence growth, nutrient utilization or body composition. In another study, dietary supplementation of a commercial proteolytic enzyme was evaluated with hybrid striped bass. This diet additive did not enhance fish growth in two separate feeding trials, nor did it increase nutrient digestibility or limit waste production. In a third study, supplementation of cholesterol and lecithin to the diet was found to have negligible effects on growth and body composition of hybrid striped bass.

Objective 2a. *Optimize feeding strategies in relation to water temperature for channel catfish production. Of particular interest is delineation of more precise feeding strategies when water temperatures are cool (spring, late fall) and extremely hot (late summer, early fall).*

Mississippi State University, Stoneville. A pond study was conducted to evaluate effects of feeding strategies related to water temperatures on optimizing nutrient utilization and reducing waste for food-size channel catfish. In March 1997, two sizes of channel catfish (22 and 225 g/fish, respectively with a 7:3 ratio) were stocked into 28, 1.0-acre earthen ponds at a rate of 10,000 fish/acre. After a 1-month conditioning period, fish were fed to satiation with a 28% protein feed once daily, once every other day, or once every third day based on water temperatures. Total nitrogen, total ammonia, nitrite, nitrate, chloride, chlorophyll *a*, and pH were measured monthly. All fish were harvested in December 1997 and samples taken for determination of carcass yield and fillet composition. Fish fed daily throughout the growing season consumed the most amount of feed and had the highest net production. Net production of fish that were not fed daily either in the spring and the fall or during the extremely hot summer were not significantly different from that of fish fed daily except when fish were fed for less days in the spring than fish in treatment 2. However, net production was significantly lower for fish that were not fed daily in the spring and the fall as well as during the extremely hot summer. Net production, feed input, feed conversion ratio, visceral fat, and total ammonia were positively correlated to the number of days that fish were fed. Net production, feed conversion ratio, visceral fat, and nitrite were positively correlated to feed input. No significant differences were observed in mortalities (based on daily recorded mortalities), carcass yield, and fillet composition among treatments. Based on these results it appears that catfish should be fed daily for maximum production. Impact of feeding strategies according to water temperatures on water quality appears to be minimal although some significant differences were found among treatments. If catfish are fed to satiation daily from spring to fall, and care is given to avoid waste of feed, they appear to reduce feed intake automatically during cool and extremely hot temperatures.

> ## *Results at a glance...*
>
> ✰ *Catfish being grown to food-size should be fed daily during the growing season for maximum production.*

A second experiment was conducted to evaluate effects of diet composition (dietary protein and energy to protein ratio) and feeding frequency (daily, every other day, or every third day) based on water temperatures on optimizing nutrient utilization and reducing waste in channel catfish farming. In April 1998, two sizes of channel catfish (30 and 165 g/fish, respectively with a 7:3 ratio) were stocked into 28, 1.0-acre earthen ponds at a rate of 10,000 fish/acre. After a 1-month conditioning period, fish were fed to satiation with diets containing different protein levels and energy to protein ratios once daily, once every other day, or once every third day based on water temperatures. All fish were harvested in November 1998. Data collection and analyses of water quality and fish samples were the same as described for 1997. Fish fed a 32% protein diet daily (treatment 1) had a lower net production than fish fed a 28% protein diet every other day in the spring and fall at water temperatures below 80°F and fed a 26%

protein diet during the rest of the growing season (treatment 4) and fish fed a 35% protein, high energy diet every third day in the spring and fall at water temperatures below 80°F and fed a 28% protein diet during the rest of the growing season (treatment 6). This may have been caused by a higher mortality observed for fish in treatment 1. Net production was positively correlated to feed input. Feed conversion ratio was positively correlated to the number of days that fish were fed and feed input. Visceral fat and chlorophyll *a* concentrations were positively correlated to feed input. Net production, feed input, visceral fat, carcass yield, and water quality parameters were not correlated to the number of days that fish were fed, which appears to contradict results from the 1997 study. Reasons for the different responses between years may be related to the fact that several different diets were used and a shorter period for water temperatures below 80°F was observed in 1998 (37 days) as compared to that in 1997 (63 days). No significant differences were observed in proximate composition of fillet samples among different treatments. Data from the 1998 study confirm those from the 1997 study in which feeding strategies based on water temperatures do not markedly affect water quality. Until the data from the 1998 study are confirmed or refuted in future studies, we recommend daily feeding of either a 28 or a 32% protein diet during the growing season.

A third experiment was conducted to evaluate feeding strategies on optimizing nutrient utilization and reducing waste in channel catfish production. The feeding schedules follow: (1) daily; (2) 5 days on -2 days off; (3) 4 days on - 3 days off; (4) 7 days on - 3 days off; (5) 6 days on - 1 day off; and (6) 4 days on - 1 day off. In March 1999, fingerling channel catfish (45 g/fish) were stocked into 28, 1.0-acre earthen ponds at a rate of 10,000 fish/acre. After a 1-month conditioning period, fish were fed to satiation with a 28% protein diet according to the feeding schedules. All fish were harvested in November 1999. Data collection and analyses of water quality and fish samples were the same as described for 1997. Fish fed once daily consumed the most amount of feed, had the highest net production, feed conversion ratio (lowest feed efficiency), carcass yield, and visceral fat. Feed input, net production, feed conversion ratio, carcass yield, visceral fat, fillet fat, total nitrogen, and chlorophyll *a* were positively correlated to the number of days that fish were fed during the growing season. These variables (except for feed input) were also positively correlated to feed input. Ammonia, nitrite, and nitrate were not correlated to the number of days that fish were fed nor feed input. These results indicate that although not feeding daily had some benefits on water quality and feed conversion efficiency, depriving fish from feed too often or too long severely decreases fish production. At current fish and feed prices, feeding daily during the growth season achieves maximum production and profits.

The University of Memphis. Channel catfish fed under the different feeding regimes reported by Mississippi State University in Stoneville responded similarly to confinement stresses. Fish were moved from ponds to aquaria receiving flow-thru well water. However, the feeding protocols resulted in different lengths of times since the last feeding, which made comparisons of the stress data difficult. As such, an alternative experimental design was developed to compare confinement stress responses between fish held on diurnally changing temperatures with fish held at constant temperature at the extremes of the diurnal oscillation.

Two patterns of diurnal temperature oscillation were used: a summer pattern which cycled between 95 and 75°F and spring/fall pattern of 80 and 60°F. The tank temperature was changed slowly over a 10-hour period and held at that temperature for 2 hours, then the pattern was reversed to the other extreme. An initial sample was taken after exposure to the temperature treatment and the fish were then stressed by confinement for 2 hours in a submerged basket, after which another sample was obtained. Eight tanks were used for each pattern (summer or spring/fall): two tanks at constant high temperature, two at constant low temperature, and four tanks with oscillating temperature. Fish in two of the oscillating-temperature tanks were stress-tested at the end of the high temperature period and fish in the other two tanks were stress-tested at the end of the low temperature period. Samples were obtained from 8 to 10 fish each, and no fish was sampled twice. Blood was taken from the caudle vessels from anesthetized fish, the plasma separated and plasma cortisol concentrations determined by radioimmunoassay.

Initial serum cortisol levels were similar in fish from all four treatments under the spring/fall pattern (80-60°F). Two hours of confinement resulted in an increase in plasma cortisol in all groups and were about 6 times higher than the initial cortisol concentration. Fish held at constant 80°F had a significantly lower cortisol concentration after 2 hours of confinement than fish held at constant 60°F or under oscillating temperature conditions, which was tested at the end of the cold (60°F) period.

Cortisol concentrations were similar to previous experiments with channel catfish and were not dramatically different among the temperature conditions. However, responses of fish held under the summertime thermal pattern showed some interesting differences. The initial (pre-confinement) cortisol concentration in fish exposed to the constant 95°F was 29.8 ± 2.2 nanograms/milliliter (mean ± standard error), and for fish exposed to the constant 75°F, the initial cortisol concentration was 20.6 ± 2.6 nanograms/milliliter. Cortisol levels in both of these constant temperature groups were higher than those from fish held in cycling conditions when sampled at the end of the heating (cortisol concentrations were 8.9 ± 0.7 nanograms/milliliter) or cooling period (cortisol concentrations were 9.5 ± 0.6 nanograms/milliliter). Confinement increased the cortisol concentration by 2 to 3 times the initial concentrations except in the fish held at a constant 95°F. In those fish, cortisol concentrations were not statistically higher after confinement than before. The increased cortisol concentration after confinement is due to increased synthesis and secretion of cortisol since little is stored by the secreting tissues. The higher initial concentrations and the lack of response to confinement in fish held at 95°F is likely due to the fish being under a constant thermal stress. Maximal synthesis and secretion during constant stress depletes the capacity to increase output due to additional stress and is described as inadequate adrenal reserve. Fish held at the lower temperature or under a cycling thermal pattern were able to respond to the confinement by increasing cortisol output. The temperature drop overnight in ponds may provide a needed stress reduction during the hottest seasons.

Texas A & M University. Two feeding trials have been conducted with channel catfish initially measuring 4-5 inches in length to investigate the effects of spring and fall photoperiod and temperature as well as dissolved oxygen on feed intake. Based on results of this study, a model was developed to describe the combined effects of temperature and dissolved oxygen in predicting feed intake of channel catfish to optimize feeding schedules and increase the efficiency of feeding.

Objective 2b. *Evaluate alternative feeding strategies including manipulation of diet composition in relation to such variables as water temperature and fish size for channel catfish, baitfish, and hybrid striped bass (sunshine bass).*

North Carolina State University. A pond trial was conducted to evaluate the effects of feeding frequency on hybrid striped bass fingerling production. Feeding frequencies of two, three, and four times per day were tested. Reducing daily feeding frequency from three or four times per day to two times per day had no effect on total production or size distribution of fingerlings.

Results at a glance...

- *Reducing feeding frequency from three or four times a day to two times a day can save costs when raising hybrid striped bass fingerlings. Feeding food-sized hybrid striped bass once a day in early morning or late afternoon results in best growth, feed conversion, and uniformity of size.*

A second pond trial was conducted to evaluate the effect of time of day of feeding on the production of hybrid striped bass foodfish. Preliminary results indicate that fish fed once per day either in the early morning or late evening had higher total production, average weight, and were more uniform in size distribution than fish that were fed either during mid-morning or mid-afternoon.

A final pond study was conducted to definitively establish the optimum feeding frequency for hybrid striped bass fingerlings. Frequencies of one, two, and four times per day were evaluated to determine the effect of feeding frequency on overall production, feed conversion and size distribution. Total production, average weight and feed conversion efficiency were significantly improved when fish were fed twice per day versus once per day. Increasing feeding frequency to four times per day did not improve measured production variables.

A series of tests was conducted to determine the acute toxicity of ammonia and nitrite to different life stages of hybrid striped bass. The 96-h LC50s (concentrations lethal to half the test organisms after 96 hours of exposure) were determined for eggs, larvae, 1-month old juveniles, 4-month old juveniles, and 18-month old adult fish. In general, the egg stage is the most tolerant of ammonia and nitrite. The larval stage was the least tolerant to ammonia with tolerance increasing by the 4-month old juvenile stage. Larval hybrid bass were highly tolerant to nitrite but this tolerance declined rapidly by the 1-month old juvenile stage.

The University of Arkansas at Pine Bluff. Diets containing different lipid sources were evaluated in terms of their effect on standard performance measures (growth, survival, feed efficiency) and also indices of stress response (cortisol, glucose, chloride). A series of aquarium experiments using purified diets with 10% lipid from different sources was conducted. The diets were formulated to be identical with the exception of the type of lipid(s) used. In trial 1, the lipid sources were: soybean oil, cod liver oil, rice bran oil, canola oil, or olive oil. In trials 2 and 3 one diet contained equal amounts of cod liver oil and soybean oil, and the diet with rice bran oil was not used. In addition, the casein, gelatin, dextrin, Celufil, and carboxymethylcellulose were extracted with boiling ethanol to remove residual lipid prior to adding dietary lipids. Fish in all trials were fed twice daily to satiation and weighed every 3 weeks.

In trial 1 (11 weeks) there were no significant differences in weight gain or survival among treatments. Whole-body lipid was higher in fish fed diets with vegetable versus animal lipids for unknown reasons. Fish from this experiment were acclimated to different tanks, then subjected to a sublethal stress test (low concentrations of dissolved oxygen). Airstones were removed and the dissolved oxygen concentrations were measured as they declined. When the dissolved oxygen concentration was 3.8 ppm, two fish died, and this was designated the end of the stress period. Remaining fish were returned to aerated tanks and mortality was tracked for 24 hours (no mortality occurred for 7 days following this 24-hour period). Cumulative mortality following the stress test was statistically higher in fish fed the diet with olive oil than in those fed all other diets. There was no mortality of fish fed the diet with soybean oil, and mortality was intermediate in other treatments.

Trial 2 was terminated after 6 weeks due to disease problems. Statistical analysis of the 6-week data showed that there were significant differences in weight gain of fish fed the non-extracted versus extracted diets. Weight gain was higher in fish fed the extracted diets. There were no differences in weight gain of fish fed non-extracted diets with different lipid sources. However, among fish fed extracted diets weight gain was highest in fish fed the soybean oil + cod liver oil diets versus those fed diets with cod liver oil, canola oil, or olive oil alone. Diets with n-6 to n-3 fatty acid ratios of 2.1 (soybean oil + cod liver oil) to 7.0 (soybean oil) supported best fish growth, while diets with ratios far below (cod liver oil = 0.3) or above (olive oil = 148; canola oil = 198) this range resulted in reduced growth. Survival did not differ among treatments.

The results of trial 3 (8 months) were not consistent with earlier trials. Weight gain was highest in fish fed non-extracted diets with olive oil or cod liver oil alone and lowest in fish fed diets with soybean oil or canola oil alone. Survival was lowest in fish fed non-extracted diets with soybean oil or cod liver oil alone. There were no differences in weight gain of fish fed non-extracted or extracted diets regardless of lipid source. However, survival of fish fed ethanol-extracted diets was significantly higher than that of fish fed non-extracted diets, regardless of lipid source. Lipid source was not associated with differences in weight gain or survival, but there were differences in appearance of fish fed diets with different sources. Fish fed the diet with olive oil had severe fin and opercular erosion, and some had exophthalmia. By contrast, fish fed the diet with canola oil maintained fin and skin integrity and exhibited little external pathology. External appearance is critical for marketing of baitfish. Fish from this trial also were subjected to a stress test (crowding) and blood was drawn for serum cortisol and electrolyte analysis (see results reported for the University of Memphis in the next section).

An outdoor feeding trial was performed (June-November 1998) to test the effects of practical diets with different lipid sources on performance of golden shiners in fertilized pools. Diets with soybean oil, cod liver oil or cottonseed oil alone, or a 50/50 mix of cod liver and soybean oils were tested. Weight gain of shiners fed diets with cod liver oil alone was significantly higher than that of fish fed diets with soybean oil or cottonseed oil at 8 weeks, but the differences were not significant by 12 weeks. Twelve-week data also showed a high negative correlation between weight gain and survival, indicating that density-dependent growth may have masked diet effects even though all diets were offered in slight excess (4% body weight daily). Serum cortisol determinations on large golden shiners fed diets from the golden shiner pool trial were conducted in July 1998 (see results reported for the University of Memphis in the next section).

A 13-week feeding trial was conducted in 1999 to compare the performance of golden shiners in ponds fed supplemental diets with 4 or 13% lipid as poultry fat, or 13% lipid as menhaden fish oil. The diet with 4% poultry fat was the control. Poultry fat and menhaden fish oil differ in fatty acid content, which could affect health and other aspects of fish performance. Diets were extruded as 4.8-mm floating pellets and crumbled to obtain smaller particle sizes as needed. Diets contained 28% protein and no vitamin or mineral supplements. Ethoxyquin (0.0125%) was added to stabilize lipids. Golden shiners (0.9-gram individual initial weight) were stocked into each of 12, 0.1-acre earthen ponds at a rate of 375,000/acre. Fish in each of four ponds were fed to satiation twice daily with one of the diets (four replicates per treatment). Subsamples from each pond were weighed to determine average weights every 3 weeks. Dissolved oxygen and water temperature were measured twice daily (7 a.m. and 3 p.m.). Secchi depth and other water quality data were collected weekly.

Average individual weights of fish fed the diet with 4% poultry fat were higher than those of fish fed diets with 13% poultry fat or menhaden oil. There were no significant differences in feed conversion or net yield (final minus initial group weight of all fish in a pond) of golden shiners between treatments. The latter implies a higher survival rate in fish fed diets with 13% lipid. Whole-body lipid of golden shiners was higher in fish fed the diet with 13% menhaden oil than in those fed diets with 4 or 13% poultry fat. This was the only measured variable associated with dietary lipid source rather than lipid amount. The reason for the production of fatty fish on the menhaden oil diet is not known. There were no consistent differences in chlorophyll *a*, ammonia, or other water quality parameters due to diet.

A companion trial comparing performance of juvenile goldfish fed supplemental diets (28% protein and no vitamin or mineral supplements) with 4 or 13% lipid as poultry fat or menhaden oil was conducted also in fertilized pools in 1999. Six hundred fish (0.4 g average individual weight) were stocked into each of 4 fertilized pools per diet (4 diets) and fed 3 to 6% of body weight daily in two feedings for 9 weeks. Subsamples of fish were weighed every 3 weeks. After 9 weeks the average individual weight gain of goldfish fed diets with 13% poultry fat or menhaden oil was higher than that of goldfish fed diets with 4% poultry or menhaden oil. Feed efficiency followed the same trend. Net yield of fish fed the diets with 13% lipid (poultry fat or menhaden oil) also was higher than that of fish fed the diets with 4% lipid (poultry fat or menhaden oil). Whole-body lipid of goldfish fed either of the diets with 13% lipid was higher than that of goldfish fed either of the diets with 4% lipid. Chlorophyll *a*, ammonia and other water quality parameters were not consistently different between treatments.

The University of Memphis. Golden shiners from the aquarium studies described in the section above were subjected to a crowding stress. Samples were taken before and after two hours of crowding stress (induced by lowering the water levels in aquaria) and then two hours after the water levels were restored. Plasma samples were collected and the cortisol concentration were determined by radioimmunoassay. The fish were very small which limited the blood sample volume. Some of the samples were combined and all samples could not be measured for cortisol and electrolytes. However, no effect of diet on the stress response was detected.

An aquarium study was conducted on large golden shiners fed 8% lipid as soybean oil, cod liver oil, soybean oil plus cod liver oil, or cottonseed oil. Each diet was fed in duplicate. The system was supplied with flow-thru water and temperature ranged from 75 to 85°F. A confinement stress similar to that above was conducted after feeding the test diets for six weeks. No effect of diet was apparent in any of the samples and low water stress resulted in a dramatic increase in cortisol concentrations (about five times the initial levels). Recovery, indicated by a lower cortisol concentration, was apparent in all groups except the group fed 4% soybean oil plus 4% cod liver oil.

A second aquarium study was conducted using large golden shiners fed diets supplemented with 4 or 13% lipid from poultry fat or menhaden oil, as described above. There were no differences in plasma cortisol samples due to diet. However, fish fed diets with 4% menhaden oil had high pre-stress and post-stress (confinement) plasma cortisol concentrations, but statistical differences were not demonstrated due to high variation in cortisol concentrations among samples.

Louisiana State University, Baton Rouge and Rice Research Station. Several studies investigating inexpensive, locally available feedstuffs for crawfish have been completed by Louisiana Agricultural Experiment Station researchers. Feeding trials conducted in microcosms and outdoor fiberglass pools that simulated pond culture environments resulted in average crawfish growth responses 7 to 72% and 30 to 173% greater when crawfish were fed supplements of rough rice seed (hull on) and whole raw soybeans, respectively, than when crawfish were fed from the cultivated rice forage system alone. Average final weights for crawfish fed agricultural feedstuffs were 60 to 103% of those fed formulated 25% crude protein crustacean feed and total yield averaged 86 to 103% of that achieved with the formulated feed. Data from field studies in earthen ponds were highly variable. It was found in one study that feeding (three days/week) while trap harvesting negatively impacted the catch, most likely by the presence of feed interfering with the effectiveness of the baited trap. In a second and third study, limiting feeding to one day per week following the last harvest day of the week failed to generate significant differences in yields, although supplements of soybean or soybean plus rice tended to provide the greatest quantity of large crawfish. In a fourth study, average total yield was significantly lower when crawfish received supplemental feeds (soybeans), although crawfish size-at-harvest was greater for that treatment. Supplemental feeds sometimes had a significant effect on sparing the forage crop and generally did not negatively impact water quality. It has become apparent that under field conditions when harvesting is accomplished by baited traps, supplemental feeding of crawfish to satiation (even once per week) may not be conducive to increased yields. Furthermore, it appears that the increased quantity of large, higher-priced crawfish at harvest often associated with feeding, may not always justify the cost of feeding, even with low-cost agricultural feedstuffs.

> ***Results at a glance...***
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> ✰ *Feeding supplemental feeds to crawfish during the harvest season reduces the catch, probably by decreasing the appeal of bait in the traps.*

Objective 3. *Develop publications to effectively extend information derived from this project to feed manufacturers and fish producers.*

See list of publications on pages 49-52.

WORK PLANNED

All work planned in the original proposal has been completed with the following exceptions:

Channel Catfish

Louisiana State University, Baton Rouge. The project has progressed as proposed, with one unanticipated problem. On an evening in July 1999, a lightning strike near the research station caused a power outage to the catfish ponds involved in this project. Power was not restored by the utility until the following morning, which resulted in a large loss of fish in production ponds due to low dissolved oxygen levels. This effectively eliminated production for the third year of the study. The experiments planned for the third year are being repeated beginning in March 2000 and will be completed in October 2000 under a 12-month, no-cost extension of the project.

Texas A & M University. The pond feeding trial in which lysine supplementation is being evaluated was completed in August 2000 and data are being analyzed. Additional feeding trials have been completed with channel catfish to further investigate the effects of temperature and dissolved oxygen as well as fish size on feed intake to augment the model being developed for improving feeding schedules for channel catfish.

Baitfish

The University of Arkansas at Pine Bluff. Proximate analysis of fish and feeds is underway, as is analysis of water quality data.

IMPACTS

Channel Catfish

- Reduction of the protein composition of fish feed to 25 or 26% without supplemental lysine should result in reduced feed costs and improve nutrient utilization. This reduction may be best achieved by decreasing the protein content by specifically decreasing the dispensable amino acid content.

- A cost savings of as much as 5% of feed production may be achieved by using an all-plant material protein source rather than animal origin protein. Vitamin and mineral supplementation may be required but sufficient methionine and phosphorus (0.2% available phosphorus) is apparently supplied by plant material protein.

- Feeding 12.5% less of a 32% protein feed resulted in the same yield as feeding 28% feed to satiation whether the fish population consisted of uniform-size fingerlings or mixed fingerlings and market-sized fish. This feeding practice results in less wasted food and may improve feed efficiency and profits.

- Reducing the phosphorus content of catfish feed has little influence on water quality, but will conserve the phosphorus adsorbing capacity of bottom soils. When ponds are drained, water should be released slowly to avoid disturbing the pond bottom and releasing sediment into the effluent. Feeding diets with different protein content had little impact on water quality, including ammonia nitrogen and oxygen demand.

- Feeding strategies based on water temperatures do not markedly affect water quality or fish production. Daily feeding to satiation, regardless of the protein content of the diet, appears to be essential to achieve maximal production. The most important factor is carefully feeding to satiation every day. There is a possibility of predicting feed intake with a model using the interaction of temperature and dissolved oxygen. Such a model might increase the planning or efficiency of feeding schedules.

- Fish exposed to a constant, high water temperature had high cortisol concentrations that were not further increased by confinement stress. Fish held at either a low water temperature or on a temperature regime cycling over 12 hours from 95 to 75°F were able to increase cortisol output during confinement stress. Exposure to constant high temperature apparently requires maximal cortisol synthesis and secretion by fish, which induces an inadequate adrenal reserve. This may contribute to handling stress at upper temperature extremes.

Hybrid Striped Bass

- Overall growth and nutrient utilization values were higher for sunshine bass reared at 90°F than at 80°F. For both temperatures, feed consumption decreased with increasing dietary energy/protein ratios. Feed efficiency, protein efficiency ratio and protein conversion efficiency were highest at a dietary energy/protein ration of 9 kcal/g protein. Whole body lipid deposition and intraperitoneal fat accumulation increased with increasing dietary lipid levels. Sunshine bass farmers should consider this if they plan to culture this species in areas where the water temperature may exceed 90°F.

- Feeding diets with 10 or 15% fat significantly enhanced weight gain over fish fed diets with 5 or 20% fat. Supplementing the diet with carnitine, commercial proteolytic enzymes, cholesterol, or lecithin had negligible effects on growth and body composition. Addition of a commercial proteolytic enzyme to the diet did not increase nutrient utilization or limit waste production.

- Reducing feeding frequency from three or four times a day to two times a day can save on labor costs and equipment wear. Feeding in early morning or late afternoon appears to improve feed conversion and total production.

- The toxicity of ammonia and nitrite changes during early life stages. The egg stage is the most tolerant of either ammonia or nitrite. The larval stage was the least tolerant to ammonia and the most tolerant to nitrite but the tolerance declined rapidly by the 1-month old juvenile stage.

Golden Shiners

- Diets with n-6 to n-3 fatty-acid ratios of 2.1 to 7.0 promoted growth better than those with low (0.3) or high (148 to 198) fatty acid ratios. Soybean oil was a better single source of lipid than canola, cod liver, or olive oils.

- Golden shiners have higher cortisol concentrations than channel catfish before and after low water exposure. Cortisol concentrations increase four to five fold after two hours and have begun to recover two hours after the water level is restored to normal. No consistent effect due to diet was detected.

- The qualitative fat requirement may vary among several factors including growth, survival and appearance and is apparently different between golden shiners and goldfish. Golden shiners grew better on 4% poultry fat than on 13%, but survival was better at the higher fat level. However, weight gain, survival, feed efficiency, and body fat of goldfish were all higher at the higher fat level.

- High-fat feed (13%) is more expensive than typical 4% fat-feed, however some producers have reported good results and intend to continue using it.

Crawfish

- The main impact has been to create awareness, among both scientists and producers, for both the potential benefits of supplementing crawfish in established forage systems of earthen ponds and the potential detriments of feeding under some conditions. Low cost agricultural feedstuffs, such as whole raw soybeans and rice grains, have been shown to be readily consumed by crawfish and contribute to increased growth. However, this project has demonstrated the practical problem whereby introduced feeds can interfere with the effectiveness of baited traps in harvesting crawfish. Furthermore, the results of this project indicate the need for, and possible direction of, further research to address the logistics of supplemental feeding in production systems that utilize baited traps as the sole means of harvesting.

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VERIFICATION OF RECOMMENDED MANAGEMENT PRACTICES FOR MAJOR AQUATIC SPECIES

Reporting Period
January 1, 1997 - August 31, 2000

Funding Level

Year 1	$31,410
Year 2	66,351
Year 3	66,925
Total	$164,686

Participants

University of Arkansas at Pine Bluff (Lead Institution) .... Carole Engle, David Heikes, Steve Killian, Pierre-Justin Kouka
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PROJECT OBJECTIVES

The overall goal of this project is to initiate verification programs in participating states. The emphasis is on developing the interdisciplinary process and internal committees within each state. While actual field results of verification trials of different management protocols will be valuable, this project is intended as a stimulus to develop and utilize verification trials as a new extension tool. The specific objectives of this project are:

1. To develop and implement recommended management practices for catfish and crawfish production systems in participating states;

2. To publish guidelines for infrastructure development, program implementation, and assessing results/benefits of aquaculture management verification. This publication will be a joint effort of participants; and

3. To publish recommended management plans and results of Objective 1.

ANTICIPATED BENEFITS

The principal benefit of verification is to determine if the total set of research-based extension recommendations produces yields, feed conversions, and costs consistent with results from research trials. Researchers and extension personnel learn whether their recommendations are valid in commercial settings and whether or not recommendations and research programs need to be adjusted based on what has been learned. Adoption of verification practices is expected to increase industry yields. The development of the verification management plan encourages open dialogue among researchers, producers, and extension specialists.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Objective 1. *To develop and implement verification programs of recommended management practices for catfish and crawfish production systems in participating states.*

Alabama. The Extension Fisheries team established recommendations for the production systems (levee ponds, watershed ponds, and cages) in the verification project. There are four cooperators and four levee ponds in West Alabama (two with channel/blue hybrid catfish and two with channel catfish), five cages (two in East Central and three in Southeast Alabama), and two watershed ponds enrolled in the verification program. Production and water quality parameters have been monitored and the Fishy '98 computer program is being used to track feeding and other data on most of the production units since 1998. Four levee-style ponds have been enrolled in the verification program for 2-3 years. Stocking rates have varied from 4,071 to 5,469 fish/acre per year. Harvested weights range from 5,241 to 10,749 pounds/acre per year, including fish scrapped from ponds. Three of the four ponds are pending final harvest and scrapping. Overall, survival has ranged from 57 to 91%. Gross feed conversion ratios (pounds of feed/pounds of fish harvested) ranged from 1.87 to 2.8. Of the two watershed ponds enrolled in the program, one was harvested completely; the second is pending harvest when a market is identified for the fish. Two cages in East Central Alabama have not yet been harvested. Fish were stocked in the spring and will be harvested later in the fall. The three cages in Southeast Alabama were successfully harvested in the first year of the project. However, in the second year, under-sized fish were delivered. This, in combination with an outbreak of columnaris disease, resulted in significant mortality and probable escape of fish from the cages. The second-year trial was abandoned.

> ***Results at a glance...***
>
> ✰ *Analysis of the production and economic data indicate that the use of recommended management practices results in greater profit to the producers.*

Arkansas. An inter-disciplinary verification committee, consisting of extension specialists, researchers, economists, and county extension agents developed specific management protocols for the verification of recommended foodfish and fingerling management practices. Record-keeping forms were developed and printed in field-booklet form on waterproof paper. A spreadsheet computer program and sampling methodology were developed to be used with the Fishy 3.2 record-keeping program. A literature search was conducted to ensure that the management protocols reflect a progressive, practical, and profitable management scenario.

Arkansas conducted verification trials on six foodfish (four in northern Poinsett and St. Francis counties and two in southern Chicot County) and two fingerling ponds across the Delta production area. Production inputs and yield data were collected on a weekly basis, summarized weekly and posted on the Arkansas CYVT web site (www.uaex.edu/aquaculture/arcyvp.htm). All ponds have been harvested completely and the complete, final report and summary of all data is in preparation.

Louisiana. The verification committee was formed, a literature review completed, and fisheries/aquaculture agents, specialists, and administration were trained in verification procedures. Management protocols have been developed for three crawfish production scenarios: 1) rice-crawfish rotation; 2) permanent crawfish pond; and 3) growing crawfish behind two successive rice crops. Five cooperators participated in the Year 2 Crawfish Yield Verification Project. Cooperators included three producers from Vermillion Parish, one producer from St. Martin Parish, and one producer from Acadia Parish. There were nine ponds enrolled in the project with six ponds evaluating the rice-crawfish rotation, one pond evaluating the permanent pond scenario, and two ponds of crawfish behind double-crop rice. The production season began in October and terminated in June. Agents collected pre-production samples of water source, forage, and soil. Forage depletion was monitored monthly. Farmers were given recommendations on trap density, bait usage, and harvest regimes. The second production season was completed and data are being summarized.

North Carolina. A five-member committee consisting of industry, university, and extension representatives established recommended catfish management protocols. These protocols have been implemented in the management of three channel catfish production ponds on three separate farms since fall 1997. Data collection continued through 2000 on different production variables (feeding, aeration, labor, etc.) on a weekly basis. Final harvests have been completed. The harvests were delayed due to extensive flooding caused by several hurricanes during the fall of 1999. A complete final report has been prepared summarizing the data collected for the cooperators' ponds over the entire three years of the project.

South Carolina. The verification committee was formed, the management protocol to be implemented developed, and a cooperator identified. Background information on financial and production performance was evaluated. A change in farm managers in the middle of the year caused some delays in the implementation of the rotational plan. The proposed phased rotation management plan was updated to work with the farm's current inventory levels, and modified to work within the farm's restrictions of capital and equipment.

In August of 1998, a 12-acre stocker pond was stocked with approximately 240,000 fingerlings weighing 60 pounds per 1000 fish. They were fed until mid-October when approximately 60,000 fingerlings weighing about 200 pounds/1000 were moved to a vacated pond. The fish were fed until August 15, 1999, and a portion of the fish were harvested (12,000 pounds) and sold to the processing plant. The fish were seined with a 1 3/8-inch sock to estimate true average size of the fish. The average size of the fish harvested was 1.38 pounds. There were personnel changes at the farm so that no accurate information was available on actual feed fed and no conversion rates could be calculated. The stockers were moved at approximately the correct time and size and were within 5% of the targeted market size at harvest that was projected by the model.

Objective 2: *To publish guidelines for infrastructure development, program implementation and assessing results/benefits of aquaculture management verification. This publication will be a joint effort of participants;* and

Objective 3: *To publish recommended management plans and results of Objective 1.*

A joint project publication will also be published in the coming year that includes guidelines for infrastructure development, program implementation, and evaluation of aquaculture management verification programs. Management protocols have been developed for use in all participating states and will be published in the near future.

WORK PLANNED

Commercial fish production requires complex management decisions on stocking, harvesting, and marketing. There are numerous factors such as market requirements, drought, disease, and hurricanes, that are outside the control of the manager that affect the operator's ability to manage the fish farm according to a pre-set schedule. These same factors and conditions have prevented final harvest of several of the ponds enrolled in the verification program. Much of the work planned for the coming year includes final harvest followed by final data summaries, and synthesis. When all data have been synthesized, complete reports with complete data will be published in each state.

Additional work planned includes review, revision, and finalization of the draft of the guidelines on yield verification. The review will be conducted by project participants. Once revised, the manuscript will be submitted to the SRAC Publications Committee for publication.

Alabama. The remaining three levee ponds in West Alabama will be drained, harvested completely, and scrapped. The second watershed pond will be harvested completely when a market is located. The cages in East Central Alabama will be harvested completely later in the fall.

Arkansas. A comprehensive report is under preparation that summarizes all data collected over the project period for all ponds and includes an economic analysis of the results.

North Carolina. Based on the data generated by this trial, a manual of recommended management practices for channel catfish farmers has been drafted. Final analysis of the data, including an economic analysis, will be completed.

South Carolina. This project had great potential to help the catfish industry and much work was put into establishing the system to collect data including a complete financial analysis of the current farm situation. However, in the middle of the project the farm experienced major personnel problems and the cooperator decided to sell the business. Therefore, the participation by South Carolina in this project has been terminated.

IMPACTS

Alabama. There has been an increased awareness of actual inputs required to produce a crop of catfish, more attention paid to tracking all the real costs of catfish production, and increased attention to and analyses of water quality in pond production. There is now an awareness that close monitoring of water quality and equipment condition (aerators, tractors, feeders, etc.) can prevent problems and decrease fish mortality. There is increased attention paid to record-keeping and tracking costs of production. One producer who normally stocked ponds in the 12 to 15,000 fish/acre range has reduced his stocking rates because of the production data in his verification pond. Also, the verification program showed that the routine use of a 1.75-inch mesh seine results in an average size of fish sold of 2 pounds, not the 1.25 pounds previously assumed. Overall, the project demonstrated without a doubt that current extension guidelines will result in profitable production.

Arkansas. Of particular interest is the impact that this program has had on producers in the northern half of Arkansas. Prior to this program, county agents had very little exposure to catfish producers and many producers have turned to non-Extension sources of information for years. Since the initiation of this program, word has spread through fish farmer networks that Extension has important information and the county agents have seen a tremendous increase in the number of aquaculture-related calls in their counties. The agent in Poinsett County has asked specifically that we continue catfish verification in his county indefinitely. Also, the number of producers from the northern counties submitting disease cases to Extension Fish Diagnostic Laboratories in Arkansas has increased, indicating an increased level of trust with Extension services.

Results at a glance...

- *The success of this project prompted one county agent in Arkansas to request that yield verification continue indefinitely.*

Louisiana. The major impact of the project to this point has been the interest of the field agents in participating in a proactive program. Field agents have increased awareness of the importance of population structure at the end of the previous season, summer management of natural forage or rice, precipitation patterns while crawfish are aestivating in burrows, pesticide use, and fall flooding protocols. Two of the cooperators reported that they realized the difference that higher trap densities had on overall catch rate. Some cooperators have shared previous years' records with agents in an attempt to further refine their production practices.

North Carolina. The cooperating farmers have expressed their satisfaction with the results of this project and, where practical, have implemented these same practices on the rest of their ponds.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

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CONTROL OF BLUE-GREEN ALGAE IN AQUACULTURE PONDS

Reporting Period
January 1, 1999 - August 31, 2000

Funding Level	Year 1	$307,574
	Year 2	281,986
	Year 3	253,326
	Total	$842,886

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PROJECT OBJECTIVES

1. Develop chemical control methodologies to prevent the establishment of noxious blue-green algal communities.

    a. Evaluate novel selective blue-green algicides identified through laboratory screening.

    b. Isolate, identify, and test allelopathic chemicals produced by competing blue-green algae and other micro-organisms found in local aquatic communities.

2. Evaluate nutrient manipulation to promote desirable phytoplankton community structure.

    a. Increase nitrogen-to-phosphorus ratios in the water.

    b. Reduce the availability of phosphorus from pond bottom muds.

    c. Enhance the availability of inorganic carbon.

    d. Manipulate trace metal availability.

    e. Increase potassium levels in the water.

    f. Increase salinity levels in the water.

3. Evaluate water circulation as a means of altering the environment to promote desirable phytoplankton community structure.

4. Evaluate the use of plankton-feeding fish to alter the environment to promote desirable phytoplankton community structure.

5. Evaluate the development of phytoplankton communities in the Partitioned Aquaculture System.

ANTICIPATED BENEFITS

The overall goal of this project is to identify methods of controlling or eliminating blue-green algae from aquaculture ponds. The ability to control algal communities in ponds could benefit farmers in several ways.

Excessive abundance of blue-green algae, especially when combined with their habit of growing in surface scums, can cause low dissolved oxygen concentrations and other water quality aberrations that affect fish growth and health. Therefore, the ability to control the composition of blooms could result in better fish growth and lower costs for aeration and other water quality management procedures.

The largest and fastest growing segment of aquaculture in the United States is farm-raised channel catfish. Catfish that are off-flavor are unmarketable, and farmers are forced to hold those fish in inventory until composition of the pond microbial community changes and flavor improves. Holding market-sized fish in inventory imposes an economic burden on farmers, and off-flavor is estimated to cost the industry well over $20 million a year.

Baitfish mortalities associated with blue-green algae are common in the early summer. Historical use of high rates of granular fertilizers may be a factor in these excessive algae blooms, especially in baitfish ponds that have been in production for years and have accumulated sediments. Documenting potential improvements in water quality as a result of pond renovation and sediment removal will provide farmers with information to make informed decisions when weighing benefits of pond renovation against costs. Alternatively, sodium nitrate has been proposed as a pond bottom treatment to improve water quality and has been shown to effectively control phosphorus release from soil in laboratory studies. Field studies in commercial ponds will provide producers with information to better evaluate the potential merits of this compound.

Most of the treatments and management practices considered in this project have been promoted for controlling blue-green algae, but their effectiveness has not been documented. It is anticipated that this research will reveal which, if any, of these treatments are beneficial. Any practice demonstrated to be effective in controlling blue-green algae has considerable potential for improving aquaculture management and enhancing profits.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Objective 1. *Develop chemical control methodologies to prevent the establishment of noxious blue-green algal communities.*

University of Mississippi. Collections have been made of nearly 500 freshwater plants and algae, and more than 300 marine algae and tropical blue-green algae. Over 4,000 extracts have been prepared from these plants, and the extracts are being tested under laboratory conditions for selective toxicity against blue-green algae that cause off-flavors in fish.

Initial extract evaluations were conducted using *Oscillatoria agardii* as the cyanobacterial test organism, and *Selenastrum capricornutum*, as a chlorophyte control for non-specific algicidal activity. These organisms are grown in continuous-flow culture by K. K. Schrader (USDA-ARS) and provide uniform algal material for biological evaluation. Lipid extracts of 33 tropical marine cyanobacteria and algae were evaluated in these assays at an initial concentration 100 ppm. Two related species of marine green algae and one species of marine brown algae were found to contain substances that were selectively algicidal against *O. agardii*.

> **Results at a glance...**
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> ✩ *Bioassay-guided fractionation of plant and algal extracts has resulted in the purification of unusual natural products that are effective against odor-producing blue-green algae in the parts per billion concentration range. The chemical structures of several of these metabolites have been solved and are undergoing additional toxicological and algicidal evaluation.*

While these results are promising, the cyanobacterium *O. agardii* is not known to produce odorous compounds that result in off-flavor problems in aquaculture ponds. Cultures of the methylisoborneol-producing cyanobacterium *O. perornata* have now been established that are suitable for high-throughput extract evaluation. Extracts from 356 collections of aquatic and marine cyanobacteria, plants and algae have subsequently been evaluated in replicate bioassays using *O. perornata*. Forty-three extracts (12%) were found to be strongly active and cyanobacterial-selective at the concentration of 100 ppm used for initial screening. Dose response data were obtained for these active extracts at half-log concentrations. Twelve extracts were found to be effective at 30 ppm or lower. The two most potent extracts were confirmed to be two of the related marine green algae species first identified in the initial bioassays that used *O. agardii* rather than *O. peronata*. Bioassay-guided fractionation of several extracts has resulted in the purification of unusual cyanobacterial-selective natural products that are effective against both *Oscillatoria* strains at concentrations in the ppb (parts per billion) range. The chemical structures of several of these metabolites have been solved and are undergoing additional toxicological and anti-microbial evaluation.

USDA-ARS. Pond work was conducted to test the effectiveness of a promising natural product derivative as a selective algicide against blue-green algae. The compound is a proprietary analog of a natural product found in plant extracts. Efficacy testing in pond mesocosms yielded promising results. Approximately 1.15 ppm of the proprietary compound killed the odor-producing blue-green alga *Oscillatoria perornata* within 24 hours. Previous laboratory studies revealed effective kill of *O. perornata* and another blue-green alga, *Raphidiopsis brookii*, at 0.57 ppm treatment with the proprietary compound. Preliminary dose-response studies conducted in catfish aquaculture ponds this summer indicate concentrations of less than 1.15 ppm of the compound are not effective in reducing abundance of *O. perornata*. Additional efficacy testing is scheduled to be performed later this summer to further evaluate the effectiveness of the natural product derivative.

Additional screening of pure natural compounds and crude plant extracts has identified several other leads for potential use as cyanobacteriocides.

Objective 2. *Evaluate nutrient manipulation to promote desirable phytoplankton community structure.*

Mississippi State University. In experiments repeated over two years, eight 1,450-gallon enclosures (limnocorrals) were placed in a small research pond or a commercial earthen fish pond in which a dense phytoplankton community dominated by *Oscillatoria agardhii* was present. The N:P ratio of four enclosures was adjusted by addition of KNO3 to provide a N:P ratio of about 30 and chelated iron was added to provide 1 ppm iron. Four enclosures did not receive nutrient additions. All enclosures were supplied with diffused aeration to produce gentle turbulence. Water samples collected every 2 to 3 days were analyzed for nutrients, solids, and indices related to phytoplankton biomass and community composition. After 2 weeks, the combination nutrient addition did not result in a shift of the phytoplankton community from dominance by *O. agardhii*. Nitrite concentrations increased and soluble phosphorus concentrations declined in nitrate-treated enclosures. Algal biomass in untreated enclosures declined, suggesting that continued nutrient supply is necessary to sustain high algal biomass. Nutrient manipulation does not appear to hold promise as a technique to effect phytoplankton community structure in hypereutrophic aquaculture ponds.

Results at a glance...

- *Studies in Mississippi and Alabama indicate that various manipulations of waterborne plant nutrients have little promise for controlling phytoplankton community composition in catfish ponds with high feeding rates.*

Auburn University. An initial laboratory study considered the effectiveness of the chelating agents ethylenediamine tetra-acetic acid (EDTA), lignin sulfonate, and citric acid for maintaining iron in solution. EDTA was the most promising of the chelating agents, because iron remained at concentrations above 0.5 ppm for 30 days in soil-water systems treated with 1 ppm iron from iron-EDTA. Thus, it was decided to use EDTA chelated metals for pond research.

Pond studies in 1999 had three treatments: (1) chelated iron (0.5 ppm) plus chopped legume hay (35 pounds/acre per week); (2) chelated iron, chopped legume hay, plus a trace element mix (1 pound/acre per week); and (3) control. The hay applications resulted in low dissolved oxygen concentrations, and no benefits related to blue-green algae control were observed. This year (2000), two treatments (legume hay at 20 pounds/acre per week and legume hay plus 0.5 ppm chelated iron and 1 pound/acre per week of trace element mix) are being compared to the control. So far, no benefits of treatments on water quality or blue-green algae control have been observed.

In 1999, potassium chloride treatments of 0, 30, 90, and 120 ppm did not result in significant differences in blue-green algal abundance. A sodium nitrate based fertilizer containing 8% N, 24% P_2O_5, and 15% K_2O was as effective as a standard, 10-34-0 liquid fertilizer in promoting sunfish production. Sodium nitrate also proved efficient as a nitrogen fertilizer in bait minnow ponds.

Soil cores from bait minnow ponds near Lonoke, Arkansas, revealed a large accumulation of inorganic and organic phosphorus over time. Studies in soil-water systems revealed that nitrate applications did not improve soil or water quality.

University of Arkansas at Pine Bluff. A laboratory study was conducted to evaluate the effects of adding sodium nitrate to pond bottom soils. Two common soils (Perry-Portland, known locally as gumbo, and Calloway-Calhoun-Loring, or crawfish) were collected from the bottoms of 20- to 45-year-old commercial baitfish ponds. A layer of gumbo or crawfish soil was added to each of 24, 3.5-gallon microcosms and sodium nitrate was soil-incorporated at rates of 0, 25, 50, or 75 g N per square meter. Buckets were filled with pond water and sodium acetate was added throughout the study as a source of organic matter to create anoxic conditions and simulate pond bottom waters. Results showed that incorporating sodium nitrate did suppress phosphorus release in both soils for 11-19 days. However, elevated nitrite concentrations were also found during this period. Nitrite levels as high as 180-250 ppm were measured in the high rate treatment. The higher the rate of sodium nitrate, the longer was the duration of phosphorus suppression, but the additional time was not proportional to the dose. Conditions in experimental microcosms differ significantly from those in ponds, and additional work in enclosures within ponds is planned to further evaluate the potential of sodium nitrate as a pond bottom treatment.

Pre-treatment water quality and plankton community data were collected from 12 commercial golden shiner ponds at monthly intervals. Study ponds ranged in size from 7 to 25 acres and represented two soil types and two ages (20-25 years and 40-45 years). Using a column sampler, water samples were collected from a single location in each pond. Results showed that for parameters linked to phytoplankton abundance (chlorophyll *a*, chemical oxygen demand, total phosphorus, and total nitrogen), seasonal water quality changes in golden shiner ponds were similar to those reported for channel catfish culture. Dissimilar results were found for dissolved inorganic nitrogen, which was highest in the fall rather than in the winter, as has been reported for catfish. This may reflect the relatively low feeding rates used for baitfish. In addition, relatively higher soluble reactive phosphorus concentrations were found in the summer months, perhaps a result of powdered feed used for young fish.

Pond renovation was evaluated as a technique to reduce problematic blue-green algae blooms and associated water quality problems. Monthly water quality and plankton community data were collected from 12 commercial goldfish ponds for a year. Pre-renovation soil samples were also taken. Six of the 12 ponds were renovated during the winter (1999-2000) and returned to production in late spring. Results to date indicate little difference in water quality in renovated ponds as compared to control ponds. Soluble reactive phosphorus was significantly higher in renovated ponds for the first two months after ponds were returned to production, reflecting pond management practices for rearing new crops of fish. Post-renovation water quality monitoring will be continued through the growing season.

Louisiana State University. Moderate increases in salinity may help control cyanobacterial populations in freshwater ponds. Concentrations of the off-flavor compounds, 2-methylisoborneol (MIB) and geosmin, were determined from March 1999 through February 2000 in 15, 0.2-acre experimental channel catfish ponds at the LSU Aquaculture Research Station. Ponds were assigned randomly to three salinity levels: 0 ppt, 1.5 ppt, and 3.0 ppt. Nominal salinities were established and maintained with periodic additions of common salt (NaCl).

All pond waters contained little or no MIB until late May. All ponds experienced off-flavor episodes in fish at some time in the production study. Trace levels of geosmin were observed throughout the year. Three major increases in the concentration levels of 2-MIB were observed; one in early June, a second in early August, and a third in late September. These increases in off-flavor-causing compounds were not observed in all ponds nor did any one pond see an increase during all three times. In several cases, concentrations of MIB increased rapidly from trace levels (<0.1 ppb) to very high levels (>30 ppb) over a 3 to 4 day period between sampling periods. In other ponds, only a slow increase in MIB concentration was observed.

MIB concentrations were slightly lower in ponds at 3.0 ppt salinity, but ponds at 1.5 ppt salinity routinely had higher levels of MIB than the ponds at 0 ppt. At this time, there is insufficient data determine the impact on the mitigation of off-flavor episodes by using low levels of NaCl. Future analysis of phytoplankton samples will see if salinity affected the presence of *Ocillatoria peronata* and other blue-green algae.

North Carolina State University. Different fertilizers and a source of organic matter were evaluated for their effectiveness in controlling blue-green algae blooms in 12, 0.25-acre hybrid striped bass (*Morone chrysops* x *M. saxatilis*) ponds. Chelated iron, a mineral mix and alfalfa pellets were applied to the ponds during an entire growing season. Water samples were taken weekly and analyzed for nutrients and phytoplankton composition. Blue-green algae began to appear in samples during the second week of September 2000 and dominated phytoplankton composition in all treatments during the month of October 2000. There were no differences in species composition, fish production, or water quality variables among any of the treatments.

Objective 3. *Evaluate water circulation as a means of altering the environment to promote desirable phytoplankton community structure.*

Louisiana State University. Twelve, 0.1-acre ponds were stocked with multiple cohorts of channel catfish at a nominal stocking density of 10,000/acre. In eight ponds, fish were restricted to approximately a quarter of the pond area by a barrier placed across the pond width.

In these ponds, a continuously-operating, horizontally-mounted pump mixed water between the area containing fish with the open area of the pond. In four of the mixed ponds, threadfin shad were stocked at 200/acre in the open area of the pond. Although the experiment is ongoing, there are no differences in water quality, phytoplankton community composition, or feeding rate among the three treatments.

Three water management practices were evaluated, each at two levels (presence or absence), alone, and in combination, to determine their effects on blue-green algal community composition and water quality in experimental mesocosms managed to simulate commercial catfish production practices. In one treatment, aluminum sulfate (alum) was applied weekly at 3 ppm to reduce phosphorus (chemical control). In a second treatment, the water column was destratified by continuous vertical mixing in contrast to conventional surface aeration (physical control). In the third treatment, planktivorous gizzard and threadfin shad (*Dorosoma* spp.) were stocked at 25,000 juveniles/acre with channel catfish, to evaluate their ability to control blue-green algae (biological control).

The eight treatment combinations, arranged in a 23 factorial design, were randomly assigned to 24, 3,000-gallon fiberglass tanks with soil bottoms (mesocosms) with three replicates per treatment combination. Catfish juveniles (mean = 52 g) were stocked in May at 10,000/acre, fed a 32% crude protein commercial feed daily at rates ranging from 40 to 150 pounds/acre, and harvested in November. Water samples were collected biweekly for nutrient and phytoplankton analysis.

Mean catfish survival was 88.5% and yield averaged 7,133 pounds/acre, with no observed differences related to water management practices. Shad biomass averaged 625 pounds/acre at harvest. The alum reduced soluble reactive phosphorus in October, but had no affect on phytoplankton density or community composition. Suspension of sediments in the water column from vertical mixing increased total nitrogen, total phosphorus, nitrate, and pH but had no discernible effect on the phytoplankton community. The presence of shad significantly reduced total algal biomass as evidence by reductions total nitrogen, total phosphorus, chemical oxygen demand, and chlorophyll *a*. Although the percentage of blue-green algae in the phytoplankton community was not significantly reduced compared to mesocosms without shad, odorous species of blue-green algae (*Oscillatoria perornata* and *Anabaena* spp.), known to cause off-flavor in catfish, were nearly eliminated by the presence of shad. Mesocosms with shad never had odorous species of blue-green algae that accounted for more than about 3% of the blue-green algal community, while mesocosms without shad were as high as 20%. Shad had no impact on catfish production.

North Carolina State University. We are currently conducting a study on the effectiveness of water circulation for controlling blue-green algae abundance in hybrid striped bass ponds.

This study has been plagued by mechanical problems with the circulators that have resulted in decreased water circulation and the loss of several replicates in the treatment receiving circulation. This study is still in progress and will not terminate until the first week of November 2000.

Mississippi State University. In two experiments, twelve, 0.1-acre ponds were stocked with multiple cohorts of channel catfish at a nominal stocking density of 10,000/acre. In the first experiment, fish in eight ponds were restricted to approximately one-quarter of the pond area by a barrier placed across the pond width. In these ponds, a continuously-operating, horizontally-mounted ½-hp pump mixed water between the area containing fish with the open area of the pond. In four of the mixed ponds, threadfin shad were stocked at 200/acre in the open area of the pond. There were no differences in water quality, phytoplankton community composition, or feeding rate among the three treatments.

In the second experiment, a baffle was placed along the long axis at the center of the pond. Four ponds were mixed with one ½-hp pump; four ponds were mixed with two ½-hp pumps; and four ponds did not contain baffles and were not mixed. Results were similar to those obtained in the first experiment. Ponds with two mixers were more turbid than ponds in the other treatments. Turbidity in ponds with two mixers was dominated by suspended mineral matter.

Objective 4. *Evaluate the use of plankton-feeding fish to alter the environment to promote desirable phytoplankton community structure.*

Auburn University. Ten, 0.1-acre earthen ponds were stocked with 9-g channel catfish at a density equivalent to 6,000/acre, with 0.3-pound grass carp at 20/acre. Five randomly selected ponds were stocked with 8-g threadfin shad at 800/acre. Each pond was fed once daily to apparent satiation with a commercial floating feed (32% crude protein). All ponds were harvested 8 November 1999 and fish were identified, sorted and weighed. One channel catfish was randomly selected from each pond for flavor analysis.

Total threadfin shad mortality occurred in one of the shad ponds (9 September 1999). One of the no-shad treatment ponds experienced a catfish kill during the final two weeks of September when they had attained an average weight of 0.7 pounds. Observed mortality during this period was 31% of the original stock and only 48% of the original stock was recovered at harvest. Following the shad mortality, that pond was eliminated for further consideration of water quality and phytoplankton analysis for the shad treatment.

The presence of shad had no effect on temperature, dissolved oxygen concentrations, pH, or total alkalinity of pond waters. Total organic carbon concentrations ranged from 6.3 ppm in April to 30.5 ppm in October in the shad treatment and from 4.7 ppm in April to 34.1 ppm in September in the no-shad treatment. Total organic carbon levels increased in both treatments during the growing season and were higher in the shad treatment on 27 April and 25 May and higher in the no-shad treatment on 19 August.

Total ammonia-nitrogen (TAN) concentrations increased in both treatments throughout the growing season, but reached higher levels in the no-shad treatment. In the shad treatment, TAN concentrations ranged from 0.03 mg/L in April to 1.92 mg/L in October. In the no-shad treatment, TAN ranged from 0.03 mg/L in June to 3.88 mg/L in September. TAN concentrations in the no-shad treatment were significantly higher than concentrations measured in the shad treatment for the period 1 September through the end of the study. Nitrite-nitrogen concentrations in both treatments remained below 2.0 ppb until July and then began to increase, reaching a maximum of 37.2 ppb in the shad treatment in September and 94.5 ppb in the no-shad treatment in October. For the period 1 September through 25 October, nitrite-nitrogen levels were significantly higher in the no-shad ponds.

Results at a glance...

☆ *In both Alabama and Georgia, stocking threadfin shad with channel catfish resulted in lower ammonia and nitrite levels late in the growing season. The improved environmental conditions apparently resulted in significantly better survival of catfish in the presence of shad in the Alabama study.*

Phytoplankton abundance (as indicated by chlorophyll *a* levels) were low initially and increased progressively throughout the study to highs of 263 ppb chlorophyll *a* in the shad treatment in September and 285 ppb in the no-shad treatment in August. Chlorophyll *a* levels were significantly higher in the no-shad treatment on only one sampling date. There were no differences overall in phytoplankton abundance when data for chlorophyll *a* levels for all sampling dates in September and October were combined. Phytoplankton community characteristics and size distribution are currently being examined.

Survival of channel catfish in ponds with threadfin shad was 92%, which was higher than the 77% survival in ponds without shad. Catfish production was also higher in ponds with shad (4,651 pounds/acre) than in ponds without shad (3,980 pounds/acre). However, average weight at harvest was similar in both treatments (overall mean = 0.80 pounds). Feed conversion was marginally better in ponds with shad (1.30) than in ponds without shad (1.40).

Off-flavor analysis was performed on one catfish from each pond. The catfish were filleted with the skin-on, microwaved, and served to a panel of three taste testers. Slight off-flavor was detected by two of the three panelists in both treatments, but no significant differences were found.

University of Georgia. Threadfin shad or fathead minnows were stocked with catfish in 0.25-acre earthen ponds at Tifton, Georgia, and compared to ponds with only channel catfish. At Cohutta, Georgia, two treatments were started comparing threadfin shad and channel catfish to channel catfish alone in 0.1-acre earthen ponds. Three replicate ponds were used for each treatment for a total of nine ponds at Tifton and six at Cohutta. Channel catfish were stocked as fingerlings in multiple sizes at 44,500/acre. Threadfin shad were stocked at about 2,500/acre and were 1.7 to 4 inches long. Fathead minnows were stocked at about 10 pounds/acre (8,900 to 10,000/acre) and were 1.4 to 2 inches long.

Threadfin shad stocking was difficult due to the fragility of this species during handling, hauling, and transfer into receiving waters. Five attempts were made to stock threadfin shad at both locations. The most successful method of threadfin shad stocking was to obtain 1.5 to 2 inch shad from local ponds in the months of January to April. Even under the best conditions, it is difficult to determine the survival of the threadfin shad after stocking. Stocking threadfin shad into holding ponds and seining after one or two weeks indicated that 30-90% of the threadfin shad could die a short time after stocking due to loss of scales during handling, temperature shock, alkalinity shock, salinity shock, or other stress due to handling or transfer. Sorting threadfin shad from gizzard shad, which often is found together with the threadfin in lakes, rivers, and aquaculture ponds, causes an increase in threadfin shad losses. Cast nets or seines can be utilized for capturing threadfin shad. However, each method of capture has disadvantages. Casting nets near paddlewheel aerators appears to be successful for capture of monospecific harvests of threadfin shad. Hauling aids should be utilized during transport and may include an anesthetic, sodium chloride, calcium chloride, antifoaming agents, or a buffer of pH to 7.0. Tempering should be extended to two hours of gradual exchange of hauling water with receiving water, even when similar water temperatures are measured in the two water sources.

Over 50 algal species have been identified from Georgia ponds during the growing season. Blue-green algal blooms are denser at the Tifton location than at the Cohutta location. Water temperatures are cooler at Cohutta and the water source is a spring from limestone caverns. The water source at Tifton is the Floridan aquifer. No differences among treatments were observed in 1999. All ponds had blue-green algae in abundant populations. Establishment of threadfin shad populations was variable and appears to affect the observed phytoplankton population densities. Off-flavors were not detected in channel catfish harvested from this study.

In 1999, total ammonia concentrations were lower in the shad and minnow treatments than in the ponds with only channel catfish. Also, nitrite concentrations were lower in the minnow treatment than in the other two fish combinations. Soluble reactive phosphorus was similar in all ponds.

In 2000 at Tifton, blue-green algae became abundant in ponds with channel catfish only as early as April, in May with fathead minnows and catfish, and in June with threadfin shad and catfish. Blue-green populations reached 100 million cells/milliliter in ponds with channel catfish only, 80 million with fathead minnows and catfish, and 35 million with threadfin shad and catfish. At the Cohutta location, blue-green algae did not become abundant until August. It was apparent that blue-green algae were less abundant in the ponds with threadfin shad, however all ponds had blue-green algae blooms by late summer.

Louisiana State University. Eighteen recently renovated experimental 0.1-acre earthen ponds at the ARS-LAES, were stocked with channel catfish fingerlings in May 2000 at 10,000 fish/acre to study the efficacy of threadfin shad for control of blue-green algae. Juvenile and adult threadfin shad were captured from a local lake for stocking in the experimental ponds, but physiological stress associated with high water temperatures resulted in over 90% mortality of shad. Catfish have been fed daily since stocking. A partial harvest of market catfish will be implemented in November-December, followed by immediate re-stocking of catfish fingerlings to replace those harvested to establish a multiple crop production system characteristic of commercial catfish ponds. Adult and juvenile threadfin shad will stocked in late November-early December 1999 when water temperatures cool to 55°F or less to minimize physiological stress associated with capture (from local lakes), transport, and stocking in experimental ponds.

Objective 5. *Evaluate the development of phytoplankton communities in the Partitioned Aquaculture System.*

Results from the 1999 Season

The 2-acre commercial scale PAS unit was brought into production in the spring of 1999 (Figure 1). The unit was stocked with 33,000 catfish fingerlings in May. Stocked fingerlings averaged 15, 31, 48, 61 and 80 g. The catfish were stocked in 2 raceways consisting of 4 sections each. In addition, 1,320 pounds of tilapia (15,000 fish at 40 g each) were stocked into the 2-acre system. An additional 216 breeding tilapia (250-300 g) were stocked into the algal basin (Figure 2). The 2-acre unit successfully produced 14,500 pounds/acre of catfish at a carrying capacity of 17,000 pounds/acre. Feed applications reached 250 pounds/acre-day. The 2-acre unit was dominated by green algal populations throughout the season and off-flavor in 1999 ranged from 1.0 to 1.5 (out of 5) at the time of harvest. This mild off-flavor was described as being "grassy".

Six, 0.33-acre PAS units were stocked with both adult tilapia alone (breeding pairs) and with tilapia fingerlings and adults to see if successful algal species control could be sustained with reducing stocking requirement through the use of breeding pairs. By midseason, four of the six 0.33-acre PAS units shifted from early blue-green dominance back to populations of more desirable green algae as the tilapia breeding pairs expanded in numbers and weight. In two of the 0.33-acre PAS units, late season algal populations shifted to predominantly blue-green populations suggesting that use of tilapia breeding pairs alone at these feed application rates is close to, or slightly beyond, the limit of blue-green population control.

Stocking density experiments were conducted showing that raceway catfish stocking could be increased from 4-5 pounds/cubic foot to

Figure 1. Overview of 2-acre (above) and six-1/3 acre PAS units at Clemson University.

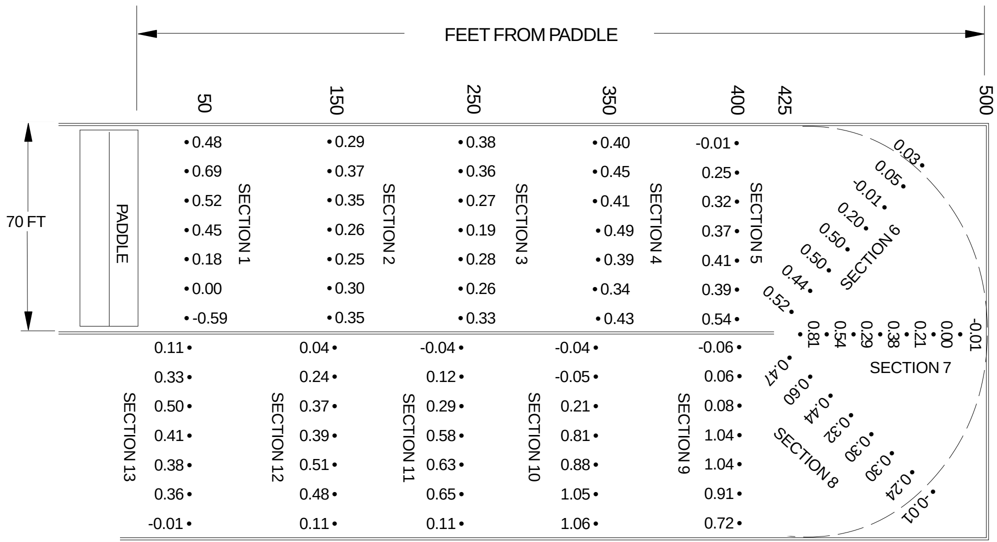
Figure 2. Two-acre PAS unit; algal growth basin.

8-10 pounds/cubic foot with no adverse effect on growth. These results demonstrated that overall system costs can be reduced by using a single high-density raceway and fewer raceway paddlewheels. A preliminary economic analysis projects that 40 acres of PAS units would produce catfish at a 5 to15¢/pound lower cost than conventional pond culture (Table 1). However, this analysis is based on the assumption the net production would exceed 22,000 pounds/acre. Because of loss of winter fish carryover as a result of spring proliferative gill disease (PGD), this potential yet has yield to be realized.

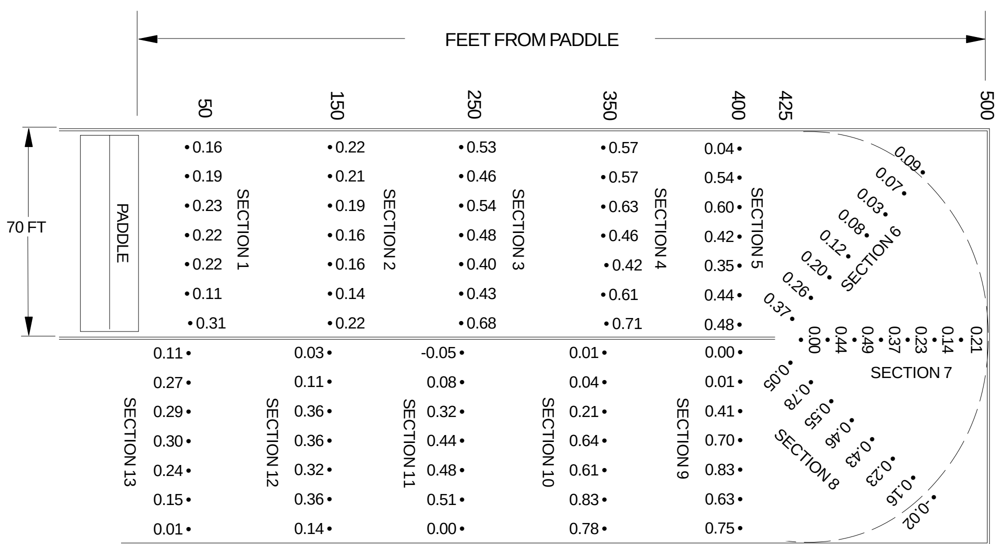
Table 1. Estimated PAS Annual Ownership Costs, Operating Costs, and Unit Costs Comparison Summary (1999).

	160-Acre Convention Ponds	20, 2-Acre PAS Unit Farm
Annual ownership cost	$ 95,301	$114,181
Annual operating cost	418,325	418,617
Total Annual Cost	513,626	532,798
Total Annual Cost/Acre	3,669	13,319
Annual pounds harvested	700,000	909,840
Ownership cost/pound	0.136	0.125
Operating cost/pound	0.598	0.460
Total Cost/Pound	0.734	0.585
TOTAL REVENUE	$525,000	$681,630

Flow experiments were conducted in 1999 to determine the uniformity of the water velocity field that can be sustained with different combinations of paddles and paddle speeds. The results suggest that sufficient mixing and flow velocity in the algal channel can be maintained with 50% of algal channel width coverage (by paddlewheels, see Figure 3).



AVERAGE VELOCITY (ft/sec)
2 PADDLES
3.4 RPM



AVERAGE VELOCITY (ft/sec)
4 PADDLES
1.3 RPM

Figure 3. Velocity profiles in 2-acre PAS with 2 paddles and with 4 paddles.

Results from the 2000 Season

In May, the 2-acre PAS unit was stocked with 33,419 catfish fingerlings (2050 pounds/acre) in size classes of 20 g , 65 g and 88 g. In addition, 110 pounds/acre of tilapia breeding pairs were stocked at large in the algal basin (150 - 250 g males and 300 - 250 g females). The 1999 carryover fish (250 g each) were lost in the spring of 2000 in the 2-acre unit as a result of proliferative gill disease (PGD). Treatment of pond sediment with hydrated lime was observed to be effective in reducing the occurrence of PGD, although treatment was initiated too late to be effective in this season.

As of August, the 2-acre PAS unit had reached a catfish carrying capacity of 9,200 pounds/acre with feed application rates surpassing 150 pounds/acre. The use of "breeding pairs" of tilapia alone in the 2-acre PAS produced marginal water quality results. By the third week of August, the algal population was completely dominated by blue-green algae. However, at the end of August, these populations were in the process of undergoing a shift to green algal populations, apparently as a result of the increased filter-feeding activity of the expanding tilapia population.

In contrast, four, 1/36-acre PAS units operating in August of 2000 at 12,000 pounds/acre catfish carrying capacity, with 1,600 pounds/acre of native mussel in two units and 2300 pounds/acre of silver carp in two units, exhibited a dominance of green algal populations. However, the mussels were not as effective as silver carp or tilapia at controlling blue-green algal populations and, as a result, the visual color difference between the mussel and silver carp unit was obvious (Figure 4).

Results at a glance...

- *A 2-acre partitioned aquaculture system unit at Clemson University produced 14,500 pounds/acre of catfish with an additional 2000 pounds/acre of tilapia. Blue-green algae were rare in the PAS and flavor scores of catfish at the end of the season were acceptable.*

Figure 4. Visual algal differences between mussel PAS unit (right) and silver carp PAS unit (left).

WORK PLANNED

Work on all objectives is proceeding on schedule and no major changes in the work plan have occurred. Refinements to the work plan or additional work is planned in some areas, as noted below.

University of Mississippi. Extracts that have shown activity against blue-green algae in primary assays are currently being evaluated following serial dilution. Bioassay-guided fractionation of the "active" extracts will be continued and the structures of new selective algaecides will be determined by spectroscopic means. Purified active compounds will be evaluated for toxicity in cell-based toxicological screens. Once the chemical structures have been established a decision can be made regarding compound sourcing for further testing and development.

USDA-ARS. Efficacy testing of the natural product derivative will continue to determine the lowest effective concentration in catfish aquaculture ponds. Determination of the half-life of the natural product derivative in catfish pond water will be performed to provide information related to environmental safety issues. Additional pond testing of the best selective compounds (as determined by laboratory testing and screening) is planned. Also, additional laboratory screening of other compounds will be performed.

Auburn University. During the current growing season (May-October 2000), commercial catfish ponds in West Alabama are being examined. Four catfish ponds with established threadfin shad populations and four similar ponds without shad were chosen for the study. Twice monthly, water and phytoplankton samples are collected for analysis. The same water quality and phytoplankton variables measured in 1999 are being measured in these large production ponds. Data on off-flavor of fish from these ponds will be gathered from tests conducted by commercial processing plants prior to harvest.

Clemson University. A detailed computer model capable of predicting PAS water quality is currently being developed. This model will be used to predict optimal PAS design and management. In 2001, experiments will be conducted to examine the costs and technical feasibility of supplementing the tilapia filter-feeding (with breeding pairs) with physical/chemical techniques for enhanced algal removal. Prophylactic treatments for control of early spring PGD in the winter carryover fish will be continued.

IMPACTS

Twelve percent of the plant and algae extracts evaluated have shown strong activity against blue-green algae. These findings indicate that natural products (small biologically active organic compounds) produced by organisms that live and compete in environments rich in blue-green algae are a valuable source of new, selective algicides and that these algicides may be of use in the control of blue-green algae in aquaculture ponds.

Results of the study of baitfish ponds have dramatically increased our understanding of water quality in this important segment of aquaculture in the southeast. Despite a history of long-term use of granular fertilizer in baitfish ponds in the study, sediment-bound phosphorus did not appear to exert a discernible effect on water quality under conditions of commercial production. The discovery of high soil sulfur levels in pond bottom samples contributed to affected baitfish farmers changing pond management practices to reduce chances of hydrogen sulfide problems.

The research at Auburn University revealed that EDTA is an excellent chelating agent for iron (and presumably other metals) for use in pond aquaculture. Application of potassium chloride, legume hay, and iron and other trace elements to ponds do not appear to be useful for controlling blue-green algae in ponds. However, sodium nitrate is a good nitrogen fertilizer for ponds.

Research at Mississippi State University showed that nutrient manipulation techniques consisting of simultaneous adjustment of N:P ratios and addition of chelated iron does not affect phytoplankton community structure. Studies also showed that some threshold level of turbulent mixing is necessary to overcome light limitation of phytoplankton production and shift phytoplankton community composition from dominance by cyanobacteria. Application of turbulent mixing should attempt to develop a uniform flow field to avoid areas of concentrated turbulence that can suspend pond soils.

Shad stocking is being considered by catfish farmers in Georgia who had off-flavor catfish and who could not utilize herbicides for control of blue-green algae. Shad stocking is expected to start in the winter of 2000 and spring of 2001 in approximately 400 acres of catfish ponds in Georgia. Information from this study has helped with the fathead minnow/channel catfish stocking program for proliferative gill disease control. Behavior of fathead minnows in channel catfish ponds indicated a need to encourage spawning by adding spawning substrate or to restock the fathead minnows at regular intervals in order to maintain at least 1500 minnows/acre.

The PAS technique offers the potential to provide a method to quadruple current fish production in a system which eliminates blue-green algal dominance and associated fish off-flavor problems, while recovering wasted nitrogen and phosphorus discharges, which currently pose an eutrophication threat to surface and groundwater supplies. Economic projections suggest that PAS catfish production costs are 5 to 15¢/pound lower than conventional pond production costs.

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MANAGEMENT OF AQUACULTURAL EFFLUENTS FROM PONDS

Reporting Period
April 1, 1999 to August 31, 2000

Funding Level

Year 1	$228,303
Year 2	237,076
Year 3 (projected)	150,740
Total	$616,119

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

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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. Studies indicated that about 53% of total suspended solids, total phosphorus, total nitrogen, and biochemical oxygen demand was associated with particles less than 5 micrometers in diameter. Preliminary studies suggest that 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.

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.*

Application of aluminum sulfate at 25 to 50 ppm did not improve the efficiency of solids removal in initial trials.

Estimates of runoff from watersheds suggest that settling basins to treat storm runoff from typical 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 for partial or complete draining would only need to be 10 to 20% of the volume of the largest pond on the farm.

Louisiana State University. Water budgets were studied in 16 experimental crawfish production ponds (0.5 acre/pond) at the LSU Agricultural Center - Rice Research Station during the 1999-2000 crawfish production season. Ponds were planted with rice as forage in mid-summer. Ground water use from fall flooding (October 1999) to pond drainage in spring (41 weeks post-flooding) was 5.41 feet. Water input from precipitation was 2.09 feet for a total water consumption of 7.50 feet. Based on historical average annual precipitation, the proportional contribution of water from rainfall was about 50% of normal because of the severe drought conditions experienced in Louisiana during 1999 and 2000. Intentional effluent discharge for management of dissolved oxygen was 1 inch during the 9-month production season. Effluent released at summer draw-down was 7.8 inches. Non-intentional effluent discharge from rainfall events was 3.2 inches. Accidental over-pumping contributed to 0.24 inches of effluent discharge. Preliminary estimate of water loss from seepage was 7.9 inches. Preliminary analysis of data on water use in the experimental crawfish ponds indicates that most of the water loss is associated with evaporation and evapotranspiration by the large standing crop of rice planted as crawfish forage.

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 had an effective diameter of less than 5 micrometers. Similar results were obtained from fractionation of solids in water collected from ponds dominated by inorganic turbidity (55% of the total suspended solids was 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 resulted in a solids reduction rate of 1.11 grams of solids per gram of alum. Size fractionation of pond water following alum treatment indicated that the proportional removal of solids less than 5 micrometers was greatest, although the greatest absolute solids reduction rate occurred in whole (not fractionated) pond water. The proportion of solids in the smallest size fractions increased with alum dose, suggesting that larger solids were selectively settled by alum treatment.

North Carolina State University. A study evaluating different fertilization regimes is currently being conducted in experimental hybrid striped bass ponds. Data on water discharge quantity and quality is being collected from a commercial hybrid striped bass farm. Data on rainfall, runoff and water use have been collected since fall 1999 on an 80-acre foodfish production farm.

Waddell Mariculture Center. The amount of suspended matter and associated nutrient and pollutants discharged during the harvest of shrimp ponds was variable and dependent on the location of the sludge pile, the accessibility of the sludge to the outgoing water flow, the slope of the pond bottom, operation of aerators during harvest, and the behavior of the shrimp.

Sedimentation treatment was effective in reducing the pollution potential of total suspended solids in pond effluent. The flocculation process of the suspended particles was of variable importance in affecting the sedimentation rate. Moreover, the importance of the flocculation process and the effectiveness of sedimentation was greatest in the pond with the most turbid discharge.

The results of this study suggest that allowing sedimentation of the latter portion of shrimp pond harvest effluent for only a few hours may significantly reduce total suspended solids, chemical oxygen demand, biochemical oxygen demand, and consequential effects on receiving waters. Thus, the simple and relatively inexpensive treatment of effluent by sedimentation may lead to a very marked reduction in the environmental effects of shrimp farming.

The results of this study suggest the possibility that resuspension of bottom mud may enhance the rate and extent of flocculation and increase the efficiency of sedimentation. An additional factor which may have affected flocculation was the stirring of the suspension, a process that enhances the probability of contact among the particles and increases the probability of efficient collision among the suspended particles. The effluents studied in this research were stirred to obtain a uniform suspension. The potential importance of this effect will also require further study and practical implications will be considered.

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

University of Arkansas at Pine Bluff. Historical streamflow daily values for hydrologic unit sampling stations near baitfish farms in Lonoke County, Arkansas have been obtained from USGS records. Water use data from golden shiner, goldfish, and fathead minnow farms has been collected to document pre-project practices. Drainage ditches are being surveyed on three baitfish farms that have agreed to cooperate with this project. Ponds scheduled to be drained within the next year (primarily late winter and early spring 2001) were identified. Data on total length, average width, slope, vegetative cover, ditch cross-sectional profiles, distance from receiving streams, and receiving stream water quality are being collected. Four ditches expected to receive pond drainage have been surveyed, and a minimum of ten ditches will be surveyed by December 2000. Presently, surveyed ditches range in length from 1,050 to 2,300 feet, and from 12 and 39 feet in width. Study ditches include both vegetated and non-vegetated types.

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.

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.*

Results at a glance...

- *Effluent volume can be reduced by increasing pond depth to increase rainwater storage capacity and linking the combined storage/production pond to adjacent conventional ponds. After one year, effluent volume was reduced by approximately 65-75% and ground-water consumption was reduced by 25-38% compared to conventionally managed ponds.*

Mississippi State University. A study was designed to evaluate the effect 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 include 3 conventional production ponds linked to 1 production/storage pond; 1 conventional production pond linked to 1 production/storage pond; and 1 conventional (control) pond. Earthwork required for increasing pond depth, modification of outflow pipes, and addition of pond linkage pipes and open channel flumes has been completed. In January 2000, ponds were filled with groundwater and data collection was initiated. During March 2000, each of the seven ponds were stocked with two sizes of commercial catfish. Catfish harvest and restocking will occur during fall 2000.

Based on 8 months of operation, effluent volume in the linked-pond systems was reduced by approximately 65-75%, and groundwater consumption was reduced by 25-38% compared to conventionally managed ponds. Reduction of groundwater use in linked ponds may have been less than expected on average because the past summer was much drier than normal. Water quality (dissolved oxygen, temperature, total ammonia, nitrate, chlorophyll *a*, conductivity, alkalinity, and hardness) was not different between ponds of the three configuration types. Disease (proliferative gill disease, enteric septicemia of catfish) epizootics have occurred in all ponds, but the spread of the disease could not be attributed to connections between ponds. The data indicate that the hydrological model used as the basis for this study will require only minor modifications to be useful for analysis of various hydrological management strategies.

North Carolina State University. A study comparing water quality in annually drained hybrid striped bass ponds compared to ponds managed for zero discharge is in its second year. Twelve, 0.25-acre ponds are being managed according to common commercial practices for foodfish production. Water quality was not significantly different between the two treatments.

Two fixed-film filters (vertical brushes and a block honeycomb medium) are being evaluated for suspended solids removal. Vertical brush filters remove approximately 20% of solids on a single pass. Solids removal by the honeycomb filter is about 20-50% less than that of the vertical brushes. Different hydraulic retention times are being evaluated to optimize solids removal.

A water budget for a commercial hybrid striped bass farm is under development. Data on rainfall, runoff, and water use have been collected since fall 1999 on an 80-acre foodfish production farm.

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. Average copepod abundance differed between 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.

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. Budgets have been developed for the sedimentation basin management option and preliminary work has also been conducted for the water-storage/production pond strategy. Construction of sedimentation basins and use of existing foodfish production ponds were considered as options for the treatment of harvest/draining effluents. Sizing of sedimentation basins within a commercial farm is complex and dependent on type of effluent to be treated, pond layout, number of drainage canals, and scope of regulations governing the release of aquacultural effluents. Factors such as farm and pond size also have an effect on the final cost of implementation. Given the wide variety of potential farm situations, multiple farm scenarios were created for which separate analyses were conducted. Sixteen different farm situations were identified for three farm-size scenarios based on combinations of pond size, drainage system, hydraulic residence time, and proportion of total effluent volume to be treated. Conversion of existing foodfish ponds to 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 in which 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. Although sedimentation basins were the only effluent treatment option examined in this study, other effluent management strategies will be considered because of the high costs associated with the excavation of sedimentation basins.

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. An environmental audit form for assessing the status of environmental management on catfish farms is under development. This instrument will be used to identify potential problems that can possibly be solved with BMPs. The audit form will be 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 has been prepared. During October and November 2000, meetings will be held with catfish farmers to obtain their suggestions for improving the practices.

Louisiana State University. The Natural Resources Conservation Service (NRCS) Code of Production was reviewed to assess their applicability to development of a set of BMP guidelines for the crawfish aquaculture industry. The review addressed the applicability of the NRCS recommended practices, clarifications or modifications recommended, economic feasibility, environmental effectiveness for water quality, and research and educational programs needed. From the information collected, a draft set of BMPs entitled "Louisiana Best Management Practices (BMPs) for Aquaculture" was completed. The draft document included a synopsis of crawfish production in ponds, a review of the NRCS conservation practices for crawfish and an individual review of BMP's for crustacean production in ponds. The draft document was distributed for review to representatives of the NRCS, USDA Agricultural Research Service, Louisiana Department of Natural Resources, Louisiana Department of Environmental Quality, Louisiana Farm Bureau, and Louisiana Crawfish Farmers Association. A final revision of the draft document is anticipated by early 2001 with publication in March 2001.

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.*

The first workshop will convene on 6-7 November 2000 in Roanoke, VA. The objectives of the workshop will be 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 potential BMPs for pond aquaculture has been extended to producers through an Extension newsletter article, and a field day abstract and a poster on BMPs. Results of the water re-use zooplankton study were presented to baitfish producers at the UAPB Aquaculture Field Day.

Additional Extension education activities on effluents and BMPs are planned for the annual convention of catfish and baitfish producers in 2001.

WORK PLANNED

The original work plan will be followed. Studies to determine the effectiveness of settling basins will be conducted during the remainder of 2000 and in early 2001. The effort to formulate BMPs will continue.

For the next two years, the field test will be continued at the seven ponds located at the DREC in Stoneville, Ms. In addition, modeling of other "drop/fill" management schemes and linked pond system schemes is underway. Also, generation and consideration of other possible management strategies is being investigated. If the primary field test provide satisfactory results, some of these other methods may also be field tested to evaluate efficiency and effects.

In the next year, cost estimates will be developed for using production/storage ponds on farms. Other treatment technologies that are under consideration in the SRAC project will be analyzed for costs. These cost estimates will be incorporated into a dynamic programming model of a catfish farm to estimate farm-level impacts.

IMPACTS

It is difficult to gauge the impacts of this research project because the first year of the project was completed only recently. However, 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.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

Boyd, C. E. and A. Gross. 1999. Biochemical oxygen demand in channel catfish *Ictalurus punctatus* pond water. Journal of the World Aquaculture Society 30:349-356.

Boyd, C. E. and A. Gross. 2000. Water use and conservation in inland aquaculture ponds. Fisheries Management and Ecology 7:55-63.

Boyd, C. E., J. Queiroz, J. Lee, M. Rowan, G. N. Whitis, and A. Gross. 2000. Environmental assessment of channel catfish farming in Alabama. Journal of the World Aquaculture Society 31:511-544.

Cathcart, T. P., J. W. Pote, and D. W. Rutherford. 1999. Reduction of effluent discharge and groundwater use in catfish ponds. Aquacultural Enginnering 20:163-174.

Cathcart, T. P., D. W. Rutherford, and J. A. Hargreaves. 2000. Field study for evaluating effluent and groundwater use reduction in catfish ponds. Water Resources Research Institute Conference Proceedings, Jackson, Mississippi, April.

Romaire, R. 1999. Management of water quality and effluents from aquacultural systems. Louisiana Agriculture 42(4):6.

Stone, N. 2000. Effluent regulations due by 2004. Arkansas Aquafarming 17(2):6.

Tucker, C. S., C. E. Boyd, J. A. Hargreaves, N. Stone, and R. P. Romaire. 2000. Effluents from channel catfish aquaculture ponds. Unpublished report prepared by the Technical Subgroup for Catfish Production in Ponds, Joint Subcommittee on Aquaculture - Effluents Task Force, 56 pp.

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SUPPORT OF CURRENT PROJECTS

Title	Yr	SRAC Funding	Other Support: University	Other Support: Industry	Other Support: Other Federal	Other Support: Other	Other Support: Total Other Support	Total SRAC + Other Support
Publications, Videos and	1	50,000	43,950	-0-	-0-	-0-	43,950	93,950
Computer Software	2	60,948	30,737	-0-	-0-	-0-	30,737	91,685
	3	45,900	35,710	-0-	1,000	-0-	36,710	82,610
	4	60,500	41,000	-0-	-0-	-0-	41,000	101,500
	5	74,000	47,000	-0-	-0-	-0-	47,000	121,000
Total		291,348	198,397	-0-	1,000	-0-	199,397	490,745
Management of	1	250,827	69,389	42,000	49,500	-0-	160,889	411,716
Environmentally-Derived	2	250,142	69,389	53,000	28,380	20,000	170,769	420,911
Off-flavors in Warmwater	3	229,266	38,329	34,000	58,483	-0-	130,812	360,078
Fish Ponds	4	80,900	25,829	26,000	36,000	-0-	87,829	168,729
Total		811,135	202,936	155,000	172,363	20,000	550,299	1,361,434
Optimizing Nutrient Utilization	1	241,476	261,465	-0-	-0-	-0-	261,465	502,941
and Reducing Wastes Through	2	257,152	263,627	-0-	-0-	-0-	263,627	520,779
Diet Composition and Feeding	3	234,817	258,545	-0-	-0-	-0-	258,545	493,362
Total		733,445	783,637	-0-	-0-	-0-	783,637	1,517,082
Verification of Recommended	1	31,410	60,286	1,000	-0-	-0-	61,286	92,696
Management Practices for	2	66,351	78,686	1,000	-0-	-0-	79,686	146,037
Major Aquatic Species	3	66,925	78,986	6,000	-0-	-0-	84,986	151,911
Total		164,686	217,958	8,000	-0-	-0-	225,958	390,644
Control of Blue-green Algae	1	307,574	171,746	27,000	172,500	-0-	371,246	678,820
in Aquaculture Ponds	2	281,986	161,882	35,000	98,380	-0-	295,262	577,248
	3	253,326	149,662	16,000	120,983	-0-	286,645	539,971
Total		842,886	483,290	78,000	391,863	-0-	953,153	1,796,039
Management of Aquacultural	1	228,303	105,319	-0-	-0-	-0-	105,319	333,622
Effluents fromPonds	2	237,076	117,051	-0-	-0-	-0-	117,051	354,127
	3	150,740	109,516	-0-	-0-	-0-	109,516	260,256
Total		616,119	331,886	-0-	-0-	-0-	331,886	948,005

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	$50,000	88-38500-4028
	Yr. 2-05/01/90-04/30/91	49,789	89-38500-4516
	Project Total	**$99,789**	
*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	$46,559	88-38500-4028
	Yr. 2-05/01/90-10/31/91	51,804	89-38500-4516
	Project Total	**$98,363**	
*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	$274,651	88-38500-4028
	Yr. 2-05/01/90-04/30/91	274,720	89-38500-4516
	Yr. 3-05/01/91-12/31/92	273,472	90-38500-5099
	Project Total	**$822,843**	
*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**	
Publications, Videos and Computer			
Software. Dr. Michael Masser, Texas	Yr. 1-04/01/95-03/31/96	$50,000	94-38500-0045
A&M University, Principal Investigator	Yr. 2-04/01/96-03/31/97	$13,405	93-38500-8393
(Continuing project)		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	$74,000	98-38500-5865
	Yr. 5-07/01/00-06/30/01	$80,550	00-38500-8992
	Project Total	**$371,898**	
*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	$56,100	99-38500-7375
	Project Total	**$867,235**	
Optimizing Nutrient Utilization and Waste Control through Diet Composition and Feeding Strategies. Dr. Kenneth Davis, University of Memphis, Principal Investigator	Yr. 1-12/01/96-11/30/97	$241,476	95-38500-1411
	Yr. 2-12/01/97-11/30/98	$47,105	95-38500-1411
		210,047	96-38500-2630
	Total Yr. 2	$257,152	
	Yr. 3-12/1/98-11/30/99	$34,365	96-38500-2630
		200,452	97-38500-4124
	Total Yr. 3	$234,817	
	Project Total	**$733,445**	
*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. 2-01/01/98-12/31/99	$7,186	95-38500-1411
		59,165	96-38500-2630
	Total Yr. 2	$66,351	
	Yr. 3-01/01/99-12/31/00	$66,925	
	Project Total	**$164,686**	

*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	$309,574	
	Yr. 2-01/01/00-12/21/00	$975	96-38500-2630
		17,394	97-38500-4124
		159,955	98-38500-5865
		103,662	99-38500-7375
	Total Yr. 2	$281,986	
	Yr. 3-01/01/01-12/31/01	$22,042	97-38500-4124
		7,202	98-38500-5865
		195,953	99-38500-7375
		28,129	00-38500-8992
	Total Yr. 3	$253,326	
	Project Total	**$842,886**	
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
		128,303	98-38500-5865
	Total Yr. 1	$228,303	
	Yr. 2-04/01/00-03/31/01	$237,076	99-38500-7375
	Yr. 3 - Projected	$150,740	
	Project Total	**$616,119**	