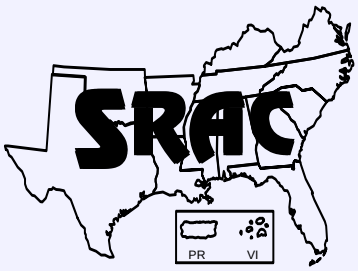

Nearly 70% of the $1 billion dollar domestic aquaculture industry is located in the southeastern United States. One of the most important programs supporting this critical sector of southern agriculture is the USDA/CSREES Southern Regional Aquaculture Center (SRAC). The Center is the only research-funding program with the flexibility to stay abreast of industry development, identify problems on a region-wide scale, and implement cooperative, interstate projects to solve those problems.

SOUTHERN REGIONAL AQUACULTURE CENTER

ARKANSAS KENTUCKY TEXAS NORTH CAROLINA FLORIDA
LOUISIANA VIRGINIA ALABAMA SOUTH CAROLINA TENNESSEE
MISSISSIPPI OKLAHOMA GEORGIA VIRGIN ISLANDS PUERTO RICO

Sixteenth Annual

SUMMARY OF PROJECTS

WHAT IS SRAC? . . . SRAC is one of five Regional Centers established by Congress and administered through the USDA Cooperative State Research, Education, and Extension Service. The thirteen states and two territories included in the Southern Region are listed in the mast-head, above. Mississippi State University serves as the Host Institution for SRAC, and the Administrative Center is located at the Thad Cochran National Warmwater Aquaculture Center, Stoneville, Mississippi.

In the simplest sense, SRAC provides an efficient means for identifying and solving problems. Priority research and education needs for the Southern Region are identified by the Industry Advisory Council, which consists of aquaculture industry representatives from throughout the region, and the Technical Committee, which is composed of research and extension scientists. These two groups recommend project areas to the SRAC Board of Directors, which then selects priority categories for project development and funding. The best scientific talent in the region is then brought together to address the problem.

IMPACT . . . Hundreds of high-quality, peer-reviewed scientific articles, graduate theses, and technical papers have been published since program inception in 1987. Through the activities of the SRAC "Publications" project, much of this work is summarized in the 170+ fact sheets and reports available at the SRAC web site (see box below).

SRAC research has also led to many technologies that benefit the aquaculture industry. For example, SRAC-sponsored research to address the impact of aquaculture on the environment—including work in the "Effluents" project reported here—was critical in recent regulatory activities and provides farmers with common-sense practices to improve environmental stewardship. As additional examples, work in our "Harvesting" project facilitated the development of a new seine at Mississippi State University and a mechanical fish grader at the University of Arkansas at Pine Bluff. These new technologies allow pond-raised fish to be harvested and graded much faster than conventional methods.

This report summarizes these projects and others funded by SRAC.

For further information on the Southern Regional Aquaculture Center and detailed reports of the results of SRAC projects, visit the SRAC web site at http://www.msstate.edu/dept/srac

December, 2003

Publications, Videos and Computer Software

THE CHALLENGE . . .

Aquaculture currently produces about 30% of the world's seafood supply and is one of the most rapidly expanding agricultural industries in the world. Domestic aquaculture production is centered in the southeast region, where more than 100 species of fish, shellfish, aquatic reptiles, and plants are cultured for food or ornamental purposes. The total farm value of southeastern aquaculture is over one billion dollars, with a total economic impact exceeding five billion dollars. Aquaculture is a young, unique, and rapidly expanding industry, and the need for information to sustain growth and development has increased dramatically over the past 20 years.

OUR RESPONSE . . .

Extension and research scientists in the southeastern United States initiated this project to produce research-based fact sheets, videos, and other educational materials to support regional aquaculture production and marketing. The SRAC publication project uses a region-wide pool of experts to develop materials for distribution through the nationwide network of Extension Specialists and County Agents. This process makes efficient use of personnel and funds at the State level, and results in timely, high-quality educational materials. Each publication contains understandable, factual information that provides guidance for producers, processors, consumers, or investors. ubject matter includes biology and life history of specific culture species, culture techniques and systems, nutrition, water quality and waste management, disease treatment, off-flavor management, consumer education, marketing, and much more.

PRINCIPAL ACCOMPLISHMENTS . . .

The Southern Regional Aquaculture Center has now published 161 fact sheets, 17 research reports, and 19 videos. These publications provide essential information for aquaculture producers, lending agencies, and consumers of aquaculture products. Educators in high schools and colleges use SRAC publications in classrooms throughout the United States and the world. One of the most popular series of SRAC publications is "Species Profiles," which provides detailed technical information on the biology and culture of marine and freshwater fish with commercial potential. Among the species included in the series are grouper, largemouth bass, southern flounder, and sturgeon.

Seven publications were printed this year, with 17 fact sheets and a video in progress. These publications were developed by 21 scientists associated with the following institutions and agencies:

- Auburn University
- Clemson University
- Kentucky State University
- Louisiana State University
- Mississippi State University
- North Carolina State University
- Texas A&M University
- University of Arkansas at Pine Bluff
- University of Florida
- University of Georgia
- USDA/ARS – Stuttgart, Arkansas

Copies of all fact sheets are available at http://www.msstate.edu/dept/srac on the Internet. ❖

Management of Aquacultural Effluents from Ponds

THE CHALLENGE . . .

Aquaculture operations in the United States have recently come under increased scrutiny because of potential or perceived environmental degradation caused by the discharge of water from production facilities. Aquaculture is now under consideration by the Environmental Protection Agency (EPA) as a candidate industry for new regulatory activity. Therefore, regulation of pond aquaculture effluents in the southeastern United States within the next few years is a distinct possibility. Regulatory requirements to operate ponds without discharge could drastically alter the way pond aquaculture facilities have traditionally been managed. Additionally, these requirements would impose additional financial burdens on existing operations and restrict further development of the regional aquaculture industry.

OUR RESPONSE . . .

The aquaculture industry has an opportunity to participate in the process of formulating regulations, because the approach and framework for the regulatory process has not yet been decided. Through this regional research project, the research community can provide information required by permit writers for the development of rational regulatory mechanisms. This project will provide important information on the character of effluents from aquaculture ponds and explore management methods for reducing the volume and improving the quality of pond effluents. This information will be used to develop a set of best-available pond water management practices which will be evaluated for environmental risks and economic performance.

Fourteen research scientists and extension agents are collaborating on a three-year project initiated on April 1, 1999. The following institutions are involved:

- Auburn University
- University of Arkansas at Pine Bluff
- Louisiana State University
- Mississippi State University
- North Carolina State University
- Virginia Polytechnic Institute and State University
- South Carolina Department of Natural Resources - Waddell Mariculture Center

PRINCIPAL ACCOMPLISHMENTS . . .

Solids in aquaculture pond effluents have been characterized, which will allow development of strategies to reduce their discharge into effluent-receiving waters. Solids in pond overflow from rainfall and in most of the water discharged during intentional drawdown of ponds are very difficult to settle because most particles are small and organic. Between 51 to 82% of the suspended solids in effluent from catfish ponds in Alabama and Mississippi, hybrid striped bass ponds in North Carolina, and baitfish ponds in Arkanasas were less than 5 micrometers in diameter.

In watershed ponds for catfish culture, marine shrimp and crawfish ponds, effluent quality deteriorates during the final 20% of water volume discharged. This effluent can be settled with sedimentation basins designed with a hydraulic retention time of 8 hours, although a settling time of 1 to 4 hours is sufficient to reduce total suspended solids in effluents to 75 to 90% of original concentrations. A large percentage of the solids is also removed as water travels down low-gradient drainage ditches. The ditches serve, in effect, as long, narrow sedimentation basins. Solids removal is associated with settling of the mineral fraction, with little change in organic solids concentrations in effluents.

Most "embankment" style aquaculture ponds are fitted with internal drains and when draining is initiated, shear forces generated by water moving into the drain pipe scour the pond bottom around the entrance to the drain pipe. The initial flush of water discharged therefore consists of pond water and a slurry of sediment that has accumulated over the screen inside the pond. Most of the solids in the "initial flush" are removed as the effluent travels down low-gradient drainage

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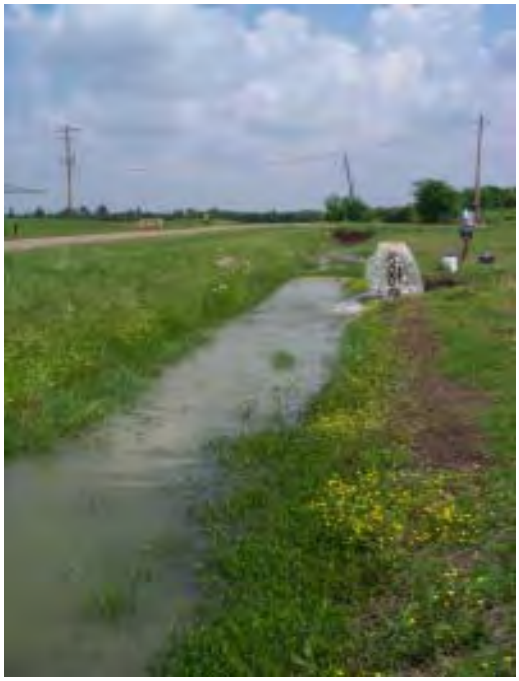
Management of Aquacultural Effluents from Ponds (continued from page 3)

ditches. Water quality improvement has been demonstrated by passing effluent from catfish ponds in Mississippi, baitfish ponds in Arkansas, crawfish ponds in Louisiana, and shrimp ponds in South Carolina through drainage ditches prior to release to receiving waters.



Effluent volume from catfish ponds can be reduced by increasing pond depth by 1 foot to increase rainwater storage capacity and linking the deeper combined production-storage pond to one or three adjacent conventional ponds. Effluent volume was reduced by approximately 50% and groundwater consumption was reduced by more than 40% compared to conventionally managed ponds. Effluent volume and groundwater use was similar in production ponds linked with one or three adjacent conventional ponds. Linking ponds and reusing stored water does not affect fish growth, occurrence of diseases, or water quality.

Effluent volume from baitfish ponds can be reduced if water can be reused. Research in Arkansas has demonstrated that water from baitfish ponds can be reused if it is filtered to remove zooplankton predators of newly stocked baitfish fry.

Economic analysis indicates that construction of settling basins or combined production-storage ponds requires a large investment of financial resources and results in a reduction in revenue. Of the methods evaluated, utilizing existing ponds as sedimentation basins is the most cost-effective approach for treatment of pond effluents.

Project scientists have been active participants in the Federal Joint Subcommittee on Aquaculture - Aquaculture Effluents Task Force. They have assembled and provided the Task Force and EPA with general information on the aquaculture sectors in the Southern region, the characteristics of pond effluents, the effectiveness of various effluent management options, and have recommended specific Best Management Practices. Best Management Practices formulated by project participants will be used as the basis for effluent regulation in Alabama and have been assembled in publications for use by fish farmers in Arkansas and Louisiana. ❖

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

THE CHALLENGE . . .

Inefficient harvesting, improper sizing, and stresses related to handling and hauling of fish are seriously affecting profitability of the finfish aquaculture industry. Market-size fish that escape harvest continue to grow and create additional inefficiencies resulting from higher feed conversion ratios and carry-over of large fish that are unacceptable to processing plants. In addition, if the stress is sub-lethal, incidence of disease increases, and growth rate, fecundity, and product quality may be lessened. Losses during transport can result in even greater overall reductions in farm profits, particularly since losses at this stage are generally market-size fish that represent investment of full production costs.

OUR RESPONSE . . .

Twelve research scientists have teamed in a three-year project that began on January 1, 2001, to investigate methods to improve harvest efficiency, grading selectivity, and transport of finfish, and thus improve profitability of finfish aquaculture. The scientists represent these institutions:

- Mississippi State University
- University of Tennessee
- University of Florida
- North Carolina State University
- University of Memphis
- University of Arkansas at Pine Bluff

Several possible solutions to this problem are under investigation:

- developing new gear and methods or modifying existing methods to improve harvest efficiency and fish grading selectivity as well as reducing fish stress associated with these activities;

- evaluating methods used for loading and transporting of fish to reduce fish mortalities and the negative effects of stress on product quality; and

- comparing new technology and current technology for harvesting, grading, and loading fish for efficiency and profitability.

PRINCIPAL ACCOMPLISHMENTS . . .

Work conducted thus far indicates that braided polyethylene mesh is a good choice for use in constructing seines and socks for harvesting and grading catfish. Also, mesh sizes of the braided material that retain fish of a certain size have been determined. In addition, a new seine design that appears to be more efficient than conventional seine designs has been commercialized. The new seine improves seining efficiency by 15 to 20% and reduces seining time by about 50%. Another result from this project is that a horizontal floating platform grader with adjustable spacing can be effectively integrated into current harvest procedures to grade catfish in ponds.

The adjustable grader allows more control over the size of fish retained than traditional grading "socks." This could lead to more harvesting flexibility and more marketing options for producers. Another advantage is that fish can be graded immediately after seining, allowing more accurate inventory estimates to be relayed to the plant. The in-pond mechanical grader removes more sub-marketable fish as compared to conventional socks, which will allow food-fish producers to retain more sub-marketable fish in the production pond while improving efficiency at the processing plant.

Fingerling producers marketing graded channel catfish fingerlings can benefit greatly from in-pond grading as it eliminates the need for costly vat grading facilities, drastically reduces the time and labor requirement of other grading methods and can eliminate costly haul-backs. To date, eight catfish fingerling facilities, three commercial catfish food-fish facilities and one hybrid

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

THE CHALLENGE . . .

One of the most serious threats to the profitability of commercial aquaculture is mortality and morbidity attributable to infectious diseases. Significant diseases include both those that seem to emerge suddenly causing major losses before the infectious agent is fully characterized, such as the "new" disease of catfish caused by a digenetic trematode, as well as those diseases that have been present from the earliest days of aquaculture, such as columnaris disease.

The "new" disease is caused by metacercariae of the trematode *Bolbophorus damnificus* that encyst in the flesh of the fish. The adult worms are in fish-eating birds (final host) and the intermediate stages are in snails and fish (intermediate hosts). In recent years the parasite has had a major impact on the catfish industry in parts of the lower Mississippi River Valley, producing both catastrophic losses and chronic infections that reduce fish growth and marketability.

Unlike trematode infestations, columnaris disease has been a significant problem in many warmwater fish species for decades but effective prevention and treatment remain elusive. Columnaris disease, caused by the bacterium *Flavobacterium columnare,* remains a very serious problem not because it is new, but because of serious gaps in our understanding of the biology of the organism.

OUR RESPONSE . . .

Twenty scientists from nine institutions are collaborating to develop methods of identification and classification of *Bolbophorus* trematodes and columnaris-like bacteria and to develop methods for their management and control. Research on *Bolbophorus* trematodes focuses on identifying and characterizing the life stages of the digenetic trematode that infects channel catfish and evaluating various methods of breaking the life cycle of the parasite by controlling the planorbid snail intermediate host.

Research on columnaris-like bacteria focuses on four issues:

1) development of standardized methods for the isolation, culture, and antimicrobial susceptibility testing;

2) characterization of archived strains of columnaris-like bacteria based on conventional and molecular techniques;

3) development of reproducible challenge models for columnaris-like bacteria; and

4) using the challenge models to correlate virulence with biotype and/or genotype of columnaris-like bacteria.

The following institutions are involved:

- Louisiana State University School of Veterinary Medicine (Lead Institution)
- Auburn University (Department of Fisheries and Aquacultures)
- Auburn University (College of Veterinary Medicine)
- Clemson University
- Mississippi State University (College of Veterinary Medicine)
- Mississippi State University (National Warmwater Aquaculture Center)
- Mississippi State University (Wildlife and Fisheries)
- North Carolina State University
- University of Tennessee
- University of Arkansas at Pine Bluff
- USDA-APHIS-WS (Starkville)
- USDA/ARS (Stuttgart, AR)

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Identification, Characterization, and Evaluation of Mechanisms of Control of *Bolbophorus*-like Trematodes and *Flavobacterium columnare*-like Bacteria Causing Disease in Warm Water Fish (continued from page 6)

PRINCIPAL ACCOMPLISHMENTS . . .

Studies of *Bolbophorus*

Confirmation of *Bolbophorus* life cycle. American white pelicans were pretreated with praziquantel, challenged with trematode *(Bolbophorus damnificus)* metacercaria to establish patent infections, and were subsequently used to artificially infect snails *(Planorbella trivolvis)*. Catfish were exposed to these infected snails and metacercaria obtained from the catfish challenge were fed to parasite-free pelicans, and patent *B. damnificus* infections were established. Each life stage of this parasite was confirmed to be *B. damnificus* morphologically and molecularly.

Description of potentially pathogenic trematode metacercariae. Three distinct types of cercariae, confirmed morphologically and genetically utilizing species-specific PCR, were obtained from snails collected from ponds experiencing mortality from trematode infestations. These were 1) *Clinostomum marginatum*, the causative agent of 'yellow grub disease', 2) *Bolbophorus damnificus,* a serious pathogen of channel catfish and 3) *B.* sp. type 2, a species not recovered from catfish but present in several other fish hosts. Interestingly, several snails were shown to be shedding both *Bolbophorus* species simultaneously or sequentially. This indicated that both species were present in aquaculture ponds and they utilized the same molluscan host.

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

Evaluation of health status and growth potential. Preliminary data collected from laboratory and field trials indicated that mild sub-lethal active trematode infections, commonly observed in channel catfish production systems, can significantly reduce production by reducing feed consumption and increasing mortality associated with the bacterial disease, enteric septicemia of catfish. These studies also indicated that the presence of fully developed metacercariae does not appear to compromise the growth performance and health status of fish. This data supports the idea that the deleterious effects of this infectious agent are associated with penetration of the parasite and initial stages of encystment.

Chemical control of pond snails. Copper sulfate and hydrated lime were applied at 589 g and 11,897 g respectively per 10 linear meters of shoreline in a 2-m swath or width. Both treatments effectively lowered the snail populations with survival ranging from 10% for $CuSO_4$ to 41% for hydrated lime. At these treatment levels copper sulfate was more effective than lime, but when the lime application was doubled snail survival was less than 2%.

Studies of *columnaris*

Standardized methods for isolation and culture. Selective cytophaga agar (SCA) has performed the best as a primary isolation medium in preliminary tests in isolation of *Flavobacterium columnare* from contaminated sites such as the gills and skin. For maintenance following isolation, flavobacterium growth medium (FCGM) or cytophaga agar (CA) slants perform well. For large batch broth culture, FCGM outperforms other formulations tested.

Antimicrobial disk susceptibility testing. Preliminary tests on disk-diffusion antimicrobial susceptibility testing of *Flavobacterium columnare*, indicate

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Development of Improved Harvesting, Grading and Transport Technology for Finfish Aquaculture (continued from page 5)

striped bass facility, have adopted this technology.

A survey was distributed to all the tropical fish farms in the state of Florida seeking information on existing technologies and practices, and suggestions for new technologies and/or practices they would recommend investigating. Results of year one observations and direct

farm input to questionnaires and site visits have been analyzed and evaluated. Based on survey results, trap design for harvesting ornamental fish was modified and the new design decreased personnel requirements and increased the quality of larger species, such as cichlids. ❖

Identification, Characterization, and Evaluation of Mechanisms of Control of *Bolbophorus*-like Trematodes and *Flavobacterium columnare*-like Bacteria Causing Disease in Warm Water Fish (continued from page 6)

dilute Mueller Hinton (DMH) plates prepared with 15 g/liter of agar in 20 g/liter Mueller-Hinton medium give the most consistent and clearly readable zones around susceptibility disks.

Molecular identification by sequencing and ribotyping. A portion of the 16s ribosomal RNA gene and the entire 16s-23s intergenic spacer region of the ribosomal RNA genes was PCR-amplified using primers to regions of the 16s and 23s ribosomal sequences that are conserved among the gram negative bacteria. One predominant product was an intergenic sequence containing the tRNA for alanine and the tRNA for isoleucine. Sequencing this spacer region should allow for discrimination among different strains of *Flavobacterium columnare*.

Outer membrane proteins. A 40 kDa outer membrane protein (OMP) has been isolated and purified from *Flavobacterium columnare*. The protein is consistently found in all *F. columnare* isolates tested thus far, including a reference strain from ATCC. A 30 kDa OMP was isolated and purified from a Clemson isolate and found to be expressed only in channel catfish *F. columnare* isolates. ❖

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

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