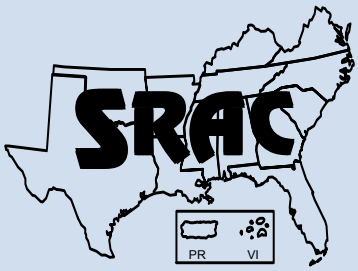

Nearly 70% of the $1 billion dollar domestic aquaculture industry is located in the south-eastern United States. One of the most important programs supporting this critical sector of southern agriculture is the USDA-CSREES Southern Regional Aquaculture Center (SRAC). The Center is the only research-funding 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 LOUISIANA MISSISSIPPI KENTUCKY VIRGINIA OKLAHOMA TEXAS ALABAMA GEORGIA NORTH CAROLINA SOUTH CAROLINA VIRGIN ISLANDS FLORIDA TENNESSEE PUERTO RICO

Eighteenth 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 masthead, 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.

SRAC provides a mechanism 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 consists of research and extension scientists. These two groups recommend project areas to the SRAC Board of Directors, which selects projects with the highest priority for development and funding. The best scientific talent in the region is then brought together to address the problem.

IMPACT . . . In the past year, four research projects funded at $2 million were in progress. The Center's "Publications" project is in its tenth year of funding and has generated more than 170 fact sheets with contributions from 158 authors from throughout the region. All publications are available at the SRAC web site (see box below).

SRAC research has led to many technologies that benefit the aquaculture industry. For example, research in the "Disease" project helped define the life cycle of a trematode parasite that has devastated catfish farms in the last few years. Of more importance to farmers, work on this project also led to the discovery of safe, inexpensive methods to control the intermediate host of the parasite, thus breaking the parasite's life cycle in ponds.

This report summarizes these projects and others currently 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, 2005

Publications, Videos and Computer Software

THE CHALLENGE . . .

Aquaculture currently produces over 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 developed this project to produce research-based fact sheets, videos, and other educational materials to support regional aquaculture education, production, and marketing. The SRAC publication project uses a region-wide pool of experts to develop materials for distribution through the nationwide network of educators, 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, students, or investors. Subject 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 171 fact sheets, 4 project reports, 19 research reports, and 20 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. In the months from April through September 2005, a total of 39,153 fact sheets were downloaded from the SRAC web site. One of the most popular series of SRAC publications is the "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, cobia, largemouth bass, southern flounder, queen conch, and sturgeon.

Eleven publications and a video were completed this year, with four fact sheets in progress. These publications were developed by 27 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 Texas
- USDA/APHIS Wildlife Services

To download fact sheets, go to <http://www.msstate.edu/dept/srac.edu> or <http://srac.tamu.edu>.

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 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 will focus 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 will focus on characterizing strains of columnaris-like bacteria and then correlating strain characteristics with virulence.

The following institutions are involved:

- Louisiana State University School of Veterinary Medicine (Lead Institution)
- Auburn University (Dept. 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, Mississippi)
- USDA-ARS (Stuttgart, Arkansas)

PRINCIPAL ACCOMPLISHMENTS . . .

Studies of *Bolbophorus*

Confirmation of Bolbophorus life cycle. American white pelicans were challenged with trematode *(Bolbophorus damnificus)* metacercariae to establish infections, and were subsequently used to artificially infect snails *(Planorbella*

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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 3)

trivolvis). Catfish were exposed to the infected snails and metacercariae obtained from the catfish were fed to parasite-free pelicans, and *B. damnificus* infections were established. Each life stage of this parasite was confirmed to be *B. damnificus*, thus confirming all stages in the life cycle.

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 indicates that both species were present in aquaculture ponds and they utilized the same molluscan host. Another species of snail, *Planorbella duryi*, has been shown to serve as a permissive intermediate host for *B. damnificus,* thus extending the potential range of the parasite.

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* has been recovered from catfish in aquaculture ponds. *Bolbophorus* species 'type 2' has been recovered from white crappie and longear sunfish and largemouth bass. Fathead minnows were found to harbor both *B. damnificus* and *Bolbophorus* sp. 'type 2.' This is the first finding of *B. damnificus* in a fish species other than catfish. Experimentally, hybrid striped bass challenged with 'type 2' cercariae developed hemorrhagic lesions similar to those observed with *B. damnificus*-challenged catfish and mortality rates were similarly high.

Evaluation of health status and growth potential. Laboratory and field trials indicate that mild, sublethal trematode infections—commonly observed in channel catfish populations—can significantly reduce production by reducing feed consumption and increasing mortality associated with the bacterial disease, enteric septicemia of catfish. These studies also indicate that the presence of fully developed metacercariae does not appear to compromise the growth performance and health status of fish. These data support the contention that deleterious effects of this infectious agent are associated with penetration of the parasite and initial stages of encystment.

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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 4)

Chemical control of pond snails. Treatment of pond margins with concentrated solutions of either copper sulfate or hydrated lime effectively and safely reduces snail populations. Whole-pond treatments with 0.75 to 1.75 ppm copper sulfate killed more than 90% of snail populations. In other trials, optimization of a slurried-hydrated lime pond-shoreline treatment proved effective in killing more than 90% of the snails. Both of these treatments may be useful in breaking the life cycle of the parasite and their use may depend on wind conditions and water temperature.

***Columnaris* studies**

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, tryptone yeast extract (TYE) performed best with some cultures remaining viable for 84 days post-inoculation. For large batch broth culture, flavobacterium growth medium (FCGM) outperforms other formulations. Clumping or autoagglutination common with other broth media is avoided with FCGM.

Antimicrobial disk susceptibility testing. Preliminary tests on disk-diffusion antimicrobial susceptibility testing of *Flavobacterium columnare*, indicate dilute Mueller Hinton (DMH) plates prepared with 17 g/liter of agar in 4 g/liter Mueller-Hinton medium supplemented with 5% equine serum 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 (ITS). Sequencing this spacer region in 50 isolates of *F. columnare* allowed for the identification of 3 distinct clusters of similar sequences representing 3 different strains of the bacterium.

Outer membrane proteins. A 30 kDa OMP was isolated and purified from a Clemson isolate and found to be expressed only in channel catfish *F. columnare* isolates. The 30 kDa OMP isolated from *F. columnare* is a potent inducer of type II nitric oxide synthase (iNOS) and inducible prostaglandin H2 synthase (cyclooxygenase-2; COX-2) in isolated catfish phagocytes. These activities can be blocked using specific antibodies against the OMP. ❖

Improving Reproductive Efficiency to Produce Channel × Blue Hybrid Catfish Fry

THE CHALLENGE . . .

Catfish farming needs to be more competitive and profitable in today's economy. Inefficiencies occurring at all phases of production need to be eliminated. Problems include high mortalities during the fry and fingerling production phase, as well as diseases and stress from poor water quality throughout the production cycle. Additional inefficiencies result when market-size fish evade harvest and continue to grow.

Application of the hybrid channel catfish female × blue catfish male could alleviate these problems, making catfish farming more competitive, sustainable and profitable. However, hybrid eggs and fry are difficult to produce. Technologies applicable to small-scale production have been developed, but they need to be improved to allow large-scale adoption of the hybrid.

OUR RESPONSE . . .

Nine scientists at five institutions are conducting research to improve the hatching rate of channel × blue hybrid catfish embryos and to improve the number of hybrid fry produced per weight of broodstock to allow economical delivery of the hybrid technology to the catfish industry.

The projects focuses on four goals to address the problem of poor hybrid egg hatching:

- Develop broodstock selection and management protocols to optimize channel × blue hybrid embryo production.
- Develop induced spawning techniques and management strategies to optimize gamete collection and storage.
- Develop techniques to identify, assess and improve gamete quality.
- Develop economically viable, standardized hatchery procedures and fertilization protocols to optimize hatching rate of hybrid embryos.

Attaining these objectives will result in techniques to induce spawning of broodstock to produce high quality hybrid embryos with improved hatch rate. The impact of wide-scale adoption of the hybrid should increase efficiency, productivity, sustainability and profitability in the catfish industry.

The project began on April 1, 2004. The following research institutions are involved:

- Auburn University
- Louisiana State University
- Mississippi State University
- University of Memphis
- USDA/ARS

Harvest Select Farms, Inverness, Mississippi has also provided resources and research facilities for this project.

PRINCIPAL ACCOMPLISHMENTS . . .

Temperature. A temperature × time model was developed to help predict the optimum time to initiate artificial spawning to produce hybrid fry. Channel catfish begin to spawn at approximately 100 degree-days after the pond water temperature reaches 21°C. This may be the appropriate time to initiate artificial spawning to produce hybrid catfish eggs. The degree-hour response time is not linear over temperatures ranging between 24 and 28°C when using LHRHa to induce ovulation in channel catfish females. The degree-hour response time is longer at cooler temperatures, and the females ovulate faster at higher temperatures. This is important so that reasonable work schedules can be formulated for commercial-scale production. Early spawning can be accomplished by heating water prior to the natural spawning season without any difference in success compared to the natural spawning season.

Ovulation rate and number of eggs released increases with increasing temperature. Hatch rate

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Improving Reproductive Efficiency to Produce Channel × Blue Hybrid Catfish Fry (continued from page 6)

of hybrid embryos is improved if LHRHa-injected channel catfish females are stripped within 2 hours of first observed egg release. Waiting longer will increase the number of eggs stripped, but this is more than offset by much lower hatch rate.

Broodstock nutrition. Feeding standard 32% protein floating catfish feed 6 days per week for 2 months prior to spawning gives equal or better fry production than feeding high-protein diets. Supplemental feeding with liver was detrimental to fry production. Supplementation of brood fish diets with menhaden fish oil and the long-chain, polyunsaturated fatty acids docosahexaenoic acid and arachidonic acid for 2 months prior to spawning can double hybrid fry output.

Broodstock strain. Strain of male blue catfish and/or strain of channel catfish female affected sperm production, hatching rate of hybrid embryos and total fry production. Genotype-environment interactions were also observed for sperm production. Utilization of genetic variation has the potential to double efficiency and productivity of hybrid embryo production.

Induced spawning At one location, no significant differences were observed between LHRHa, carp pituitary extract and catfish pituitary extract for production of hybrid catfish fry. At a second location LHRHa was superior to carp pituitary extract, other forms of GnRH and ovaprim for producing hybrid catfish fry. Injections of 30 Fg/kg female body weight of LHRHa followed by 150 Fg or the utilization of 100 Fg/kg implants generates the greatest number of fry per weight of female during the early and peak spawning periods. Late in the spawning season, implants are superior to injections. Early in the season latency of time from initial introduction of the LHRHa until the time of ovulation for implants is longer than that for injections, but later in the season latency is the same for both injections and implants. Ovulation of individual females in aquaria or bags resulted in greater fry production than females mass-ovulated in tanks.

In general, plasma estradiol, plasma testosterone, cathepsins D and L and mean egg sizes of channel catfish females increased from May/June of one year and then plateaued at various time periods until spawning in May of the second year. Activity of cathepsin B was variable from month to month, and mean protein content of eggs was highest in October when eggs appeared and decreased for the remainder of the year (November through April) when eggs were present. These measurements may allow screening of females most likely to produce high-quality eggs. No large differences in these variables were observed among four strains during each month.

Gamete quality. Spectrophotometric assays were used to determine sperm concentrations from crushed testis of catfish. Utilization of this tool should result in more efficient use of sperm, and more consistent fertilization rates. The anterior testis of channel catfish produced more sperm and more concentrated sperm with better motility than the posterior testis. This relationship should hold true for blue catfish testis and will be tested. Increased sperm concentrations gave increased fertility, and fresh sperm had almost double the fertilization rate of frozen sperm. Sperm concentrations can be reduced in currently used fertilization protocols by 100-fold, with little reduction in subsequent hatch rate.

Automated transparency scanners imaged catfish oocytes and embryos during oocyte maturation

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Innovative Technologies and Methodologies for Commercial-scale Pond Aquaculture

THE CHALLENGE . . .

Aquaculture operations in the Southern Region of the United States are finding it difficult to maintain profitability. Production costs are increasing, but the prices that producers receive for fish, shrimp, and other cultured aquatic animals are not keeping pace. The problems are especially troublesome for channel catfish farming, the major aquaculture activity in the region. Increasing profitability of channel catfish farming is a long-term, complex, multifaceted problem. Nevertheless, methods for reducing production costs would provide an immediate improvement in profitability.

OUR RESPONSE . . .

Eleven research scientists from seven institutions have joined in a four-year project to investigate new technologies and methodologies to improve the efficiency and enhance the profitability of aquaculture in the Southern Region. The scientists represent the following institutions:

- Auburn University
- Clemson University
- Louisiana State University
- Mississippi State University
- University of Arkansas at Pine Bluff
- USDA-ARS (Pine Bluff, Arkansas)
- USDA (Stoneville, Mississippi)

Several possible methods for improving efficiency and profitability of aquaculture are under investigation:

- Evaluation of new production systems and improvements in existing production systems for channel catfish;
- Improvement in equipment used for mechanical aeration and for fish harvesting in channel catfish culture;
- Evaluation of energy, material, and economic efficiency of production systems.

PRINCIPAL ACCOMPLISHMENTS . . .

This is the first year of this multi-year project, and 2 to 3 years of work will be needed to achieve major accomplishments. Work has been initiated on all objectives, and some useful findings have resulted. For example, a partitioned aquaculture system can be used to produce larger fingerlings at a lower cost than possible in traditional fingerling ponds. Design and initial testing of a motor-powered U-tube aerator, a low-speed paddlewheel aerator, and an electrically-enhanced seine have been accomplished. These improvements in equipment will be further evaluated in commercial-scale ponds to determine if they can reduce production costs for aeration and harvesting. ❖

Improving Reproductive Efficiency to Produce Channel × Blue Hybrid Catfish Fry (continued from page 7)

and embryogenesis, respectively. Animations of time-lapse image stacks revealed a surprising amount of cell movement in cleavage stage embryos. Other details of embryonic development included gastrulation/epiboly, neurulation, initiation of motility, and hatching. Arrested development and subsequent cytolysis of abnormal embryos could also be clearly documented, including the developmental events prior to arrest and death.

Cleavage-arrested embryos continued to show movements in spite of failed development.

Developmental arrest is not necessarily followed immediately by cytolysis and death. The cause of this developmental arrest needs to be ascertained and corrected. Hopefully, this can be adapted for practical application of predicting egg and embryo quality.

Ultrasound was able to identify ovarian development differences between females that ovulated and those that did not following injections of LHRHa. However, no predictive differences were observed prior to injection. After injection, use of ultrasound enabled identification of females that were at the correct stage of ovulation to allow stripping of eggs.

Hatchery practices. Various chemotherapeutants were tested to improve egg hatching success. Hatching success was high in the untreated controls (82.8%) and highly variable within treatments.

A tendency toward increased hatching success was observed among eggs treated with 100 ppm formalin (87.7%), 100 ppm iodine (88.1%), and 2.5 ppm copper sulfate (87.0%). The frequency of formalin treatments should be three times per day to maximize hatch rate of hybrid embryos and four treatments per day is excessive. At 28°C, hybrid embryos are chemically sensitive to formalin between 42 to 46 hours post-fertilization, and formalin treatments should be avoided during this period to maximize hatch rate. ❖

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