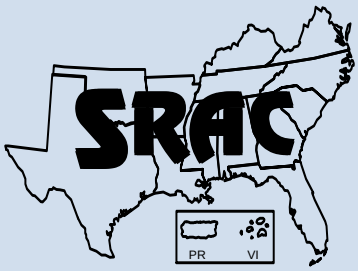

Nearly 70% of the $1 billion dollar domestic aquaculture industry is located in the southeastern United States. One of the most important programs supporting this critical sector of southern agriculture is the USDA-CSREES Southern Regional Aquaculture Center (SRAC). The Center is the only research-funding 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

Seventeenth 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.

In the simplest sense, 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 $1.9 million were in progress. The Center's "Publications" project is in its ninth year of funding and has generated more than 170 fact sheets with contributions from more than 135 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 led to the discovery of safe, inexpensive methods to control the intermediate host of a trematode parasite that has devastated catfish farms in the last few years. In the future, the disease may be considered no more than a manageable nuisance thanks to these findings. As additional examples, work in our "Harvesting" project aided in 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 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, 2004

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 publications 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. 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, 17 research publications, and 20 videos. More than 135 authors from across the United State have contributed to SRAC publications. SRAC fact sheets and reports 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.

Eleven fact sheets and one video were completed this year, with 11 fact sheets in progress. These publications were developed by 20 scientists associated with the following institutions and agencies:

- Auburn University
- Clemson University
- Kentucky State University
- Louisiana State University
- Mississippi State University
- USDA/ARS – Stuttgart, Arkansas

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

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 . . .

A new catfish seine. Braided polyethylene mesh is the best material for 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 is more efficient than conventional seines has been commercialized. The new seine improves catfish harvest efficiency by 15 to 20% and reduces seining time by about 50%.

In-pond grading of catfish. Major advances were made in the development of in-pond fish grading technology. The resulting

continued on page 4

Development of Improved Harvesting, Grading and Transport for Finfish Aquaculture (continued from page 3)

basic design consists of an adjustable horizontal bar grader, a trailer with built-in PTO-driven water pump, and an eduction pump system that delivers fish to the grading panel. Various grading panel designs have been developed for fingerlings, stockers, foodfish, and brood catfish. An overlapping split panel design was also developed to effectively sort fish into three groups with a single pass across the grading system. An additional piece of equipment, a live car reel system, was developed to facilitate the process of crowding fish while grading. This reel system can be added to any standard seine boat and allows one person to easily grade large quantities of food-fish.

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

An economic analysis was performed using data from previously reported field trials to determine whether farmer adoption of this grader is economically feasible. Results indicated that the UAPB grader is a profitable short- and long-term investment for small and large farms. The increase in profitability results from eliminating the inefficiency of live-car grading by returning sub-marketable fish to the pond. Annual yields and weight of fish sold will generate greater returns to the investment in fingerlings and feed by not selling sub-marketable fish prematurely.

In-pond grading of hybrid striped bass. The portable in-pond grader designed for use in the catfish industry performed poorly when used to grade phase II hybrid striped bass. Most fish did not enter the eductor box even after extreme crowding. Those that did enter the box were buffeted around before being deposited on the grader with obvious signs of trauma. Based on the discouraging results of these trials, a series of modifications were made to the eductor box design and these modifications were eventually successful in moving fish from the net holding pens to the grader section without trauma.

Harvesting and transport of ornamental fish. Ornamental fish growers in Florida were surveyed to obtain information on current harvesting and transport practices About 20 taxonomic groups of fish were represented by farms responding to the survey.

Dosage rates of compounds needed to reach light transport sedation were determined for blue gourami and results should have applications to other species. Of the compounds tested (metomidate, quinaldine, tricaine methanesulfonate, salt, and Hypno®), all but salt resulted in reduced blood-cortisol levels relative to controls following a one-week post-treatment period. Also, trials showed that fish fared better when transported in aerated well water or a mixture of half pond water and half aerated well water (or system water) compared to using pond water exclusively.

Studies were conducted to evaluate the benefits of holding fish in waters amended with tetracycline,

continued on page 10

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 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 four issues:

- development of standardized methods for the isolation, culture, and antimicrobial susceptibility testing;
- characterization of archived strains of columnaris-like bacteria based on conventional and molecular techniques;
- development of reproducible challenge models for columnaris-like bacteria; and
- 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 (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)

continued on page 6

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 5)

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 the 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 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 active trematode infections, commonly observed in channel catfish production systems, can significantly reduce production by reducing feed consumption and increasing mortality associated with the

continued on page 7

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)

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.

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 and may prove to be the most effective method of breaking the life cycle of the parasite.

***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. These fish, which should have been marketed, are exposed to additional risk factors and cause higher population feed conversion ratios. Increased carcass yield would also benefit processors and the industry.

Application of the hybrid channel catfish female × blue catfish male could dramatically alleviate these problems, making catfish farming more competitive, sustainable and profitable. However, only a few farmers use the hybrid because fingerlings are not available due to difficulties in producing hybrid eggs and fry. Artificial hand stripping and fertilization technologies have been developed that allow small-scale production of the channel × blue hybrid. But this technology and reproductive efficiency needs to be improved to optimize hybrid fry production and 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 brood stock to allow economical delivery of the hybrid technology to the catfish industry.

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

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

Attaining these objectives should result in improved techniques to induce spawning of broodstock to produce high quality hybrid embryos, with improved hatch rate, resulting in improved efficiency and total production of fry, fingerlings, foodfish, and processed product. 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 . . .

A temperature × time model was developed to help predict the optimum time to initiate artifical spawning to produce hybrid fry. Channel catfish begin to spawn at approximately 100 degree-days after the pond water temperature reaches 21EC. 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 28EC when using

continued on page 9

Improving Reproductive Efficiency to Produce Channel × Blue Hybrid Catfish Fry (continued from page 8)

LHRHa to induce ovulation in channel catfish females. The degree-hour response time is longer at cooler temperatures, and the females ovulate much faster at higher temperatures. This is important so that reasonable work schedules can be formulated for commercial scale production. The ovulation rate and number of eggs released also increased with increasing temperature. Hatch rate 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. 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.

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. 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. Implants produced more fry than injections. Ovulation of individual females in aquaria or bags resulted in greater fry production than females mass-ovulated in tanks.

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.

Automated transparency scanners imaged catfish oocytes and embryos during oocyte maturation 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

continued on page 10

Development of Improved Harvesting, Grading and Transport for Finfish Aquaculture (continued from page 4)

acriflavine neutral, salt, potassium permanganate, nitrofurazone, formalin, methylene blue, or Quick Cure®. Overall, tetracycline yielded the best appearance and behavior. Fish treated with potassium permanganate, Quick Cure®, formalin, and nitrofurazone also had significantly better appearance and higher behavior scores than controls, indicating a benefit to using certain additives when treating fish coming in from a pond.

When transporting fish to a distributor, certain additives can improve the quality of the fish shipped. Of the additives tested, salt was the most consistently beneficial additive for appearance and behavior. Acriflavine neutral yielded lower appearance scores than other treatments, including the control.

Baitfish transport. Ammonia production by baitfish was measured to develop predictive models of final ammonia concentrations in transport tanks. Ammonia excretion rates increased with increased temperature and the anesthetic tricaine methanesulfonate resulted in a slight eduction of excretion rates. If ammonia can be controlled on fish haulers, fish densities might be doubled and the cost of shipping fish cut in half. ❖

Improving Reproductive Efficiency to Produce Channel × Blue Hybrid Catfish Fry (continued from page 9)

arrest and death. 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.

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 28EC, hybrid embryos are chemically sensitive to formalin between 42 to 46 hours post-fertilization, and formalin treatments should be avoided during this period to maximize hatch rate. ❖

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