SUMMARY OF RESEARCH, EXTENSION, AND DIAGNOSTIC ACTIVITIES

2006

Thad Cochran
NATIONAL WARMWATER
AQUACULTURE CENTER

SUMMARY OF EXTENSION, DIAGNOSTICS, AND RESEARCH ACTIVITIES 2006

This document summarizes current extension, research, and diagnostic activities at the Thad Cochran National Warmwater Aquaculture Center, MSU Department of Wildlife and Fisheries, MSU Department of Agriculture Economics, USDA Southern Regional Aquaculture Center, and the USDA Catfish Genetics Research Unit. Projects are listed by specific subject area and include work that was completed or published within the last year and projects that are planned for 2007.

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PERSONNEL

MSU Extension Service (MSU-ES)

Dr. Jimmy Avery, Extension Aquaculture Leader and Extension Professor, is housed at the Thad Cochran National Warmwater Aquaculture Center. Dr. Avery is responsible for statewide assistance as an Extension Aquaculture Specialist. He has a 90% extension appointment and a 10% research appointment. In addition to his catfish responsibilities, he also serves as contact for information concerning crawfish and aquatic weeds.

Charlie Hogue, Jr., Extension Associate III, is housed at the Black Belt Experiment Station in Brooksville. Mr. Hogue is responsible for providing educational efforts in the East Mississippi catfish producing area. He also provides presumptive disease diagnosis based on pond visits and laboratory examinations.

Dr. Jim Steeby, Associate Extension Professor, is housed at the Humphreys County Extension office in Belzoni. Dr. Steeby has a 100% extension appointment and is assigned to the Delta counties as an Extension Aquaculture Specialist. In addition to his catfish responsibilities, he also serves as contact for information concerning freshwater prawns.

MSU Mississippi Agricultural and Forestry Experiment Station (MSU-MAFES)

Dr. Craig Tucker, Research Professor and NWAC Director, is housed at the Thad Cochran National Warmwater Aquaculture Center. Dr. Tucker is also the Director of the USDA Southern Regional Aquaculture Center program. His appointments are split 80% MAFES; 11% CVM; and 9% MSU-ES. In addition to his administrative responsibilities, Dr. Tucker conducts research on off-flavor management, environmental impacts of aquaculture, and general water quality management.

Dr. Lou D'Abramo, Professor, is a faculty member in the MSU Department of Wildlife and Fisheries on the Starkville campus. Dr. D'Abramo conducts research on crustacean nutrition and aquaculture production related issues.

Dr. Terry Hanson, Associate Professor, is a faculty member in the MSU Department of Agricultural Economics (MSU-AEC) on the Starkville campus. Dr. Hanson has a 71% MAFES appointment, 20% teaching appointment, and a 9% Extension appointment. He conducts research on aquaculture production economics, marketing, and risk management.

Dr. Terry Greenway, Assistant Research Professor, is housed at the Thad Cochran National Warmwater Aquaculture Center. Dr. Greenway has a split research appointment with MAFES and MSU-CVM and conducts research concerning immunology, vaccine development, and vaccine delivery strategies.

Dr. Menghe Li, Research Professor, is housed at the Thad Cochran National Warmwater Aquaculture Center. Dr. Li has a 100% research appointment with MAFES and conducts research on nutrition, feeds, and feeding of catfish.

Dr. Doug Minchew, Associate Research Professor, is appointed to the Thad Cochran National Warmwater Aquaculture Center and is housed at the Starkville campus. Dr. Minchew has a 100% research appointment with MAFES and conducts research in fish physiology, spawning behavior, harvest technology, and pond resource management.

Dr. Chuck Mischke, Associate Research Professor, is housed at the Thad Cochran National Warmwater Aquaculture Center. Dr. Mischke has a 100% research appointment with MAFES and conducts research on fry and fingerling pond management, general pond ecology, and innovative pond production systems.

Dr. Ed Robinson, Research Professor, is appointed to the Thad Cochran National Warmwater Aquaculture Center and is housed at the Starkville campus. Dr. Robinson has a 45% research appointment with MAFES and conducts research on nutrition, feeds, and feeding of catfish.

Dr. David Wise, Research Professor, is housed at the Thad Cochran National Warmwater Aquaculture Center. His appointments are split 63% MAFES; 24% CVM; and 13% MSU-ES. Dr. Wise conducts research on fish diseases and fish health management, and he is the leader of the applied fish health research program at NWAC. While Dr. Wise's extension appointment is small, his on-farm work with producers has developed many management techniques used by the catfish industry.

MSU College of Veterinary Medicine (MSU-CVM)

Dr. Lester Khoo, Professor of Aquatic Animal Health, is a veterinary pathologist and Director of the Aquatic Diagnostic Laboratory at the Thad Cochran National Warmwater Aquaculture Center. In addition to his primary diagnostic role, Dr. Khoo participates in variety of research projects, investigation of new and emerging diseases, and intern training.

Dr. Pat Gaunt, Associate Professor of Aquatic Animal Health, is a boarded veterinary toxicologist. Dr. Gaunt's time is split evenly between diagnostic responsibilities and research. Dr. Gaunt has worked closely with pharmaceutical companies seeking antibiotic approval for use in catfish by the FDA.

Dr. Michael Mauel, Associate Professor of Aquatic Animal Health, is a microbiologist. Dr. Mauel shares in diagnostic responsibilities at the NWAC; however, research in the area of molecular biology is his primary responsibility.

USDA/ARS Catfish Genetics Research Unit (USDA/ARS-CGRU)

Dr. Ken Davis is a Research Physiologist and serves as Research Leader for the Unit. His personal research involves catfish reproduction, physiology, and gender control.

Dr. Brian Bosworth is a Research Physiologist and uses quantitative genetics approaches to coordinate and analyze data from all the researchers to identify the best performing catfish. His personal research focuses on understanding how muscle growth and development is regulated, and improving carcass yield through selective breeding.

Dr. Brian Peterson is a Research Physiologist whose research focuses on hormonal control of growth and developing tests to predict growth potential. He is also investigating possible roles for growth hormone in regulating the immune system.

Dr. Sylvie Quiniou is a Research Microbiologist whose research has focused on understanding the Major Histocompatibility Complex class I and class II molecules. These molecules are proteins that function in the immune system to recognize self vs. foreign and understanding how they work in catfish will help us in our selection program.

Dr. Brian Small is a Research Physiologist working in the area of fish stress as it impacts disease susceptibility, growth, and reproduction. This includes research to identify genetic mechanisms that control growth and spawning success. He is also working on methods to improve egg hatching rates.

Dr. Les Torrans is a Research Fisheries Biologist working in the area of intensifying catfish production through improvements in the pond environment. He is developing new aeration technology and identifying optimal oxygen requirements for catfish.

Dr. Geoff Waldbieser is a Research Molecular Biologist whose research focuses on catfish genomics and identifying genes that control important traits such as growth rate, carcass yield, disease resistance, and reproduction.

Dr. Paul Zimba is a Research Microbiologist whose research is focused on optimizing pond water quality. His research involves identification and control of harmful algal species and the use of remote sensing for identification of algal blooms.

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PUBLICATIONS

NWAC Publications

The following NWAC publications may be viewed by visiting the publications page on the NWAC website (http://www.msstate.edu/dept/tcnwac/publications.htm):

- **Annual Report** – A current summary of research, extension, and diagnostic activities at the NWAC is available at http://www.msstate.edu/dept/tcnwac/Seraieg/2006msu.pdf
- **Research Publications -** Mississippi State University scientists at NWAC have authored or co-authored more than 750 publications from 1981 to the present. A list of publications by discipline is available at http://www.msstate.edu/dept/tcnwac/publist9-6-07.pdf
- **NWAC News -** The Thad Cochran National Warmwater Aquaculture Center Newsletter is published twice a year. The Newsletter contains research findings, diagnostic summaries, Extension recommendations, industry updates, and notice of upcoming events at the NWAC. The Newsletter is distributed to farmers, researchers, Extension agents, and suppliers in 27 states. The 10 newsletters are available at http://www.msstate.edu/dept/tcnwac/nwac_news.htm
- **Producing Hybrid Catfish Fry: Workshop Manual** – The manual is a compilation of materials presented at the May 25, 2005 workshop developed by NWAC and USDA Catfish Genetics Research Unit scientists. (http://www.msstate.edu/dept/tcnwac/HybridManual05.pdf)
- **MSU-CVM Aquatic Diagnostic Laboratory Annual Reports -** Annual reports are available for 2001 - 2006. These reports may be accessed at http://www.msstate.edu/dept/tcnwac/Diagnostics.htm

MSUCares.com Aquaculture Website

Web-based information on catfish, prawns, and additional aquaculture species is available through the Aquaculture Pages on the MSUCares.com network at http://msucares.com/aquaculture/index.html. Each species section provides links to MSU-MAFES and MSU-ES publications on aquaculture subjects as well as links to other University sites.

MSU Department of Agriculture Economics

Catfish industry statistics, production budgets, cash flows, and Trade Adjustment Assistance curriculum can be accessed at http://www.agecon.msstate.edu/research/catfish.php. Information on aquaculture risk management is available at http://www.agecon.msstate.edu/aquaculture/pubs.php.

USDA Southern Regional Aquaculture Center Factsheets

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 Center has now published 179 factsheets, 4 project reports, 19 research reports, and 20 videos. The complete collection of SRAC publications may be accessed at http://srac.tamu.edu/

WATER QUALITY

Off-Flavor Management

Pond aquaculture is profitable because natural processes provide many of the resources needed to sustain the aquaculture crop. However, rates and magnitudes of all natural processes are variable in time and space, resulting in unstable and unpredictable environmental conditions. Ecosystem instability increases as nutrient loading rates increase, and, in the extreme example of catfish culture ponds, environmental conditions can rapidly deteriorate and affect productivity, product quality, or the environment outside the pond. For example, populations of odor-producing blue-green algae may become established in catfish ponds causing fish to develop undesirable "off-flavors." These fish are unmarketable until flavor quality improves, causing an economic hardship on farmers. Problems related to off-flavors cost catfish farmers $15 to 75 million per year, and, additionally, have economic impacts far beyond the farm because inconsistent product quality may adversely affect market demand. Therefore a series of studies were conducted to 1) develop dependable and safe treatment protocols for algae-related off-flavors using currently available algicides and 2) develop alternatives to currently available algicides.

Low dose applications of copper sulfate for controlling off-flavor (Craig Tucker, Terry Hanson, and Kevin Schrader, USDA Natural Products Lab)

Copper-based algicides have a long history of safe use in drinking water supplies and are currently the only phytoplankton-control agents approved for use in aquaculture. Although there is some evidence that cyanobacteria are relatively more sensitive to copper than are eukaryotic phytoplankton, it is difficult to take advantage of differential toxicity under field conditions because the difference in copper tolerance among algal taxonomic groups is not great. Furthermore, there is relatively little margin of safety between phytotoxic concentrations of copper and concentrations that are dangerous to fish, and the toxicity of copper to all aquatic organisms is strongly affected by complex interactions with environmental variables such as pH, water temperature, and concentrations of calcium and dissolved organic matter. These interactions, which are largely unquantified, make it impossible to formulate consistently safe and effective treatments for algae control. Successful use of copper algicides in commercial aquaculture is therefore based largely on trial-and-error. In the early 1990s, commercial catfish producers in northwest Mississippi began using copper sulfate to manage algae-related off-flavors with a perceived measure of success. Their practice, which had evolved over many years of experience, involved relatively low doses of copper sulfate (0.5 ppm as copper sulfate pentahydrate) that were applied weekly by placing the chemical in a burlap bag and then suspending the bag in the current produced by an aerator. The aerator current helped dissolve the chemical and distribute it throughout the pond. This technique was therefore evaluated in a series of laboratory and filed studies, culminating in a 3-year study on commercial farms in Mississippi. Overall prevalence of off-flavor was reduced by 50% based upon fish flavor analysis. Copper sulfate treatment reduced potential harvest delays by nearly half and reduced costs associated with off-flavor by 35%. Based upon these results, weekly low-dose applications of copper sulfate appear to be beneficial in mitigating musty off-flavor problems in commercially produced catfish. This treatment is now considered the standard protocol for managing off-flavors using this chemical.

Diuron efficacy and evaluation of other herbicides for control of blue-green algae (Paul Zimba, Craig Tucker, and Chuck Mischke)

Selective control of problematic algae in aquaculture ponds has been an elusive goal. Diuron is presently available for control of off-flavor producing algae, however little information on the selectivity of this compound is available. Paul Zimba and collaborators conducted field and laboratory studies to assess

trophic impacts of diuron. Diuron reduced off-flavor occurrence, but was not effective in controlling species of algae capable of forming toxins (i.e. microcystin). Efficacy of various compounds was tested and one compound has been identified that exhibits desirable control at reasonable application rates (0.3 mg/L to kill 50% of algae present). This chemical should provide a cost effective alternative to copper compounds and diuron for control of off-flavor and toxin-producing algae if EPA permitting is obtained.

Diuron residues in catfish exposed to the chemical over multiple years (Craig Tucker)

Diuron has been a useful off-flavor management tool for almost 10 years, yet EPA continues to question the environmental and health impacts of its use in catfish ponds. A study was conducted to determine whether multi-year exposure of channel catfish to diuron resulted in tissue residues exceeding the EPA tolerance level of 2.0 ppm. The study showed that diuron residue levels in fillets remained well below 1 ppm after treatment and there was no carryover of residues in fish from one year to the next. Results show that this algicide can be safely used to manage this important problem in catfish farming. Data from this study were critical in the recent favorable EPA ruling to allow a full registration for this chemical in the catfish industry.

Using sodium carbonate peroxyhydrate for managing off-flavor (Craig Tucker, Chuck Mischke, and Kevin Schrader, USDA Natural Products Lab)

An alternative algicide for managing blue-green off-flavors has recently been approved for aquaculture use by EPA. This chemical, sodium carbonate peroxyhydrate (SCP), is an oxidizing agent that works by releasing hydrogen peroxide when dissolved in water. The chemical is attractive because it degrades to water, oxygen, and carbon dioxide, leaving no residue in the pond or fish. However, field trials showed that effective dosages varied considerably from pond to pond, but were generally in the range of 5 to 10 ppm. Effects were short-lived and multiple treatments would probably be needed to effectively eliminate odorous blue-green algae and treat off-flavors. The cost and logistics of using SCP do not appear favorable at this time.

Evaluation of silver carp polyculture to reduce blue-green algal off-flavors (Craig Tucker)

At present, using chemical algicides to eradicate odor-producing algae is the most dependable means of preventing off-flavors in catfish. Nevertheless, using algicides to control flavor problems has several disadvantages and there has been interest in biocontrol of plankton communities using planktivorous fishes. In this study, low densities (0, 25, or 100 fish/acre) of silver carp were co-cultured with catfish in ponds. Silver carp did not eliminate odor-producing blue-green algae from pond phytoplankton communities and did not reduce the incidence or intensity of off-flavors in catfish. The results of this study will help eliminate inducements to use this non-native fish for water quality management in catfish ponds.

Evaluation of a bacterial pathogen of blue-green algae as a biocontrol agent for off-flavor (Kevin Schrader, USDA Natural Products lab, Craig Tucker, and Lynn Walker, Louisiana Tech)

Current off-flavor management is based on using chemical algicides to eliminate odorous blue-green algae. All chemical algicides suffer the disadvantages of leaving residues in the pond and fish. Biological control of odorous algae using natural pathogens is an attractive alternative. A bacterium that kills odor-producing blue-green algae has been isolated by Louisiana Tech scientists. The bacterium is strikingly effective under laboratory conditions but results have been highly variable in 2 years of field testing at Stoneville. Additional work is planned to identify the basis for the mixed success when using this organism for algal management.

Evaluation of sensory versus analytical detection of off-flavors (Paul Zimba)

Fast through-put analytical methods were developed for routine use in off-flavor assessment. Flavor checkers in processing plants are used to assess fillet quality and often their opinion of quality has been debated. Four processing plants provided fillets that were processed for analysis by flavor checkers; samples for gas chromatography/mass spectrometry were also obtained from each fish. The sensory detection limit for flavor checkers was determined to be 0.1–0.2 ppb for MIB and 0.25–0.5 ppb for geosmin. Results from both analyses were highly correlated; suggesting flavor checkers provided reliable evaluations. The present practice of flavor testing is a reasonable alternative to instrumental detection.

Dissolved Oxygen and Aeration

Effects of low oxygen on channel catfish production (Les Torrans)

Catfish farmers have long known that aeration allows them to feed more and grow more fish. Over the last 30 years, farmers have progressed from having only tractor-powered aerators that could be moved to a pond with low D.O. concentration, to having over 2-hp/acre of electric paddlewheels permanently installed. Production has increased concurrently from 1,000 lbs/acre to 5,000-10,000 lbs/acre. However, prior to the research of Les Torrans (USDA/ARS Catfish Genetics Research Unit), most aeration recommendations were based on minimizing visible oxygen stress in fish, not on maximizing fish performance or profit. He utilized computerized technology that enabled him to both control the D.O. concentration and record aerator usage in a multi-pond research facility. In several production studies (the first of which has been published), he determined that allowing the D.O. concentration to fall to 1.5 mg/L (as compared to 4.5 mg/L) reduced food consumption, growth, and net production by 45%, 31% and 54%, respectively. At higher dissolved oxygen concentrations, a net production of 23,547 kg/ha was achieved, a record for pond production systems. For the first time, D.O. management can be based on empirical data. In addition to increased profits resulting from increased food input, Dr. Torrans demonstrated that catfish can be grown to market size in two years instead of three with increased feed consumption made possible by increased aeration. These data have been used by the Catfish Farmers of Mississippi in an on-going process to negotiate “off-peak” electric rates from the Mississippi Electric Power Association. Following a meeting of the Association with representatives of the catfish industry and Dr. Torrans (who provided technical data on electrical usage and fish production), the Association provided a $3 million rebate to Mississippi fish farmers while they continue to develop an off-peak rate structure.

Comparative oxygen requirements of blue and channel catfish (Les Torrans and Brian Bosworth)

Blue catfish have potential as a commercial culture fish but it was believed that their oxygen requirements were higher than channel catfish. Drs. Les Torrans and Brian Bosworth (USDA/ARS CGRU) conducted a study at the Delta-Western Research Center to determine the relative impact of low oxygen on food consumption and production of channel and blue catfish. It was determined that while channel catfish food consumption decreased 9.5% and net fish production decreased 851 lbs/acre when the dissolved oxygen concentration dropped below 3.0 mg/L, blue catfish actually consumed slightly more feed (+0.9%) and had slightly higher net production (+156 lbs/acre) than blue catfish maintained at a dissolved concentrations above 4.0 mg/L. Blue catfish may have more potential as a commercial culture species than previously thought, and certainly merit further examination.

Oxygen requirements of channel catfish eggs and fry (Les Torrans, Brian Small, and Jim Steeby)

Approximately 2 billion catfish eggs are incubated each year by the channel catfish industry. The process is simple, and the methods have changed little since the inception of the industry nearly 75 years ago. With no empirical data available, recommendations on egg and fry oxygen requirements vary widely, and accordingly the D.O. concentrations observed in commercial hatcheries range from 40-100% saturation. In 2005 Les Torrans (USDA/ARS Catfish Genetics Research Unit) conducted a study to determine for the first time both metabolic rates and critical oxygen concentrations for catfish eggs, sac fry, and swim-up fry. It was determined that eggs require over 90% oxygen saturation during the last day of incubation. At lower concentrations, the eggs hatch prematurely, and suffer a higher mortality during the sac fry stage. It was recommended that hatcheries run supply water through a packed column to bring the D.O. concentration up to air saturation. LOX should then be used to maintain D.O. at or above air saturation through incubation. In addition to eliminating premature hatch and higher mortality, the LOX also provides a margin of safety during electrical outages. The incumbent worked with five hatcheries to improve oxygen management during the 2006 hatching season. These hatcheries represent approximately 15% (350 million eggs incubated yearly) of the total industry capacity. Dr. Torrans' recommendations are being rapidly accepted by the industry due to the large improvement in hatchery production, low cost, and ease of implementation, resulting in a 15-20% increase in industry fry production.

Effects of paddlewheel placement on fish production (Les Torrans and Paul Dees, Dillard and Co., Inc.)

Les Torrans (USDA/ARS Catfish Genetics Research Unit) has devoted several years of research to determine D.O. requirements of pond-raised catfish. The focus of his research has now shifted to finding economical means of meeting those requirements. He initiated a non-funded cooperative agreement with Dillard & Company, Inc. to evaluate a new aerator placement strategy on a commercial catfish farm. Ten 17-acre ponds were selected and brought into the study in pairs, with one pond using the conventional aerator placement strategy and the other pond using the new strategy. The ponds were stocked similarly and managed according to normal commercial practices. This was the first study to determine how aerator *placement* in commercial ponds can affect oxygen concentrations. This new aerator placement strategy was a radical departure from conventional practices and shows great potential for increasing production while reducing energy costs. Commercial ponds using the new strategy had higher morning D.O. concentrations, higher net production, with fish consuming more feed and converting that feed more efficiently. Ponds using the new aerator placement strategy used 18% less electricity for routine aeration, and used tractors 22% less frequently for emergency aeration. Overall savings on this farm from use of this new strategy is estimated to be $100,000. Publication is pending the results of two additional farm-scale research studies now underway.

Development of a new aerator: the Powered U-tube (Les Torrans)

Development of more efficient aeration systems is critical for the future of the catfish industry. Les Torrans (USDA ARS Catfish Genetics Research Unit) collaborated with John Carradine of Southern Machine Welding Inc. ("Big John Aerators") to design a prototype commercial-scale powered U-tube aerator which was installed in a pond at the National Warmwater Aquaculture Center in Stoneville, MS. The initial prototype shows promise, moving over 8300 gpm. If the Phase-II design can meet all of the criteria necessary for commercialization, this aeration system could greatly increase the efficiency of catfish production in the US.

Pond-age water column trophic relationships in catfish production ponds (Paul Zimba and Chuck Mischke)

Long-term changes in nutrients, phytoplankton and zooplankton have not been previously investigated in production ponds. We analyzed the relationships of pond age with nutrients, zooplankton and phytoplankton populations, and the incidence of off-flavor occurrence, as well as synoptically assessed off-flavor and algal toxin prevalence in Mississippi, Alabama, Arkansas, and Louisiana. Nutrients accumulated in ponds during the first 3 years of production, but leveled off after this time. After year 4, the algal composition became dominated by filamentous cyanobacteria, and zooplankton composition was dominated by larger copepods and cladocerans. Younger ponds have lower incidence and intensity of off-flavor relative to older ponds. Results of this study may be used to help predict off-flavor occurrence, and management practices can be altered in older ponds. Around 60% of ponds in the 485-pond survey had detectable levels of the toxin microcystin, whereas less than 30% had detectable levels of off-flavor compounds present. A PCR method was designed for detection of algae capable of forming this toxin - this method has been used in over 25 published studies from Europe, Australia, Canada, and the United States.

Optimization of remote sensing data analysis methods to detect harmful algal blooms (Paul Zimba and Chuck Mischke)

High suspended solids and chlorophyll concentrations are typical in aquaculture grow-out ponds. High fish stocking density requires both rapid mineralization of nitrogenous waste and production of oxygen by algae. To rapidly and accurately monitor algae, remote sensing methods offer utility. We have used both airborne and handheld remote sensing spectral radiometer/cameras to detect algal biomass in ponds. Digital imagery is less useful than high resolution radiometers for detecting specific wavelengths critical for accurate biomass assessment. Using handheld units, we were able to model measured field concentrations with 80% accuracy using pond-specific models. We have also modified ground-truthing methods to more accurately extract cyanobacterial pigments.

Evaluation of two bio-stimulants for improving water quality (Chuck Mischke)

Two commercial products (LASE and PhytoMax) are formulations of organic acids, enzymes, vitamins and micronutrients designed to stimulate microbial activity. The manufacturer of the products claims the products maintain phytoplankton diversity and stimulate bacterial and fungal communities to break down waste products. The specific claims for the products include consistently higher levels of dissolved oxygen, healthy algae blooms, reduced aeration costs, and overall improvement in water quality. However, these products had not been scientifically tested under commercial channel catfish conditions. Many producers were purchasing the products based on the manufacturer’s claims; the results of this study showed no benefits of adding either product to production ponds.

Managing dissolved oxygen during harvest with the Sock-Saver (Les Torrans)

Catfish are typically held in a net "sock" overnight to grade out small fish prior to delivery to the processor in the morning. Over 70,000 pounds of catfish may be held at densities exceeding 20 lbs/ft^3. This is an extremely risky situation; especially when water temperatures are high and D.O. (dissolved oxygen) concentration is low. Most farmers have had at least one disaster in which an entire sock of fish was lost; more typical situations may result in several hundred pounds of "weighbacks", dead fish arriving at the plant for which the farmer is not paid. Les Torrans (USDA/ARS Catfish Genetics Research Unit) believed that LOX (liquid oxygen) could remedy this situation. He sought out and collaborated with a commercial catfish farmer to build the "Sock-Saver", a portable trailer capable of holding and delivering 150 gallons of LOX to remote farm areas where fish are being held in a sock overnight. This equipment was thoroughly tested over the summer by a commercial harvest crew and proved useful in reducing fish mortality. Dr. Torrans developed this concept and the initial plans in collaboration with farmer; an area aquaculture extension agent was added to the team during the testing phase to help with subsequent technology transfer. This new technology has been widely publicized through numerous newsletters, abstracts, posters, and one journal article, and with over 15 units in use the industry has adopted the technology.

Comparison of the Sock Saver with conventional aeration (Doug Minchew and Rachael Beecham, Miss. Valley State University)

The aeration effectiveness of a diffused oxygen system was compared to that of a tractor-powered paddlewheel during eight harvest trials conducted at a commercial farm in Arkansas during August of 2004. Dissolved oxygen and water temperature data were collected from two live cars (one aerated by a diffused oxygen system; one aerated by a paddlewheel) and from the open pond between the two live cars. Conditions were monitored from the time the fish were forced into the live cars until they were crowded for loading on to live haul trucks. The results of the study indicated that the diffused oxygen system is a viable alternative to the paddlewheel for aeration of channel catfish held in live cars (grading nets).

Effects of transport water quality on subsequent fillet quality (Brian Bosworth)

Exercise and stress during transport negatively impact meat quality in most livestock species, including channel catfish. We conducted research to determine the effects of transport water temperature and oxygen on subsequent fillet quality. Water temperature had little effect on meat quality, but increasing dissolved oxygen levels during transport resulted in improved filet color and reduced drip-loss. Increasing oxygen levels during transport can improve meat quality, and future work is focusing on the cost/benefits of increasing transport oxygen levels.

Effluent management systems (Craig Tucker)

Effluents from aquaculture facilities have come under scrutiny by non-governmental organizations and regulatory agencies as potential sources of pollution. A wide variety of culture systems are used to grow aquatic animals, and each type of system has unique waste discharge characteristics. For example, flow-through culture systems (raceways) produce a constant effluent stream that, for individual facilities, varies little in volume and quality over time. Such systems are physically amenable to traditional "end-of-pipe" approaches to waste treatment, in which performance can be easily documented by periodic sampling of the effluent. By contrast, pond culture systems discharge water intermittently (only after heavy rains or when ponds are drained) and effluent quality varies widely over time and among individual ponds. These characteristics make it difficult to identify suitable waste treatment technologies and monitor their performance. The intermittent and unpredictable discharge from ponds impacts the cost and potential effectiveness of nearly all "end-of-pipe" treatment options. Treatment systems for pond effluents would be idle for many more days than they are used and the average annual hydraulic loading to the system will be low. However, when discharge occurs, the volume may be relatively large for a brief period. This is a difficult engineering problem because the system must be designed to rapidly treat a large volume of dilute wastewater. The intermittent nature of pond effluent discharge also makes it impossible to assess the performance of waste treatment technology by simply monitoring waste concentration. Therefore, approaches that reduce effluent volume and improve water quality prior to discharge will more effectively reduce mass discharge from aquaculture ponds. Adoption of an Environmental Management System (EMS) consisting of a set of management practices to minimize environmental impacts is one such approach. A set of effluent-management practices was therefore evaluated over 3 years in 2.4-ha earthen ponds in northwest Mississippi. Mass discharge—which is the product of concentration and volume—is usually more important than concentration alone in determining the impact of an effluent on the environment. Practices were therefore implemented that addressed both factors affecting mass discharge. One practice, water-level management, was implemented to reduce effluent volume. The other three practices were implemented to reduce the concentration of substances in effluents: 1) limiting daily feed inputs to 110 kg/ha per day, 2) using a low-protein (28%) feed, and 3) maintaining a modest fish density (18,500 fish/ha). Pollutant discharge and fish production from the "managed" ponds were compared to "traditional" ponds managed without these practices. Concentrations of total nitrogen, total phosphorus, 5-day biochemical oxygen demand, and total suspended solids were only slightly lower in effluents from managed ponds relative to traditional ponds. However, mass discharge (kg/ha) of those substances from managed ponds was reduced by more than 60%, primarily because water-level management reduced effluent volume to half of that from traditional ponds. Water-level management was originally developed in catfish farming as a water conservation tool, so it is not surprising that average annual groundwater use was only 18 cm in managed ponds while 45 cm of water was added to traditional ponds. Average annual fish harvest was 6,425 kg/ha in managed ponds and 6,250 kg/ha in the traditional ponds. This shows that a simple management system can be used to dramatically reduce pollutant discharge and water use in catfish ponds without affecting fish production. The centerpiece of the management system is pond water-level management, which can reduce overflow volume by 50% and groundwater use by more than 60%. Combining water-level management with practices to improve water quality inside the pond reduces pollutant discharge to less than 40% of that from unmanaged ponds. This study demonstrates that improved environmental performance of catfish culture can occur without sacrificing profitability because large-scale and costly changes to the existing production system are not required. This is the first study to quantify the effectiveness of a farm-level environmental management plan to reduce potential pollution from aquaculture ponds. Components of this plan have been implemented into comprehensive sets of environmental best management practices for pond aquaculture.

Effluents from channel catfish hatcheries (Craig Tucker)

Effluents from aquaculture facilities have come under scrutiny by non-governmental organizations and regulatory agencies as potential sources of pollution. In response, considerable research has been conducted to characterize effluent quality and potential environmental impacts, especially for pond facilities. However, no assessment has been made of catfish hatchery effluents despite the fact that hatcheries can discharge significant volumes of water. Assuming a 10-week spawning season and a water flow of 400 L/min for a hatchery designed to produce 10 million fry/year, about 40×10^3 m^3 of effluent will be discharged from the hatchery each year. This study was conducted to describe the solids, phosphorus, nitrogen, and organic matter content of effluents from typical commercial channel catfish hatcheries in northwest Mississippi. Net pollutant loads (effluent concentration minus inflow concentration) were low for all variables. Concentrations of all potential pollutants are lower than corresponding concentrations in effluent-receiving streams in northwest Mississippi and total effluent volume from catfish hatcheries constitutes less than 0.02% of total annual streamflow in the region. It is therefore highly unlikely that catfish hatchery effluents will have a negative effect on receiving stream water quality. These results have been incorporated into effluent-management plans for warmwater aquaculture.

Quality of effluents from catfish ponds during draining (Craig Tucker)

Development of effective waste management plans for pond aquaculture depends on characterization of the water discharged from ponds. This study assessed the quality of pond effluents when ponds are drained and characterized the nature of the material discharged. When ponds are drained, the initial flush of water discharged consists of pond water and a slurry of sediment that has accumulated over the screen inside the pond, but the effluent clears in 5 to 30 minutes and all water subsequently discharged is simply pond water. Since only a small proportion (1 to 4%) of the total solids discharged during pond draining was contained in the initial flush, it will be uneconomical to design elaborate treatment facilities to remove the material in that small volume of water.

Characterization of solids discharged from catfish ponds (Craig Tucker)

Processes that rely on gravity settling are the most economical method of removing solids from the initial flush of water released when ponds are drained. We measured the settling characteristics of solids in catfish pond effluent and used equations to calculate design criteria for settling basins. For average pond discharge rates, removal of 95% of solids requires a basin area ranging from 95 to 125 square meters. We also assessed the capacity of common drainage ditches to remove solids and nutrients from pond effluents. At typical pond effluent discharge rates, more than 95% of the solids in initial pond draining effluent was removed after the effluent traveled 120 to 220 meters downstream in a 1-meter wide ditch. This was consistent with the results of basin-design modeling and show that it is unnecessary to build elaborate facilities to improve catfish pond effluent quality because considerable improvement in quality occurs as effluent flows down the simple drainage ditches that are common features of most commercial catfish farms.

PRODUCTION SYSTEMS

Catfish Fry Nursery Pond Management

Survival of catfish fry after stocking into ponds is not well documented, and reported averages among farms range from about 55% to 80%; survival within a farm can range from 0 to100%. This variable, unpredictable return leads to decreased efficiency in the fry to fingerling production stage of culture. Several studies were conducted to identify areas of management that could improve fry survival.

Fertilization of catfish nursery ponds (Chuck Mischke, Paul Zimba, David Wise, and Menghe Li)

Commonly used nursery pond fertilization practices were developed over 40 years ago and had not been studied in Mississippi ponds. MSU and USDA researchers evaluated responses to several combinations of organic and inorganic fertilizer. Ponds responded more to nitrogen additions than phosphorus additions, and organic fertilizer did not produce a response. Increasing nitrogen application stimulated the algal bloom more quickly and significantly increased preferred zooplankton densities. We are now evaluating different nitrogen application levels. Changing the previous fertilization methods can increase densities of zooplankton and provide a healthy algal bloom which should lead to improved fry growth, survival and health. Large zooplankton are readily consumed by catfish fry, but the nutritional contribution of these zooplankton was not known. We determined the nutritional value of zooplankton from catfish nursery ponds. The zooplankton consumed meet or exceed all nutritional requirements of channel catfish fry. These zooplankton are high in protein (65%), contain essential amino and fatty acids, and are excellent sources of vitamins and minerals. Because of the high nutritional value of zooplankton present in catfish nursery ponds, the importance of maintaining high densities of zooplankton is confirmed. The new fertilization recommendations have been adopted by many farmers and are now considered the standard practice for catfish nursery ponds in the Mississippi Delta. Farmers using the new recommendations have reported higher fry survival and less labor costs than when using the previous recommendations; thus improved economic efficiency of fry production.

Zooplankton size and taxonomic selectivity of catfish fry (Chuck Mischke, David Wise, and Paul Zimba)

The zooplankton selection patterns by catfish fry were determined. Catfish fry selectively feed on cladocerans from 450 to 1000 micrometers, copepods greater than 650 micrometers, and ostracods greater than 450 micrometers. Rotifers and copepod nauplii were not consumed by the catfish fry. Collectively, cladocerans, copepods, and ostracods met or exceeded all nutritional requirements of catfish. These large zooplankton are high in protein, contain essential amino and fatty acids, and are excellent sources of vitamins and minerals. In laboratory feeding studies, catfish fry grew 50% more over a 20-day feeding study when fed commercial feeds supplemented with zooplankton compared to commercial feeds alone. In preliminary studies, catfish fry performed better in challenges to a bacterial pathogen (*Edwardsiella ictaluri*, the cause of enteric septicemia of catfish) when fed a diet supplemented with zooplankton. Impacts of this research should help fish farmers adopt management practices to maximize profitability of fry culture through more efficient utilization of natural pond productivity. Many catfish farmers have adopted the practice of monitoring zooplankton and basing their stocking decisions on the numbers of large (preferred) zooplankton taxa present. Utilizing more of the ponds' natural productivity is providing both economic and environmental advantages to the farmers.

Importance of pH tempering for catfish fry (Chuck Mischke, David Wise, and Jim Steeby)

Variability in fry survival may also be related to handling and stocking methods currently used. When stocking, fry are transferred relatively quickly from hatchery to pond water. Generally, temperature differences are monitored between hatchery and pond water, but little attention is paid to pH differences. Studies conducted to determine the tolerance of catfish fry to pH changes showed that catfish fry tolerate decreasing pH values relatively well, but have low tolerance for increasing pH values. It is estimated that a rapid increase in pH of 0.7 units will cause mortality in catfish fry of 10%, and an increase of 1.4 pH units will cause 50% mortality. We recommend that farmers monitor pH before stocking fry and stock ponds that tend to increase in pH throughout the day early in the morning. By monitoring pond pH at stocking, the major problem of poor, variable fry survival should be mitigated.

Intensification of Catfish Pond Culture

Evaluation of a modified partitioned aquaculture system (Craig Tucker)

Most United States aquaculture production comes from ponds which have the advantage of low capital cost and the relative reliability of fish production. However, traditional ponds need continuous management of oxygen concentrations and are susceptible to algae-related fish off-flavors, losses to avian predators, difficulties in disease control, inefficient fish harvesting, and the finite limit on fish production. Even so, catfish ponds should be capable of producing 15,000 kg/ha per year, yet average fish production across the industry falls far short of that potential. Engineers at Clemson University developed a culture system that allows routine fish production at the limits of the potential for culture systems with no water exchange. The partitioned aquaculture system (PAS) as currently configured at Clemson consists of an extensive, shallow “waste-treatment” basin representing about 95% of the total system area and an intensive fish-confinement area in which fish are crowded at about 20 to 40 times the density of traditional ponds. A low-speed, energy-efficient paddlewheel circulates water between the fish-holding section and the waste-treatment section. A modified PAS system was constructed at Stoneville that confines fish at a much lower density than the Clemson system. The overall concept is to take advantage of the fish confinement benefits of the PAS (facilitation of inventory, harvest, health management, and protection against predation) while avoiding the need for intensive system management. In two years of optimization studies, the simple version of the PAS found capable of sustained fish feeding rates of 150 to 200 kg/ha per day without deterioration of water quality. Net fish production (harvest weight minus stocking weight has been 12,000 to 16,000 kg/ha at feed conversion of less than 1.9. This system shows promise as an alternative to traditional pond aquaculture.

Evaluation of the three phase production system for the channel catfish (Lou D’Abramo, Jim Steeby, Terry Hanson, and Craig Tucker)

A fingerling to stocker phase (2^{nd} phase) evaluated as part of a three phase (modular) channel catfish production system was experimentally shown to be feasible and economically practical. The per kilogram total cost of production for two higher stocking rates was respectively eight and fifteen cents less than corresponding production costs for the multiple-batch production system. In addition, to being a cost effective, alternative management strategy for the farming of channel catfish, the modular system offers several advantages based upon better inventory control and minimizing loss due to bird depredation. The fingerling to stocker phase also affords flexibility in the choice of management options to meet an array of different goals of individual operations.

Organic catfish production (Les Torrans, Menghe Li, Ed Robinson, Brian Bosworth, and Chuck Mischke)

Farm-gate channel catfish prices hit an all-time low of $0.53/lb in 2002, primarily affected by illegal "dumping" of Vietnamese basa on the U.S. market. Farmers were in dire straights, and were looking for alternatives. NWAC scientists were asked by industry representatives about the possibility of producing channel catfish organically. The irony is that although fish can be produced in Europe, certified by various European agencies, and sold in the U.S. as organic, our farmers cannot produce and sell organic channel catfish, since the USDA does not yet have standards in place. In collaboration with other scientists from Mississippi State University (NWAC), Les Torrans (USDA/ARS Catfish Genetics Research Unit) participated in a two year study to determine the potential of producing channel catfish fingerlings (year 1) and food fish (year 2) under presumptive organic standards. This research demonstrated that catfish *can* be produced under conditions that will likely be approved by the USDA NOSB. In fact, catfish will likely be both the first US-produced fish to be certified as organic, and the major aquaculture species to be produced and marketed in the future as organic in the U.S. This project will be the benchmark scientific study facilitating that endeavor.

FISH HEALTH

Diagnostics

The Aquatic Diagnostic Laboratory (ADL), administered by the College of Veterinary Medicine, offers a comprehensive disease diagnostic service to Mississippi catfish producers. In 2006, the Aquatic Diagnostic Laboratory (ADL) at Stoneville received a total of 845 fish diagnostic cases. These cases were received from 73 farms which represents approximately 20% of the Mississippi industry. This is a 39% increase in the number of submissions over the 607 cases in 2005. There were 954 water quality samples from 226 farms analyzed. This number represents a 38% increase over the 681 samples received from 186 farms submitted in 2005. The laboratory is fully accredited by the American Association of Veterinary Laboratory Diagnosticians, which sets rigorous standards for quality assurance and supports guidelines outlined by the World Organization for Animal Health (OIE). Routine services performed include a visual examination, plus testing for the presence of bacterial, viral, fungal, and parasitic diseases. Every effort is made to provide farmers with a summary of preliminary findings on the day services are rendered, updates as needed, and a final written report when a case is completed. Whenever possible, treatment suggestions are also offered.

In conjunction with its primary diagnostic role, the ADL works closely with MAFES fish health professionals to offer treatment recommendations to producers, monitor disease trends, provide surveillance for and investigation into the causes of new and emerging diseases, provide field service investigation, serve as an archive for bacterial isolates, and maintain a database for epidemiologic information relating to diseases of catfish. A unique opportunity is offered to student preceptors, interns, and graduate students seeking advanced training in fish disease diagnostics and research. The ADL supports the research efforts and diagnostic needs of other NWAC units, including MAFES, MSU Extension Service, MSU College of Veterinary Medicine and USDA-ARS Catfish Genetics Research Unit. Furthermore, the laboratory provides an outlet for the dissemination of information gained from research efforts back to producers.

In June 2006 the ADL laboratory director Dr. Al Camus left MSU for the University of Georgia, and Dr. Pat Gaunt assumed the role of interim director. Dr. Lester Khoo returned to MSU as laboratory director in the summer of 2007.

Table 1. Trends in disease diagnosis as a percentage of diagnostic case submission over time.

Disease	Average	2006	2005	2004	2003	2002	2001	2000	1999	1998
Columnaris	44.1%	68.4	49.4	40.9	44.7	44.5	37.2	42.6	45.5	44.8
ESC	37.2%	56.5	31.1	30.8	34.7	39.8	36.4	33.5	41.2	41.2
PGD	21.7%	17.8	8.4	10.7	10.8	16.3	20.1	29.8	30.0	16.3
Saprolegnia	8.6%	8.4	4.0	3.7	5.3	10.1	10.4	10.5	8.7	8.6
CCV	4.6%	5.9	9.1	10.8	8.9	5.8	7.3	2.3	1.8	3.1
Anemia	4.0%	4.9	4.6	2.1	5.2	5.3	5.0	4.9	2.8	3.0
Ich	1.3%	0.8	1.3	5.0	0.5	2.2	1.8	2.7	0.7	0.5
Bolbophorus	2.9%	0.7	3.4	2.6	1.1	2.0	4.4	5.6	1.5	-
VTC	2.7%	3.1	0.9	3.2	3.7	2.0	2.5	-	-	-
No Pathogens	16.1%	20.3	12.8	20.8	18.3	16.2	19.2	15.0	15.2	11.4
No. of Cases	1339	845	602	778	832	1057	1602	2189	2007	1647

Methods for predicting potential epizootics (David Wise and Terry Greenway)

Control of bacterial infections in catfish culture is dependent on early detection and prompt treatment. In general, once significant mortalities occur treatment intervention is not successful. A method of predicting potential epizootics would aid in the control of disease. Several approaches are being developed to identify sources of pathogens prior to overt disease. An "immuno-capture" system to extract *E. ictaluri* or *F. columnare* cells from pond water has been developed, and is being optimized. In this assay, antibody coated magnetic beads are used to extract the target organism from the water. The organisms can then be quantified by plating on microbiological media or by using molecular techniques (real-time PCR). The assays will be used to study how numbers of these disease organisms fluctuate with temperature throughout the year and if there are minimum numbers required to initiate outbreaks in ponds. It may then be possible to avoid outbreaks by maintaining numbers below this threshold by chemical treatment or other means. To date the ESC and columnaris assays have been successfully used to measure the number of bacteria in filtered pond water to which known numbers of bacteria have been added. These procedures will be combined with real-time PCR assays and used to quantify number of bacterial pathogens in the water. The goal is to determine the threshold number of bacterial cells necessary to initiate mortalities. Knowledge of this threshold level and monitoring of bacterial cell numbers in farm ponds will permit the prediction of disease outbreaks and provide farmers an opportunity to initiate treatments before overt losses occur.

Another approach for monitoring pathogen levels in pond water will rely on a novel antibody based technique that can potentially raise detection signals 1000 to 10000 fold over conventional antibody based detection systems (currently under development). The number of fish that seroconvert along with the magnitude of the immune response will be examined in conjunction with the concentrations of pathogens in the pond water. Attempts will be made to establish threshold antibody values indicative of protective immunity and determine pathogen levels necessary for triggering disease outbreaks.

If the various parameters have predictive value as when outbreaks may occur production strategies could be altered to minimize losses. Preliminary trials focusing on ESC have been conducted on a commercial fingerling farm in 2005. This data demonstrates a strong correlation between sero-positive populations of fish and increased disease resistance. This information was used to make recommendations on feeding practices to maximize growth in resistant populations of fish and minimize losses in susceptible populations of fish.

The epidemiology of bacterial diseases in food-size channel catfish (David Wise, Lester Khoo, Chuck Mischke, and Michael Mauel)

The epidemiology of bacterial diseases was evaluated in cooperation with the USDA National Animal Health Monitoring System which is tasked with continual surveillance of animal health in the US. A logistic regression model identified four risk factors associated with increased reporting of bacterial diseases: operation size, pond draining interval, and source and size of fish. This study was conducted to generate a hypothesis describing possible managerial and environmental interactions that represent significant risks to production. A data collection and analysis system is being developed to evaluate production and economic efficiency on individual farms. Diagnostic information integrated into the database will permit the analysis of relationships such as the cost of disease related losses and control programs, and correlations between disease incidence and specific production practices. At present the basic structure of the database has been constructed, although it is expected that it will continue to undergo modifications over time. In a pilot program, approximately 24 months of "back" records on

hundreds of ponds has been entered into the database. The data includes stocking and harvest information, feed consumption, water quality, mortality estimates, and where available disease diagnostic information. The project is being used to study risk factors associated with visceral toxicosis of catfish, channel catfish anemia along with other disease losses.

Stress, Immunology, and Models of Disease Development

Effect of sedatives on fish health and fillet quality (Brian Small and Brian Bosworth)

Hauling channel catfish can induce a significant stress response in both fingerlings and foodfish as a result of high densities and poor water quality. Two new fish sedatives were evaluated for use under the extreme conditions of high water ammonia, low water oxygen, and fish crowding. Results of this research indicate that sedation for up to 24-hours with either compound reduces circulating levels of cortisol, glucose and lactate. Ongoing research indicates that reduction of these compounds in circulation would likely lead to improved overall fish health and fillet quality.

Identification of genes involved in immune response (Geoff Waldbieser, Sylvie Quiniou, Brian Small, and Brian Peterson)

In order to identify more catfish genes involved in immune responses, we used the newly developed catfish microarray to measure levels of expression of 19,000 genes in the spleen after injection of bacterial cell wall components that elicit immune responses. The results showed expression of 64 genes was significantly increased and expression of 74 genes was significantly decreased after injection. These experiments provided researchers with candidate genes for further analysis to determine whether differences in responses correlate with improved survival after pathogen challenge.

Other candidate genes involved in immune responses were explored in cooperative research with the University of Mississippi Medical Center. The Major Histocompatibility Complex (MHC) contains genes whose products regulate vertebrate immune systems, and the MHC class I and II molecules were identified in channel catfish. Unlike mammals, the catfish class I and II gene regions were found on different chromosomes. Preliminary functional studies showed spontaneous non-specific cell killing responses between cells of different fish were mediated by the MHC class I region molecules. The CD45 gene, whose product plays in important role in communication within immune cells, was also identified. This research provided insights into the control of catfish immune responses.

Impact of dietary components, mycotoxins, and feeding practices on disease resistance (Bruce Manning, Menghe Li, Ed Robinson, Pat Gaunt, and David Wise)

Feed contaminated with mycotoxins were shown to reduce growth and disease resistance. This research was used to determine tolerance levels in fish feeds. Dietary supplements of trace minerals, vitamins, and menhaden oil were not shown to improve resistance of fish to *E. ictaluri* infection. These data suggests that dietary supplements will not dramatically improve disease resistance and that other strategies for improving fish health should be pursued. Pre- and pro-biotics at different inclusion rates are currently being investigated as a practical method of improving fish health and growth. Improvements in disease resistance through genetic selection are also being investigated as method of improving fish health.

Manipulation of feeding regimes to control disease losses (Menghe Li, Ed Robinson, Terry Greenway, Al Camus, and David Wise)

Manipulation of feeding strategies has been shown to be very effective in controlling ESC-associated losses in channel catfish fingerlings. Laboratory studies demonstrated withholding feed from fish during infection can reduce losses by 50-80%. Field trials support these observations where the implementation of restricted feeding regimes reduced ESC mortality by approximately 75%. The mechanism responsible for this response appears to be related to the ingestion of bacteria present the water by feeding fish. The program is developing management strategies that will maximize both size and survivorship and decrease the use of medicated feeds. Implementation of restricted feeding practices on commercial farms has dramatically reduced losses associated with ESC. This information is also being used to develop strategies for controlling disease related losses in food fish production ponds. Current projects focus on the judicious use of antibiotics to clear subclinical infections and enhance the development of acquired immunity before the onset of losses.

Use of pulsed-field gel electrophoresis for typing disease organisms (Michael Mauel)

Flavobacterium columnare and *Edwardsiella ictaluri* are the most often cultured bacterial disease agent of pond raised catfish in the southeastern United States. Pulsed-field gel electrophoresis (PFGE), in which the entire genome can be represented as a distinct pattern of DNA restriction fragments, is a particularly powerful tool in epidemiology and is now regarded as the gold standard for molecular typing of microorganisms. Methods were developed for conducting PFGE on *F. columnare*, and its efficacy determined for characterizing *F. columnare* strains isolated from different locations in the Southeastern United States. On the basis of PFGE-derived profiles, similarity dendrograms were constructed for more than 30 *F. columnare* isolates from the Southeast, resulting in two major genetic groups with more than 60% similarity. Virulence diversity was observed in two different immersion challenge experiments conducted with 16 different isolates in channel catfish fingerlings. A direct correlation was found between the PFGE clustered groups and virulence. Challenged fingerlings with PFGE - Group A isolates resulted in average percent mortalities higher than 60%, whereas challenged fish with PFGE-Group B isolates resulted in average percent mortalities of less than 9%. These results suggest that two genetic divisions of *F. columnare* channel catfish isolates exist, one that contains strains that are “primary” pathogens of channel catfish (Group A), and another that are “secondary” or opportunistic pathogens of catfish (Group B). Ultimately, these studies will identify the mechanisms used by highly lethal strains to cause disease that can be applied to improved vaccine technology in the future. This information will also aid in diagnostic evaluations by separating virulent from less virulent strains of *F. columnare* isolated from diseased fish.

An *E. ictaluri* genome sequencing project has discovered the presence of numerous mobile elements. The sheer number of these mobile elements has encouraged the investigation of how these *E. ictaluri* strains may have changed molecularly. An epidemiological analysis of archived *E. ictaluri* isolates was performed. A total of 88 isolates of *E. ictaluri*, isolated between 1978 through 2006 from Mississippi, Louisiana, Alabama, Arkansas, Georgia, and South Carolina, were analyzed by repetitive sequence PCR using BOX and GTG-5 primers. 20 of these strains were subsequently analyzed by restriction enzyme digest with *Pme*I and *Spe*I followed by separation by pulsed field gel electrophoresis. While the analysis is still ongoing the presence of three or greater molecularly distinctive groups have been observed. The recognition of genetic groups within *E. ictaluri* will for the first time allow epidemiology studies into the maintenance of isolates from year to year in ponds/farms and into the variation of virulence between genetic groups. The development of vaccines will be enhanced by allowing the characterization of high and low virulent strains.

Preventing disease through vaccination (David Wise)

Enteric septicemia of catfish (ESC), caused by a gram negative enteric, and columnaris disease, caused by *Flavobacterium columnare,* are the most serious diseases affecting the culture of channel catfish fingerlings. Traditional means of controlling these diseases have relied on the use of medicated feeds or implementing restricted feeding regimes. Although these practices can significantly reduce losses, there are inherent problems associated with their use. Feed restriction is only effective in controlling ESC-related losses and results in a significant reduction in growth through lost feeding days. Medicated feeds can be used to target both bacterial pathogens but are expensive and prolonged use can result in the development of antibiotic resistance strains. A more efficient method of controlling disease is preventing disease through vaccination. Two vaccines are commercially available for control against ESC (AQUAVAC-ESC) and columnaris (AQUAVAC-COL), but evidence supporting vaccination as an effective control measure has been inconsistent.

Commercial field trials showed significant increases in fish size, feed conversion, and net production of fish vaccinated with AQUAVAC-ESC. This increase in production efficiency was shown to increased net revenue by $500 to $1,000 per acre. While field trials for the most part have been positive, laboratory trials are needed to substantiate these observations. In controlled laboratory settings, vaccination improved survivability but not all trials have been successful. These inconsistencies have made final assessment of the vaccine difficult. In 2006, the method of commercially producing the vaccine was altered. Since changes in processing could affect the characteristics of the vaccine, it was necessary to re-evaluate AQUAVAC-ESC. The new manufacturing process resulted in improve efficacy of the vaccine. All experimental trials indicated vaccination improved survivability compared to non-vaccinated cohorts following lethal challenge. Furthermore, this efficacy was demonstrated for up to 65 days post vaccination. These data demonstrated that vaccination with AQUAVAC-ESC can improve production efficiency and economic returns. Field and laboratory tests are also being conducted to evaluate the efficacy AQUAVAC-ESC used in combination with AQUAVAC-COL in 7 to10-day old post-hatch channel catfish fry. Information garnered from these studies is being used to develop cost effective disease management practices. Additional work is being conducted to investigate the use of adjuvants and vaccine delivery strategies to identify factors contributing to enhanced immune function and increased disease resistance.

DNA-based detection method for ESC (Geoff Waldbieser and David Wise)

Infectious disease in channel catfish causes significant losses to the U.S. catfish industry, and enteric septicemia of catfish (ESC) is the most prevalent and costly bacterial disease affecting commercial channel catfish farms. We developed and patented a DNA-based detection method for the bacteria, *Edwardsiella ictaluri,* that causes ESC. This permitted rapid, specific, and sensitive detection of *E. ictaluri* in catfish blood and solid tissues. The test demonstrated catfish that survived an experimental challenge with *E. ictaluri* contained lower levels of the bacteria than diseased catfish from the challenge. Other research involving experimental challenge with E. ictaluri showed some evidence that plasma cortisol levels during an *E. ictaluri* challenge correlated to the genetic predisposition of a family for *E. ictaluri* resistance or susceptibility. Catfish administered recombinant bovine growth hormone grew faster and cleared *E. ictaluri* to a greater extent than controls. Toll-like receptor 5, involved in cell signaling in the immune system, was identified as a potential biomarker for ESC exposure because it showed high levels of gene activity in challenged catfish. When exposed fish were compared to controls, expression of the TLR5 gene in stomach and liver was elevated 200 fold in fish with low susceptibility to ESC

compared to only a 2-fold increase in fish with high susceptibility. The patterns of gene expression after bacterial exposure can differ, however, between channel catfish, blue catfish, and their hybrid. These experiments provided valuable tools for measuring genetic differences in immune responses between catfish populations.

Selective breeding for resistance to ESC (Brian Bosworth, David Wise, and Brian Peterson)

Selective breeding of channel catfish for resistance to a bacterial pathogen has shown some success in the laboratory. A multitrait selection index was utilized to select for growth, carcass yield, feed efficiency, and resistance to enteric septicemia of catfish (ESC). After two generations of selection, 62 families of USDA303 catfish were screened for ESC susceptibility and were compared to 29 families of the parent strain USDA103 in two replicate experimental challenges. The USDA303 catfish population showed a 10% improvement in resistance to ESC than NWAC103 catfish during replicate challenges. This suggests that resistance to ESC has a genetic component and selective breeding could be useful in providing some increase in ESC survivorship in the pond environment.

Effect of cortisol and stress on mortality due to ESC (Ken Davis, Brian Small, and Brian Bosworth)

The bacterial pathogen *Edwardsiella ictaluri* is a prevalent source of substantial economic loss to catfish farmers, and stress has been shown to increase *E. ictaluri* susceptibility. Trials were conducted to determine the effect of cortisol in the presence and absence of stress on mortality during an *E. ictaluri* challenge. Cortisol is the primary stress hormone in fish, and may adversely affect feeding, growth, disease resistance, reproduction, and overall fish health. Increased plasma cortisol levels due to stress correlated to increased mortality, however, stress-free cortisol administration had no effect on mortality. These results suggest that although cortisol is considered the primary stress hormone in fish, it in itself is not immunosuppressive. Understanding the relationship between stress and disease resistance is crucial to the development of biomarkers and management strategies for improving disease resistance.

Cortisol secretion and clearance rates were investigated in channel catfish as an initial step toward elucidating the regulatory mechanisms involved in cortisol metabolism. The results of this research suggest that cortisol secretion can continue for some time after removal of the stressor, and that the continued increase is due to continued secretion. Catfish broodfish fed cortisol-treated feeds during the spawning season demonstrated higher plasma cortisol levels, reduced growth and smaller livers; however, these fish had a 41% increase in spawning success compared to controls, suggesting a potential function for corticosteroids in final oocyte maturation. A study was also conducted to determine the effect of sedation with a relatively new fish anesthetic (AQUI-S^{TM}) on sexually mature channel catfish. AQUI-S had suppressive effects on blood-born stress factors, and proved to be an efficacious sedative. This research provided the foundation for developing stress-reducing handling methods, selecting broodfish with improved stress tolerance, and improving spawning success.

Real-time PCR assay for *Edwardsiella ictaluri* (Lanie Bilodeau, Geoff Waldbeisser, and David Wise)

Edwardsiella ictaluri, the cause of enteric septicemia of catfish (ESC), and *Flavobacterium columnare*, the cause of columnaris disease are the two most serious diseases affecting the culture of catfish fingerlings. A patent was awarded in 2005 for a a real-time PCR assay for the identification and detection of *Edwardsiella ictaluri*, the causative agent of ESC. The assay is designed to detect genetic material of bacterial cells in small amounts of blood or other tissue samples. This assay is capable of detecting the equivalent of as few as 2.5 cells of E. ictaluri and is species specific. Also in development is a real-time PCR assay for the detection and identification of *Flavobacterium columnare*, the causative agent of columnaris disease. These technologies will be used for monitoring bacterial levels in diseased fish and pond water samples.

Evaluation of AQUAVAC-ESC® vaccinated fish fed fry diets supplemented pond biota using single- and continuous-dose challenge models (David Wise, Terry Greenway, and Chuck Mischke)

Experiments were conducted to develop laboratory protocols that better reflect the growth characteristics of pond-raised fish and pathogenesis of natural *E. ictaluri* infection. Channel catfish fry were fed commercial diets supplemented with natural pond biota and vaccinated 10-12 days of age post-hatch. Following vaccination resistance of fish to *E. ictaluri* infection was evaluated using standard single- and continuous-dose challenge models. Mortality differences were not detected between vaccinated and non-vaccinated fish or among dietary treatments using a standard single-dose challenge model. In contrast, vaccination and diet significantly affected mortality when disease resistance was evaluated using the continuous-dose model. Using this method mortality was lowest in vaccinated fry fed supplemented diets. This data also suggests that diet is critical in the growth and development of fry and that the methods used to induce disease in the laboratory can influence the outcome of disease challenge trials.

High-temperature tolerant strains of *E. ictaluri* (Lester Khoo, Michael Mauel, and Pat Gaunt)

Enteric septicemia of catfish is a highly seasonal disease, occurring primarily in the spring and fall when temperatures are permissive for growth of the pathogen. Several high-temperature tolerant *E. ictaluri* strains have been isolated from diseased fish. These isolates differ markedly in their ability to confer protection and induce death upon lethal challenge. Bacterial growth can occur at higher temperatures considered non-permissive to virulent field strains. Serologically, antibody responses to these temperature tolerant E. ictaluri strains exhibit little or no cross reactivity to virulent field isolates after primary immunization. We are currently investigating whether prior exposure to these temperature tolerant strains alters the kinetics and magnitude of antibody responses as well as protection from challenge with virulent *E. ictaluri*. Differences in antigenic determinants will be determined to identify factors responsible for the induction of protective immunity (or lack of) and the development of vaccines.

Efficacy of Aquaflor® (Schering Plough Animal Health) against *Edwardsiella ictaluri* (Pat Gaunt)

Enteric septicemia of catfish (ESC), caused by *Edwardsiella ictaluri*, is one of the most serious diseases affecting the culture of channel catfish fingerlings. Preventative measures such as vaccination and feeding restriction can help control disease, but these measures are not 100% effective. An effective and palatable antibiotic incorporated in floating feed is needed by the catfish industry to combat these diseases when preventative measures fail.

Laboratory and field efficacy trials were conducted in support of FDA approval of Aquaflor®, a new antibiotic for fish. Results showed that Aquaflor® is palatable, effective, and safe for use in catfish. Efficacy and residue-depletion studies were used to establish a dose rate of 10 mg active ingredient/kg of body weight that was approved by the U.S. FDA for control of mortality associated with *E. ictaluri*. Pharmacokinetic studies are being proposed to define drug uptake and clearance rates and are needed to substantiate the current recommended dose rate for ESC treatment in catfish.

Efficacy of Aquaflor® (Schering Plough Animal Health) against *Flavobacterium columnare* (Pat Gaunt)

Columnaris disease, caused by *Flavobacterium columnare*, is one of the most serious diseases affecting the culture of channel catfish fingerlings. Preventative measures such as vaccination and feeding restriction can help control disease, but these measures are not 100% effective. An effective and palatable antibiotic incorporated in floating feed is needed by the catfish industry to combat these diseases when preventative measures fail.

There are no approved antimicrobials approved for use in catfish for combating columnaris. Aquaflor® is approved for use in catfish to control mortality associated with ESC. Extra-label use of Aquaflor® for other bacterial infections is prohibited. Efficacy trials have demonstrated Aquaflor® is effective against columnaris infections and the data was used to obtain a provisional license for control of columnaris in catfish. As opposed to Romet® and Terramycin®, Aquaflor® is effective against both bacterial pathogens. Diagnostic and field observations indicate Aquaflor® has significantly reduced industry losses associated with bacterial infections.

Previously unknown streptococcal bacterial disease (Al Camus, Michael Mauel, and David Wise)

A previously unknown streptococcal bacterial disease has been diagnosed in brood-sized channel catfish on three Delta farms. The disease is characterized by severe weight loss, arching of the back due to progressive destruction of the vertebral column, and draining sores located primarily at the angle of the jaw and fin bases. Challenge trials have been conducted to fulfill Koch's postulates demonstrating the organism in question is a true pathogen. Complete characterization of the bacterium is being conducted in collaboration with the Centers for Disease Control in Atlanta. Findings to date indicate this is a previously unknown species of bacteria. At present the significance of this emerging disease is unknown, but monitoring efforts are ongoing to determine its prevalence in the industry.

Efficacy of Aquaflor® (Schering Plough Animal Health) against *Streptococcus iniae* in Nile tilapia (Pat Gaunt)

There are no approved antibiotics for use in most warmwater fish in the U.S. By "crop grouping" (i.e. selecting 2-3 representative species of warmwater fish to represent warm water fishes), the FDA will grant approval for all warmwater fish if efficacy and safety can be demonstrated in each of two to three representative species. Dose titration and dose confirmation studies were conducted to assess the efficacy of Aquaflor® against *Streptococcus iniae*. Results showed that Aquaflor® is effective against *S. iniae* in tilapia. This work will be submitted to FDA in support of an approval package for use in all warmwater fish in the U.S.

Susceptibility of various catfish lines to CCVD (Geoff Waldbieser and David Wise)

Channel catfish virus (CCVD) accounts for approximately 5% of the annual losses to disease on commercial farms. However, localized CCVD outbreaks can produce large losses of fingerlings on individual farms. In cooperation with investigators from Mississippi State University, an extensive series of viral challenges was performed to determine the relative susceptibility of various catfish lines to CCVD, especially the NWAC103 line that was developed at the Catfish Genetics Research Unit. NWAC103 performance was average for the catfish strains tested, with no significant increase or decrease in susceptibility to CCVD compared with other strains. A quantitative DNA test was optimized for detection of CCVD in fish showing no symptoms of infection, and 10-50% of broodfish and fingerlings from commercial farms and research populations were determined to be carriers of CCVD.

Visceral toxicosis of catfish (Pat Gaunt and Lester Khoo)

Visceral toxicosis of catfish (VTC) is a disease seen primarily in market size and brooder fish. Mortality associated with this disease is variable but most commonly results in severe losses that can approach 100%. Disease transmission and fractionation studies demonstrated the disease was non-infectious and likely associated with a 50 kd heat labile protein present in the serum of affected fish. Evidence suggested the involvement of a naturally occurring toxin and that fish can develop anti-toxin and resistance to subsequent exposures. Neutralization bioassays and mass spectrometric analysis defined botulism (type E) as the cause of VTC. Further analysis demonstrated fish are extremely sensitive to botulinum toxin and levels of the toxin in the serum are below the levels of detection by standard analytical methods. The epidemiology of this disease is currently being investigated to help identify commonality in management and environmental factors among farms that have experienced outbreaks. Identification of risk factors associated with VTC will help develop management practices that reduce or prevent losses. Immunological based assays are being investigated as a diagnostic method for the rapid detection of the toxin.

Channel catfish anemia (Al Camus)

Channel catfish anemia (CCA) is a disease of unknown etiology and is characterized by severe anemia. The severity of the disease is variable but can result in losses approaching 100%. On-going studies will confirm the results of preliminary trials that indicate fish affected with channel catfish anemia (CCA), suffer from iron deficiency and recover rapidly following iron injection. The lifespan of the channel catfish erythrocyte is currently being determined to estimate the length of time required to deplete body iron stores and allow for removal of aged erythrocytes from circulation.

Hepcidin, a key regulator of iron uptake from the intestine, was shown to be present in catfish and responsive to levels of stored iron in the body. Hepcidin levels were found to decrease in catfish affected by CCA, indicating a normal physiological response to anemia. These findings eliminate the possibility of

a role for hepcidin in development of the disease. Current studies focus on dietary inhibitors of iron uptake from feeds.

Limited quantities of catfish grow-out feeds were purchased through commercial sources to conduct experiments on ways to improve the health status of juvenile catfish through nutritional intervention. During the course of the initial aquaria study, early recurring morbidity and mortality were encountered. Examination of dead and impaired fish revealed the presence of anemia symptoms as characterized by pale gills, white-lip syndrome, and depressed hematocrits. In many specimens, hematocrit values were less than 10% with some individuals as low as 2-5%. Summary of hematocrits from one experiment showed that approximately 14% of fish sampled had hematocrit values of 10% or less compared to none for catfish fed a standard reference diet. This condition is experimentally repeatable using the same feeds. The condition appears to mimic the characteristics of feed-related anemia that has been observed in pond-raised channel catfish. Research is continuing to determine the cause of the condition and ways to prevent it from occurring in pond-raised channel catfish.

Real-time PCR for proliferative gill disease (Michael Mauel, David Wise, and Linda Pote, MSU Vet School)

Proliferative gill disease (PGD) caused by the myxozoan parasite *Henneguya ictaluri* is one of the most devastating parasitic infections in channel catfish (*Ictalurus punctatus)* aquaculture. Currently, there is no effective treatment for *H. ictaluri* and outbreaks can result in >50% mortality in commercial channel catfish ponds. To aid in the diagnosis and prediction of disease outbreaks, A real-time PCR assay has been developed to identify and quantify *Henneguya ictaluri* the agent of Proliferative Gill Disease (PGD) in catfish. The real-time PGD PCR is now being utilized to characterize the disease progression through the catfish tissues and to quantify levels of the PGD agent in pond water and correlate those levels to disease outbreaks. The goal is to determine the threshold number of spores necessary to initiate mortalities from PGD. Knowledge of this threshold level and monitoring of PGD spore numbers in farm ponds will permit the prediction of PGD outbreaks. Predicting PGD outbreaks will provide farmers an opportunity to market or move fish before losses ensue. The assay could also be used to screen ponds prior to stocking, to evaluate the efficacy of control measures aimed at eliminating spores or the oligochaete PGD vector *Dero digitata* from ponds, and would eliminate the need for live sentinel fish. Applied to gill tissue samples, the assay could be used to refine light microscopic techniques that are currently used to grade the severity of infection in clinical samples.

Comparisons of proliferative gill disease channel catfish, blue catfish, and channel x blue catfish hybrids (Brian Bosworth and David Wise)

Proliferative gill disease (PGD) caused by the myxozoan parasite *Henneguya ictaluri* is one of the most devastating parasitic infections in channel catfish (*Ictalurus punctatus)* aquaculture. Currently, there is no effective treatment for *H. ictaluri* and outbreaks can result in >50% mortality in commercial channel catfish ponds. Challenge studies have shown that blue catfish (*Ictalurus furcatus*) do not exhibit as severe an inflammatory response to *H. ictaluri* infection and mortalities are significantly lower than in channel catfish. Comparisons of PGD severity and *H. ictaluri* infection in channel catfish, blue catfish and channel x blue catfish backcross hybrids were carried out by gross examination of gill clip wet-mounts, histopathology and real-time PCR (QPCR). This study was conducted in an attempt to elucidate factors attributing to variation in host response amongst susceptible and unsusceptible species of catfish. Our study showed significant gill damage and *H. ictaluri* development in channel catfish and channel x blue

catfish hybrids but not in blue catfish. No significant gill damage was observed grossly in blue catfish and parasitic organisms were absent from histological preparations of blue catfish tissues. *H. ictaluri* DNA was detected in blue catfish gills, however this could be attributed to the presence of the organism in the water and does not necessarily signify infection. Presently, it is unclear whether *H. ictaluri* actinospores are able to penetrate blue catfish tissue and are subsequently cleared by host defenses, or if *H. ictaluri* is unable to penetrate and enter the blue catfish altogether. On-going projects focus on identifying the mechanisms utilized by blue catfish to prevent infection.

Prevalence, seasonal occurrence, and severity of proliferative gill disease (David Wise, Lester Khoo, Chuck Mischke, Michael Mauel, and Jim Steeby)

Studies were conducted to gain a better understanding of the prevalence, seasonal occurrence and severity of PGD in catfish production ponds and determine environmental factors that contribute to severe epizootics. Infection rates were monitored in commercial production systems by quantitatively grading the severity of gill pathology occurring in sentinel fish. Monitoring efforts indicated that fish in essentially all intensively managed production systems develop some degree of PGD and that the most severe manifestations of the disease occur during the spring when fish are initially stocked into the ponds. Laboratory and field studies indicate that severe manifestations of the disease are dependent on the rate spores are shed into the water and the susceptibility of the fish to infection. This information is being used to modify current management practices in efforts to reduce the impact of PGD. The protocol used in these studies appears an accurate and quantitative method of monitoring infection rates. These procedures are being used to evaluate potential disease treatments and predict the occurrence of PGD related losses. It has been demonstrated on commercial operations that the use of these procedures can effectively eliminate PGD related losses in fish that are stocked in production ponds during the spring.

A molecular-based “PCR” assay capable of quantifying numbers of infective PGD spores in pond water and fish tissue has been validated for specificity. These procedures are being used to develop rapid methods of assessing the risk for the occurrence of PGD related losses. Initial experimental trials have demonstrated that the disease causing agents are evenly dispersed in the pond environment and validate the use of single point monitoring procedures in a risk assessment program.

Trematodes

Snail control using native fish species (Les Torrans and Conrad Kleinholtz and Greg Luker, Langston University)

Conrad Kleinholtz and Greg Luker (Langston University) collaborated with Les Torrans (USDA/ARS CGRU) to develop techniques to spawn and rear freshwater drum (*Aplodinotus grunniens*). Drum feed on mollusks and are being evaluated as a potential control of the ram’s horn snail, an intermediate host of the catfish trematode (*Bolbophorus confusus*). Drum were successfully spawned both in open ponds and in laboratory tanks in sufficient numbers to begin field trials of pond culture techniques and efficacy. Fingerlings (5 g) were acclimated to eating frozen blood worms. Fingerling drum (15 g) were stocked in 0.04 ha catfish ponds at 125/ha to control snails. Drum survival ranged from 0-80%. *Physa* snails >6 mm were controlled by the drum, but small snails were not. Larger fingerlings (50+ g) were stocked in commercial catfish ponds. Drum were stocked at 50/ha in 0.8 ha ponds, and at 33/ha in 0.3 ha ponds. Snails were significantly reduced ($P<0.05$) in ponds with drum, but were not eliminated.

***Bolbophorus damnificus* infections in channel catfish (David Wise and Al Camus)**

Infection caused by the trematode, *Bolbophorus damnificus*, is a relatively new disease and was first reported in Louisiana in 1994 and later in Mississippi in 1999. While diagnostic case reports suggest the disease is associated with poor production efficiency and high mortality, there are no scientific studies addressing these relationships. Studies were conducted to determine the production potential and disease resistance of fish infected with the trematode *Bolbophorus damnificus*. Light trematode infections reduced feed consumption and overall production by approximately 30%. These experimental results are comparable to decreases in production observed on commercial farms. Aquaria studies demonstrated that once fish are removed from the source of infection, the growth potential of trematode infected fish is equal to that of non-infected fish. Data indicates that breaking the life cycle of the trematode is an effective cure for this disease and that the long term implication of the disease are negligible once the source of infection has been removed. Experimental investigation concerning the interaction of *E. ictaluri* and *Bolbophorus* sp. infection demonstrated that concurrent exposure to both pathogens significantly increased mortality associated with ESC. However, no significant increase in mortality was observed when exposure to *Bolbophorus* sp. cercarieae occurred 28 days prior to *E. ictaluri* exposure. This data supports field observations and experimental growth data that the presence of the mature parasite in fish alone does not have a negative impact on fish health. These studies described experimental procedures that reflect on farm conditions and the pathogenesis of natural infections.

Impact of trematode infections (David Wise, Terry Hanson, Lester Khoo, Chuck Mischke, and Jimmy Avery)

The trematode identified as *Bolbophorus damnificus* is a significant cause of production losses in commercially raised channel catfish. This disease has been associated with high mortality rates, decreased feed consumption and poor production efficiency. To assess the economic impact of this disease, disease monitoring and production efficiency studies have been conducted on commercial catfish operations. Sampling protocols were established to determine presence and severity of trematode infections. The number of ponds containing infected fish and the overall severity of the infection was evaluated and used to assess the potential impact of the disease. To date, approximately 2000 ponds in 8 counties in Mississippi have been sampled. From these sampling efforts it is estimated that 20-30% of the ponds used in the commercial production of channel catfish contain infested populations of fish. The severity of infection ranged from mild to severe with most of the farms sampled testing positive for this disease. Farms that were unable to implement an effective bird management program and were within close proximity to pelican loafing sites were much more likely to suffer from severe infections. Trematode infections have resulted in severe losses as a result of mortality and decreased feed consumption and conversion. Compared to trematode negative ponds, ponds in light, moderate and severe category produced 13.8%, 36.0%, and 40.5 % less pounds of fish per acre, respectively. Decreases in production were associated with significant decreases in net returns. Net returns for ponds in the light category were reduced by 80.8% ($87 net return/acre) and production from ponds in the moderate and severe categories were not shown to cover variable costs of production. Ponds in the moderate category produced a net loss of $506 per acre and severely infected ponds produced a net loss of $631 per acre. The overall economic impact of this disease to the catfish industry in the southeastern United States is estimated to be $45.4 million annually.

Use of copper sulfate to eliminate snails (David Wise, Chuck Mischke, Terry Greenway, and Linda Pote, MSU Vet School)

The overall economic impact of trematode infections to the catfish is estimated to be $45.4 million annually and severe infestations at the farm level can lead to bankruptcy. This work was conducted to limit the economic impact of this disease by developing procedures for breaking the life cycle of the

parasite by eliminating the intermediate host (ram's horn snail) that is commonly found in catfish production ponds. Hydrated lime and copper sulfate shoreline treatments were developed and shown effective in reducing snail populations that are present in the aquatic vegetation around the perimeter of the ponds. While effective, these treatments had little impact on snails that are not within close proximity to the pond bank. Uniform application of copper sulfate was evaluated as treatment against snails in catfish production ponds. This work demonstrated that copper sulfate applied as a solution across the pond surface to deliver between 2.5 and 5.0 ppm was effective in killing snails throughout the pond environment. On average pond treatments killed greater than 95% of the test snails. Treatments were also shown to reduce the natural populations of snails along the pond margin by 98.3%.

Hydrated lime and copper sulfate are being evaluated as practical control measures against ram's horn snails on commercial catfish farms. Fish populations have been evaluated for the trematode infections. Ponds containing trematode infected fish have been treated with hydrated lime and copper sulfate. In the second year of the study, long term treatment efficacy will be evaluated by monitoring production parameters and infections rates. Comparison of pre and post treatment feeding rates indicates the chemical treatments were effective in controlling new infections and improving feeding rates. This data will be used to develop cost effective management programs to control this disease. It is anticipated that this program will dramatically reduce the economic impact of this disease

Hatchery-Associated Disease Problems

Chemotherapeutant treatments for maximal hatching success of hybrid catfish (Brian Small)

Fungal and bacterial egg infections can be a significant problem in commercial catfish hatcheries. Studies were conducted to optimize chemotherapeutant treatments for maximal hatching success of hybrid catfish. Hybrid catfish eggs were treated with increasing doses of hydrogen peroxide, formalin, copper sulfate and povidone iodine, and the optimal frequency of treatment application was determined. Hatching success was greatest among eggs treated with 100-ppm formalin, 100-ppm iodine, or 2.5-ppm copper sulfate. The optimal frequency of treatments was determined to be thrice daily, and a treatment-sensitive, developmental stage was identified. These data were used to formulate an optimal chemotherapeutic treatment regime for managing maximal hatching success of hybrid catfish and increasing production efficiency.

Treatment of catfish eggs with hydrogen peroxide (Brian Small)

Catfish eggs often become infected with aquatic fungi or bacteria that lower hatching success. The optimal concentration of hydrogen peroxide was determined for use as a daily therapeutant in static and continuous-flow situations. Treatment with hydrogen peroxide significantly improved hatching success by ~30% compared to iodine and formalin treated eggs. Hydrogen peroxide treatment methods were optimized to account for differences between channel and hybrid catfish under different culture temperatures, and dependant upon developmental stage at the time of treatment. By following management recommendations, commercial catfish producers could realize as much as 30-40% increase in annual fry production.

Impact of pathogens on fry survival (Chuck Mischke, David Wise, and Terry Greenway)

Certain pathogens may also play a role in early fry mortality. Chemical control of host organisms (e.g., snails and *Dero* worms) may reduce the spread of these pathogens and increase survival. Uniform pond application of copper sulfate at 5.0 ppm is an effective treatment against snails. Rotenone, Bayluscide,

chloramine-T, formalin, and potassium permanganate may be useful as pond sterilization strategies for control of *Dero* worms before stocking fry. Application of these study results have provided farmers experiencing chronic disease problems an additional management tool.

NUTRITION AND FEEDING

Evaluation of animal protein concentration on production of channel catfish stocked at various densities (Ed Robinson, Menghe Li, Bruce Manning, and Brian Peterson)

Since fish meal is of limited supply and more expensive than most plant protein sources, reducing its use in catfish feeds while maintaining optimum fish performance will reduce feed cost, increase profit, and maintain sustainability of the catfish industry. Several aquarium and pond studies on replacement of fish meal with plant protein sources have be conducted, but yielded inconsistent results. Some studies show that fish meal in catfish diets can be completely replaced with all plant proteins without affecting fish growth and feed efficiency, while others show a small amount of fish meal is needed for maximum growth and feed efficiency. However, one conclusion is that fingerling and food-sized channel catfish require no more than 4% in the diet for maximum growth. Our aquarium and pond studies have shown that fish meal could be replaced with other animal proteins, such as meat and bone/blood meal and poultry byproduct. Reducing or eliminating fish meal in catfish diets for food fish grow out has reduced feed cost by $2 to 4 per ton and $1.5 to 3 million annually for the catfish industry.

Use of cottonseed meal, distiller's grains, and supplemental lysine to partially or totally replace soybean meal in catfish diets (Menghe Li, Ed Robinson, Bruce Manning, and Brian Peterson)

The main protein source used in catfish feed is soybean meal. Since other commodities tend to follow soybeans in the market, catfish feed prices are dictated by the price of soybeans. As the price of soybeans increases, there are often more economical protein sources that may be used to replace soybean meal in catfish feeds. Two products that are of interest are cottonseed meal and distillers dried grains with solubles (DDGS). Cottonseed meal is a local product that is generally priced competitively (on a protein basis) with soybean meal. Distillers dried grains with solubles, though currently not locally available, may become abundant as ethanol plants come on line as a result of new energy policies and an abundant corn crop in the U.S. Two pond studies were conducted to evaluate the use of DDGS, cottonseed meal, and supplemental lysine to partially or totally replace soybean meal in channel catfish diets. Results show that about 50% of soybean meal can be replaced with cottonseed meal + supplemental lysine in channel catfish diets without negatively affected fish performance. Further, DDGS can be used in channel catfish diets up to at least 30% of the diet when the diet is supplemented with lysine. Use of DDGS in the diet appears to improve feed efficiency. Use of cottonseed meal and DDGS will reduce feed cost by $1 to 2 per ton and $0.75 to 1.5 million annually for the catfish industry.

Use of high protein finishing diets to improve processing yield of pond-raised channel catfish (Menghe Li, Ed Robinson, Bruce Manning, and Brian Bosworth)

Although when fed to satiation, dietary protein levels as low as 24% can provide essentially the same growth and production for catfish as higher protein levels during food fish grow out. However, feeding low protein diets generally results in lower processed yield and higher body fat levels due to higher digestible energy to protein ratios in the low protein diets. It appeared that it might be beneficial to feed a high-protein finishing diet before harvest to improve processing yield and reduce body fat levels in pond-raised catfish, but research data do not support this contention. Also, because of various constraints including occurrence of off flavors, disease outbreaks, and market constraints, one cannot predict with certainty when the fish will be harvested. Thus, it would be difficult to judge when to start using a finishing diet to optimize processed yield. Any economic advantage gained from using finishing diets

diminishes with an increased finishing period since the high-protein finishing diets are more expensive. If maximum processing yield is the desired goal, a diet properly balanced in respect to energy and protein should be used for the entire growing season. This project will allow catfish food fish producers to feed one diet through out the growing season.

Effects of dietary protein levels and use of carnitine on growth and body fattiness of channel × blue catfish hybrids (Ed Robinson, Menghe Li, and Brian Bosworth)

Previous research has shown that channel x blue catfish hybrids out-perform channel catfish for growth. However, the hybrids appear to deposit more fat than channel catfish. A pond study was conducted to evaluate effects of dietary protein concentration and carnitine supplementation on growth and body composition of channel x blue catfish hybrids. Carnitine is a product that is naturally occurring in the animal body and is approved for adding to animal feeds to reduce fattiness. Results demonstrated that growth, feed conversion, and visceral fat were not affected by dietary protein levels ranging from 28% to 36% or by addition of carnitine. As dietary protein increased fillet fat generally decreased. Fish fed the 28% protein diet containing carnitine contain 7.8% fat in the fillet while the fish fed the same level of protein without carnitine had 8.6%. Use high-protein diets appear to reduce body fatness, but may not be economical since high-protein diets are more expensive. This project allows better understanding of carnitine and dietary protein effects on catfish body fatness. Carnitine can be potentially used in catfish feeds, but presently it is uneconomical.

Effects of various dietary protein levels and sources on meat yield of channel catfish (Menghe Li, Ed Robinson, Bruce Manning, Chuck Mischke, Brian Peterson, and Brian Bosworth)

Development of low-cost diets for farm-raised catfish could lower production costs, but it is important to understand the effects of diet on important traits such as meat yield (the percentage of whole fish weight that is saleable meat). Effects of various dietary protein levels and sources on meat yield of channel catfish were conducted in cooperative research between Mississippi State University and the USDA, ARS, Catfish Genetics Research Unit. Meat yield was generally not affected by diets with protein levels ranging from 28 to 32 % or diets with protein sources that were all plant in origin versus a combination of plant and animal proteins. The research indicates that it may be possible to lower diet costs without negatively impacting meat yield of channel catfish.

Effects of dietary protein level and frequency of feeding on meat yield of channel catfish (Menghe Li, Ed Robinson, Bruce Manning, Chuck Mischke, Brian Peterson, and Brian Bosworth)

It is important to understand the effects of dietary protein and feeding frequency on important traits such as meat yield of catfish (the percentage of whole fish weight that is saleable meat) to determine the most profitable combination of dietary protein and feeding frequency. Effects of dietary protein level and frequency of feeding on meat yield of channel catfish were determined. Meat yield increased as dietary protein level and feeding frequency increased. Catfish farmers need to consider the impact of dietary protein level and feeding frequency on fish growth and meat yield in determining the optimal protein level and feeding frequency. However, the optimal combination of dietary protein level and feeding frequency changes with the price of feed and the price of fish.

Replacement or reduction of inorganic phosphorus (Ed Robinson, Menghe Li, and Bruce Manning)

Phytase, an enzyme produced by fungi, has been shown to be effective in improving phosphorus bioavailability in animals with a single stomach. Results from our laboratory and pond studies demonstrated that a level of 250 units phytase per kg of diet could be used to completely replace inorganic phosphorus supplements in channel catfish diets without affecting fish growth and bone phosphorus deposition. Using phytase to replace inorganic phosphorus supplements reduced fecal phosphorus concentrations and thus reduced phosphorus input to the pond. We recommend a level of 500 units phytase per kg of feed and a total phosphorus level of 0.6% and above be used to ensure adequacy. This research has led to commercialization of the use of supplemental phytase in catfish feeds. In terms of feed cost, the use of phytase to replace inorganic phosphorus in catfish diets does not increase or decrease feed cost. However, the long term effects of reduced phosphorus loads in pond waters may increase production and profits.

Dietary phosphorus and pond effluents (Craig Tucker)

Compared to other agricultural animals, fish require phosphorus at relatively high dietary levels and a significant fraction of the total phosphorus in practical fish diets is not assimilated. Dietary phosphorus in unavailable forms or supplied in excess of fish metabolic requirements will be excreted into the culture water. If the water is discharged from the culture unit, phosphorus may cause eutrophication in effluent-receiving water bodies. One approach to reducing mass discharge of waste is to modify feeding strategies or feed formulations to increase the assimilation and retention of nutrients so that less waste is generated per unit of feed consumed. Although the environmental benefit of optimizing phosphorus retention by fish raised in flow-through and net-pen culture systems is obvious. However, the value of optimizing dietary phosphorus retention by fish in ponds is less clear because the long hydraulic residence time in ponds provides ample opportunity for various biological, chemical, and physical processes to affect the amount of phosphorus that is eventually discharged. To assess the effect of dietary phosphorus on potential waste discharge from ponds, three studies were conducted. In experiment 1, a basal diet with 0.20% available phosphorus was compared to diets supplemented with 0.5% or 1.0% dicalcium phosphate to provide 0.27% or 0.35% available phosphorus. In experiment 2, fish were fed diets supplemented with either dicalcium phosphate or defluorinated rock phosphate to contain 0.40% available phosphorus. In experiment 3, fish were fed one of three diets containing 250 or 500 FTU (phytase units) phytase/kg (0.27% available phosphorus) or 0.75% dicalcium phosphate (0.39% available phosphorus). Husbandry practices in all three experiments were typical of commercial culture conditions. Quantitative and qualitative modifications of dietary phosphorus did not affect waterborne phosphorus concentrations or phytoplankton abundance and, therefore, will not reduce phosphorus or organic matter mass loading in pond effluents. Lack of effectiveness is the result of high baseline nutrient loading from phosphorus contained in practical feed ingredients combined with high internal phosphorus loading (recycling) within ponds. These factors overwhelm any effect of small changes in external phosphorus loading associated with diet modification. Therefore, the source and level of dietary phosphorus in channel catfish feeds should be based primarily on nutritional and economic considerations rather than potential environmental impact. These results have been incorporated into environmental management plans for pond-raised fish.

Organic catfish feeds (Menghe Li, Ed Robinson, Chuck Mischke, Les Torrans, and Brian Bosworth)

In recent years, there has been growing interest in organically grown foods. The USDA National Organic Standards Board is currently working on standards for aquaculture, which are expected to be approved soon. Channel catfish, being the most important aquaculture species in the country, possess several good qualities, such as rapid growth, easy spawning, tolerance to wide ranges of temperature and water quality, less stringent dietary requirements, consistent quality, delicate flavor, year around availability, and higher nutritional value. These traits make it a suitable candidate for organic production, which may be adapted by producers who are willing to raise channel catfish for niche markets under strict guidelines. A study was conducted to evaluate effect of organic diets on growth and body composition of food-sized channel catfish. There were no differences in production characteristics and carcass yield between conventionally and organically grown fish. However, organic fish had lower fillet yield and higher body fat than control fish. The slightly higher body fat observed in organic fish resulted from the slightly higher fat content in the organic diet. This was because of the higher fat content of mechanically extracted organic soybean meal compared with solvent extracted conventional soybean meal that is typically used. However, the amount of deposited fat in organically grown fish was within the range typically found in channel catfish. This project provides information that will allow catfish producers to grow organic fish products and potentially increase farm income and improve the competitiveness in seafood markets.

Increasing omega-3 HUFA levels in catfish fillets (Ed Robinson, Menghe Li, Bruce Manning, and Brian Peterson)

Omega-3 fatty acids have several beneficial effects on human health, which include improvement of cardiovascular health, reducing the effects of autoimmune diseases, such as rheumatoid arthritis and psoriasis, and for normal eye and brain development of infants. A project was conducted to boost the omega-3 highly unsaturated fatty acid (HUFA) content of catfish fillets through dietary manipulation. Results from aquarium and pond studies have shown that it is feasible to substantially increase omega-3 HUFA levels in catfish fillets by including 2-4% refined menhaden oil in the diets while maintaining the natural, mild flavor of farm-raised catfish. Feeding pond-raised catfish a diet containing 2.5% refined menhaden oil for a growing season resulted in a 3-fold increase in fillet omega-3 HUFA contents compared to catfish fed 2.5% catfish offal oil. Development of omega-3 enriched catfish products could allow catfish to be marketed as a value-added product, and thus potentially increase profitability.

Feeding Strategy

Effects of feeding frequency on production and feed efficiency of channel catfish fed various levels of protein (Ed Robinson, Menghe Li, and Brian Bosworth)

Channel catfish fed dietary protein levels as low as 24% can achieve essentially the same growth and production as fish fed high protein diets (32% to 40%) when fish are fed daily to satiation. However, it is not clear how much dietary protein is optimal for fish fed every other day (EOD) to satiation, a feeding strategy that may be used when harvest is delayed because of off-flavor or other issues. A study was conducted to evaluate dietary protein requirement for fish fed once daily or EOD. Results demonstrated that feeding EOD to satiation improved feed efficiency and used less aeration compared with fish fed daily. However, feeding EOD resulted in lower growth, net production, and processed yield. For fish fed

daily, a diet containing 24% protein provided the same growth and production as a 36% protein diet. However, for fish fed EOD to satiation, net production and weight gain per fish generally increased as dietary protein levels increased, suggesting that channel catfish may require higher dietary protein when fed EOD. Use of optimum dietary protein levels under different feeding regimens will improve catfish production efficiency and increase profitability.

Evaluation of daily feeding time and frequency on production and feed efficiency for pond-raised channel catfish (Ed Robinson and Menghe Li)

Daily feed consumption of fish may be influenced by several factors including water temperature, water quality, weather, fish size, and feeding frequency. Feeding fish at an optimal time during the day and at proper frequencies may optimize growth and feedefficiency. Channel catfish were fed to satiation with a commercial feed once daily in the morning, once daily in the afternoon, or twice daily in both morning and afternoon for two growing seasons. Results indicated that there were no improvements in weight gain and net production by feeding catfish twice daily from advanced fingerlings to market size. Feed efficiency appears to be reduced for fish fed twice daily probably because of more wasted feed. Although fish fed twice daily appear to have a better processing yield, feeding twice daily would increase feed and labor costs; thus, feeding twice daily may not be economical. Data also indicate that pond-raised catfish can be fed either in the morning or in the afternoon during the growing season. Feeding once a day will reduce feed waste, improve feed efficiency, and reduce labor cost compared with feeding twice a day.

Effects of maintenance feeding regimens on weight gain, feed efficiency, and body condition of pond-raised channel catfish (Ed Robinson and Menghe Li)

Catfish are normally fed daily as much as they will eat with minimum wastage and without adversely impacting water quality. However, under unusual circumstances, such as when harvest is delayed because of off-flavor or other issues, it may be desirable to feed fish a restricted ration or even at maintenance levels. A study was conducted to determine weekly feeding frequency for maintaining fish body weight for different sizes of catfish in ponds under a single-batch cropping system. Based on results from this study, it appears that feeding once weekly to satiation can maintain the body weight of advanced fingerling and larger-sized catfish. However, condition factor, which is a measure of body condition, is reduced for fish fed once a week compared with fish fed two or three times a week. If a multiple-batch cropping system is used, feeding more frequently than once weekly will also increase the chances of smaller, less aggressive fish to feed. Maintenance feeding can be used as a management tool to maintain fish body weight when harvest is delayed.

Comparison of feeding channel catfish seven days per week vs. six days per week (Ed Robinson, Menghe Li, Bruce Manning, and Brian Bosworth)

During the growing season, most catfish producers feed their fish seven days a week, but there are some producers who feed six days a week. Our data show that feeding six days a week (not feeding on Sundays) reduced net production by 3.3% and feeding five days a week (not feeding on both Saturdays and Sundays) reduced net production by 6.9%, compared with fish fed seven days per week for a growing season. Feed conversion ratio (feed/gain) was reduced by 4.8% and 7.9%, respectively for fish fed six days and five days a week, compared with fish fed seven days per week. Considering the slight decrease in net production, slight improvement in feed efficiency, and possible reduction in other costs associated with feeding, feeding six days per week may reduce production cost for food-sized catfish. However, it should be noted that in our study, we used a single-batch cropping system and the fish were fed to satiation on days fed. If feed is restricted, one would expect a further reduction in net production by feeding five or six days per week compared with fish fed seven days per week. Also, if this strategy is used in a multiple-batch system, skipping feed days may have a more negative impact than in a single-

batch system because the smaller fish may gain less weight than was shown in our study. Feeding catfish five or six days a week may improve feed efficiency.

Effect of feed restriction on subsequent growth (Menghe Li, Ed Robinson, Bruce Manning, Brian Small, and Brian Bosworth)

Catfish are occasionally subjected to feed restrictions due to economic or environmental conditions. A study was conducted to determine the effects of feed restriction and the age at which the restriction was imposed on subsequent growth of channel catfish. A 1 week feed restriction was imposed at 7 or 28 days post-hatch, followed by return to satiation feeding. Compared to unrestricted controls, weight at 1 year post-restriction was lower in both feed restricted treatments and lower for fish for which feed restriction was imposed at 7 days than 28 days. Results demonstrate that short-term feed restriction has long term impacts on growth and producers should avoid feed restriction, especially in catfish fry.

Feed Conversion

Feed conversion by various sizes of channel catfish (Ed Robinson, Menghe Li, Bruce Manning, Brian Peterson, and Brian Bosworth)

Feed efficiency of fish is influenced by several factors including diet composition, feeding rate and frequency, fish size and age, water temperature, and other environmental factors. Generally, as fish grow larger, the efficiency of converting feed into body mass becomes less efficient. However, there have been no comprehensive studies on the feed efficiency of various fish sizes of pond-raised channel catfish. A pond study was conducted to evaluate feed conversion ratios of channel catfish varying in initial sizes from 0.23 to 1.4 kg. Results showed that as fish sizes increased feed conversion ratio increased. For example, fish raised from 0.23 to 0.45 kg had a feed conversion ratio of 1.79, whereas fish raised from 1.36 to 1.82 kg had a feed conversion ratio of 3.74. This indicates that raising larger fish may not be economical. Raising catfish to optimum market size will improve feed efficiency and profits.

Freshwater Prawn Diets

Using low input management practices for pond culture of freshwater prawn (Lou D'Abramo and Terry Hanson)

A preliminary economic analysis of a low input management practice using low cost forms of inorganic fertilizers, corn gluten pellets and/or wheat midds pellets (as an alternative to sinking catfish feed), for pond culture of freshwater prawn reduced feed costs per pound of shrimp produced to $ US 0.30 to $ 0.62/lb, (up to 56 % decrease) thereby reducing production costs to US $ 3.04 to 2.94/lb, respectively. These production costs dramatically increase the prospects for economic success of freshwater prawn farming in the United States because selling prices would be highly competitive with foreign imports. Organic fertilization as part of the low input strategy eliminates the need of comparatively expensive formulated feeds, allows flexibility in the selection of sources of organic material used to fertilize, produces a large proportion of a large, highly value product, and is an example of highly sustainable aquaculture.

GENETICS, BREEDING, AND REPRODUCTION BIOLOGY

Genomics

Efficient use of genomic technologies (Sylvie Quiniou and Geoff Waldbieser)

Efficient use of genomic technologies in catfish production requires the development of species-specific genomic resources. Toward this goal, we have identified several thousand DNA sequences from actively expressed genes and added them to public databases. We produced a first-generation microarray of 19,000 catfish sequences and used it to identify genes that were differentially expressed in the catfish spleen after exposure to bacterial cell wall components. This microarray was used as the backbone for a second-generation microarray used by a cooperator to measure gene expression after exposure to pathogenic bacteria. We produced the full DNA sequence of the catfish mitochondrial genome which is useful for identification of broad range population structure and removal of contaminating mitochondrial sequences from nuclear DNA libraries. We produced a genomic library (BAC library) consisting of 55,000 bacterial clones that each contained a large fragment (160,000 base pairs) of catfish DNA, so that the entire catfish genome was covered 7-fold in the library. We also developed a technique to efficiently identify variable DNA sequence markers that were within or near genes of interest, and our existing first-generation catfish genetic map was enhanced by the addition of many genes that are conserved between species. Not only did this provide more genetic markers for the map, but it also permitted direct comparisons of genome structure and gene order between catfish and species with sequenced genomes such as zebrafish and pufferfish. Use of cross-species comparisons will permit more efficient identification of genes that are controlling important production traits.

While the catfish genetic map showed relative positions of genes in the genome, it did not provide information on the absolute distance between these genes so we produced the first physical map of the catfish genome. By adapting existing ARS methodology to a high-throughput DNA analysis system, one scientist was able to efficiently perform DNA fingerprinting on the clones of the BAC library, then utilize specialized software to join the overlapping clones based on similar DNA fingerprints. These adaptations were incorporated into a user bulletin by the vendor of the DNA analysis system. Also, more than 30,000 genomic DNA sequences were obtained from these clones to provide information on the repetitive structure of the catfish genome and also provide several thousand potential DNA markers for the genetic map. Understanding the composition and function of the catfish genome, identification of DNA markers linked to beneficial gene sequences, and integration of genomic technologies with selective breeding methods will aid breeders in the efficient identification of genetically superior broodstock.

The similar physical characteristics of all channel catfish confound methods to identify individuals, families, and strains. Using DNA markers identified in the catfish genome, we developed a catfish strain identification system based on DNA fingerprinting. This method was used by the U.S. catfish industry, via the Mississippi Seed Improvement Association, to manage populations of the NWAC103 strain and maintain its genetic integrity on commercial farms. The DNA fingerprinting technology can be used by catfish producers to genetically define their catfish populations.

Comparison of feed efficiency of Norris and USDA103 line channel catfish (Ed Robinson, Menghe Li, Bruce Manning, Chuck Mischke, Brain Peterson, Brian Small, and Brian Bosworth)

USDA103 line channel catfish grow 10-20% faster than average industry catfish, but at the expense of greater feed consumption. USDA 103 strain catfish were compared to Norris strain catfish. USDA 103 catfish had superior feed efficiency compared to Norris catfish, with observed difference between families. Comparison of USDA 103 strain catfish with the average industry fish showed no differences with regard to nitrogen and energy utilization. Selection for catfish with greater feed efficiency will result in marked savings for catfish producers.

Comparison of disease resistance of crossbred channel catfish lines (Brian Bosworth, David Wise, and Brian Peterson)

Economic losses associated with diseases are an increasing problem for farm-raised catfish producers. A study to compare production traits of USDA 103 strain channel catfish, USDA 102 strain channel catfish, USDA 103 × USDA 102 crossbred, and USDA 103 strain channel catfish × blue catfish hybrids is underway. Hybrids had the best fingerling performance of all groups. Of the channel catfish groups, the USDA 103 × USDA 102 crossbred was superior to the USDA 103 line and USDA 102 line catfish for fingerling production traits. The same crosses are currently being evaluated for foodfish production and processing traits. Initial results indicate crossbreeding and hybridization could enhance catfish production.

Comparison of meat yields of purebred channel catfish, blue catfish, and channel × blue hybrids (Brian Bosworth, Menghe Li, Bruce Manning, and Ed Robinson)

Channel catfish × blue catfish hybrids exhibit favorable meat yield and resistance to some diseases compared to purebred channel catfish, but reproductive barriers limit large-scale production of hybrids. F1 channel × blue hybrids were backcrossed to purebred channel catfish and then intercrossed to close this population in order to produce a reproductively viable synthetic, hybrid catfish line that retains the superior growth, disease resistance and meat yield of the hybrid. After two generations of backcrossing the population successfully spawned in ponds and offspring had superior resistance to ESC, a bacterial disease of catfish. Another trial was conducted to compare production traits of blue catfish, channel × blue catfish hybrids, and two channel catfish strains (Norris and NWAC103). Fingerling growth and survival were better for blue catfish and channel × blue catfish hybrids than for the channel catfish strains. Feed records indicated hybrids consumed the most feed, followed by blue catfish, then NWAC103s, and then Norris strain channel catfish during the fingerling to market weight portion of the study.

Selective breeding for increased meat yield will lead to the development of catfish germplasm with higher meat yield and increased economic value. Meat yield was measured in offspring from more than 200 channel catfish families, and the results demonstrated a positive response to selection for increased meat yield. Development of catfish lines with improved meat yield could reduce costs of production to benefit catfish producers, processors, and consumers.

Meat yield, an economically important trait for farm-raised catfish, is affected by catfish strain/species and season of the year. A project comparing effect of season on meat yield of blue catfish, channel catfish and channel × blue catfish hybrids was completed. Hybrid catfish had higher whole carcass and fillet yield than blue or channel catfish during both the spring and fall. Blue catfish had higher carcass yield than channel catfish during both the spring and fall. Channel catfish had higher fillet yield than blue catfish in the fall, but blue catfish had higher fillet yield than channel catfish in the spring. Blue catfish

had higher nugget yield (lower valued rib-meat) than channel or hybrid catfish. Catfish processors could increase processing yields by processing different genetic groups of catfish at certain times of the year.

Growth

Effect of recombinant bovine growth hormone (rbGH) on growth performance (Brian Peterson)

Since feed costs account for about 50 % of the operating costs of commercial catfish production, any improvement of growth rate and or feed efficiency could potentially decrease the costs of catfish production. Experiments were conducted to study the growth promoting effects of recombinant bovine growth hormone (rbGH) on growth performance, body composition, and endocrine responses in two strains of channel catfish. The rbGH increased final weight and improved feed conversion ratio in the USDA103 catfish line without a change in body composition due to treatment. Identifying hormones and growth factors involved in fish that are grown under an accelerated growth regime will provide insight into producing faster growing fish.

Insulin-like growth factor hormonal pathway in catfish (Brian Peterson, Brian Small, Geoff Waldbieser, and Brian Bosworth)

The growth hormone-insulin like growth factor hormonal pathway is central to tissue growth in mammals, but little was known about this pathway in catfish. We identified the gene and protein sequences for several members of this pathway, including growth hormone, growth hormone receptor, insulin-like growth factors I and II (IGF-I, IGF-II) and their receptors, and an insulin-like growth factor binding protein. An IGF-I radioimmunoassay was developed and validated in collaboration between the USDA, ARS, Catfish Genetics Research Unit and the USDA, ARS, Stuttgart National Aquaculture Research Center. This research provided tools to measure gene expression and protein levels for these hormones in catfish.

Growth hormone was injected into channel catfish as a step toward elucidating the regulatory mechanisms involved in the GH-IGF system. In mammals, injection of growth hormone stimulates IGF-I gene expression, but our experiments showed injection of growth hormone in catfish stimulated both IGF-I and IGF-II gene expression. In fact, IGF-II gene expression was induced three-fold greater than IGF-I. Levels of IGF-II mRNA were higher in faster growing catfish families compared to slower growing families while IGF-I mRNA levels were unchanged. However, IGF-I protein levels in plasma were higher in faster growing families. As expected, levels of IGF-I protein decline in response to bacterial infection. Faster growing catfish strains also showed higher levels of plasma IGF-I protein. Variation in growth hormone or insulin-like growth factor levels could lead to differences in catfish growth and muscle development, and measurement of protein levels and gene activity may be useful in selection of catfish with superior genetic potential.

Effect of dietary cortisol on growth (Brian Small and Brian Peterson)

Glucocorticoids are known to impede growth in a number of vertebrate species. In order to better understand the mechanisms through which they may act in channel catfish, we examined the effects of feeding cortisol on the growth hormone (GH)/insulin-like growth factor-I (IGF-I)/IGF-binding protein system. The results showed that cortisol administration increased a particular IGF binding protein found in blood and also decreased plasma IGF-I and hepatic growth hormone receptor (GHR) levels. One mechanism through which cortisol may impede growth of catfish is through an increase in this IGF

binding protein and a decrease in GHR which may inhibit the action of IGF-I. IGF binding protein and growth hormone receptor may be useful as markers of growth or stress.

Growth hormone receptor and ghrelin in catfish (Brian Small, Geoff Waldbieser, and Brian Peterson)

Molecular genetic markers have the potential to improve the selection for desired traits, such as growth and feed efficiency, in catfish by serving as predictors of genetic merit. Two genes known to regulate growth and feeding in other animals are growth hormone receptor and ghrelin. Research was conducted to identify these genes in catfish, determine their roles in catfish growth, and identify differences in sequence and gene expression patterns. Both genes were identified in catfish, found to be potent regulators of growth hormone, and to play central roles in the regulation of catfish growth. This new insight into the regulation of catfish growth together with observed differences in sequence and expression patterns of the two genes may prove to be useful tools for identifying superior families of catfish.

Processing Traits

Identification of candidate genes for meat yield (Ken Davis, Geoff Waldbiesser, Brian Bosworth, and Brian Small)

Identification of candidate genes for meat yield permits more efficient selection to increase catfish meat yield. Molecular genetic analyses have determined channel and blue catfish DNA sequences for myostatin, myogenin, myoD, and follistatin, whose products influence muscle growth and development in other species. DNA sequence variants were found in these genes in order to determine if these polymorphisms are associated with meat yield and growth. Myostatin is a negative regulator of muscle growth, but its role is poorly defined. We determine the effects of passive immunization against myostatin on muscle growth in developing USDA103 line catfish embryos. There was no increase in weight gain due to passive immunization against myostatin, but muscle fiber number and type are currently being analyzed. Identifying mechanisms that regulate muscle growth will provide strategies for producing fish with higher fillet yields.

Relatively little is known about the mechanisms responsible for muscle protein breakdown in the channel catfish, and stressors experienced in catfish production could alter muscle growth in developing catfish. Catfish were exposed to glucocorticoids or a bacterial lipopolysaccharide (LPS). Expression of the myoD gene was upregulated in response to LPS, while expression of the myostatin gene was downregulated in response to glucocorticoids and LPS. These results were opposite the responses seen in mammalian models of muscle growth and metabolism. This research demonstrates the need to further characterize the mechanisms involved in muscle growth and breakdown in catfish, rather than just extrapolating from mammalian models.

Jar hatching of channel × blue catfish hybrid eggs (Brian Bosworth and Les Torrans)

Channel catfish × blue catfish hybrids are superior to channel catfish for several production traits, but poor fertility and hatch are factors which impede large-scale production of hybrids. Catfish eggs are adhesive and are typically hatched as a large egg mass, but poor fertility typical of hybrid egg masses leads to increased bacterial and fungal infection and reduced hatch. We compared jar-hatching of hybrid eggs after chemical elimination of the adhesiveness to traditional hatching of egg masses and found use of jars increased hatch to 6.8 hybrid fry per gram of eggs compared to 4.8 fry per gram with traditional hatching methods. Increased hatching rate can help offset inherent inefficiencies in production of catfish hybrids.

PHYSIOLOGY

Stress Physiology

Effects of rested-harvesting on physiological responses and fillet quality of channel catfish (Brian Bosworth, Brian Small, and Chuck Mischke)

Exercise and stress during harvest negatively impact meat quality in most livestock species, including channel catfish. Research was conducted with isoeugenol (a product currently undergoing FDA approval for use in foodfish with zero withdrawal time) at the USDA/ARS Catfish Genetics Research Unit in cooperation with researchers from New Zealand Crop and Food to determine effects of rested-harvesting on physiological responses and fillet quality of channel catfish. Trials demonstrated that rested-harvesting reduced serum cortisol, blood lactate, blood glucose and increased blood and muscle pH. Thus the detrimental physiological responses associated with harvesting were reduced. The color and texture of catfish fillets were also improved compared to current harvest methods. Rested harvesting of catfish has the potential, if implemented on a practical, commercial scale, to provide substantial improvements of meat quality in farm-raised catfish.

Effect of harvest stress on blood physiology and fillet quality (Doug Minchew, Brian Small, and Rachel Beecham, Miss. Valley State Univ.)

Processors and commercial producers have begun to place increased significance on the health and physiological condition of catfish from capture to unloading at the plant as all strive to further improve the quality of catfish products sold to the public. To begin to address these issues, a study was conducted at a commercial fish farm between late August and early September of 2004 to determine how the stresses related to routine harvest procedures affect the blood physiology and filet quality of commercially grown food-sized channel catfish. Blood samples were taken from ten fish in each at four sampling periods (pre-socking, after-socking, pre-loading, and pre-unloading) during each of 10 harvest events. The combined mean cortisol levels in captured catfish after-socking, at pre-loading, and at pre-unloading were approximately 28, 45, and 43 times higher than the mean pre-harvest levels (3.02 ng/mL) and had dropped only slightly just prior to their unloading at the plant. Secondary stress responses were also elevated during harvest and transport. The combined mean glucose levels in captured catfish after-socking, at pre-loading, and at pre-unloading were approximately 2.8, 3.9, and 3.9 times higher than the mean pre-harvest levels (34.7 mg/dl); similarly, the mean combined lactate levels after-socking, at pre-loading, and at pre-unloading were approximately 5, 4.7, and 3.2 times higher than the mean pre-harvest levels (2.43 mmol/L). In addition, it was determined that capturing catfish by seine and holding them overnight in a grading sock aerated by a tractor-powered paddlewheel or a diffused oxygen system did not result in significant quality differences in fillet meat immediately after slaughter. There were significant changes within treatment over 7 days of refrigerated storage for fillet pH, color, and drip loss, however there were no consistent trends between treatments. The results of this study will be helpful to scientist and producers seeking to improve fish health by reducing the harvest related stress levels in pond cultured catfish.

Effects of exhaustive exercise on the blood physiology of channel and blue catfish (Doug Minchew and Rachel Beecham, Miss. Valley State Univ.)

The ability to recovery from exhaustive exercise is important because it determines the performance limits of a fish. The potential frequency of maximal performance in a fish is set by the time required for recovery and restoration of energy stores. Therefore, we conducted a series of swim-tunnel studies to

determine the time needed for blue and channel catfish to recover from bouts of exhaustive exercise. No differences were found between blue and channel catfish blood lactate, glucose, or cortisol concentrations following exhaustive exercise. As expected, pre-exercise blood levels of blood lactate, glucose, and cortisol were significantly lower than levels determined at the time of fatigue. All fish recovered from exhaustive exercise within four hours.

A comparative study of the swimming endurance of channel and blue catfish fingerlings (Doug Minchew and Rachel Beecham, Miss. Valley State Univ.)

This study compared the swimming endurance blue and channel catfish fingerlings at current speeds ranging from 0 to 120 cm/sec. The channel catfish fingerlings had significant better swimming endurance at all current speeds above 40 cm/sec. The magnitude of difference was about 10 cm/sec. Differences in the swimming performance of fish have inheritable components which might be important in establishing the superiority of closely related species, families, or lines of fish.

Using portable lactate and glucose meters for catfish research: An inexpensive alternative to established laboratory methods? (Doug Minchew, Brian Small, and Rachel Beecham, Miss. Valley State Univ.)

The objective of this study was to determine the accuracy, precision, and cost effectiveness of using portable, handheld lactate and glucose meters in the field for channel catfish research and to provide a prediction model for comparing meter values to values determined using established spectrophotometric analyses in the laboratory. Data collected in this study demonstrated that both the Accutrend lactate meter and the Accu-Chek Advantage glucose meter can be useful tools for measuring plasma values in the field or laboratory. Both meters had good precision but consistently yielded values lower than those obtained by established laboratory methods, and should only be used in situations where relative measurements are suitable. The use of these instruments has the potential to make field sampling more accessible and less time consuming, which may lead to a better understanding of fish metabolism outside of the laboratory environment.

PRODUCTION TECHNOLOGY

Development of a more efficient commercial seine (Doug Minchew and Ed Robinson)

This completed project focused on the development and evaluation of a new commercial seine and live car system with improved fish handling and grading characteristics designed to greatly reduce the stresses imposed on fish during harvest as well as reducing the overall harvest time. The new seine was constructed by modifying a conventional seine based on recommendations of the National Marine Fisheries Service' Harvest System Team. Specific modifications are as follows: 1) use of braided, polyethylene mesh hung in a square pattern rather than twisted polyethylene mesh hung in a diamond pattern; 2) significant enlargement (width and height) of the funnel section of the seine; 3) use of long marine zippers instead of standard small metal frames to attach the live cars to the seine; 4) use of a "traveling" mud line with mud rollers instead of the conventional mud line; 5) use of braided, polyethylene mesh hung on a square pattern to build the live cars; 6) use of larger, more closely spaced floats with grommets on the seine. Several versions of the prototype seine with some or all of the modifications were built and tested in commercial and research catfish ponds in Mississippi, Arkansas, and North Carolina. Catfish producers participating in the project liked the marine zippers for attaching the live car and seine funnel and the larger, more closely spaced floats with grommets but preferred a convention rolled, mesh mud line instead of the newer "traveling" mud-roller mud line. Although farmers and seining crews disliked the "traveling" mud-roller mud line system, they agreed that the system had merit in ponds with very muddy bottoms; they indicated that the primary advantages of the experimental seine and live car were; 1) ponds could be seined more quickly with less pushing needed to dump mud; 2) fish transitioned into the live car more easily and appeared to be in better condition; 3) fish graded out of the live car faster; 4) little if any aeration was needed when loading the fish into the live car; and 5) the braided, polyethylene webbing appeared to be much more durable. Since the conclusion of this project in 2004, one large net manufacturer now puts enlarged funnels and marine zippers in 80 to 90 percent of the new seines and live cars built; the manufacturer has also installed 400 to 500 of marine zippers and funnels in existing seines and live cars. These changes have resulted in reduced harvest costs (shorter harvest times) and improved conditions of harvested fish. Farmers indicate using the larger throat and marine zipper system maintains the fish in much better condition

Harvest of channel catfish with an electrically enhanced seine (Doug Minchew)

Initial studies used a programmable commercial backpack electrofisher to evaluate over 600 varying combinations of wave-form types, voltages, pulse frequencies, pulse widths, and pulse sweeps to determine their effectiveness in moving channel catfish away from electrodes used to "seine" catfish held in concrete vats. The studies demonstrated that an electrical system using a low to high frequency waveform with a 6.0-60 Hz sweep, and a 2-millisecond pulse width over a 4 second sweep time could be used to capture 92% of the fish present in a concrete vat in a single pull. These results were used to construct a 70 feet long by 9 feet deep electrically enhanced seine for use in a comparative harvesting study. The comparative studies were conducted in twelve rectangular 0.1-acre ponds which had been stocked with 1200 or 1800 fingerling the previous spring. Six ponds were harvested with a conventional seine and 6 with the electrically enhanced seine. The results demonstrated for the first time that an electrical stimulus can be used to significantly improve the catch efficiency of a conventional seine when used to harvest catfish from ponds. The electrically enhanced seine captured an average of 22% more fish than the conventional seine. The results of tests conducted in concrete vats indicate that the system is effective in repelling adult catfish away from the attached electrodes. Future design changes will involve

increasing the effective electrical output of each module, further reducing the weight and size of the electrical components and power supply, and determining the most effective design for the electrodes which will be built into the seine.

Post-Harvest Technology

Machine settings required for processing of blue catfish (Brian Bosworth)

Growth trials conducted at the CGRU have shown that blue catfish may have potential as an alternative to channel catfish for commercial production. However, little is known about machine settings required for commercial processing of blue catfish. We worked with a commercial processor and found that minor adjustments to fillet machines set for channel catfish were required for processing of blue catfish. This information was made available by a presentation to the industry at the Catfish Processors Conference held at Mississippi State University, Starkville, MS in May, 2006.

Composting fish wastes (Les Torrans)

Disposal of fish wastes from research facilities or small-scale processors is problematic. Les Torrans (USDA ARS CGRU) studied various methods of fish disposal for use on the research facility. A modular, reusable composter was developed for a cost of less than $40.00 that can convert 5000 pounds of fish and fish waste into garden fertilizer. Composting is a simple, low-cost, “green” method of fish waste disposal that has widespread application for disposal of small volumes of fish and fish waste.

Automated Farm Management Systems

Farm resource management system (Doug Minchew)

The size and complexity of modern catfish farms make it difficult to track and manage people, equipment, and data in real time. We are developing a wireless networking infrastructure with specialized hardware and software products that enable custom–designed and “traditional” instruments or equipment to become networked and web-enabled distribution devices. A prototype wireless network with the embedded software for data handling and analysis was installed and a satellite link was installed to allow the system to be accessed and controlled remotely. A GPS-enabled prototype wireless integrator (PWI) has been built and connected to the oxygen meter used during routine oxygen checks on the farm. The PWI automatically tags the data for time and the GPS determined location and forwards it to the central server for storage and processing. The infinite scalability of the system, its capability to interface with off-the-shelf instruments, and its ability to store, analyze and display complex data are features that are normally not available on proprietary commercial systems. When fully developed, the system will reduce the cost and simplify the management of electronic information and equipment on fish farms which will greatly improve the ability of a producer to make timely, well informed management decisions.

ECONOMICS

Aquaculture Marketing

Mississippi "MarketMaker": A nationwide food marketing data base (Ken Hood and Terry Hanson)

This project looks at how small- to medium-sized aquaculture producers can sell more of their product at potentially higher prices. "MarketMaker" is a marketing tool aimed at connecting food producers, distributors, buyers, and sellers to specific consumer markets. The website program maintains multiple data bases on-line for easy access by users of the system. The on-line information can be used to generate maps so market concentrations can be located visually. Fact sheets, customized demographic profiles and supporting research are also features of the site. The more states that join in the program the better the data base becomes and the greater the benefit to users of MarketMaker. Mississippi's MarketMaker web site came on-line in October 2007 and can be found at: http://ms.marketmaker.uiuc.edu/.

Risk Management

Reducing aquaculture risk through feed purchase management (John Anderson and Terry Hanson)

This work addresses strategies to lessen the impact of increasing feed costs in the aquaculture industry. Since corn and soybeans make up 60% to 80% of most aquaculture feeds, developing strategies to mitigate production cost risk, i.e., input cost increases, through knowledge and use of grain price trends and/or use of grain futures and options markets is valuable to producers. A basic understanding of futures/options markets and timely knowledge of corn and soybean price trends can save producers money by being aware of price swings and strategically forward pricing their feed inputs.

National aquaculture risk feasibility study for aquaculture (Terry Hanson and Keith Coble, MSU-AEC)

This four-year project involves determining the feasibility for insurance product development for the four major U.S. aquaculture produced species, namely catfish, salmon, trout and baitfish. This research has focused on characterizing aquaculture risks and perils in an actuarially accepted manner. Methodologies have been developed to cope with the challenges of estimating magnitudes and frequencies of losses for the numerous perils faced by aquaculture producers. Accurate determination of these estimates is critical in developing actuarially sound premium rates, without which there would not be a sustainable insurance product. A draft report has been submitted to the USDA/RMA includes proposed aquaculture insurance policies for catfish and trout. The next step is to work with RMA personnel and their Board of Review process to address concerns and comments about the policies.

Use of economic principals in catfish farm decision-making (Terry Hanson, Jimmy Avery, John Anderson, Gregg Ibendahl, Jim Steeby, and Charlie Hogue)

Following up on the 2004 Trade Adjustment Assistance (TAA) program for catfish producers additional workshops were conducted in east and delta regions of Mississippi. Workshop topic areas included presentations and examples of economic evaluation tools for use in determining the costs and benefits of potential farm production changes; and how to evaluate a farm's financial condition and what to do once you know your farm's financial situation. Partial budgeting extension publications with examples for the delta region and east Mississippi have resulted from this project and can be found at, http://msucares.com/pubs/publications/p2437.pdf and http://msucares.com/pubs/publications/p2436.pdf.

Catfish stocker cost of production estimates from commercial-scale verification trials (Terry Hanson, Lou D'Abramo, and Jim Steeby)

The cost of producing catfish from the fingerling-to-stocker phase of the modular system has been estimated using research results from small replicated experimental ponds (0.05ha) conducted in 2004 and 2005. To address the question of whether commercial-scale production of the fingerling-to-stocker phase would be comparable to smaller experimental pond results was addressed in this research and economic analysis. In early April 2006, 13.2-14.3g fingerlings were stocked at 122,888 to 124,518/ha and fed 35% crude protein fingerling feed into 1.6 ha ponds. The trial was harvested in late January 2007 and an economic analysis of stocker and foodsize production from the modular system was estimated and compared to multiple-batch production costs. Stocker production cost in the commercial-scale verification study was higher than in small research pond results, but overall foodfish cost was $0.013/lb less than food size fish production from the multiple-batch system.

Economic analysis of trematode infections in channel catfish in a commercial pond setting (Terry Hanson, David Wise, and Craig Tucker)

The trematode identified as Bolbophorus sp can cause significant production losses in commercially raised channel catfish. This disease has been associated with high mortality rates, decreased feed consumption and poor production efficiency. To assess the economic impact of this disease, a disease monitoring and production efficiency study was conducted on a commercial catfish operation with reported trematode infestations. Compared to trematode negative ponds, ponds in the light, moderate and severe categories produced 14%, 35% and 40% less fish weight per acre, respectively. Net returns for ponds in the light category were reduced by 39% and production from ponds in the moderate and severe categories were not shown to cover variable costs of production. Ponds in the moderate category produced a net loss of $781 per acre and severely infected ponds produced a net loss of $1,123 per acre. While heavy infestations are usually recognized, low grade and even moderate trematode infections can remain undetected for years, slowly decreasing production and profitability. In some ways, low-grade infections represent a greater risk to production in the long run because they can go undetected. A mild infestation usually does not cause immediately obvious production problems.

Freshwater prawn cost of production - results from a 2005 multi-state freshwater prawn grower survey (Terry Hanson and Steve Sempier)

Results from a mail survey of freshwater prawn producers were used to estimate the variable, fixed and marketing cost to produce prawns in the central region of the U.S. Highlights of findings, based on 27 useable include: ten states had FWP producers; respondents grew a total of 47,202 pounds of FWP in 81 acres of ponds; of the FWP harvested: 45% were 7-11 per pound, 45% were 12-22 per pound, 10% were over 22 per pound; FWP sold heads-on received prices ranging from $4.50 to $10.00 per pound and heads-off from $7.20 to $15.00 per pound; stocking costs in dollars per pound produced in Kentucky and Mississippi were about 65% less than in other regions, probably due to hatcheries being located in these two states; all regions had positive incomes when only operating costs were subtracted from receipts; two out of the four regions and the national average had positive values for net returns above operating and fixed costs. Survey results were sent to each cooperating producer along with a financial analysis of their operation. A MAFES bulletin (Bulletin 1162) will soon be coming out with detailed analysis and comparison of freshwater prawn costs across southern states. Results should be helpful to producers in analyzing their production costs and profitability.

Impact of stocking density on dressout percentages of harvested freshwater prawns (Lou D'Abramo)

The dressout study of female and male prawns in four different tail categories used for marine shrimp sale shows a consistent percentage for females while dressout percentages for males decreased by 8 %. The overall dressout percentage was 47.5 %. At a stocking density of 11,500/A, the percentage of headless prawns that were 21 to 30 count was 68 %. The percentage by weight of headless imported shrimp (352 million lb, 2005) of the same count category is 19.5. Weight loss of whole prawns after an individually quick frozen process was 2.8 % (range = 0.0 to 4.9 %) This post-harvest processing information provides farmers with important guidelines in establishing prices for whole and deheaded product and determining the appropriate stocking densities to meet the desired product size for their specific market.

EXTENSION

Workshops and Seminars

Workshops were developed to educate producers and infrastructure personnel on a variety of production-related issues. Seminars were developed for the Delta and East MS to present Center research findings. Speakers generally included NWAC scientists, extension personnel, and other MSU faculty.

- Fishy 2006 Workshops (2). Catfish Farmers of America and NWAC conducted computer-based, pond record keeping workshops for a total of 20 catfish producers. Extension faculty assisted Dr. Killcreas in teaching participants.
- Pesticide Applicator Training for Catfish Farmers. Pesticide applicator certification is a legal requirement for persons using restricted-use pesticides. Over 20 catfish producers passed the training.
- Fall Seminar - DREC. Information from selected programs at the NWAC were presented by NWAC, MSU-CVM, and USDA personnel. Over 75 people were in attendance.
- Winter Seminar - Campus. Information from selected programs at the NWAC were presented by NWAC, MSU-CVM, USDA, and industry personnel. Over 80 people were in attendance.

Special Projects

Mississippi Aquatic Invasive Species Task Force (Craig Tucker and Jimmy Avery)

A task force formed by the Governor and administered by the Dept. of Environmental Quality was given the responsibility to develop an Aquatic Invasive Species Plan for Mississippi. Represented the aquaculture industry concerning use of non-native species and potential impacts to the aquaculture industry.

Catfish Farmers of America Research Symposium (Jimmy Avery)

Organized a 20-presentation symposium representing 14 institutions. Responsible for making call for submissions, recruiting review/selection panel, developing an agenda, compiling/editing the book of abstracts, recruiting moderators, and facilitating the visual presentations. Over 100 farmers and infrastructure personnel attended the symposium.

EcoTour (Jimmy Avery, Jim Steeby, and Charlie Hogue)

Approached The Catfish Institute about developing an educational tour and technical session for the environmental non-governmental community. Was successful in attracting participation from World Wildlife Fund, Sustainable Fishery Advocates, and Monterey Bay Aquarium. Developed a technical session that included experts from Auburn University, USDA National Wildlife Research Center, University of Arkansas – Pine Bluff, and the NWAC. Organized a tour of feed mills, processing plants, and farms in Mississippi and Alabama. The event was responsible for a joint letter from two of the organizations stating their support of catfish aquaculture.

Disaster Assistance (Jimmy Avery, Jim Steeby, Terry Hanson, and Charlie Hogue)

The Mississippi Department of Agriculture and Commerce informed Mississippi aquaculture producers that the USDA – Farm Service Agency would require supporting documentation of losses along with the application for hurricane assistance. In an effort to assist farmers in providing the required information, Extension Aquaculture Specialists, along with Catfish Farmers of Mississippi representatives and MSU Dept. of Agriculture Economics faculty, met with MDAC officials to discuss this issue. Based on those discussions, the group developed a set of documentable loss calculations for farmers to use. The response by the Extension Service made a dramatic impact on the industry's ability to receive the $10 million in disaster assistance funds.

Trematode Awareness (Jimmy Avery, Jim Steeby, and David Wise)

In response to the research finding that even light trematode infestations can cause economic loss, we initiated a media blitz to raise awareness of this issue. We taped a radio spot, drafted and reviewed a news article, and scripted and organized a *Farmweek Television* segment.

Sustainability Panel (Jimmy Avery)

Asked by The Catfish Institute and Lewis and Neal, Associates to participate in a panel discussion of sustainability issues in aquaculture and capture fisheries. Made a presentation on sustainability of catfish farming and answered questions from the audience. This presentation lead to an Associated Press article titled *"Catfish Growers Face Global Competition"* about sustainability and foreign competition.

USFWS Asian Carp Task Force (Jimmy Avery)

Participated in development of a national plan to deal with the management of invasive Asian carp species.

Publications / Materials Developed

- *Partial Budgeting as a Decision-Making Tool for Catfish Producers: Analysis of Raising Catfish in a Multiple-batch or a 3-phase Modular Production System*. 2007. T. Hanson, J.D. Anderson, G. Ibendahl, J. Steeby, and J. Avery. MSU Extension Service Pub. 2437.
- *Partial Budgeting as a Decision-Making Tool for Catfish Producers: Analysis of Purchasing 6" Fingerlings for Growout or Purchasing 3.5" Fingerlings and Growing to 7" and 8" Stockers for On-Farm Growout*. 2007. T. Hanson J.D. Anderson, G. Ibendahl, C. Hogue, and J. Avery. MSU Extension Service Pub. 2436.
- *Effects of Smallmouth Buffalo and Potassium Permanganate Treatment on Plankton and Water Quality*. 2006. Mischke, C.C., D.J. Wise, J.A. Steeby, and P.V. Zimba. North American Journal of Aquaculture 68: (1) 36-46.
- *Oxygen Management at Channel Catfish Hatcheries*. 2006. Torrans, L. and J. Steeby. Global Aquaculture Advocate Magazine 9: (3) 56-58.
- *Catfish Nutrition: Feeds.* 2006. Ed Robinson, Menghe Li, and Charlie Hogue. MSU Extension Service. Publication 2413.
- *Catfish Nutrition: Feeding Food Fish.* 2006. Ed Robinson, Menghe Li, and Charlie Hogue. MSU Extension Service. Publication 2414.

- *Catfish Nutrition: Nutrient Requirements.* 2006. Ed Robinson, Menghe Li, and Charlie Hogue. MSU Extension Service. Publication 2412.
- *NWAC Newsletter.* Volume 9, Number 1 (July 2006). J. L Avery, editor. It can be accessed at: http://www.msstate.edu/dept/tcnwac/nwac_news.htm
- *Diuron-based herbicides obtain emergency exemption.* 2006. J. L. Avery. NWAC News, Vol. 9 (1).
- *Use of smallmouth buffalo (*Ictiobus bubalus*) to reduce the incidence of proliferative gill disease.* 2006. J.A. Steeby, D. Wise, T. Byars, and L. Thompson. NWAC News, Vol. 9 (1).
- *Use of Fintrol to remove scaled fish in catfish ponds.* 2006. J. L. Avery. The Catfish Journal, Vol. 19 (9).
- *Catfish Broodstock Trends in the Southeastern United States*. 2006. J.A. Steeby, N.Chatakondi and B. Wagner. NWAC News, Vol. 9 (1).
- *Impacts of Stocking Size and Protein Content of Feed on Stocker Production.* 2006. L. D'Abramo, Terry Hanson, and J.A. Steeby. NWAC News, Vol. 9 (1).
- *Stocking Mississippi Farm Ponds.* 2006. J.L. Avery and H. Huffstatler. Mississippi Gamefish Suppliers and Consultants Association publication. 2 pp.
- *Research and Review: A compilation of Abstracts of Research on Channel Catfish 2006*. 2006. J.L. Avery, Editor.

Fish Health Demonstration Projects

On-site demonstration projects developed from laboratory and field research are used to evaluate potential disease treatments and determine the potential for infectious diseases to develop in commercial ponds. Farmer interactions and demonstration projects have proven an efficient method of demonstrating the benefits of various disease management techniques. Technology transfer plays a vital role in helping producers cope with new and emerging fish health management issues.

Field Visits

Field visits continue to be a vital component of the Extension effort. Numerous field visits were made to farms, hatcheries, manufacturers, and processors across the Delta and East MS. Visits are routinely made to catfish hatcheries to evaluate poor egg / fry survival and to ponds to conduct presumptive diagnosis.

Increasing the Exposure of the NWAC

A continuing goal is to increase the exposure of the NWAC to catfish farmers and infrastructure personnel. One approach is increasing mass media exposure through press releases, radio programs, and television segments.

- *MSU Better Farming Radio program*. Four radio programs were developed discussing catfish financial situation, NWAC programs. and the Catfish Festival. Archive list can be found at: http://msucares.com/news/radio/betterfarming/archive_05.html
- *Farmweek Television- Catfish trematodes,* Segment #3018, show 1469. Aired November 3, 2006. Farmweek Show Line Up can be seen at: http://msucares.com/news/farmweek/Lineups/lineups2006/fmwk3018nov3-2006.pdf.

- *Farmweek Television- Fall Aquaculture Seminar*, Segment #3018, show 1469. Aired November 3, 2006. Farmweek Show Line Up can be seen at: http://msucares.com/news/farmweek/Lineups/lineups2006/fmwk3018nov3-2006.pdf.
- *Educational Booth* at The Fish Farming Trade Show. Extension personnel manned an educational booth representing NWAC. (Over 1,300 people attended.)
- *Tour* for the Vanderbilt Program. A presentation and tour was provided for 18 students from 11 developing countries along with three staff members from Vanderbilt University.
- *NWAC News*. A newsletter about current projects at the NWAC was sent to over 1,000 producers and infrastructure personnel.
- *Quarterly articles for Northeast Mississippi County Directors.* County Director in 8 counties were provided articles for local newspapers or direct mail outs. Subjects included hurricane preparedness, workshop announcements, and impact of soybean rust treatments.

Industry Support and Input

Extension personnel continue to serve in an advisory capacity with the Mississippi Farm Bureau Federation, Northeast Mississippi Catfish Farmers Advisory Panel, Catfish Farmers of America, Catfish Farmers of Mississippi, The Catfish Institute, Southern Regional Aquaculture Center, Mississippi Gamefish Suppliers and Consultants Association, US Freshwater Prawn and Shrimp Growers Association, USDA/NASS, and Delta Council on issues affecting Mississippi aquaculture industries.

SOUTHERN REGIONAL AQUACULTURE CENTER
Craig Tucker, SRAC Director

The Regional Aquaculture Centers encourage cooperative and collaborative research and extension education programs in aquaculture having regional or national application. Center programs complement and strengthen existing research and extension educational programs using funds provided by the USDA-CSREES and other public institutions.

Project Updates

Publications, Videos, and Computer Software

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, County Agents and the World Wide Web. 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, consumer education, marketing, and much more. The Center has now published 179 fact sheets, 4 project reports, 19 research reports, and 20 videos. These publications were developed by 16 scientists across the region.

Innovative Technologies and Methodologies for Commercial-scale Pond Aquaculture

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. 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.

Several possible methods for improving efficiency and profitability of aquaculture are under investigation: 1) Evaluation of new production systems and improvements in existing production systems for channel catfish; 2) Improvement in equipment used for mechanical aeration and for fish harvesting in channel catfish culture; and 3) Evaluation of energy, material, and economic efficiency of production systems.

Among the significant findings so far:

- **Partitioned Aquaculture System.** Several variations of the partitioned aquaculture system (PAS) concept are being evaluated and all show some promise for commercialization. The PAS concept is based on physically dividing the pond into sections for holding fish and treating waste produced during culture. The two sections are hydraulically connected by water flowing between the two systems. Research at Clemson University showed that the PAS has promise for accelerating the growth of channel catfish fingerlings and appears to have potential for commercial use. A less intensive approach, called the semi-confinement system, also appears to have potential for use in channel catfish fingerling production.

- **Biofloc System.** Maintenance of water quality in most pond aquaculture systems depends on plant growth (autotrophy). However, under super-intensive culture conditions, autotrophy cannot provide adequate waste treatment and is increasingly supplemented by microbial-based (heterotrophic) processes. A heterotrophic, biofloc system was tested by USDA scientists at Pine Bluff, Arkansas, and successfully produced channel catfish under experimental conditions. Further testing is needed to ascertain the commercial possibilities of this system. At Stoneville, Mississippi, USDA scientists tested a motor-powered U-tube aerator and confirmed that the device is highly efficient in moving water, but the oxygen transfer rate must be improved through design modifications. Research at Auburn University has focused on developing indicators of economic and resource use efficiency. These indicators will be further refined in the next project year.

Improving Reproductive Efficiency to Produce Channel × Blue Hybrid Catfish Fry

The hybrid channel catfish female × blue catfish male possesses many of the best qualities of both parents, including fast growth, disease resistance and easy harvest. However, only a few farmers use the hybrid because fingerlings are not available due to difficulties in producing hybrid eggs and fry. Nine scientists at five institutions are conducting research to improve the hatching rate of 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 project began on April 1, 2004, and among the significant findings are:

- **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 the hormone LHRHa to induce ovulation in channel catfish females. The degree-hour response time is longer at cooler temperatures and females ovulate faster at higher temperatures. This is important because reasonable work schedules can now 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. If warm water is available, channel catfish can be spawned as early as the first of January. Ovulation rate and number of eggs released increases 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.

- **Brood stock 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 increase hybrid fry output 33% to 100% depending upon the initial condition of the females. Availability of forage fish, even at low levels, improves hybrid fry production.

- **Brood stock genetics.** 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. Use of crossbred channel catfish females did not improve hybrid fry production.Selection for body weight or

inbreeding in channel catfish reduced hybrid fry production in some, but not all lines of channel catfish.

- **Induced spawning**. At one location, no significant differences were observed between LHRHa, carp pituitary extract, and catfish pituitary extract to induce spawning 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 of LHRHa at a rate of 100 μg/kg generates the most consistent and the greatest number of fry per weight of female. At the end of the season this dose needs to be reduced to 75 μg/kg. 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. Indirect exposure of channel catfish females to the scent of channel catfish males increases number of hybrid fry produced per kg of female. LHRHa implants had minimal, but positive effects on blue catfish male reproduction.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. Using 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 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. Water activated, but unfertilized, eggs showed the characteristic movements seen previously in normally fertilized embryos. Blastodisc enlargement and protrusion also took place mimicking normal development, however, none of the activated, unfertilized eggs underwent gastrulation or cleavage. 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

Feed Formulation and Feeding Strategies for Bait and Ornamental Fish

The overall goal of this project is to assess changes in diet composition and feeding strategies on the growth, health, and body composition of freshwater baitfish and ornamental fish. Market sizes of bait and ornamental fishes are relatively small and specific sizes are needed for specific purposes, so that repeated grading and handling during production are often required. After harvest, fish must withstand the additional demands of distribution and sales, and must survive for extended periods. Significant losses occur when fish are transported on trucks from the production facilities to distribution sites. A combination of handling stress and suboptimal environmental conditions can result in high mortality when fish are transferred between facilities. Six scientists from four institutions will collaborate to develop diets and feeding practices that enhance stress resistance and prolong survival of bait and ornamental fishes.

The project addresses the following objectives: 1) manipulation of diet composition and feeding strategy for economical production of "jumbo" golden shiners; 2) manipulation of diet composition and feeding strategy to increase immunocompetence and resistance to stress in bait and ornamental fish during production, transport and live display; and 3) determination of the relative contribution of natural foods and prepared diets to growth, response to low dissolved oxygen, and other health indices for bait and ornamental fish in different production systems.

Among the significant findings so far:

- **Feeds and feeding for "jumbo" golden shiners.** There is an unmet demand for large baitfish ("jumbos") and there is currently no good way to produce these fish in one year. Our first task was to determine the best stocking density for juvenile golden shiners to maximize the production of jumbos (larger than 12g) within a single growing season. In this trial, golden shiner juveniles were stocked at four densities and grown for 105 days while being fed once daily with a commercial 42% protein feed. Average fish weight decreased with increasing fish density, but gross yield (total pounds of fish produced) increased with density. Survival was not different among treatments. Fish stocked at 30,000/acre resulted in about 54% jumbos by weight. Stocking juvenile golden shiners in late July resulted in lower single-season yields of jumbos compared to direct stocking of hatchery fry at low densities. Direct stocking of fry in early May resulted in higher yield of jumbos in a single season. However, the extra production of jumbos must be balanced against other uses for the ponds. In a second trial, we are evaluating the effects of diet composition and feeding frequency on the growth and production of golden shiners stocked at 30,000 fish/acre. Fish are being fed either once or twice daily with one of two diets (42% protein diets with or without fish meal). The most recent data shows that feeding frequency is affecting fish weight (fish fed twice daily are larger), but there is no effect of the different diets on growth.

- **Feeds and feeding to enhance health of bait and ornamental fish.** Multiple feeding trials with dietary additives that may stimulate fish health have been attempted at Texas A&M University and the University of Arkansas at Pine Bluff. Methodological problems have hindered progress, but overall there have been few pronounced effects of these feed additives on general performance (growth, survival, feed conversion) of golden shiners in tanks. Better methods of measuring immune and stress responses of small fishes must be developed to assess these diet additives fully, and concentrated efforts are being made in this area. One promising technique is the measurement of cortisol (a stress hormone) in the whole body of the fish instead of the blood. Also, some of the feed

additives may be more effective in protecting the fish against specific pathogens (such as the bacterium that causes columnaris disease), rather than enhancing the general immune response. The physical form of diets used may also need adjustment. We are using cold-pelleted diets, but in commercial production extruded (floating) pellets are produced using steam. Diet form as well as composition can affect experimental results, so this is another area for additional research. We have also examined the effects of diets with 4 or 10% lipid (fat) and diets with or without fish meal on performance of golden shiners in tanks. Previous studies showed some beneficial effects of high lipid levels in diets for golden shiner and goldfish. However, in this trial there were no differences in growth, survival, or whole-body lipid of fish fed diets with 4 or 10% lipid. In general, feed conversion of fish fed 10% lipid diets was higher than that of fish fed 4% lipid diets, except for fish fed diets with a prebiotic (Grobiotic®-A). It is likely that fish did not respond to higher dietary lipid in this study because they were larger at the beginning of the trial (larger fish grow more slowly), and the diets were lower in protein than previous studies. Therefore, more protein may have been used for energy instead of tissue growth, and the benefits of extra lipid were not realized. There were no major differences in performance of fish fed diets with or without fish meal, consistent with previous studies. Removal of fish meal from production diets for baitfish would reduce cost. We also performed a bacterial challenge on groups of golden shiners fed the standard diet (4% poultry fat), a 10% poultry fat diet, or a diet with both 10% poultry fat and 2% Grobiotic®-A. The Grobiotic®-A is a prebiotic, a non-living product containing indigestible carbohydrates that stimulate "good bacteria" in the gut. Prebiotics can enhance fish performance under stress, such as exposure to pathogens. Golden shiners fed the diet with Grobiotic®-A had higher survival than fish fed the other diets when exposed to the bacteria that cause columnaris disease. This is a significant pathogen of golden shiners and other bait and ornamental fish. At the University of Georgia, whole-cooked soybeans are being compared to soybean meal in diets for golden shiners, feeder goldfish and fathead minnows. During initial trials in aquaria, it was necessary to increase the salinity of the water to 3 parts per thousand using artificial sea salts to get good survival of golden shiners. All remaining trials in aquaria will be conducted at this higher salinity. Goldfish and fathead minnow trials are in progress, and pond trials will begin after the aquarium trials have been completed.

- **Contribution of natural foods to growth and health of bait and ornamental fish.** It is difficult to separate the effects of prepared diets and natural foods in outdoor systems (pools, ponds) on performance of fish, but most commercial production of bait and ornamental fishes is in outdoor systems. Studies are underway to determine the best diets to use in ponds where bait and ornamental fish will have access to both food sources. In three feeding trials conducted in recirculating systems at Texas A&M with golden shiners, we were unable to maintain enough natural productivity to assess the relative contributions of natural and prepared foods to fish performance. A modified culture system has been developed and is currently being tested for this purpose. A feeding trial in outdoor pools is in progress at the University of Arkansas at Pine Bluff using the same diets described in Objective 2 (diets with or without Grobiotic®-A, with or without fish meal, or with 4 or 10% lipid). We are measuring chlorophyll *a* in the pools to assess algal abundance (indicator of natural foods). At 4 weeks, there were no differences in weight gain among treatments and survival has been good. The feeding trial will last a minimum of 8 weeks, or as long as water temperature will support active daily feeding. Growth, survival, feed conversion, body composition and health indices will be determined to assess performance of the fish. Feeding trials at the University of Florida were conducted with ornamental fish species (swordtails and zebra danios). There was a significant difference in the growth and perhaps survival of zebra danios produced in ponds receiving treatments of liquid fertilizer, cottonseed meal, an unprocessed meal diet, and a processed (pelleted and reground) diet. A significant difference in primary productivity (algae, based on chlorophyll *a*) was also observed. Zebra danios fed an unprocessed and a processed diet in tanks also demonstrated a difference in growth. The low dissolved oxygen stress test was inconclusive, and other protocols are needed to assess stress in these species also. Other preliminary results include an apparent difference in growth,

survival, and production (i.e. number of offspring produced) of swordtails based on pond trials with the 4 treatments. The data also have suggested the need for another study which would classify and quantify secondary productivity in these ponds (i.e. crawfish, tadpoles, insects, etc.) based on the different treatments. Studies for unprocessed and processed diets in tanks for swordtails are in progress. Based on this data, several producers have altered their stocking densities and feeding regimes for zebrafish and swordtails. The producers were enticed by the rapid growth and high survival rates demonstrated by feeding a processed diet twice daily.

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