Aquaculture (NP 106) Annual Report for 2009

Vision: The vision for ARS aquaculture research and technology transfer is to support a thriving domestic industry based on improved genetic stocks and scientific information on biotechnologies and management practices to ensure a high quality, safe supply of healthful seafood and aquatic products.

Mission: The mission of the Aquaculture National Program is to conduct high quality, relevant, basic and applied aquaculture research, to improve the genetic foundation of domesticated aquaculture species, and to transfer technology to enhance the productivity and efficiency of U.S. producers and the quality of seafood and other aquatic animal products.

The primary aim of the ARS Aquaculture Program, as described in the National Program 106 Action Plan, is to help develop and ensure an abundant, safe, and affordable supply of seafood products within a healthy, competitive, and sustainable aquaculture sector; this sector is supported by over 4,300 aquaculture farmers that produced in excess of $1 billion dollars worth of goods in 2005 (NASS, 2005 Census of Aquaculture).

Over the past year, research plans for the next 5 year cycle were developed and submitted, and the newly written and externally peer reviewed project plans will be implemented and run from 2010 through FY 2014.

Recruitments: New permanent scientists welcomed to the program in FY 2009 include Julia Pridgeon (Auburn, Alabama); Nagaraj Chatakondi (Stoneville, Mississippi); and Sixin Liu and Beth Cleveland (Leetown, West Virginia).

Awards and Recognitions:
Scientists in the Aquaculture National Program were well recognized nationally and internationally over the past year, with many invited presentations. The following scientists in the Aquaculture National Program were recognized with prominent awards:

> Drew Mitchell, Stuttgart National Aquaculture Research Center,
> (Service Award from the Catfish Farmers of Arkansas)
>
> Les Torrans, Catfish Genetics Research Unit, Stoneville, Mississippi
> (2008 Excellence in Technology Transfer Award from the Southeast Region Federal Laboratory Consortium)

During 2009, 61 full time scientists working at 14 locations across the United States were engaged in 26 research projects in the program. In total, scientists produced more than 120 peer reviewed publications in 2008 and 2009. Technology transfer activities included 8 new Cooperative Research and Development Agreements (CRADA) and Material Transfer Agreements (MTA). These agreements cover transfer of Atlantic salmon germplasm to commercial producers, supply of rainbow trout families to University collaborators, transfer of specific regions of rainbow trout DNA (in bacterial artificial chromosomes), and transfer

of specific pathogens strains for vaccine work. Researchers maintained beneficial collaborations with a wide range of international investigators and laboratories to leverage research and resources, which is critical in this rapidly expanding field. In salmonid research, for example, the aquaculture program maintains solid ties with Canada, France, and Norway.

New Facilities: The broodstock facility for the National Center for Cool and Cold Water Aquaculture in Leetown, West Virginia, was completed in the summer of 2008 and in full use this year, holding the rainbow trout brood fish for the Center's selective breeding program.

Funding: During fiscal year 2009, ARS operated under a budget that was mostly unchanged from the previous year, with the exception of a new project focused on control and eventual eradication of Viral Hemorrhagic Septicemia virus (VHSv). Total funding in the Aquaculture National Program for 2009 was approximately $38 million. Over $200,000 in funding came through extramural sources, with the Catfish Genetics Research Unit (Stoneville, MS), Aquatic Animal Health Research Unit (Auburn, AL), and the Stuttgart National Aquaculture Research Center (Stuttgart, AR) receiving extramural funds.

Research Results

The following section of the report summarizes the specific high impact research results addressing objectives in the current National Program Action Plan.

Genetic and Genomic Resources

- ***Improved Atlantic salmon released to industry***. U.S. commercial salmon producers use fish stocks that are not many generations removed from wild, unselected stocks, and must be certified stocks of North American salmon. In collaboration with industry, scientists at the National Cold Water Marine Aquaculture Center in Franklin, Maine, evaluated the growth of salmon from their breeding program in commercial sea cages, and selected a salmon line with increased growth traits. Eggs from this improved line were provided to commercial producers. The use of improved fish will increase the profitability and sustainability of coldwater marine aquaculture and provide a quality seafood product to U.S. consumers.

- ***Breeding trout with better growth performance and disease resistance***. The U.S. rainbow trout industry wants fish with improved growth and disease resistance. After three generations of selective breeding, scientists have produced trout that are approximately 25 percent larger at one year of age (about 750 grams versus 940 grams). In a separate line of fish, scientists have produced a variety with greater resistance to bacterial cold water disease, the most damaging bacterial disease for trout culture, with survival increasing approximately 45 percentage points. These lines will be tested in farm situations and should offer breeders a genetic resource for improving growth and disease resistance in commercial populations.

- ***Immune response genes in rainbow trout identified.*** The development of genetic markers for immune response genes will enable breeders to select and develop fish with improved disease resistance. ARS scientists identified, mapped, and thoroughly described nine toll-like

receptor (TLR) genes—a family of proteins linked to immune response—and surrounding markers in rainbow trout. Breeders could use these TLR genes in marker assisted selective breeding programs for improved disease resistance in rainbow trout and other salmonids, helping reduce viral and bacterial disease epidemics that cost salmon and trout farmers millions annually.

- ***Tumor necrosis factor (TNF) gene expression identified as candidate marker for disease resistance in catfish.*** In order to better understand why some families of catfish are more resistant than others to enteric septicemia of catfish (ESC), ARS scientists have developed a standardized screening protocol to distinguish between families with high or low levels of susceptibility. (Resistant families repeatedly demonstrate a mortality rate of less than 30 percent following experimental challenge, while the mortality rate in susceptible families is more than 70 percent.) In analyzing gene expression in tissues from infected and non-infected fish following experimental challenge, scientists found that the tumor necrosis factor (TNF) gene is expressed differently in the two family types, with resistant families showing a significantly higher peak in expression in spleen tissue at 48 hours post-challenge and at 72 hours post-challenge. This research paves the way for further experiments to determine the correlation between the TNF expression and resistance to *E. ictaluri* and other pathogens.

Animal Performance, Well-being and Efficiency

- ***Higher feed consumption linked to improved hybrid performance.*** There is increasing demand by farmers for the hybrid catfish (cross between female channel and male blue catfish), and strategies to maximize production are still being developed. In an intensive study by scientists at the Harry K. Dupree Stuttgart National Aquaculture Research Center, research demonstrated that the hybrid catfish produced higher yield than channel catfish, an improvement that was attributable to higher feed consumption. Because the higher consumption produced increased yield, there was no loss in growth efficiency and but rather improved overall performance. Identifying a superior performing fish is an important factor in ensuring sustainable catfish production in the United States.

- ***Low Tolerance for Sudden Increases in Environmental pH Demonstrated in Catfish Fry***. Early-life stage survival in catfish farming is variable, and low survival often cannot be attributed to diseases or malnourishment but may be related to currently used fish handling and stocking methods, which involve rapid transfer from the hatchery to nursery ponds for further growth. Scientists found that catfish fry have high tolerance for sudden decreases in water pH, but low tolerance for increasing water pH, with both channel catfish and hybrid blue/channel catfish demonstrating this sensitivity. Sac fry are the least tolerant, followed by swim-up fry, and then 3-4 inch fingerlings. As a result of this research, farmers have been advised to monitor pH before transferring fry and fingerlings to a new environment, a simple practice that is being adopted and should have significant impacts on fry survival in catfish farming.

- ***Bromelain enzyme shown to remove adhesiveness of catfish eggs and improve hatch.*** Catfish eggs are adhesive and stick together after being spawned, which requires manual egg stripping of catfish for hybrid catfish production but typically results in poor fertilization of the resulting eggs, degradation of the unfertilized eggs, and reduced hatching for the entire mass of eggs. ARS scientists determined that the eggs from manually stripped females would be easier to care for if the eggs were free of the adhesive matrix and demonstrated that the bromelain enzyme could be applied to prevent adhesion of catfish eggs after fertilization; scientists determined the concentration, time of application, and duration of application necessary. Researchers showed that removal of egg adhesion reduced the need for chemical treatment of eggs and improved hatch relative to trough hatching of egg masses, and developed protocols and scaled-up versions of standard hatching jars that could make hatching of catfish eggs commercially feasible on a large scale. Four commercial facilities producing hybrid catfish will test this procedure for jar hatching in spring 2010, which may lead to new strategies to improve fertilization.

Nutrient Requirements, Nutrient Composition of Feedstuffs, and Expanding Alternative Ingredients

- ***Soy oil identified as replacement for fish oil in diets for Pacific threadfin***. Fish meals and fish oils are limited resources and replacement ingredients are being sought for these commodities. In a trial by ARS scientists in Hilo, Hawaii, fish oil was replaced by graded levels of soy oil in diets fed to Pacific threadfin, showing that soy oil could replace up to 75 percent of fish oil in the basal diet with no reduction in growth rate or feed efficiency. Thus, research has provided an economical alternative to the limited fish oil resource.

- ***New ingredient choices identified for Atlantic salmon and Arctic char diets***. Worldwide fishmeal production has reached a maximum sustained level while demand is increasing, so new feed ingredients are needed from environmentally sustainable sources. Scientists in Franklin, Maine, and Aberdeen, Idaho, evaluated sun dried algae, barley protein, a bacterial fermentation product, corn gluten, canola protein, soybean, and soy protein concentrate as feed ingredients for Atlantic salmon and Arctic char. Barley protein and corn gluten meal, in particular, show potential as feed ingredients for salmon and char diets. This is critical information for feed manufacturers to develop cost-effective, reduced fish meal feeds for salmon and char.

- ***Heart healthy long chain omega-3 oils from salmon byproducts stabilized***. Valuable salmon oils can be extracted from fish processing wastes, but they must be stabilized immediately to prevent oxidative damage to long-chain polyunsaturated fatty acids. Smoke-processing was evaluated by ARS scientists at Fairbanks, Alaska, in cooperation with the University of Alaska scientists, as a technology to reduce oxidation of salmon oil. Research found that salmon heads exposed to the hot smoking technology produced oils with decreased oxidation and superior antioxidant potential, including higher levels of anti-oxidant tocopherols, than their non-smoked counterparts. This work shows that smoking byproducts prior to oil extraction will allow non-refrigerated transportation of oils without the addition of costly antioxidants.

- ***New vitamin premix developed for extruded, plant-based feeds for rainbow trout.*** Although feed processing and nutritional research for fish have made many improvements over the past 2 decades, the last open-formula premix for trout was released 20 years ago before modern feed processing methods were in use. ARS scientists in Aberdeen, Idaho, have developed a new vitamin mix—ARS 702—that accounts for modern feed processing methods and new sources of vitamins. This vitamin premix is formulated to account for vitamin potency losses due to heat during processing and is designed for use in modern alternative feeds. The formulation is now being commercially produced, fed to a variety of species, and used by several commercial feed manufacturers.

- ***Macro-minerals found to be lacking in trout feeds***. As dietary fish meal is reduced in the diet of carnivorous fish such as rainbow trout, several nutrients become limiting. ARS scientists in Aberdeen, Idaho, conducted a study demonstrating that rainbow trout fed plant based, fish meal-free diets required supplementation with potassium chloride, sodium chloride, and magnesium oxide, to improve feed efficiency and fish health. These minerals are abundant in fish meal, but limiting in plant-based feeds. Supplementation of these minerals is now done in all fish meal-free trout feeds, for both research and commercial production of ARS formulations.

- ***Nutrient availability for feedstuff alternatives quantified***. Feed mills need accurate digestibility data for feedstuffs to allow development of cost-effective, sustainable, adequate diets for all life stages of hybrid striped bass. Collaborative studies by scientists from the Stuttgart National Aquaculture Research Center, in Stuttgart, Arkansas, and Kentucky State University identified the best method to determine feedstuff digestibility in market-size hybrid striped bass and used this method to quantify essential nutrient and amino acid availability for feedstuff alternatives in marine fish meal. These data are being used by feed mills to formulate more cost-effective, sustainable diets for hybrid striped bass and will advance efforts to develop life-cycle-specific diets for fish as is done for other livestock species.

Improving Health

- ***Vaccine developed for motile* Aeromonas *septicemia disease of catfish and tilapia***. No vaccine is currently available for the bacterial pathogen *Aeromonas hydrophila*, which causes motile *Aeromonas* septicemia disease in many species of cultured fish, including catfish. In a cooperative agreement with a U.S. biologics manufacturer, ARS scientists developed a modified live *A. hydrophila* vaccine and demonstrated its effectiveness in preventing motile *Aeromonas* septicemia disease in channel catfish and tilapia. The studies also demonstrated the efficacy of bath immersion immunization for both juvenile channel catfish and Nile tilapia, which is an attractive option for use in aquaculture. A U.S. patent application was filed for this vaccine.

- ***Improved methods developed for the genetic manipulation and analysis of* Edwardsiella ictaluri.** *Edwardsiella ictaluri*, the cause of enteric septicemia in channel catfish (*Ictalurus*

punctatus), has considerable economic impact on the cultured catfish industry. Molecular genetic manipulation of this bacterium can be used to determine virulence factors, invasive pathways, and mechanisms of host-pathogen interactions. ARS scientists completed the first successful genetic manipulation of this bacterium, using chemical and electric current to transform seven strains. This improved technique will aid in determining virulence traits and other host-pathogen interactions that may lead to new vaccine strategies to protect farm raised catfish from *E. ictaluri*.

- ***Therapeutic benefits of* E. icaluri *vaccine demonstrated.*** In general, animals are vaccinated far in advance of exposure to a disease so that the immune system can prepare itself for exposure to the disease at a future time. Through a series of experiments, ARS scientists found that catfish injected with the modified live *E. ictaluri* vaccine just one day prior to virulent challenge were protected, and that an important immune gene (toll like receptor 5) was up-regulated within hours after exposure to the vaccine strain. Consequently, research shows that this vaccine confers adequate protection for therapeutic use in treating exposed fish during a disease outbreak, illustrating its utility beyond advanced prevention.

- ***Public health implications of* Streptococcus agalactiae *infection identified***. *Streptococcus agalactiae* is a cause of infectious disease in numerous animal species, having become recognized as an emerging pathogen of wild and cultured fish and bottlenose dolphins. ARS scientists examined the genetic relatedness of fish, dolphin, cattle, and human *S. agalactiae* isolates from different geographical regions, finding that the dolphin and fish lineage was also found in human infections in Japan. These findings suggest that *S. agalactiae* derived from different animal species may have the potential to cause disease in humans, which has important public health implications.

- ***Pathogenesis of new enteric redmouth biotype revealed.*** Enteric redmouth has long been controlled in fish culture by using an effective vaccine against *Yersinia ruckeri*. Recently, a new "biotype II" of the disease has emerged, causing disease outbreaks of enteric redmouth disease on farms using the traditional vaccine. ARS scientists have revealed a potential molecular basis for the emergence of this new biotype, finding that a simple change to the DNA prompts the switch from biotype I to II. The bacterial strains developed in this study will be useful for developing a vaccine that is effective against both biotype strains and for understanding and predicting vaccine failure.

- ***Disease spreading capacity of fish-eating birds shown to be doubtful***. There has been concern that fish-eating birds could spread disease organisms by eating sick fish and then passing viable disease organisms through their feces. Using a recognized *in vitro* model that replicates the gastric and intestinal systems of birds, reptiles, and crawfish, ARS scientists showed that microbial pathogens, especially viruses, are extremely sensitive to digestive tract fluids, especially at warm temperatures. This shows that birds are unlikely to serve as significant vectors of these fish pathogens.

Production Systems and Products

- ***Moving bed biofilters developed for low-cost energy-efficient production of marine fish***. The low energy, water, and capital cost requirements of a "moving bed biofilter" make it an ideal component for the removal of toxic ammonia and nitrite from recirculating aquaculture systems. However, operational protocols and design criteria for optimal performance are lacking. In a series of collaborative studies by researchers from the ARS Sustainable Marine Aquaculture project and Harbor Branch Oceanographic Institute of Florida Atlantic University in Fort Pierce, Florida, scientists established operational protocols and design criteria, including optimal airflow, media flow, and media type, for moving media bed biofiltration technology. These protocols will reduce capital and operational costs for farmers utilizing recirculating aquaculture systems for the production of marine fish in underutilized inland rural sectors.

- ***New catfish egg mass incubator designed.*** Catfish eggs have been incubated in essentially the same way for the past 50 years, with current technologies requiring significant water flow and space to ensure adequate exchange of oxygen and carbon dioxide across the egg shell. An ARS scientist developed an idea for improving gas exchange at higher egg densities with less water flow. While standard troughs are loaded with not more than 18 pounds of eggs and require at least 2 ½ gallons per minute (GPM) of water flow, the new design incubated up to 70 pounds of eggs on as little as 2 GPM of water flow. While development of this incubator is still in the preliminary stages, it appears that it will save both space and water compared to existing equipment.

- ***Membrane biological reactor system shown to enhance performance of recirculating aquaculture systems***. Recirculating aquaculture systems reduce water use by filtering and removing fish wastes and then re-using the water. Efficient removal of the wastes to maximize recovery of water, heat, and salt in the water is a major engineering challenge. ARS scientists evaluated treatment of a high strength aquaculture wastewater using a pilot-plant membrane biological reactor to determine overall treatment performance, especially the removal of nutrients and metals. Researchers found that the treatment process removed more than 99 percent of the solids and most of the heavy metals, more than 99percent of the total phosphorus, and 97 percent of the total nitrogen, while recovering 93 percent of the wastewater discharge. This research identifies better waste management technologies and practices that can be implemented to improve waste capture and disposal while allowing reclamation of water, heat, and salts.

- ***Shell pigmentation in Pacific oysters improved***. Markets for cultured oysters are rapidly shifting from shucked meats destined for cooking toward the live half-shell trade, making traits such as shell shape and color more economically important. Yet, the genetic basis of these traits is not well understood. In collaboration with the NIFA-funded Molluscan Broodstock Program, ARS scientists from the USDA-ARS Shellfish Genetics Program in Newport, Oregon, studied the genetic basis of shell pigmentation in Pacific oysters and demonstrated that this character is under very strong genetic control. This strong genetic determination of shell color will enable selective breeding to rapidly develop oyster strains

with desirable shell pigmentation patterns and thus increase the profitability of oyster farming in the Pacific Northwest.