National Program 106 Aquaculture
National Program Annual Report: FY2010

Vision: The vision for ARS aquaculture research and technology transfer is to support a thriving domestic industry based on improved systems developed through research and application of better genetic stocks, improved diets and nutrition, enhanced aquatic animal health and consistent water quality. Our program supplies scientific information on processes, 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, fundamental, and applied aquaculture research; to improve the systems for raising 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 for the program cycle 2010 to 2014 was initiated, following project plan development and external review and approval.

Recruitments: Benjamin Beck joined the Stuttgart National Aquaculture Research Center in Stuttgart, Arkansas, as a research physiologist. Tom Welker moved from the Auburn, Alabama, unit to the group at Hagerman, Idaho, as a research physiologist (animal), so while Tom isn't new to ARS, he is new to the Hagerman location.

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:

- Keshun Liu, Small Grains and Potato Germplasm Research Unit, Aberdeen, Idaho
  (Award of Merit from the American Oil Chemists Society)

- Dave Straus, Drew Mitchell, and Ray Carter, Stuttgart National Aquaculture Research Center, Stuttgart, Arkansas
  (2010 Winners of Excellence in Technology Transfer from the Mid-Continent Federal Laboratory Consortium)

- Geoff Waldbieser, Catfish Genetics Research Unit, Stoneville, Mississippi (2010 Distinguished Alumni Award from Purdue University Department of Animal Sciences)

During 2010, 61 full time scientists working at 14 locations across the United States were engaged in 23 research projects in the program. In total, scientists produced more than 75 peer reviewed publications in 2009 and 2010. Technology transfer activities included 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. Across the program, researchers maintained beneficial collaborations with a number of international investigators and laboratories. Canada and Norway lead the list in terms of the number of collaborations. Work ranges across cooperation on salmon breeding efforts and information sharing on recirculating aquaculture systems with Canadian counterparts to exchange with the Norwegian Aquaculture Protein Center on feed processing and with NOFIMA (Norwegian Institute of Food, Fisheries and Aquaculture Research) on fish health and well-being in recirculating systems.

Funding: During fiscal year 2010, ARS operated under a budget that included funds to initiate a new project on shellfish breeding on the East Coast in conjunction with industry and a group of universities. A new scientist will be hired in 2011 for that project and will be located at the University of Rhode Island. There was loss of funds to the Aquatic Animal Health Research Unit in Auburn, Alabama. Total funding in the Aquaculture National Program for 2010 was approximately $37 million. Over $300,000 in funding came through extramural sources, with the Trout-grains project (Hagerman, Idaho), Aquatic Animal Health Research Unit (Auburn, Alabama) and the Catfish Genetics Research Unit (Stoneville, Mississippi) 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:

Arctic charr selected for increased growth

Arctic charr have a flavor many consumers feel is superior to trout and salmon. Scientists at the National Cold Water Marine Aquaculture Center in Franklin, Maine, evaluated the growth of two different arctic charr stocks for culture in recirculating aquaculture systems. Development of a breeding program for U.S. arctic charr stocks could alleviate some of the production problems limiting expansion of arctic charr culture and provide a source of germplasm with improved production traits. A charr line selected for fast growth and delayed sexual maturity will be released for cooperative research evaluations and commercial production in the fall of 2010.

Construction of the first genome-wide integrated genetic map for rainbow trout
ARS Scientists at the National Center for Cool and Cold Water Aquaculture (NCCCWA) in Leetown, West Virginia, are using molecular genetic technologies to enhance selective breeding of important aquaculture production traits. This requires the development of tools such as genetic maps, which characterize the order and distance between genetic markers, and physical maps which contain overlapping DNA fragments from chromosomes. A comprehensive map integrating the genetic and physical maps was constructed by connecting 274 reference points throughout the 29 chromosomes of the trout genome. This integrated mapping resource will be used to reveal associations in the inheritance of superior production traits with the genes which underlie them.

Molecular tests for stress tolerance genes in Pacific oysters
Selective breeding of Pacific oysters to improve agronomic performance is in the early stages. Scientists from the ARS Shellfish Genetics Program in Newport, Oregon, demonstrated that higher expression levels of stress-response related genes in Pacific **oysters** are associated with poor growth and survival in field trials. This research indicates that molecular testing in the laboratory may provide a rapid, low-cost method for identifying superior performance in the field. These assays will help scientists predict performance and accelerate genetic improvement efforts.

Animal Performance, Well-being and Efficiency:

Rainbow trout health and performance and carbon dioxide concentration
Elevated carbon dioxide levels (over 12.5 mg/L) in aquaculture settings have been linked to poor growth, feed conversion, and reduced survival. At the same time, water pumping measures to increase water exchange and suppress carbon dioxide levels represent significant cost to the producer in the form of energy required for pumping and in fixed equipment costs. ARS researchers at Leetown, West Virginia, raised rainbow trout in water recirculating systems for six months, in either high (25 mg/L) or low (10 mg/L) carbon dioxide concentrations. Performance, survival, and numerous fish health outcomes were unaffected by the high carbon dioxide treatment. The findings of this study demonstrate that rainbow trout can be raised to market size at carbon dioxide concentrations of 25 mg/L without detrimental consequences, and therefore water pumping and pumping costs can be lowered substantially.

Improved Catfish Feed Conversion through Pond Oxygen Management
Dissolved oxygen (DO) is the most critical water quality parameter in warm-water aquaculture. Controlled studies on the impact of DO fluctuations on channel catfish have been lacking. ARS researchers at Stoneville, Mississippi, conducted a long-term research program examining the impact of DO concentrations on catfish growth, food consumption, and food conversion. Results showed that higher DO concentrations (2.5-3.0 mg/L) are required for optimum food conversion and growth, and this improved growth will significantly shorten the production cycle. Increased growth resulting from improved DO management can reduce food conversion ratios from an estimated industry-wide 2.5-3.0 down to 2.0, greatly improving the profitability of catfish farming.

Year-round spawning achieved with pompano
Lack of sustained year-round production of juveniles for grow-out operations is one of the foremost bottlenecks of marine finfish aquaculture. Spawning induction protocols were developed and tested. Spawning performance of Florida pompano broodstock, measured as number of eggs, fertilization, egg quality, and hatch rate was quantified over a 12 month period. Spawning was achieved in 10 months of the year having an average production rate of 1.9 million eggs/year with no discernable diminishment in egg quality over time. This work demonstrates Florida pompano seedstock can be produced year-round from a small population of broodstock, and overcoming one of the key bottlenecks to marine finfish aquaculture.

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

Improved soy concentrate for aquafeeds
Soy protein concentrate (SPC) is a highly nutritious ingredient in aquafeeds, but is currently too expensive to be practical. ARS researchers in Aberdeen, Idaho, have developed a modified method for the production of feed grade SPC and are working with a Cooperative Research and Development Agreement (CRADA) partner in pilot scale testing and possible commercialization. Availability of a feed grade SPC will make fish meal free diets more cost effective and the aquaculture industry more sustainable.

Catfish Pond Fertilization Practices Improved
Catfish juveniles are moved from the hatchery into a fry pond and depend on natural plankton in the ponds for some of their nutrition. Scientist in Stoneville, Mississippi, showed that natural productivity of catfish fry nursery ponds in Mississippi are not phosphorus-limited but are nitrogen-limited. By fertilizing with inorganic nitrogen fertilizers, desirable phytoplankton blooms develop more quickly and preferred zooplankton numbers are increased. The new fertilization recommendations from this research are being quickly adopted by catfish producers, and producers of other aquaculture products like shrimp, and private recreational pond owners. The new fertilization practices are producing more consistent, efficient, and economical fry production.

Determination of nutritional requirements for Pacific threadfin (Moi)
Pacific threadfin, known in Hawaii as moi, is a fish that has gained considerable aquaculture interest, however the nutrient requirements are not well defined. Protein is the most expensive ingredient in feed. Therefore it is important to optimize the protein and energy levels in feed, and meet the requirements for all essential amino acids in order to reduce feed costs. This will also minimize use of dietary protein for energy and decrease ammonia discharge that impacts the environment. Scientists at the Oceanic Institute conducted feeding studies that showed that diets containing 35% protein and 14% lipid generated the best growth performance. Additionally, the requirement for lysine, often the first limiting amino acid in ingredients used in fish feed is 1.79% (5.1% protein). The results provide basic information to estimate nutritional requirements, which are essential for developing cost effective diets for Moi culture.

Clean fish co-products from Alaska
The presence of contaminants can affect the healthfulness and marketability of fish processing co-products. Scientists with ARS in Fairbanks, Alaska, and the Institute of Environmental and Human Health, Texas Tech University, screened a number of fish oils and meals made from Alaskan fishery co-products. Persistent organic contaminants (organochlorine pesticides and polychlorinated biphenyl) were not detected in any of the samples analyzed from Alaska. These results suggest byproducts from cold water marine fish caught in Alaska are free of persistent organic pollutants.

Improving yellow perch fingerling production through techniques to enhance survival and health
A new feeding regimen consists of a variety of larval diets from natural (live) to nonliving commercial specialty microdiets (SMD) for larval fish. Compared with a semi-natural freshly processed high-protein diet (5% survival for larval perch), yellow perch larvae fed the SMD exhibited a 45% survival at the fingerling stage. These results were used to determine the best management practice for reducing diet components and maintain high fingerling survival and cost effectiveness. In a separate study, we found that young-of-the-year perch that were produced from 2- and 6-year-old broodstock exhibited different survival rates: 70% for those with 6-year-old parents and 17% for those with 2-year-old parents. We will continue to evaluate different diets and the best management practice approach for yellow perch fingerling production.

Free Gossypol (FG) Present in Cottonseed Meal (CSM) is the Major Factor Limiting the Use of CSM in Fish Diets
We found that CSM is very palatable for tilapia and FG was not toxic to tilapia even at 1,800 mg/kg diet. Nevertheless, for catfish, FG higher than 300 mg/kg diet depressed growth and feed efficiency. A dietary level of 15% CSM can be included in tilapia and catfish diets without the harmful effect of FG. The increased use of CSM in tilapia and catfish diet would reduce feed costs and increase the demand of CSM.

Improving Health:

Discovered genetic basis for Biotype 2 (BT2) disease caused by Yersinia ruckeri
Biotype 2 variants of the bacterium *Yersinia ruckeri*, the causitive agent of enteric redmouth, are an emerging disease problem in the United States and European finfish aquaculture. Scientists at the National Center for Cool and Cold Water Aquaculture, Leetown, West Virginia, identified four mutations, each in a different bacterial strain, that cause the loss of motility and enzyme (lipase) secretion that define Biotype 2 *Yersinia ruckeri* strains. All four mutations were in genes that encode essential components of the flagellar apparatus which allows the bacterium to swim and secrete certain enzymes. Our results demonstrate that the Biotype 2 phenotype has emerged separately at least four times. This differentiation of pathogen lineages is being used to assess the risk of BT2 disease and for development of specific vaccines and treatment strategies.

Hydrogen peroxide treatments effective for fungus control on catfish eggs
Water molds or fungi can destroy entire channel catfish egg masses in commercial hatching troughs, causing huge economic losses and shortages of fish. An experiment was designed to compare hydrogen peroxide and copper sulfate pentahydrate treatments for their ability to control fungus and increase egg survival. All treatments were observed to limit egg fungus and increase egg survival, but the highest egg survival and least fungal development were achieved using a static hydrogen peroxide treatment. This treatment could allow hatcheries to produce more juvenile channel catfish and reduce economic losses.

Development of modified live Edwardsiella tarda and Aeromonas hydrophila vaccines for prevention of diseases in aquaculture
Losses caused by these bacterial pathogens result in severe economic problems to farmers worldwide and both these pathogens are re-emerging strongly in the United States. Currently, no vaccines have been successfully developed to protect against these pathogens. ARS researchers at Auburn, Alabama, developed and patented these vaccines under a Cooperative Research and Development Agreement (CRADA) with a vaccine manufacturer. These modified live vaccines can be effectively used by a bath immersion method to cost effectively immunize large numbers of fish. The new A. hydrophila vaccine may be especially useful in preventing huge losses of food size catfish (over one million lbs. in 2009) that caused considerable economic hardship to Alabama catfish producers.

Characterization of the Genetic Response of Catfish to Vaccination
Catfish immunized with vaccine against *Flavobacterium columnare* have effective protection against this economically important bacterial pathogen. The nature of the genetic response of fish immunized with vaccine is important to the future development of new fish vaccines. A total of 32 genes were isolated from a channel catfish 10 minutes post vaccination; 28 of these genes were induced by vaccination. The 28 induced genes have putative functions in the following five major categories: (1) immune response (46%); (2) signal transduction (21%); (3) transcriptional regulation (11%); (4) cell maintenance (11%); and (5) unknown (11%). This research has resulted in the identification of genes important to the immune response and protection against this major pathogen and will provide researchers with new genetic information to develop reliable vaccines to reduce fish diseases that cause severe economic loss to fish producers.

Production Systems and Products:

Development of protocols to simplify control of sexual maturation in salmonids
In several states and regions, only non-reproductive (i.e. sterile) fish can be used for stocking or fee fishing operations. This rule is meant to control spread of genes from non-native fish stocked into public waters. Procedures were developed by ARS Scientists at Leetown, West Virginia, to simplify and standardize generation of sterile rainbow trout and Atlantic salmon, and these procedures were shown to also work well with brook trout and brown trout. Production of sterile fish for stocking is important to many small rural aquaculture businesses where only sterile fish can be used due to environmental considerations. A commercial Atlantic salmon farming operation and a state hatchery have successfully applied the procedures to produce sterile salmon and brook trout, respectively.

Development of a highly efficient Catfish Egg Incubator
Traditional catfish egg incubators (moving paddles to circulate water) have been used for nearly a century but require a high water exchange rate and lose efficiency at egg loading rates above 15 lbs per trough. ARS researchers at Stoneville, Mississippi, in collaboration with industry partners, have developed a new incubator (the see-saw) which can incubate more eggs and use less water and labor. In comparison with the traditional incubator, the see-saw produced 2.32 times as many swim-up fry. Additional studies indicate that over three times as many eggs can be hatched in the see-saw using 10-20% as much water, saving considerable labor, space, ground water, and energy for pumps and heaters. Thus far, in commercial field trials over 100 M eggs have been hatched.

Split-pond aquaculture systems may increase catfish production by 2 to 3 times over traditional earthen ponds
A split-pond aquaculture system has been developed that may increase channel catfish production by 2 or 3 times that archived in traditional earthen ponds. The new system splits an existing earthen pond into two unequal sections with an earthen levee and then links the two systems by circulating water that is pumped with a large, efficient, slow-turning paddlewheel. Fish are held in the small section and the larger section provides waste treatment and oxygen production. Annual catfish production has averaged more than 15,000 kg/hectare during the study. Several commercial growers have implemented similar systems.

High efficiency, low energy requiring bio-filters for recirculating aquaculture systems
Energy cost is a top operational cost in recirculating aquaculture systems. The low energy, water, and capital cost requirements of a moving media bed bio-filter make them an ideal bio-filter for the removal of toxic ammonia in recirculating aquaculture systems; however, their operation in low salinity conditions was not known. Studies by scientists at the ARS location in Fort Pierce, Florida, indicated total ammonia removal rates were equivalent or greater than conventional bio-filters in a low-salinity environment. Operational and design criteria for moving media bed bio-filters were determined for recirculating aquaculture systems, reducing capital and operational costs of inland marine fish producers.

Physical and chemical properties of commercial salmon oil
Oil extracted from salmon processing co-products may add value to the commercial fishing industry. A study was conducted by ARS scientists in Fairbanks, Alaska, and industry collaborators to examine the physical and chemical properties of oil collected from two different salmon oil and meal processing plants over one salmon fishing season. Results indicated the oils were good sources of vitamins A, D, and E and there were only minor changes seen in the fatty acid profiles or vitamin levels over the course of the fishing season, though steps may be needed to protect the oils collected early in the season from oxidation. Over the course of a fishing season, these salmon oils are of high and consistent quality.

Reducing the environmental impacts of catfish farming
Catfish aquaculture is known for environmentally sustainable production practices. A set of best management practices (BMPs) was developed to reduce the environmental impacts of catfish farming even further. Practices include water-level management, limiting daily feed inputs, using a low-protein feed, and maintaining a modest fish density. Discharge volume

was reduced by 50% and pollutant discharge reduced by 60%, while groundwater use was reduced by more than 60%. This is the first study to show 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. Components of this plan have been widely implemented into comprehensive sets of environmental best management practices for pond aquaculture.