National Program 106 Aquaculture Annual Report for 2014

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, 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 aim of the ARS Aquaculture Program, as described in the National Program 106 (NP 106) Action Plan: http://www.ars.usda.gov/SP2UserFiles/Program/106/NP106_Action%20Plan%2011-03-08_FINAL.pdf, is to support a safe and affordable domestic supply of seafood products for the 330 million U.S. consumers, that is produced in a healthy, competitive, and sustainable aquaculture sector; a sector supported by more than 3000 aquaculture farmers producing in excess of $1.35 billion worth of goods annually. This year the USDA National Agricultural Statistics Service published the Census of Aquaculture (2013) updating these statistics for the first time since 2005. The report details many features of aquaculture in the United States, and shows that since 2005, the overall number of farms has dropped (from about 4300 to 3090) and the sales have increased from just over $1billion to over $1.37 billion in 2013.

Fiscal year 2014 was the fifth and final year of externally-reviewed five-year project plans (2010-2014). The aquaculture laboratories and the aquaculture scientists were involved all year in developing the new five year plan which has just begun and will run from 2015-2019. The new Aquaculture Action Plan is available at: http://www.ars.usda.gov/SP2UserFiles/Program/106/NP%20106%20Action%20Plan%202015-Final2.pdf. Although these project plans guide most of the efforts of the laboratories, we remain flexible to respond to unanticipated challenges and opportunities. NP 106 research covers the spectrum from fundamental to applied research, and is focused on solving problems through long term high impact research. NP 106 scientists published over 100 articles over the past two years in peer-reviewed scientific journals.

NP 106 People in 2013

During 2014, we added two new scientists to the NP 106 roster, Miles Lange, Ph.D. was hired at the Harry K. Dupree Stuttgart National Aquaculture Research Center, Stuttgart, Arkansas. Miles is a research microbiologist and has extensive research training and experience in immunology. Miles completed his Ph.D. at Kentucky State University and has post-doctoral experience working with primary B-cell development and B-cell immune response to HIV infection and immunization.

Allyn Spears, Ph.D. joined our ARS team at the University of Wisconsin-Milwaukee. Allyn is a research molecular biologist with considerable experience in virology. Allyn came to Milwaukee from a post-doc with ARS at the National Animal Disease Center in Ames, Iowa.

This year we had three retirements. Phil Klesius, Ph.D. retired in January 2014, after an extremely productive 42 years of Federal service with ARS. Phil achieved Super-grade (ST) status as a scientist and served as Research Leader of the Aquatic Animal Health Research Unit and Location Coordinator in Auburn, Alabama, for 26 years. Dr. Klesius' team research on discovery, patenting, and licensing of vaccines for bacteria pathogen control in fish has been recognized repeatedly in technology transfer awards from ARS and the Federal Laboratory Consortium (FLC). Phil retired with 356 publications, book chapters, and proceedings including 177 original and referred publications and 16 patents. Joyce Evans, Ph.D. and Natha Booth, Ph.D. retired from our Stoneville, Mississippi, Warmwater Aquaculture Research Unit (WARU). Joyce started with ARS in 1998 and worked at the Chestertown, Maryland, worksite of the Auburn, Alabama, Aquatic Animal Health Research Unit. In 2012 she transferred to WARU. Throughout her career with ARS, Joyce contributed expertise involving vaccine development, pathogen identification, diagnostic tool development, and immune responses of fish. She was author and co-author on over 100 publications and contributed to 7 patents related to vaccine development. Natha began at WARU as a post-doc in 2006 where she focused on physiological and immunological studies on catfish, and research contributing to the breeding program.

Awards and Recognitions:

Scientists in the Aquaculture National Program were well recognized nationally and internationally over the past year, with successful grant applications for external funds, several cooperative research and development agreements (CRADAs), and many invited presentations.

Dr. Benjamin Beck with the Stuttgart National Aquaculture Research Center, Stuttgart, Arkansas, was selected as the Early Career Scientist of the Year from the Southern Plains Area. Kevin Schrader and co-authors won honors for the best poster at the International Congress of Pesticide Chemistry. Steve Summerfelt, of the Conservation Fund Freshwater Institute, Shepherdstown, West Virginia, is a close collaborator in the Aquaculture program and was recognized with the 2014 "Award of Excellence" by the Aquaculture Engineering Society. The headquarters post-doctoral research associate program award went to Ken Overturf (Hagerman, Idaho) for *Enhancing aquaculture sustainability by improving plant oil utilization* and Peter Bechtel (New Orleans, Louisiana) for *Postharvest processing to add value to catfish*.

National Program 106 involves efforts in 10 different locations on 20 projects performed by approximately 85 scientists (41 ARS scientists and an equal number of collaborating scientists). Technology transfer activities included invention disclosures, 1 new patent application, and 18 new Material Transfer Agreements (MTAs). A number of additional activities to transfer technologies to other scientists and to industry partners were also completed. Among the outstanding examples is the ongoing effort to improve hybrid catfish production at Stoneville, Mississippi, where scientists are working closely with a number of

hatchery operators in the Mississippi Delta region having tremendous success increasing the number of hybrid fry produced across the industry. Another example is the rapid development of numerous fish diets including new ingredients. The Trout Grains project has worked with multiple collaborators to improve, develop, and test new ingredients including pistachio and almond processors, leading to new feed formulations and opportunities for ingredient manufacturers. In addition, the Atlantic salmon industry on the East Coast of the United States and Canada use the improved germplasm developed at the National Coldwater Marine Aquaculture Center, Orono, Maine. Scientists' research on kaolinic clays at Stuttgart National Aquaculture Research Center, Stuttgart, Arkansas, has had far reaching applications. See below for a sampling of some of the top research accomplishments from our scientists during 2014.

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 active 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. Our aim is to form real partnerships that have benefit to the United States and to cooperating countries. These relationships increase the depth of our intellectual capital with original ideas from different perspectives.

Funding: During fiscal year 2014, total funding for aquaculture research at ARS was approximately $30 million. Balancing the need for additional funding with the maintenance of our core mission is a constant challenge and the resulting dialog is a big part of the innovative process.

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

Atlantic salmon evaluated and selected for multiple traits. Commercial salmon producers in the United States use stocks that are not many generations removed from wild, unselected stocks. Because salmon are an endangered species, producers are legally required to culture certified stocks of North American salmon. ARS researchers at the National Cold Water Marine Aquaculture Center in Franklin, Maine, in collaboration with industry, generated a broodstock of fish with North American origin and compared the growth of 4-year classes of salmon from their breeding program with a control line of fish in commercial sea cages. Salmon that were selected for greater growth, resistance to sea lice, and better fillet color, averaged approximately 90 percent larger than the control fish. Using improved salmon germplasm is increasing the cost-effectiveness, profitability, and sustainability of cold water marine aquaculture in the United States and providing a quality seafood product to consumers.

The development of a new tool to speed genetic improvement in rainbow trout. Technology to use genomic information for improving selective breeding in dairy cattle and poultry has not yet been developed for rainbow trout. ARS researchers at the National Center for Cool and Cold Water Aquaculture in Leetown, West Virginia, worked with international partners to develop a commercially available genetic marker tool (SNP chip) for rainbow trout. By incorporating genome information, this tool improves performance prediction accuracy for individual fish versus traditional family-based estimates. Commercial producers will find this information useful and it will accelerate genetic improvements in trout.

Improvement of catfish growth. Producers and processors of farm-raised catfish want to improve catfish growth. ARS scientists at the Warmwater Aquaculture Research Unit in Stoneville, Mississippi, have conducted a selective breeding program over several catfish generations to improve this trait, and showed that catfish in the breeding program were more than 25 percent larger at harvest than catfish that were not in the breeding program. These efforts are part of a long-term breeding program to improve channel catfish germplasm for release to the United States catfish industry.

Powerful genomic resources developed for blue catfish. The blue catfish, *Ictalurus furcatus,* is native to North America and serves as the paternal species of the blue x channel catfish hybrid that is highly valued for U.S. aquaculture production. Little was known about the extent of genetic diversity in blue catfish, which impeded genetic selection/improvement in this species. ARS scientists in Stuttgart, Arkansas, and Stoneville, Mississippi, collaborated with Auburn University researchers to identify genetic diversity between individuals and between populations. The research demonstrated many DNA sequence variants within and between five populations, and revealed potential shared ancestry among populations. This research resulted in highly informative DNA markers useful for parentage and kinship determination in blue catfish and hybrid catfish populations.

Animal Performance, Well-being and Efficiency

Lysine supplementation of fishmeal-free diets influences muscle genes. As part of a strategy to reduce feed costs, research on fish feeds is targeted at reducing reliance on expensive fish meal, and supplement diets directly with limiting amino acids. The mechanisms by which dietary amino acid supplementation affects nutrient utilization and muscle growth in fish is unclear, but elevations in amino acids in the blood after a meal have been shown to stimulate protein synthesis in the muscle. Researchers at the H.K. Dupree Stuttgart National Aquaculture Research Center, Stuttgart, Arkansas, demonstrated that dietary lysine supplementation of a commercial fishmeal-free diet influences the expression of two genes, myostatin, a growth inhibiting gene, and myogenin, a growth promoting gene, that contribute to muscle growth in hybrid striped bass. The relative expression of myostatin to myogenin was highly correlated to muscle protein accumulation. This is an important discovery of genetic markers that may be useful to select hybrid striped bass with improved growth performance on fishmeal-free diets.

Causes of variation in hybrid catfish growth rates. Hybrid catfish grow rapidly but not uniformly. This size variation is undesirable and some processors pay a discounted price for larger and smaller fish. A study was conducted at the Warmwater Aquaculture Research Unit in Stoneville, Mississippi, to examine the effects of size variation of fish on growth rates of hybrid catfish. Small fish raised in an ungraded (mixed sizes) population grow 15% slower than small fish raised in a graded (uniform size) population. Stocking graded fingerlings is an important aspect of producing a faster growing, more uniform sized population of harvest-sized fish.

Pre-spawning stress affects reproductive performance of channel catfish and subsequent progeny performance. Routine handling procedures like seining, transportation, crowding and handling may affect the quality of eggs that are stripped from gravid channel catfish. ARS scientists at the Warmwater Aquaculture Research Unit in Stoneville, Mississippi, determined the effects of pre-spawning handling on the performance of mature channel catfish and of their eggs and subsequent progeny. Gravid channel catfish females were exposed to lowered oxygen levels that are often experienced in commercial culture during warmer weather. Low oxygen stress reduced the hatching success of eggs by 6.3 percent. Furthermore, offspring from treated parents had higher mortality in challenges of enteric septicemia of catfish (ESC) disease (9.6%). This research points to the importance of careful pre-spawning handling procedures to improve reproduction and progeny performance.

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

New processing method to improve nutritional value of ethanol production co-products. The primary method for producing fuel ethanol from grains results in a co-product known as distiller's dried grains with solubles (DDGS), which is a low-value ingredient for animal feeds. The standard method results in distiller's grains of high fiber and low protein digestibility, and only a single product being produced. These problems reduce the total revenue from ethanol production and limit the use of the co-product in animal feeds. To improve the value of this co-product, ARS scientists in Aberdeen, Idaho, determined the fate of many nutrients during traditional processing and devised a new method to recover multiple co-products, including a high-protein feed ingredient, a high-ash fraction for mineral supplementation, an oil fraction, and a glycerol fraction. Greater value of co-products from ethanol production increases the value generated by using renewable energy and provides valuable nutrients for animal production.

Improving water quality and lowering the price of diets for hybrid striped bass. Temperature and ammonia levels (ammonia is a waste product secreted by fish) often increase dramatically in ponds during summer production of hybrid striped bass in the southern United States. Extended periods of high ammonia result in fish stress, disease, mortality, and significant loss of feeding days as producers attempt to reduce ammonia to manageable levels by reducing feeding or using lower protein diets. Along with participants from the Trout-Grains Project, ARS scientists in Stuttgart, Arkansas, and Hagerman, Idaho; and U.S. Fish and Wildlife Service scientists in Bozeman, Montana, demonstrated better

growth and nutrient retention by hybrid striped bass fed a 35 percent protein diet supplemented with limiting amino acids compared with fish fed a higher protein diet. Overall protein levels were decreased, whereas dietary nutrient retention increased, thereby lowering feed costs to producers and reducing negative environmental effects on water quality.

Method to produce a protein concentrate from barley is commercialized. Feeds costs are a significant production item in commercial aquaculture. Barley that is too high in protein to be used for malting has the potential to be developed as a protein-based fish feed. A method was developed and patented by ARS scientists in Aberdeen, Idaho, for producing a protein concentrate that can be incorporated into aquaculture feeds. Feeding studies with rainbow trout and Atlantic salmon demonstrated the concentrate is highly digestible and supports rapid fish growth, reducing the need for more expensive ingredients such as fishmeal. A pilot plant for barley concentrate production has been built and is delivering the product to commercial farms in Idaho. Two more plants are in development.

Improving Health

Development of a vaccination platform to protect catfish against Enteric Septicemia. Enteric septicemia is the most devastating disease affecting the catfish industry. The development of a new vaccine and feed-based delivery platform has resulted in providing exceptional protection against enteric septicemia of catfish with dramatic increases in production efficiency and economic returns. Mississippi State University scientists working in collaboration with ARS scientists developed a mechanized vaccine delivery system that consistently delivered target-immunizing doses in experimental pond trials. The vaccine delivery system was used in commercial field trials during the 2013 production season with excellent results; 2014 production season trials are ongoing. This vaccine will support catfish producers in efforts to control diseases and manage production costs.

Effective vaccine candidates to prevent *Aeromonas* disease in catfish. From 2009 to the present, outbreaks of motile aeromonad septicemia (MAS) in market-size catfish have occurred in western Alabama and eastern Mississippi with losses estimated to be greater than $12 million. Currently, there are no methods available, such as vaccination, to prevent MAS, but they are urgently needed. ARS scientists in Auburn, Alabama, identified secreted extracellular proteins of the bacterium that trigger protective immune responses. Their results show that catfish immunized with the extracellular proteins are resistant to infection and that this immunity persisted for at least 7 weeks. This research provides the foundation for developing an effective vaccine to prevent MAS disease and may help catfish producers contain production losses.

The development of a molecular test for the detection of significant catfish pathogens in environmental and tissue samples. Researchers from Mississippi State University (MSU), Mississippi Agricultural and Forestry Experiment Station (MAFES), and the College of Veterinary Medicine (CVM)), working with ARS, have developed and validated highly sensitive assays for the detection and quantification of numerous pathogens. These assays

provide a reliable method for the detection and quantification of pathogens in pond environments and are being used for rapid diagnostic evaluations and evaluation of treatment efficacies associated with recommended disease management strategies and research. In addition to their diagnostic benefits, these assays provide a means to conduct epidemiological and environmental studies and evaluate how management strategies alter pathogen loading rates in commercial catfish ponds. This novel and proactive strategy is being used to optimize disease management practices aimed at maximizing production efficiencies and economic returns.

Standard protocol for determining *Flavobacterium columnare* type. *Flavobacterium (F.) columnare* is the causative agent of columnaris disease which severely impacts channel catfish production in the United States and is emerging in the rainbow trout industry. The assay previously used to identify different genetic types of *F. columnare* was found to be flawed. Research at the Aquatic Animal Health Research Unit in Auburn, Alabama, developed a protocol for the genetic typing of *F. columnare*. The knowledge obtained from this research allows for the proper assignment of an unknown isolate to a genetic type, which is important because there is an association between genetic type of *F. columnare* and virulence.

Production Systems and Products

Scaling up recirculating aquaculture systems. Although production of Atlantic salmon in land-based recirculating water systems offers an environmentally sustainable approach to meeting domestic demands for seafood, some off-flavors can develop in such systems, and harvest strategies must be developed that ensure the product quality that consumers expect. Researchers at the Conservation Fund's Freshwater Institute in Shepherdstown, West Virginia, working with an ARS researcher in Oxford, Mississippi, determined that concerns about off-flavor in 4-kg Atlantic salmon can be eliminated with a purging step in which fish are transferred to tank systems pre-disinfected with hydrogen peroxide and that do not contain hard-to-clean locations, such as aeration media, during the final 6–10 days of fish rearing. Compounds associated with off-flavor were consistently reduced to levels that are below human tasting limits. These standard operating practices maximize product quality for Atlantic salmon and other species that have been cultured in water recirculating systems.

Minimal impacts of oyster aquaculture to eelgrass at the landscape scale. Submerged aquatic vegetation such as eelgrass provides valuable habitat for fish and invertebrates in estuaries, particularly for juvenile salmon on the west coast of the United States. ARS researchers in Newport, Oregon, used layers of geographic information on tidal heights, cumulative wave stress, salinity, distance to the river mouth, and distance to the nearest channel to quantify the distributions of eelgrass and bivalve aquaculture in Willapa Bay, Washington. The effect of bivalve aquaculture on eelgrass at the landscape scale was measured over a period of 5 years. Although oyster harvest methods had demonstrable effects on eelgrass over time at the individual bed scale, oyster aquaculture reduced eelgrass cover by less than 1 percent in any given year over the entire estuary. This information

promotes sustainable shellfish culture and is enabling managers and regulators to evaluate the potential effects of existing and expanded oyster aquaculture on estuarine habitat.