

# National Program 106 Aquaculture Annual Report for 2017

The **vision** for ARS aquaculture research and technology transfer is *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 is to support a safe and affordable domestic supply of seafood products for 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 \$1.4 billion worth of goods annually. In 2013 the USDA National Agricultural Statistics Service published the Census of Aquaculture 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). However, sales in 2015 increased to over \$1.4 billion.

Fiscal year 2017 was the third year of externally-reviewed five-year project plans (2015- 2019) that fall under the five Components of the National Aquaculture Action Plan which are:

1. Selective Breeding, Directed Reproduction, and Development of Genomic Tools
2. Nutrient Requirements and Alternative Sources of Protein and Lipid
3. Health of Aquatic Animals
4. Sustainable Production Systems
5. Product Quality and New Products

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 73 articles in peer-reviewed scientific journals in fiscal year 2017.

National Program 106 conducts research through nine different locations on 17 projects performed by approximately 41 ARS scientists and University or private cooperators on 9 congressionally mandated agreements.

During fiscal year 2017, ARS base funding for aquaculture research was approximately \$28.3 million, not including \$504K incoming funds from grants and agreements. Technology transfer activities included: 1 new patent awarded, 1 new CRADA, 10 new Material Transfer Agreements (MTAs), and 3 new Material Transfer Research Agreements (MTRAs). Outgoing agreements to research partners/collaborators totaled \$6.7 million.

NP 106 scientists were active in serving on committees and as advisors/mentors for undergraduate and post-doctoral students, and serving as adjunct/affiliate faculty members (Table 1).

**TABLE 1.** NP-106 scientist participation in student engagement activities.

| <b>Student and Other Outreach:</b> | <b>No.</b> |
|------------------------------------|------------|
| Undergraduate students             | 4          |
| Post-docs                          | 9          |
| SYs Serving as Advisors            | 3          |
| Mentorships                        | 2          |
| Adjunct or Other Appointments      | 7          |
| Other Outreach Activities          | 29         |

In 2017, NP 106 scientists participated in research collaborations with scientists in the following countries:

**BELGIUM:** Identifying the host-derived factors that govern the susceptibility or resistance to *Flavobacterium columnare* infection to better understand how this bacterium infects an array of aquaculture species.

**BRAZIL:** Evaluating immune responses and immune gene expression in South American catfish (*Pseudoplatystoma* spp.) after vaccination against fish parasite, *Ichthyophthirius multifiliis*.

**CHINA:** Evaluating efficacy of plant extracts for controlling and preventing parasite *Ichthyophthirius multifiliis* in cultured fish.

**DENMARK:** Providing information on peracetic acid used in aquaculture. Collaboration takes place by phone and email exchanges.

**FRANCE:** Gaining a better understanding of immunity in catfish and ultimately design better vaccines through shared ideas and novel techniques.

**GERMANY:** Studying the toxicity/effectiveness of peracetic acid to fish and the effectiveness of this compound to control, *Ichthyophthirius multifiliis*, *Ichthyobodo necator*, *Flavobacterium columnare* and *Saprolegnia* spp. on fish.

**NORWAY:** Determining the feasibility of selectively breeding Nile tilapia for resistance to *Streptococcus* species.

**SPAIN:** Identifying genotypes and determining the population structure of *Saprolegnia parasitica* world-wide.

## **Personnel in NP 106**

### **New addition to the NP 106 team in 2017:**

**Jason Abernathy**, Stuttgart, Arizona, joined the National Aquaculture Research Center as an Scientist Research Physiologist.

### **The following scientists retired from the ranks in NP 106:**

**Carissa Li**, Food Processing and Sensory Quality Laboratory, New Orleans, Louisiana.

### **The following scientists in NP 101 received prominent awards in 2017:**

**Brian Peterson, Sylvie, Quiniou, Monica Wood and Corrin Flora**, Stoneville, Mississippi, were awarded best “Applied Studies” paper of 2016 for their paper Vaccination of Full-sib Channel Catfish Families against Enteric Septicemia of Catfish with an Oral Live Attenuated *Edwardsiella ictaluri* Vaccine by the World Aquaculture Society (WAS).

**Craig Tucker**, Stoneville, Mississippi, received the 2017 Auburn University College of Agriculture Outstanding Alumni Award; awarded Researcher of the Year at the 2017 Annual Meeting of the Delta Council; received the award for best “Review” paper of 2016 by The World Aquaculture Society for the paper “Alkalinity and Hardness: Critical but Elusive Concepts in Aquaculture”; and was inducted as a Fellow of The World Aquaculture Society.

## RESEARCH RESULTS

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

### **Component 1: Selective Breeding, Directed Reproduction, and Development of Genomic Tools**

#### **Problem Statement 1A: Genomic Tools and Genotype-to-Phenotype**

##### *Atlantic Salmon*

***Improving fillet quality through genomics.*** Improved fillet quality traits, such as color and omega-3 fatty acid levels, will provide economic benefit to Atlantic salmon producers and also potentially increase health benefits to consumers. ARS scientists in Franklin, Maine, determined that the heaviest fish families generally consumed the most feed, had the highest fillet color scores, omega-3 fatty acid concentrations, and that fillet color is a function of feed intake and time. Furthermore, our industry partner has developed markers for fillet color and verified these markers with fish from the National Cold Water Marine Aquaculture Center, Orono, Maine. Using genetic markers developed in collaboration with industry partners, salmon broodstock can now be screened to ensure that the offspring have the markers for the highest retention of carotenoids in the fillet.

##### *Catfish*

***Production of high-throughput channel catfish genotyping platform.*** Efficient selection of genetically superior broodstock depends on the ability to identify DNA sequence variants that are correlated with superior trait performance. In an effort to identify genome-wide DNA sequence variants in channel catfish, ARS scientists in Stoneville, Mississippi, sequenced genomic DNA from the Delta Select strain of catfish and aligned those sequences with the catfish reference genome. Using computer algorithms, the scientists identified 6 million sequence variants in this population and developed a genotyping platform that contained 660,000 probes for potential sequence variants. Subsequent genotyping demonstrated that 75 percent of the probes detected DNA sequence variants in other channel catfish. A result of this research is a platform for interrogating DNA sequence variation throughout the genome, which will support more efficient genetic selection in the Delta Select catfish population.

***Testis development in blue catfish.*** Testes from male blue catfish are used to obtain sperm to fertilize eggs from channel catfish females for production of hybrid catfish fry. Testis development in blue catfish varies greatly between individuals and understanding the sources of this variation will be useful for improving the efficiency of hybrid catfish fry production. ARS scientists at Stoneville, Mississippi, found that blue catfish strain, fish size, and time of the year (early vs. late spring) all affected testis development. The D&B strain of blue catfish had the largest, best quality testes of the strains tested; larger fish tended to have larger, better quality testes; and testis size and quality were better early in the spring than later in the spring. These

results were presented to hybrid catfish producers through workshop presentations and one-on-one meetings to inform their blue catfish broodstock management practices.

### ***Rainbow Trout***

***Improving the rainbow trout genome sequence.*** A high-quality genome sequence is important for facilitating a meaningful understanding of fish biology. ARS researchers in Leetown, West Virginia, worked closely with national and international cooperators to assemble a reliable and high-quality reference genome sequence for rainbow trout. The portion of the new assembly that aligns to chromosome sequences increased from 5% to 88%, dramatically improving the effectiveness of genomic approaches to selective breeding and gene discovery. The new reference genome is available for browsing through the online interactive databases of the National Center for Biotechnology Information. Knowing the rainbow trout genome assembly and chromosome sequences will lead to major improvements in aquaculture genetics and all other aspects of fish quality improvement and fish biology research.

***Fast-growing rainbow trout.*** Fast growth to market-size is one of the most important economic traits in rainbow trout aquaculture that can be improved through selective breeding. ARS researchers at Leetown, West Virginia, selectively bred a pedigreed, commercial-scale rainbow trout population to market weight for five generations. Compared to the unselected control line, body weight in the growth-selected line increased by approximately 12% per generation through 13 months of age, resulting in fish that grow approximately 60% faster to and beyond standard market weight. The improvement in growth over contemporary commercial lines was consistent when fish were grown in different environments. This genetically-improved and highly-characterized population has been released for commercial propagation.

### **Problem Statement 1B: Define Phenotypes and Develop Genetic Improvement Programs**

#### ***Catfish***

***Genomic selection for growth and carcass yield in catfish.*** ARS scientists in Stoneville, Mississippi, in cooperation with scientists at the University of Georgia, employed a new genomic method that can analyze 50,000 genetic markers of the 2000 Delta Select strain catfish. These genetic data, combined with trait measurements from more than 20,000 fish from current and previous generations, were analyzed. The scientists were able to produce genome-based breeding values that demonstrated a 7% increase in accuracy compared with traditional estimates. This is the first use of genomic breeding values in a catfish selection program.

### **Problem Statement 1C: Enhance Aquatic Animal Reproduction**

#### ***Catfish***

***Incubation temperature affects the hatching success and progeny performance of catfish.*** Incubation temperature affects the hatching success and progeny performance of catfish. Channel x blue hybrid catfish are exclusively produced in hatcheries by fertilizing the stripped eggs of induced spawn channel catfish with pooled blue catfish sperm. Hybrid catfish fry production is

inconsistent and variable, hence the need for consistent and increased embryo production is desired to meet the needs of U.S. catfish producers. ARS scientists at Stoneville, Mississippi, evaluated the influence of incubating temperature (to mimic optimum and extreme temperatures in a spawning season) on hatching success and progeny performance. Fertilized eggs were incubated either at 80°F or 90°F and the time to hatch and percent hatching success were lower at the higher water temperature. However, incubation temperature did not affect the survival and progeny performance. This research contradicted an impression by some producers that late-season fry were less viable than early- and mid-season fry.

## **Component 2: Nutrient Requirements and Alternative Sources of Protein and Lipid**

**Problem Statement: Determine Nutrient Requirements and Evaluate the Nutritional Value of Alternative Sources of Protein and Lipid**

### *Bluegill*

***Winter feeding for bluegill, an emerging food fish.*** Demand for bluegill has moved beyond sport fishing to an emerging food-fish market for this and other related Centrarchid fish (bluegill, crappie, and largemouth bass). Little is known about optimum feeding or nutrient requirements of these fish. Fingerling growers lose millions of dollars yearly to a phenomenon called winter mortality. ARS researchers at Stuttgart, Arkansas, and university collaborators, evaluated alternate feeding schedules in commercial stocks of bluegill. Monthly, weekly, and twice weekly feeding schedules were evaluated in cold temperature-controlled tanks using fish survival, growth, and whole-body fatty acid profiles as markers of fish performance. Feeding frequency did not improve fish performance significantly, but body fat profiles suggest diet composition is a more important factor to optimize for future improvements. This collaborative work has motivated commercial Centrarchid growers to rethink feed and feeding practices, and has prompted future research to improve diet formulas, eliminate winter mortality, and enhance production success in this emerging industry.

### *Catfish*

***Alternate feeding regimes for hybrid catfish.*** Rapidly growing hybrid catfish can often exceed the size-range preferred by processors when grown under management practices developed for channel catfish. ARS and Mississippi State University scientists in Stoneville, Mississippi, determined that feeding hybrid catfish to satiation once per week was the best strategy to prevent weight loss and maintain carcass yield in market-size hybrid catfish. This information was provided to catfish feed producers who altered diet compositions and thus lowered feed costs for catfish farmers.

***Reducing feed costs using alternative feed ingredients.*** Soybean meal is the main protein source for pond-raised catfish because of its high-quality protein and balanced essential amino acid profile. However, soybean meal prices have increased dramatically in recent years and at times prices have exceeded \$600/metric ton. ARS and Mississippi State University scientists in

Stoneville, Mississippi, showed that soybean meal could be replaced with other, less expensive dietary protein sources (cotton seed meal, corn distillers dried grains with solubles, corn germ meal, peanut meal, or porcine meat and bone meal) with no effect growth, processing yield, or proximate composition of fillets from channel and hybrid catfish. Based on soybean meal prices at the time of the study, replacing all or part of the soybean meal in catfish feeds reduced feed-ingredient costs by \$32 to \$78/metric ton (a cost reduction of 8 to 20% compared to a soy-based diet). These results provide flexibility in formulating cost-effective diets for pond-raised catfish, especially during periods of high soybean meal prices.

***Reducing feed costs in catfish nursery ponds.*** Nursery pond management practices were evaluated in efforts to reduce feed costs by promoting optimal zooplankton populations to serve as a natural food source during the initial stages of fish development. Previous work from this project demonstrated natural pond biota could support optimal growth of channel catfish fry for 6 weeks without supplemental feed. However unlike channel catfish, noticeable deficiencies in hybrid catfish fry growth were observed by ARS scientists at Stoneville, Mississippi, after only 2 weeks. Although, compensatory growth was observed, and restricting feed did not result in differences in production variables observed 6 weeks post-stocking, delayed feeding of hybrid catfish is not recommended past 2 weeks. Data indicate hybrid catfish do not have to be fed fry diets for the first 2 weeks of development, provided the recommended fertilization schedule is followed to promote optimal pond productivity. At the feeding rate and prices during this study, we observed feed savings of \$38.75/ha by delaying feeding for 2 weeks.

### ***Hybrid Striped Bass***

***Improving palatability for plant-based diets for hybrid striped bass.*** Plant-based ingredients are a sustainable option for protein sources in fish feeds. However, as fish diets become more plant-based, hybrid striped bass reject their flavors and feed intake drops dramatically. ARS researchers in Stuttgart, Arkansas assessed the feed intake of an all-plant diet supplemented with one or more flavor attractants and compared performance with consumption of an unsupplemented plant diet and a well-accepted commercial diet (containing fish meal). The attractants included natural plant and animal extracts, or blends that have increased feed consumption in some land animals. In feeding trials using juvenile hybrid striped bass, several attractants were identified that resulted in a dramatic increase in consumption of the all-plant diet to levels that were comparable to those of fish that ate the commercial diet. This research paves the way for significant advancements in developing all-plant diets for hybrid striped bass that have the potential to save \$30 to \$400 per ton of feed and reduce dependence on fish meal.

***Female white bass females preferentially incorporate fatty acids from their diet into eggs.*** The type of feed fed to commercial broodfish prior to the spawning season, as well as the length of time the feed is fed, tremendously influences the quality, composition, and performance of the resulting eggs and newly hatched fish (fry). In particular, the composition of the dietary fat, i.e., the fatty acid (FA) profile, is one of the most influential factors that determine spawning success. ARS Researchers in Stuttgart, Arkansas, evaluated the fatty acid composition of broodfish white bass ova fed one of six commercial diets which varied significantly in FA composition for 4 and 8 weeks prior to sampling. Fatty acid profiles of eggs from brooders fed each of the six diets

were significantly altered. There were also some fatty acids that were preferentially, and rather uniformly, stored in the eggs even though there were large variations in the availability of those FA in the diets, particularly n-3 polyunsaturated FA (so called "heart healthy" fatty acids) such as docosahexaenoic acid (DHA; 22:6n-3). By formulating broodfish diets using these results, increases in fry survival and performance are expected by providing 1) greater energy stores for developing fry, e.g., monounsaturated fats, as well as 2) building blocks for brain, eye, and nervous system development (e.g., long chain omega 3 polyunsaturated fatty acids).

### ***Rainbow Trout***

***Improved conversion of plant oils to healthy omega-3 fatty acids in fish.*** Fish oil has been a critical ingredient in feeds for carnivorous fishes, but it continues to increase in price and decrease in availability. ARS scientists and their University of Idaho collaborators in Hagerman, Idaho, evaluated the ability of certain fish species to biosynthesize and convert plant oils to omega-3 fatty acids when reared on feeds free of fish meal and fish oil (i.e., marine free). Using genetic selection procedures, the researchers selectively bred these fish, and the animals showed an 11 percent increase in omega-3 fatty acids compared with the previous generation. This strategy raised the fatty acid percentage of EPA and DHA to 6.61 percent, whereas fish that were fed fish oil typically have 18 percent. Continued development of fish strains that do not require fish meal or fish oil, yet maintain the same health benefits and taste would significantly improve the sustainability and economics of aquaculture production in the United States.

***Identification of ash content in algae.*** Algae are a valuable source of both protein and lipid for farmed fish, yet algae are known for having high ash content (some as high as 70% of dry matter). ARS researchers in Aberdeen, Idaho, recently characterized the ash component in algae, documenting that silica-containing materials are important contributors of the ash component for algae, particularly those with high ash contents. Three types of silica materials were identified in algae: cellular structures of non-diatoms, diatom cell walls, and sandy particles of geologic origin. Contamination by diatoms and sandy particulates are the two major contributors to high ash content of algal samples. Several measures were proposed to produce algae with reduced ash content, which will be more suitable for fish feed.

## *Shrimp*

***Improved feed for shrimp.*** Shrimp is one of the largest globally produced aquaculture species, but commercial losses during rearing can be extremely high. Evaluation of a new formulated shrimp feed containing an additive was performed in ponds at eight separate commercial facilities. Shrimp on the ARS feed had an average survival of 82% versus 40% for standard commercial feed. Use of this feed could potentially double production at commercial shrimp facilities with minimal expense.

## *Tilapia*

***Mapping gut microbial communities during tilapia larvae culture with probiotic.*** Bacteria in the gut can have a significant impact on animal health and are heavily influenced by the aquaculture environment. Probiotics are beneficial bacteria that have been demonstrated to improve economically-important traits in fish during continuous administration, such as feed value and enhanced immune response. However, the potential for lasting effects of probiotics administered during early fish development is not fully understood. ARS researchers at Stuttgart, Arkansas, and Hagerman, Idaho, and university collaborators examined the composition of gut bacteria during initial life-stages of tilapia cultured in various environments and with the addition of a probiotic. Probiotic was found to be successfully transferred to tilapia gut via water with high relative abundance and could influence microbial communities. Probiotic was not lasting, however, and did not colonize the gut after discontinuation of administration when transferred to culture conditions. This information is useful for our understanding of the early colonization of gut bacteria along with the persistence of specific populations after probiotic treatment in an effort toward more cost-effective administration practices in aquaculture.

# **Component 3: Health of Aquatic Animals**

## **Problem Statement 3A: Improve Understanding of Host Immunity, Immune System Evasion by Pathogens, and Disease-Resistant Phenotypes**

## *Catfish*

***Catfish feeding practices may impact susceptibility to virulent *Aeromonas (A) hydrophila* (vAh).*** An emerging bacterial pathogen called virulent *Aeromonas hydrophila* (vAh) has been responsible for widespread fish mortalities in the U.S. catfish industry over the last eight years. While the genetic and biochemical understanding of vAh has been greatly enhanced during this period, the environmental or host-derived factors leading to disease outbreaks have remained elusive. Taking cues from observed farm conditions associated with outbreaks, ARS scientists in Auburn, Alabama, in collaboration with Auburn University, evaluated nutritional factors tentatively associated with the vAh disease process. Investigators determined that the time between the last feeding and an experimental infection was a critical driver of catfish susceptibility to vAh. Indeed, fish recently fed and with a full gastrointestinal tract had dramatically lower survival than those in a fasted state where food was withheld for 24 hours prior to the challenge. Taken together, these results not only provide a more robust challenge

model, but offer actionable insights into pond level host-pathogen interactions underlying vAh disease development.

***Sensing of iron by virulent *Aeromonas (A ) hydrophila (vAh) may trigger increased ability to kill catfish.**** Iron is a vital nutrient for bacteria, so it is no surprise that virulent bacterial pathogens are characterized by a vast array of mechanisms by which they can acquire iron from host animals, but also at the expense of other bacteria in their aquatic environment. Among these mechanisms are the production and secretion of siderophores which are small molecules with a high binding affinity for iron that are also regarded as key virulence factors for a number of bacteria. ARS scientists in Auburn, Alabama, in collaboration with Auburn University, determined that vAh possesses a defense mechanism whereby the presence of siderophores from other microbes, such as those in production ponds, constitute a danger signal, alerting the pathogen to the presence of competing bacteria or algae and oncoming iron limited conditions. In response to the presence of competing microbial siderophores, the investigators found that vAh virulence dramatically increased which doubled mortality of catfish in laboratory challenges.

### ***Rainbow Trout***

***A key protein of the VHS virus has been shown to suppress the fish immune response.*** Viral hemorrhagic septicemia virus (VHSV) is a pathogenic fish virus found in locales throughout the northern hemisphere that leads to up-regulation of the host's virus detection response, but the virus quickly suppresses interferon production and antiviral gene expression. Using systematic screening methods, scientists from the University of Toledo, Ohio, the University of Maryland, and ARS researchers in Milwaukee, Wisconsin, identified matrix protein (M) as the most potent host immunosuppressive protein. This protein indicated one mechanism by which the pathogen suppresses the host immune response. This work demonstrates that small changes in the sequence of the VHSV M gene can dramatically affect the interaction of the virus with the host cell, suggesting the individual involvement of the VHSV genome with pathogen virulence. A greater understanding of how viral proteins impact cellular antiviral recognition and response pathways, and how the virus might evade or suppress the host immune response, will enable rational design of methods to combat VHSV infection in commercial finfish species.

***Development of efficient techniques for genetic modification of *Flavobacterium columnare*.*** Columnaris disease is an emerging problem for rainbow trout farmers, causing losses in both fry and harvest-size fish. University of Wisconsin scientists and ARS researchers at Leetown, West Virginia systematically explored genetic components that contribute to the virulence of *Flavobacterium columnare*. By using gene transfer, gene deletion, and complementation, an *F. columnare* mutant deficient for protein secretion was created; this mutant failed to cause columnaris disease in rainbow trout. The understanding of the genetic components that contribute to virulence and toxicity of this pathogen in commercially bred finfish will enable development of safe candidate vaccine strains to treat columnaris disease and reduce losses.

***Development of a new assay for measuring immune system activation in rainbow trout.*** Infectious disease causes appreciable losses in aquaculture, and knowledge of the immune response is incomplete. ARS researchers at Leetown, West Virginia, developed a rapid and standardized assay that simultaneously measured multiple immune genes that were identified

from the recently sequenced rainbow trout genome. Using this assay, fifteen genes were identified that exhibited altered expression following pathogen exposure. Analysis of a commercially-available disease-resistant rainbow trout line developed by ARS, and comparison to a reference susceptible line identified three genes that contribute to a survival difference between lines. These findings have allowed fish health workers to rapidly measure the inflammatory response and identify genes associated with disease resistance, and provides a new means for evaluating fish health on-farm.

### **Problem Statement 3B: Control of Pathogens and Prevention of Disease**

#### ***Catfish***

***A novel vaccine against Enteric Septicemia of Catfish.*** Enteric septicemia of catfish (ESC) is one of the most problematic bacterial diseases affecting the production of channel catfish fingerlings. ARS scientists and collaborators from Mississippi State University in Stoneville, Mississippi, developed an effective vaccine and delivery method against this disease. To date, approximately 180 million stocked catfish have been orally vaccinated in field trials on commercial farms. Improved survival of vaccinated catfish increased the average value of farm production by \$1,800-\$2,500 per acre.

***Naturally occurring non-antibiotic compounds kill bacterial fish pathogens.*** Fish producers are eager for new non-antibiotic strategies to prevent and treat costly diseases on farms. Three of the most problematic pathogens of the United States farmed catfish industry are *Aeromonas hydrophila*, *Edwardsiella ictaluri*, and *Flavobacterium columnare*. ARS scientists in Auburn, Alabama, evaluated the antibacterial activity of two naturally occurring non-antibiotic compounds called chitosan and chitosan oligosaccharide lactate, which are derived from the shells of invertebrates such as shrimp. The ARS investigators found that both of these compounds were effective at halting bacterial growth and directly killing bacteria in laboratory tests. It was determined that these compounds act by coating bacteria and disrupting the protective barrier function of their outer membranes. These findings are a crucial first step in developing strategies to exploit the antibacterial activity of chitosan-based approaches for combating disease outbreaks in aquaculture.

***Development of a recombinant protein vaccine to protect catfish against columnaris disease.*** *Flavobacterium (F.) columnare* is the causative agent of columnaris disease which severely impacts channel catfish production in the United States. ARS researchers in Stuttgart, Arkansas, had previously identified *F. columnare* proteins which activate the adaptive immune response in fish. A new recombinant protein vaccine has been developed resulting in excellent immune protection against columnaris disease when subjected to a laboratory disease challenge. The ARS scientists will build on this research and continue to both optimize and develop effective recombinant protein-based vaccines to prevent columnaris disease and help catfish producers reduce overall production losses, potentially increasing profitability, and thereby allowing for industry expansion.

***Identification of genomic variation linked to resistance to Proliferative Gill Disease.*** There is no vaccine against *Henneguya (H.) ictaluri*, the organism that causes Proliferative Gill Disease

(PGD) in catfish. Because blue catfish are more resistant than channel catfish to PGD, ARS scientists at Stoneville, Mississippi, evaluated genetic variation in PGD resistance in a backcross family produced by mating a channel x blue catfish F1 hybrid female to a channel catfish male. Fish were challenged with *H. ictaluri* in a pond. Variation in PGD resistance among the offspring was correlated with their genotype. The analysis revealed two chromosomal regions associated with PGD resistance that explained 20% of the resistance trait variation. Refinement of these loci will provide targets for marker-assisted selection.

***Rotation of channel and hybrid catfish in ponds reduces occurrence of disease.*** Infections of the parasite *Henneguya (H.) ictaluri* lead to Proliferative Gill Disease (PGD) in channel catfish and significant losses to producers. Mississippi State University research previously found that channel x blue hybrid catfish were a dead-end host in the *H. ictaluri* life cycle, thus preventing propagation of this parasite in ponds. ARS scientists at Stoneville, Mississippi, demonstrated significant reduction in *H. ictaluri* numbers and incidence of PGD reduced in channel catfish grown in ponds that had previously contained hybrid catfish, rather than channel catfish, through a production cycle. These results have led to field trials on commercial catfish operations as a method of reducing losses associated with PGD. This data has been presented at scientific meetings and producer workshops.

### ***Rainbow Trout***

***Development and commercialization of a Lactococcus vaccine for rainbow trout.*** *Lactococcus garvieae* infection is a major cause of on-farm losses of rainbow trout in the state of Washington. ARS researchers at Leetown, West Virginia, successfully developed a vaccine against *L. garvieae* and validated the safety and efficacy of a commercially-manufactured version of the vaccine. Field evaluation results demonstrated that vaccination induced a strong antibody response and robust protection against experimental pathogen exposure. Mortality was dramatically reduced the first year after vaccination, and the disease has not been detected in vaccinated fish since program initiation. The commercial vaccine is in large-scale use at affected farm sites with 6 million fish vaccinated since 2015. The rapid development and implementation of a *Lactococcus* vaccine prevented substantial rainbow trout losses due to this emerging disease.

***Copper sulfate controls fungus on rainbow trout eggs.*** Fish hatcheries often experience egg mortality resulting from *Saprolegnia*, a fungus, prompting producers to communicate the need for inexpensive and effective control strategies. A range-finding study by ARS researchers who work in Stuttgart, Arkansas, and Leetown, West Virginia, determined that treating rainbow trout eggs daily with copper sulfate at a concentration of 10 parts per million until the eyes develop will prevent the fungus from growing and destroying the eggs. This strategy will provide direct benefits by resulting in higher survival rates in rainbow trout hatcheries.

### ***Tilapia***

***Breeding for disease resistance in Nile tilapia.*** Worldwide tilapia aquaculture is valued at about U.S. \$8 billion with a U.S. industry producing nearly 30 million pounds per year. However, production is hindered by two bacteria, *Streptococcus iniae* and *S. agalactiae*, which are responsible for around \$1 billion in annual losses. ARS scientists in Auburn, Alabama, in

collaboration with industry partners verified that response to infection with *S. iniae* is heritable, therefore selective breeding of superior individuals resulted in increased disease resistance of progeny. They also demonstrated that resistance to *S. agalactiae* was also heritable. Industry partners are now including selection for resistance to the two *Streptococcus* sp. in their breeding program along with increased harvest weight for a fast growing fish with reduced risks of disease. The improved tilapia are being sold throughout the Americas and abroad. Based on current production statistics and available models, representative gains from growing the improved tilapia on an average sized farm are U.S. \$635,000 in additional revenue. This research not only helps U.S. fish farmers, but paves the way globally for reducing antibiotic use on farms leading to safer products entering the United States.

## **Component 4: Sustainable Production Systems**

### **Problem Statement 4A: Improve Technologies for Recirculating and Flow-through Production Systems**

#### *Atlantic Salmon*

*Establishing safe limits for environmental parameters in Atlantic salmon recirculating aquaculture systems.* Determining optimal environmental parameters for raising Atlantic salmon in water recirculation aquaculture systems is critical to supporting the growth of land-based salmon production in the United States. ARS extramural researchers in Shepherdstown, West Virginia, defined the safe upper limit of nitrate-nitrogen that Atlantic salmon can be exposed to without negative effects on growth performance, survival, or welfare indicators. Nitrate-nitrogen is an end-product of biofiltration that accumulates as the level of water reuse increases. Researchers also identified optimal levels of dissolved oxygen and carbon dioxide and described ideal swimming speeds that produce the best Atlantic salmon performance. Collectively, these findings defined acceptable ranges for water quality information that producers depend on.

### **Problem Statement 4B: Enhance Control of Pond-Based Ecosystems to Maximize Production and Product Quality**

#### *Catfish*

*Limits to intensive production of catfish in ponds.* It has been assumed that at very high fish stocking and feeding rates, ammonia would become limiting. Recent studies by ARS scientists in Stoneville, Mississippi, using catfish stocking rates up to 36,000/acre now question that assumption. Data indicate that at high aeration rates, high feeding rates do not result in high ammonia concentrations. Farmers are advised that intensive production limits may be determined by aeration capacity and general economic considerations, but issues with ammonia concentrations should not be a factor.

## ***Hybrid Striped Bass***

### ***Hybrid striped bass advanced fingerlings produced successfully in biofloc production system.***

Stocking an advanced, i.e., larger, hybrid striped bass (HSB) fingerling into production ponds improves fish yield and survival, but production of advanced fingerlings has not been optimized. During advanced fingerling production, a certain percentage of the HSB fingerling population gains an early growth advantage and becomes cannibalistic towards conspecifics. These larger, cannibalistic HSB must be sorted from the population before advanced fingerlings can be stocked for grow-out to market size. Biofloc technology production systems can be used as a water quality management tool to increase fish productivity per unit volume of water because the community of microorganisms suspended in the water quickly consumes the waste excreted by the intensively fed fish. However, HSB had never been grown in the biofloc production system. Researchers at Stuttgart, Arkansas, quantified the relationship between advanced fingerling yield and stocking rate in an outdoor intensive biofloc system. High yields and survivals of advanced HSB fingerlings were obtained at all stocking rates tested; additionally, the proportion in the population at harvest of larger, cannibalistic fingerlings decreased from 40% to 6% as stocking rate increased. This accomplishment demonstrates, for the first time, that advanced HSB fingerlings can be produced in the biofloc production system and paves the way for HSB farmers to intensify production of advanced fingerlings.

## ***Tilapia***

***Dietary protein can be reduced in tilapia biofloc production.*** There is a trade-off between feeding for high fish yields vs. water quality deterioration in all aquaculture production systems. This is because fish only convert a portion of the protein in their feed into edible flesh. Feed protein not converted to flesh is excreted as waste that deteriorates water quality in the system and can become toxic to the fish. Biofloc technology production systems, on the other hand, contain not only the fish being grown, but a community of microorganisms suspended in the water that quickly consume fish waste, thus removing it as a threat to the fish. Also, certain fish like tilapia, may be able to get some of their nutritional needs by eating the biofloc. Researchers at Stuttgart, Arkansas, quantified the relationship between diet formula, particularly diet protein content, and tilapia yield and water quality in an outdoor intensive biofloc system. Fish were raised to market size on considerably less protein (5% less than stated protein needs) than previously thought while maintaining better water quality and optimum diet nutrient retention in the fish. This diet formulation represents a good trade-off between economic and environmental risks and maximum production potential for intensive tilapia farmers.

## **Problem Statement 4C: Develop Shellfish Systems to Maximize Productivity and Environmental Compatibility**

### ***Pacific Oyster***

#### ***Underwater video platform to quantify fish and crab use of intertidal longline oyster***

***aquaculture beds.*** Regulations developed by management agencies currently constrain new or expanded shellfish aquaculture operations because they are designed to protect eelgrass as valuable estuarine habitat for fish and crab. However, these policies do not consider aquaculture

as habitat and instead simply minimize its potential effect on eelgrass. The shellfish aquaculture industry has at least in part shifted from on-bottom culture operations to using off-bottom structures (longlines, floating bags) in order to minimize impacts to eelgrass. Off-bottom culture structures are impossible to sample for mobile fish and crab with most traditional sampling methods which use nets. ARS researchers in Newport, Oregon, and project collaborators at the Pacific Shellfish Institute designed underwater video assessment methods to evaluate the functional value of this off-bottom culture as habitat and compared it to that for adjacent eelgrass. Examination of video allowed for behavioral observations (e.g feeding, taking refuge) which can be used to examine the function of these habitats for these aquatic animals.

## **Component 5: Product Quality and New Products**

### *Catfish*

*Evaluation of catfish texture.* Methods for determining textural differences between catfish fillet samples were needed in order to evaluate variation resulting from differences in fish gender, geographical location, harvest season, and processing. ARS scientist at New Orleans, Louisiana, developed methods to analyze the texture of cooked catfish fillets using both a trained sensory panel (7 attributes) and a mechanical texture analyzer (6 attributes). Catfish fillets from processors in Mississippi and Alabama were collected and differences in several texture attributes were found with a greater difference seen in male fillets for female fillets. Correlations were found between several sensory and mechanical texture attributes; validating the much faster and cheaper mechanical texture measurements. This information will be used by the catfish industry to improve the quality of U.S.-grown catfish.