National Program 106 Aquaculture Annual Report for 2019

The **vision** for ARS aquaculture research and technology transfer is *to enable science-based use of our natural resources to meet the seafood demands of a growing global population.*

Mission: The mission of National Program (NP) 106, Aquaculture, is to conduct research and deliver technologies that improve domestic aquaculture production efficiency and product quality while minimizing impacts on natural resources.

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 almost 3000 aquaculture farmers producing more than $1.5 billion worth of goods annually. In 2019 the USDA National Agricultural Statistics Service (NASS) published the 2018 Census of Aquaculture updating these statistics for the first time since 2013. 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 3093 to 2932 from 2013 to 2018). However, sales in 2018 increased to over $1.51 billion from 2013 level of $1.37 billion.

Fiscal year 2019 was the fifth and final year of externally-reviewed five-year project plans (2015- 2019) that fall under the five Components of the 2015 – 2019 National Program NP 106 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; and
5. Product Quality and New Products

In 2019 NP106 conducted research through nine different locations on 18 projects performed by approximately 53 ARS scientists and University or private cooperators on 10 congressionally mandated agreements. During fiscal year 2019, ARS base funding for aquaculture research was approximately $37 million, not including approximately $650K from grants and agreements.

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. The 2020-2024 NP106 Action Plan was approved in 2018 and amended in 2019 in response to new funding provided for research in warmwater marine finfish. The new components of the 2020-2024 Action Plan (available here) are:

1. Improving the Efficiency and Sustainability of Catfish Aquaculture
2. Improving the Efficiency and Sustainability of Salmonid Aquaculture
3. Improving the Efficiency and Sustainability of Hybrid Striped Bass Aquaculture
4. Enhancing Shellfish Aquaculture
5. Developing Marine Finfish Seedstocks

In 2019 NP106 scientists developed thirteen new project plans that align with one or more components of the 2020-2024 NP106 Action Plan. Each plan was reviewed by one of four external peer-review panels and rated as requiring Minor Revision (9), Moderate Revision (2) or Major Revision (2). These new projects have

been revised to address reviewer comments and will be initiated in fiscal year 2020. In 2019 the Congress provided new funding for warmwater marine finfish research, these funds were used to support a cooperative agreement with the Florida Atlantic University-Harbor Branch Oceanographic Institute. In the future three new ARS scientists will be hired and hosted on their campus.

2019 NP 106 Technology transfer activities are summarized in **Table 1** below.

Mechanism	# New
Peer Reviewed Journal Articles	78
Trade Journals	2
Book/Book Chapters	5
Material Transfer Research Agreements	1
Material Transfer Agreements	11
Invention Disclosures*	2
New Patent Applications Filed	1

NP 106 scientists were also active in serving on committees and as advisors/mentors for undergraduate and post-doctoral students and serving as adjunct/affiliate faculty members as outlined in **Table 2** below.

Undergraduates	Graduates	Post-Docs	Scientist Advisors	Mentors	Adjunct Professors/Other
1	3	8	5	4	9

NP 106 scientists were active in with student-related outreach activities as summarized in **Table 3** below.

	Hosting Use of Facilities and Equipment for Educational Purposes	Presentation to Schools	Science Fair Participation	Student Tours/Visits to ARS Locations
Number of Activities	1	4	3	17
Number of Students	30	329	50	1249

NP 106 scientists were also active in with outreach to stakeholders and the public summarized in **Table 4** below.

Type of Activity	Number of Activities	Number of Participants
Field Day	1	200
Tours	10	226
Presentation to Local/Community Groups	5	119
Career Fairs	2	52
Webinars	1	18
Presentation to Other Scientists	10	828
Presentation to Practitioner/Industry/Producer	2	160
Workshops	2	89
Stakeholder Meetings	1	20

In 2019, NP 106 scientists participated in research collaborations with scientists in the following countries:

BELGIUM:

- Conducting collaborative research with Faculty of Veterinary Medicine at Ghent University to identify the host-derived factors that govern susceptibility of fish to columnaris disease.

- Working with scientists at Ghent University Veterinary School to study how biofilms are formed by different clinical isolates of *Flavobacterium columnare* to better understand how this bacterium infects an array of aquaculture relevant species, and through gene expression studies which coincide with the formation of *F. columnare* biofilms.

CHINA:

- Working with researchers from Nanchang University to evaluate the metatranscriptomic data from the microbiome of the selected and unselected fish reared on either plant or fishmeal-based diets, analyzing the metatranscriptomic data and running the comparison with proteomic and microbiome data taken from these fish.

- Conducting collaborative research with Jinan University to evaluate and develop effective parasiticides for controlling and preventing parasite *Ichthyophthirius multifiliis* in cultured fish and study fish immune responses against parasites.

DENMARK:

- Collaborating with scientists at the Technical University of Denmark to provide information on peracetic acid used in aquaculture.

FRANCE:

- Collaborating with the French Research Institute for Exploration of the Sea (IFREMER) to use the IFREMER assay test to conduct resistance screening to OsHV 1 µvar in juvenile oyster spat.

- Conducting collaborative research to evaluate clays for treating or preventing bacterial diseases of fish and shellfish including *Aeromonas hydrophila*, *Flavobacterium columnare*, and *Vibrio parahaemolyticus*.

- Collaborating with the National Institute for Agricultural Research to gain a better understanding of immunity in catfish and ultimately design better vaccines through shared ideas and novel techniques.

GERMANY:

- Collaborating with scientists at the Leibniz-Institute of Freshwater Ecology and Inland Fisheries to study 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.

JAPAN:

- Collaborating with the Japan Fisheries Research and Education Agency to examine the effects of recent changes in ocean climate on oyster condition, gametogenesis and spawning in both countries.

NORWAY:

- Conducting collaborative research with Akvaforsk Genetics Center to determine the feasibility of selectively breeding Nile tilapia for resistance to *Streptococcus* species and other tilapia pathogens.

- Collaborating in the CtrlAQUA project, 7-year research initiative run by Nofima, to make closed-containment aquaculture systems a reliable and economically viable technology and identify biological solutions for producing Atlantic salmon.

PORTUGAL:

- Collaborating with researchers from the University of Coimbra to analyze tissue and plasma samples from the ARS and control lines of rainbow trout to determine the physiological mechanisms responsible for the enhanced utilization of plant-based feeds.

SPAIN:

- Collaborating with the Department of Mycology, Real Jardín Botánico to identify genotypes and establish the population structure of *Saprolegnia parasitica* world-wide.

THAILAND:

- Conducting collaborative research with Chulalongkorn University to develop and validate new molecular assays for detecting the fish pathogen *Flavobacterium columnare* and determine the genotypes of this bacterium in Thailand aquaculture species.

UGANDA:

- Working with researchers from the National Agricultural Research Organization for Aquaculture Research and Development Center to assist in the design and testing of diet formulations for tilapia incorporating locally available protein sources.

PERSONNEL

In 2019 the following NP106 scientists retired:

Dr. Eugene (Les) Torrans, Research Fish Biologist, Warmwater Aquaculture Research Unit, Stoneville, Mississippi.

Dr. Craig Tucker, Research Leader, Warmwater Aquaculture Research Unit, Stoneville, Mississippi.

The distinguished record of these scientists is recognized world-wide and they will be missed.

The following scientist in NP 106 received prominent awards in 2019:

Dr. Benjamin Beck received an award for the Best Paper in the *North American Journal of Aquaculture* 2018 for his article entitled "*Influence of Kaolin Clay on Aeromonas hydrophila Growth, Chemotaxis, and Virulence to Channel Catfish*" by Mediha Yildirim-Aksoy, Haitham Mohammed, E. Peatman, S. Adam Fuller, and Benjamin H. Beck (Volume 80 pages 427-435).

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

Catfish

Sex determination in channel catfish. Channel catfish have an XY sex determination system in which XY fish are male and XX fish are female, but the gene controlling sexual differentiation is unknown. ARS scientists in Stoneville, Mississippi, and scientists at Auburn University determined that disruption of the BCAR1 gene in genetic males leads to a female phenotype. These results will be used to develop accurate markers to identify genetic sex at an early age and provide a target for identification of the gene in blue catfish determining sex. As the paternal contributor in hybrid catfish, culture of only blue catfish males would increase the efficiency of hybrid catfish production.

Oyster

Sequencing the eastern oyster genome. Genomic resources will speed selective breeding strategies that can keep pace with industry priorities and consumer demands. In collaboration with the Eastern Oyster Genome Consortium, ARS researchers produced a high-quality, chromosome-level genome assembly for the eastern oyster. Using the reference genome, ARS scientists and university colleagues identified millions of polymorphic markers distributed throughout the genome. These markers will facilitate the development of high-throughput genotyping tools that will be used to investigate the genetic basis of commercially important traits.

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

Catfish

Comparison of growth and carcass yield of Delta Select and Delta Control strains of channel catfish. Improved catfish germplasm will allow U.S. catfish farmers to reduce their production costs and remain competitive in the global seafood market. ARS scientists in Stoneville, Mississippi, initiated a selective breeding program to develop the Delta Select strain of channel catfish, which demonstrates a superior growth rate and meat yield, traits that are important to catfish producers and processors. A series of performance trials were conducted to compare the growth and meat yield of the Delta Select strain with those of the Delta Control strain, an unselected strain representative of channel catfish currently being grown by U.S. farmers. The Delta Select strain grew 30 percent faster and had 0.25 to 0.80 percent higher meat yield than the Delta Control strain, demonstrating that selection has improved both traits in the Delta Select strain. Approximately 150,000 2-year-old Delta Select strain channel catfish will be available for release to farmers during fiscal 2020 to allow U.S. catfish farmers to be more efficient and profitable.

Blue catfish germplasm for release to U.S. catfish farmers. Over the last 15 years, U.S. catfish production has shifted from predominant use of purebred channel catfish to the production of F1 hybrids between channel catfish and blue catfish. ARS scientists in Stoneville, Mississippi, established the most diverse collection of blue catfish in existence and initiated evaluations of these strains for purebred blue catfish and hybrid catfish performance. Initial research revealed that purebred and hybrid progeny of the Rio Grande strain of blue catfish showed superior growth and meat yield relative to other blue catfish strains. Approximately 10,000 4 to 6-year-

old Rio Grande fish, 20,000 2-year-old Rio Grandes, and 100,000 Rio Grande fingerlings will be released to farmers during fiscal 2020.

Rainbow Trout

Large scale commercial use of trout selected for plant protein diets. Fishmeal is a limited protein source that is increasing in cost. Sustainable aquaculture production requires the development of feeds formulated with alternative protein sources, of which plant proteins are the most abundant and cost effective. ARS researchers in Aberdeen, Idaho, selected rainbow trout for increased growth on aquaculture feeds in which plant proteins have completely replaced dietary fishmeal. Non-selected trout reared on the plant-based feed develop the intestinal condition enteritis. However, the selected fish fed the plant-based feed do not develop enteritis and demonstrate improved performance, compared to conventional commercial trout strains fed fishmeal-based diets. In 2019 the third largest rainbow trout producer in the United States stocked 1 million of these selected fish in production net pens for commercial production.

ARS trout germplasm selected by a U.S. producer for use in commercial egg sales and production. Most rainbow trout farmers do not manage their own broodstock, but instead purchase eggs for production from outside sources. Riverence, the second largest commercial egg retailer in the United States obtained trout germplasm noted for growth and utilization of plant protein feed from ARS researchers in Aberdeen, Idaho, and is now selling eggs from these lines. The company is expressly marketing eggs from the ARS line as being hardier and has demonstrated their improved growth rate under different environmental conditions compared with eggs supplied by other egg vendors in the United States and abroad. In addition, the company is the second largest commercial producer of rainbow trout and uses ARS germplasm almost exclusively in its production farms.

Selective breeding improves resistance to bacterial cold-water disease and columnaris disease. Antibiotics are routinely used to control these diseases of rainbow trout because few alternative control strategies currently exist. ARS researchers in Leetown, West Virginia, evaluated the genetics of resistance to both diseases in two rainbow trout populations. Resistance was found to be heritable and favorably linked, suggesting that a rainbow trout's resistance to both diseases is due, at least in part, to the same genes. Based on these studies, molecular genetic techniques are now being used to identify the actual genes that affect disease resistance. Commercial breeders who select rainbow trout strains for their improved resistance to only one of the diseases can expect to reduce the impacts of both diseases in their fish populations.

Improved growth performance in triploid rainbow trout. Rainbow trout are diploids (possess two copies of each chromosome) like terrestrial livestock, but unlike terrestrial livestock they are tolerant to triploidy (three copies of each chromosome). Triploid rainbow trout are reproductively sterile and are used extensively to avoid negative impacts of sexual maturation on performance and to avoid their breeding with native populations. However, this sterility complicates selective breeding programs because genetically-superior triploids cannot be used to produce offspring, and scientists have been uncertain if breeding for improved diploid growth performance also results in improved triploid performance. ARS researchers in Leetown, West Virginia, evaluated long-term growth performance of diploids and triploids from a growth-selected line and an unselected control line. They demonstrated that selection on diploid growth performance is effective for improving triploid growth performance, thereby simplifying commercial breeding programs that market triploid rainbow trout.

Atlantic salmon

Improved North American Atlantic salmon germplasm. Commercial salmon farming in the United States is expected to increase 5-fold over the next 3 years, and an Atlantic salmon breeding program is needed to support this industry expansion. ARS researchers in Franklin, Maine, developed a selection index in the St. John River strain of Atlantic salmon for carcass weight, fillet color, the conversion of α-linolenic acid into the omega-3 fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), and resistance to sea lice. Eggs from the improved strain have been provided to industry stakeholders for integration and propagation on commercial farms. Development of salmon germplasm with increased growth, enhanced processing characteristics, and disease resistance will improve the production efficiency and sustainability of the U.S. salmon industry.

Problem Statement 1C: Enhance Aquatic Animal Reproduction

Catfish

Blue catfish sperm cryopreservation. The F1 hybrid between the blue and channel catfish represents 75 percent of current U.S. farm-raised catfish production. However, the blue male catfish must be sacrificed to obtain sperm for use in hybrid production. ARS scientists in Stoneville, Mississippi, in cooperation with ARS scientists in Fort Collins, Colorado, and Louisiana State University, established a collection of cryopreserved blue catfish sperm. This collection is a crucial component of efforts to produce improved blue catfish germplasm for release to U.S. catfish farmers. Currently sperm from approximately 300 blue catfish males has been cryopreserved and is used for breeding.

Electrosedation reduces catfish handling stress and improves post-spawning survival. Channel x blue hybrid catfish are increasingly raised in commercial catfish ponds in the Southeastern United States because of superior production traits. Obtaining eggs for hybrid production requires unavoidable handling stress on channel catfish and can contribute to high losses of broodfish after spawning. Only one FDA approved chemical sedative, Tricaine Methanesulfonate (MS222) is used to reduce physical damage and handling stress for routine procedures. Under farm conditions, broodfish are often exposed to higher concentrations and held for a longer duration in sedative solution than required. ARS scientists in Stoneville, Mississippi, collaborated with scientists at the University of Arkansas to identify effective parameters for electrosedation of catfish broodstock. The scientists found that electrosedation of mature channel catfish was as effective as MS222 sedation but avoided bioaccumulation of MS222 and provided a more controlled exposure than MS222. Field testing showed that catfish producers preferred using electrosedation to improve broodstock survival and reduce losses due to handling stress.

Component 2: Nutrient Requirements and Alternative Protein and Lipid Ingredients

Problem Statement 2A: Determine Nutrient Requirements and Evaluate the Nutritional Value of Alternative Sources of Protein and Lipid

Rainbow Trout

Improved methods for removing anti-nutritional factors from soybeans. Soybeans are an important oilseed source and provide edible oil, defatted protein meals, and related products to the food and feed industries. However, soybeans contain antinutritional trypsin inhibitors (TI), which can cause digestive and metabolic diseases and retard growth in animals. It is vitally important to have an analytical method that can accurately measure TI levels in soybean products. ARS researchers in Aberdeen, Idaho, developed two improved methods that give more accurate results with less variation and reduced reagent usage. The two methods can be used for measuring TI levels in soy products and many other TI-containing products as well.

Component 3: Health of Aquatic Animals

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

Rainbow trout

The pathogen *Yersinia ruckeri* can sense its host. Disease-causing bacteria have evolved systems to recognize their hosts and respond by turning off functions that might trigger an immune response. ARS researchers in Leetown, West Virginia, demonstrated that the pathogen *Yersinia ruckeri* shuts off production of the flagellum when it senses its rainbow trout host. The flagellum is a whip-like structure that bacteria use for locomotion, but it also is a potent immune stimulator. By creating a mutant *Yersinia ruckeri* strain that cannot shut off flagellum expression, the researchers demonstrated that absence of the flagellum during infection is critical for the bacteria to avoid recognition and subsequent destruction by the fish's immune system. This work provides a better understanding of the factors leading to infection and will guide development of new vaccines for disease control.

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

Catfish

Waterborne exposure to select clay minerals protects catfish against virulent *Aeromonas hydrophila* infections. *A. hydrophila* is one of the most widespread bacterial pathogens affecting freshwater fish, and a new strain has severely impacted the catfish industry over the last decade. ARS scientists in Auburn, Alabama, evaluated the effect of treatment with kaolin, an inert clay, for controlling *A. hydrophila* outbreaks. Tests revealed that kaolin clay significantly blocked the movement and binding ability of *A. hydrophila* to catfish mucus. Kaolin treatment at a level of 1 gram of Kaolin in 1 liter of water to a significant improvement in survival (66.7 percent) of experimentally infected catfish compared with survival (28.9 percent) among untreated fish. Kaolin treatment did not alter the growth of *A. hydrophila*, but bacterial levels in test suspensions were significantly reduced within 15 minutes after kaolin treatment, indicating the rapid formation of complexes that settle between kaolin and bacteria. These findings suggest that integrating kaolin into some production settings may be beneficial, particularly in scenarios where the use of antibiotics is not possible, or when it is likely that an *Aeromonas* outbreak could occur following stressors such as grading, stocking, or transport of fish.

Iron fortified diets to control catfish anemia. Ever since the catfish industry began, it has been plagued by catfish anemia (CCA), a disease of unknown etiology that costs the industry between $5-10 million annually. Clinically, these fish are often lethargic and show signs of respiratory distress in the face of adequate dissolved oxygen concentrations. ARS collaborators in Stoneville, Mississippi, developed a cost-effective treatment that is being employed throughout the catfish industry. Initial clinical trials demonstrated diets fortified with iron increase red blood cell production in anemic fish. The use of iron supplements was validated in field trials and was proven effective in stopping and preventing the development of catfish anemia. As a result, catfish operations with recurrent anemia have begun using diets fortified with modest ferrous sulfate levels to promote red blood cell production. To date this practice has resulted in a dramatic decrease in the incidence of CCA.

A vaccine protects catfish against motile Aeromonas septicemia. Outbreaks of motile Aeromonas septicemia (MAS) in West Alabama and East Mississippi have cost U.S. catfish aquaculture an estimated $60-70 million due to death, lost feeding days and costly chemical and antibiotic treatments. Control of virulent *Aeromonas hydrophila* (vAh) is problematic because fish kills on farms are often rapid and the mortality is typically seen in larger and valuable market-sized fish. Little time is available to initiate antibiotic therapy and the withdrawal period after antibiotic feeding requires additional time and economic input prior to harvest. Alternative control strategies such as vaccination are desperately needed at the farm level. ARS scientists in Auburn, Alabama,

designed and evaluated the effectiveness of a simple vAh bacterin (killed vaccine) delivered via immersion to hybrid catfish. Results demonstrated strong protection of hybrid catfish for at least 7 weeks following vaccination with this simple preparation against 2 vAh strains.

Component 5: Product Quality and New Products

Problem Statement 5A: Product Quality and New Products

Catfish

Spatial analysis of fat deposition in catfish fillets. The compounds responsible for catfish off-flavor and yellow off-color are very soluble in fat, so the distribution of fat across the catfish fillet is important in detecting and treating flavor and color quality issues. Rapid methods to determine spatial fat distribution in fillets did not exist. ARS researchers in New Orleans, Louisiana, developed a new method that uses time-domain nuclear magnetic resonance (TD-NMR) to overcome these limitations. The TD-NMR method allowed a smaller sample to be analyzed, which provided a more focused map of the fat content within the fillet. Also, the analysis time was reduced from days in the previous method to seconds for each point in the map. The new method is non-destructive, so samples can be used for other studies, such as color and possibly off-flavor analysis. The spatial analysis of fat and its correlation with off-flavor or color can help flavor checkers and processors identify which fillet region is best for determining quality problems or how to more efficiently trim the fillet.