USDA ARS National Program 106 Accomplishment Report 2018 – 2022

Executive Summary

In 1980 Congress declared through the National Aquaculture Act "… that aquaculture has the potential for reducing the United States trade deficit in fisheries products, for augmenting existing commercial and recreational fisheries, and for producing other renewable resources, thereby assisting the United States in meeting its future food needs and contributing to the solution of world resource problems. It is, therefore, in the national interest, and it is the national policy, to encourage the development of aquaculture in the United States." The USDA ARS National Program (NP) 106 – Aquaculture conducts research and transfers technologies to facilitate the economically and environmentally sustainable expansion of the U.S. domestic aquaculture industries producing finfish, shellfish, and seaweeds.

This report summarizes achievements of NP106 scientists during fiscal years 2018 – 2022. Research in fiscal years 2018 and 2019 fell under the previous National Program Action Plan with Components and Problem Statements focusing on scientific disciplines such as breeding, nutrition, health, and production systems. The 2020 update of the Plan primarily aligns Components by species, with Problem Statements focusing on improving production efficiency, health, and product quality. In response to new funding and guidance provided by Congress, this plan was amended in 2019 and 2021 to reflect new projects in warmwater marine finfish and aquaponics, respectively. As resources for each Component allow, research themes include genetics and breeding, genomics, growth and reproductive physiology, nutrition, health, product quality and production systems. This report aligns scientific achievements under the framework of the amended National Program Action Plan for Aquaculture 2020 – 2024.

As documented in the National Program Action Plan, ARS Aquaculture scientists conduct stakeholder driven research within the guidance and funding provided by Congress. Although the program adheres to a five-year strategic planning cycle, ARS is also able to direct research capacity towards new and emerging industry challenges and lend expertise to other programs or organizations that support the responsible expansion of domestic aquaculture. Our formal projects focus on catfish, Atlantic salmon, rainbow trout, hybrid striped bass, shrimp, oysters, and marine water finfish, however this report will also highlight results of collaborations targeting additional aquaculture species.

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Introduction

Aquaculture production is growing because demands for healthy seafood products are increasing even as stocks of wild-caught seafood are dwindling from overfishing and other factors. Developing technologies that reduce production costs and maintain or improve product quality will help U.S. aquaculture producers meet that increasing demand. Producers, processors, and breeders need systems that maximize aquatic animal production, reduce environmental impacts, increase market competitiveness, sustain producers, and earn consumer confidence.

Aquaculture research is important for informing the development of science-based environmental policies that:

- sustain aquaculture production while maintaining healthy and productive freshwater, coastal, and marine ecosystems;
- protect special aquatic areas;
- rebuild overfished wild stocks;
- restore populations of endangered species;
- restore and conserve freshwater, coastal, and marine habitat;
- balance competing uses of aquatic environments;
- create employment and business opportunities in rural inland and coastal communities; and
- enable the sustainable production of safe, nutritious and affordable seafood.

Research conducted in NP106 supports efforts to ensure that a healthy, competitive, and sustainable aquaculture sector can produce an abundant, safe, and affordable supply of seafood products. NP106 work advances the efforts of more than 4,300 aquaculture farmers producing more than $1 billion dollars' worth of goods annually to meet the potential market demand generated by ~300 million U.S. consumers. Furthermore, the trade deficit in seafood products, 50% of which come from aquaculture has grown to $17 billion (in 2020), indicating a huge market opportunity for domestic producers.

Since the 1980s, capture fisheries production has been relatively static and has shown no signs of increasing. The **2020–2025 Dietary Guidelines for Americans** suggests Americans should increase their seafood intake from an average of about 5.9 ounces per week to 8 ounces or more per week (8 to 12 ounces is recommended for pregnant or breastfeeding women). Aquaculture production could fill in the gap between current supplies of wild-caught seafood and increasing demands for seafood and become the most readily available source of safe and sustainable seafood. In fact, aquaculture is now the source for about half of all fish produced for human consumption, and its contribution will probably increase as the demand for seafood increases.

U.S. consumers spent an estimated $102 billion on fishery products, making it one of the top three seafood markets worldwide. Yet, U.S. marine and freshwater aquaculture production ranks 18th worldwide, producing 298,463 metric tons of fishery products with a farm gate value approaching $1.5 billion annually. As a result, 90 percent of the seafood consumed in the United States (by value) is imported.

Seafood suppliers and producers recognize that wild-caught fisheries are producing at peak capacity and that aquaculture will be a critical component in meeting the nutritional requirements

of a growing global population and in protecting natural resources. The United States is well-positioned to expand aquaculture production because of the following:

- the *Dietary Guidelines* recommendations for increasing seafood consumption;
- the United States is a major producer of plant-based fish feeds and feed ingredients;
- there is an abundance of underused water resources, including the Exclusive Economic Zone and the Great Lakes;
- Recirculating Aquaculture Systems support local production of native and non-native species;
- the U.S. regulatory framework ensures aquaculture production is sustainable, has minimal impacts on the environment and that harvested food is safe for consumption; and
- the United States is a net seafood importer with a trade deficit of $17 billion in 2020, there is an existing market demand for seafood products.

The U.S. capacity for innovation and technology development will enable the use of science-based approaches to expand responsible use of the Nation's natural resources and contribute towards meeting the nutritional demands of a growing global population. Therefore, the ARS National Program for Aquaculture focuses on research that supports the production of quality seafood products for human consumption. Research in the disciplines of genetics, nutrition, health, and physiology will support the production of aquatic animals, while studies in ecology, water quality, engineering and food science will support the improvement of systems and products to ensure sustainability. Research teams that integrate scientific disciplines and approaches to meet the needs of aquaculture industries is critical, therefore the 2020 – 2024 Action Plan was structured by species.

COMPONENT 1: IMPROVING THE EFFICIENCY AND SUSTAINABILITY OF CATFISH AQUACULTURE

Problem Statement 1A: Improve Catfish Aquaculture Production Efficiency

Problem Statement 1B: Reduce the Impacts of Disease in Catfish Aquaculture

Problem Statement 1C: Improve Catfish Product Quality

COMPONENT 2: IMPROVING THE EFFICIENCY AND SUSTAINABILITY OF SALMONID AQUACULTURE

Problem Statement 2A: Improve Salmonid Aquaculture Production Efficiency and Ensure Product Quality

Problem Statement 2B: Reduce the Impacts of Disease in Salmonid Aquaculture

COMPONENT 3: IMPROVING THE EFFICIENCY AND SUSTAINABILITY OF HYBRID STRIPED BASS AQUACULTURE

Problem Statement 3A: Enhance Hybrid Striped Bass Aquaculture Production

COMPONENT 4: ENHANCING SHELLFISH AQUACULTURE

Problem Statement 4A: Enhance Shellfish Aquaculture Production

COMPONENT 5: DEVELOPING MARINE FINFISH SEEDSTOCKS

Problem Statement 5A: Develop Warmwater Marine Finfish Seedstocks Optimized for Aquaculture Production Efficiency

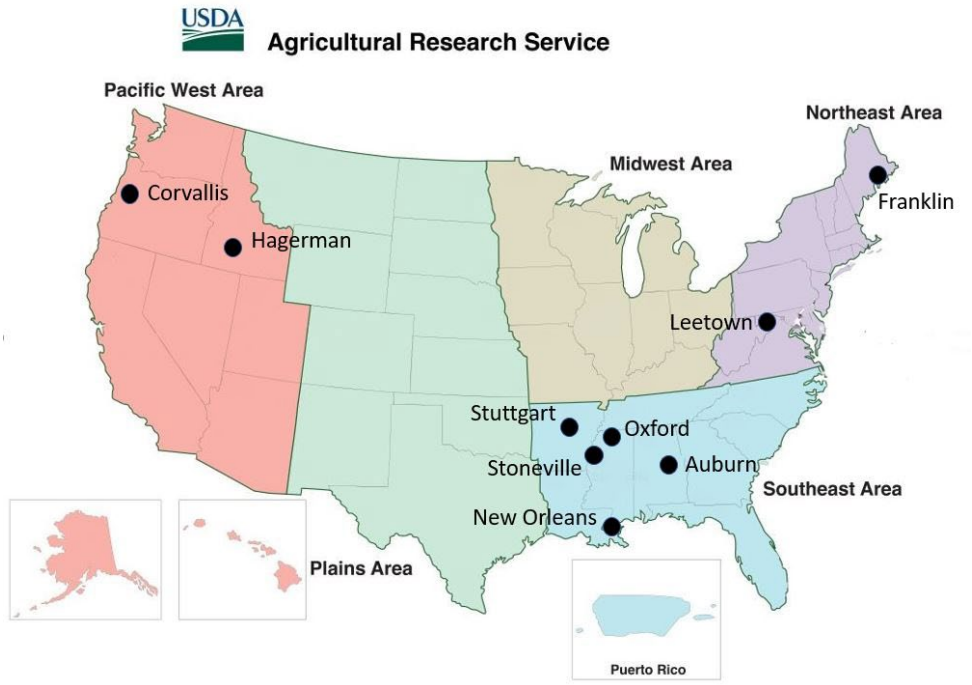
COMPONENT 6: DEVELOPING SUSTAINABLE AQUAPONIC PRODUCTION SYSTEMS

Problem Statement 6A: Optimize Aquatic Animal Species Production Systems for Aquaponics

Problem Statement 6B: Optimize Plant Production Systems for Aquaponics
Problem Statement 6C: Optimize the Integration of Fish and Plant Production Systems

ARS Aquaculture Research Capacity

NP106 - Aquaculture is 1 of 15 ARS National Programs, aquaculture relevant research is also conducted in National Programs for Food Safety, Crop Production, Human Nutrition, and Food Animal Production. As of 2022, NP106 research is conducted by 65 scientists and their staffs on 15 permanent projects in 11 main laboratories. ARS also supports 15 funded collaborations with university or non-profit institutions.



The Small Grains and Potato Germplasm Research Center includes four scientists located in Hagerman or Aberdeen, Idaho, and Bozeman, Montana, who conduct fish nutrition research to enhance rainbow trout production nationwide and increase rainbow trout production efficiency using plant-based feeds.

The National Center for Cool and Cold-Water Aquaculture includes eleven scientists at Leetown, West Virginia and collaborators at the Conservation Fund Freshwater Institute who enhance U.S. aquaculture production by developing improved germplasm and technologies that increase farm efficiency, product quality, and environmental sustainability. Research focuses primarily on rainbow trout and encompasses genetics, genomics, physiology, aquatic animal health, and aquaculture engineering.

The National Cold Water Marine Aquaculture Center includes nine scientists in Franklin, Maine and Kingston, Rhode Island and collaborators at the University of Maine and University of Rhode Island, who conduct research that will solve problems limiting production efficiency of cold-water marine aquaculture. The current primary research focus is genetic improvement using an applied selective breeding program to increase efficiency and sustainability of Atlantic salmon and eastern oyster culture.

The Pacific Coast Shellfish Research Unit includes four scientists at Corvallis, Oregon, and collaborators at Oregon State University who improve the sustainability of shellfish production systems in Pacific Northwest estuaries.

The Warmwater Aquaculture Research Unit includes eleven scientists in Stoneville Mississippi, and their Mississippi State University collaborators who develop technologies that improve the efficiency, profitability, and sustainability of fish farming in the United States through development of improved fish strains and hybrids and by developing better production technologies. The unit includes one scientist in Oxford, Mississippi, who conducts research to reduce the impacts of off-flavor in recirculating aquaculture systems and pond production.

The Aquatic Animal Health Research Unit includes twelve scientists in Auburn, Alabama, and their collaborators at Auburn University, who conduct research to develop control strategies to prevent large economic losses in the aquaculture industry caused by diseases and parasites and research to develop sustainable aquaponic production systems.

The Harry K. Dupree Stuttgart National Aquaculture Research Center includes seven scientists in Stuttgart, Arkansas, who conduct research on hybrid striped bass with two aims: 1) development of feeds and improved culture strategies; and 2) disease therapeutics evaluation and control.

The Food Processing and Sensory Quality Research Unit includes two scientists working in New Orleans, Louisiana, to develop technologies that optimize the nutritional, functional, and sensory qualities of catfish.

A project aiming to develop marine finfish seedstocks includes four scientists and collaborators at Florida Atlantic University's Harbor Branch Oceanographic Institute in Fort Pierce, Florida.

Recent changes to NP106 Research Capacity

Since 2018 Congress has provided new guidance and funding for NP106 research:

- 2019 and 2020 funding increases to develop warmwater marine seedstock in partnership with the Harbor Branch Oceanographic Institute, adding 4 ARS scientist positions;
- 2019 and 2020 funding increases for Pacific shellfish genetics in collaboration with Oregon State University, adding 3 ARS scientist positions;
- 2020, 2021, 2022 funding increases to expand eastern oyster genetics research, adding 2 scientist positions;
- 2020 and 2021 funding increases to initiate research in precision aquaculture in partnership with The Conservation Fund's Freshwater Institute, adding 1 scientist position;
- 2021 addition of aquaponic research in partnership with Auburn University, adding 4 scientist positions;
- 2020, 2021, 2022 funding increases to support an Aquaculture Experiment Station in partnership with the University of Maine, adding 3 scientist positions; and
- 2022 termination of aquaculture research in Milwaukee, Wisconsin.

Impact of the Global Covid-19 Pandemic

Without question the Covid-19 pandemic had a profound negative impact on NP106 research activities, including:

- Scientists unable to travel to workshops and conferences;
- Scientists and staff unable to travel for field work, sample collection, training and collaboration activities;
- Reduction in laboratory progress due to social distancing requirements for staff; and
- Reduced access and use of research facilities (laboratories, ponds, etc…) due to pandemic response measures.

These impacts are reflected in trends observed in the accomplishment and publication metrics reported in tables below.

Alignment of National Program 106 – Aquaculture with USDA Strategic Plans

National Program (NP) 106- Aquaculture envisions of science-based use of our natural resources to meet the seafood demands of a growing global population, therefore its mission is to conduct research and deliver technologies that improve domestic aquaculture production efficiency and product quality while minimizing impacts on natural resources. Within this context NP106 directly contributes to the mission of USDA as outlined in Departmental, Mission Area and Agency level strategic plans.

Relationship of this National Program to the USDA Strategic Plan 2022 - 2026
NP106 - Aquaculture supports the USDA FY2022-2026 Strategic Plan by contributing to Strategic Goal 1. Combat Climate Change to Support America's Working Lands, Natural Resources, and Communities; and Strategic Goal 2. Ensure America's Agricultural System is Equitable, Resilient, and Prosperous.

Relationship of this National Program to the USDA Research, Education and Economics (REE) Action Plan
NP106 - Aquaculture supports the 2014 REE Action Plan by contributing to Goal 1: Sustainable Intensification of Agricultural Production, specifically including the following Subgoals:
Subgoal 1A. Crop and Animal Production
Subgoal 1B. Crop and Animal Health
Subgoal 1C. Crop and Animal Genetics, Genomics, Genetic Resources, and Biotechnology

Aquaculture research in this Action Plan also relates to the following REE Action Plan Goals:
Goal 2: Responding to Climate and Energy Needs
Goal 3: Sustainable Use of Natural Resources
Goal 4: Nutrition and Childhood Obesity
Goal 5: Food Safety
Goal 7: Rural Prosperity/Rural-Urban Interdependence

Relationship of this National Program to the USDA Science Blueprint – A Roadmap for USDA Science from 2020 to 2025
NP106 – Aquaculture directly supports the themes outlined in the USDA Science Blueprint, primarily Sustainable Ag Intensification.

Relationship of this National Program to the USDA Agricultural Research Service 2018 - 2020 Strategic Plan
Research outlined in this Action Plan falls under Strategic Goal Area 4, Animal Production and Protection, in the 2018-2020 ARS Strategic Plan. This plan specifically outlines research that supports Goal 4.2: Improve Domestic Aquaculture Production Efficiency and Product Quality While Minimizing Impacts on Natural Resources.

Performance Measure: Provide scientific information to maximize the production efficiency of our food animal production systems. Develop new technologies and tools contributing to improved systems to meet current and future food animal production needs of diversified consumers while ensuring economic and environmental sustainability and animal well-being.

Relationship to the ARS Grand Challenge Synergies
Farmers, consumers, and citizens share the intertwined goals of ensuring sufficient food supplies for a growing population, ensuring the production of wholesome food, and improving agriculture's substantial environmental footprint. ARS research leadership recognized these issues require holistic and synergistic approaches and developed an innovation platform known as Grand Challenge-Synergies (GC-S) to encourage and facilitate collaboration across projects, locations, and programs within the Office of National Programs (ONP). The goal of GC-S is the development of systems approaches for addressing national and international agricultural research needs.

ARS aquaculture research can contribute to the ARS GC-S and other cross-disciplinary opportunities by developing technologies that increase the availability of healthy dietary protein from seafood through environmentally sustainable production that protects and enhances natural resources. This will require collaborating with scientists working on other ARS goals that affect aquaculture production systems, including:

- Developing crops and insects optimized for use as fish feed ingredients and reduce the demand on ingredients from wild-caught fisheries;

- Modifying production systems and developing technologies that ensure product quality, healthfulness, and food safety;

- Modifying production systems and developing technologies that optimize agricultural water use and identify beneficial uses of fish waste; and

- Developing technologies that improve fish health and welfare by developing alternatives to antibiotics and reducing on-farm antibiotic use.

NP106 – Aquaculture Research Achievements

ARS designs projects to be multidisciplinary teams of 1-8 scientists, including funded collaborators where appropriate, to plan and execute integrated research approaches that will directly address 1 or more challenges presented by stakeholders. Every year the Lead Scientists for each NP106 project submits an annual report to communicate research progress and achievements from the prior fiscal year. This report includes accomplishment statements that provide a narrative on significant scientific advances, development of critical resources, transfer of technologies, and/or science that informs policy. Projects are not necessarily expected to report accomplishments every year though progress toward accomplishments is expected and several accomplishments are anticipated over the 5-year timeframe of the project cycle.

Along with accomplishments, annual reports document publications and progress towards milestones, which are a part of each project plan and reflect significant progress events over the life of the project. Following publication of project annual reports, the Office of National Programs publishes an NP106 annual report to highlight ***selected*** accomplishments and notable activities across the National Program. This section will report metrics for accomplishments, milestones and publications in summary tables and then under the Component and Problem Statement framework of the Action Plan (select accomplishments only). ***All*** project accomplishment statements and publications are reported in Appendices 1 – 7.

The previous Action Plan was organized by scientific discipline and included research on tilapia and yellow perch, for this report those accomplishments and publications are reported under "Additional Accomplishments," which also reports achievements from NP106 scientists using their research expertise and capacity for impact beyond the scope of the 6 components listed.

National Program Metrics
This section is meant to provide a broad overview of NP106 research progress by reporting research metrics for the National Program. Tables 1 – 4 show the numbers and distribution of NP106 accomplishments, publications, technology transfer mechanisms, and milestones. Table 1 breaks down accomplishments by Component, and Problem Statement wherever relevant.

Table 1. Accomplishment Summary

	2018	2019	2020	2021	2022	Total
Component 1 Catfish	18	25	17	6	9	75
1A Production	11	11	8	3	3	36
1B Health	6	12	6	3	4	31
1C Product Quality	1	2	3		2	8
Component 2 Salmonids	9	13	16	10	16	64
2A Production and Quality	7	9	15	7	9	47
2B Health	2	4	1	3	7	17
Component 3 Hybrid Striped Bass	1	2	3	8	6	20
Component 4 Shellfish		2	3			5
Component 5 Marine Warmwater Finfish			3	6	5	14
Component 6 Aquaponics					1	1
Additional Accomplishments	6	3	3			12
Total						**191**

NP106 scientists are required to publish peer-reviewed journal articles on an annual basis, however they write other types of articles of interest to scientific, industry, and/or general audiences. Table 2 reports NP106 publications by type and year for 2018 – 2022.

Table 2. Publication Summary

	2018	2019	2020	2021	2022	Total
Abstracts	49	45	19	1	6	120
Book Chapters	2	5	2	1	1	11
Database/Datasets	2	2	1	3	3	11
Exper. Station Publication	1					1
Patent Application	1					1
Peer Review Journal Articles	93	87	77	81	67	405
Popular Press Articles	1	3	4		2	10
Proceedings	4					4
Review Articles	2			2	1	5
Trade Journal Articles	9	4	3	1	1	18
Other	1	2	1	1	1	6

Peer-reviewed publications are the primary method of technology transfer for ARS scientists, however there are other mechanisms, Table 3 reports other technology transfer activities for NP106 initiated during 2018 – 2022.

Table 3. Technology Transfer

Mechanism	2018	2019	2020	2021	2022
Cooperative Research and Development Agreement	1				
Material Transfer Research Agreements	3	1	26	5	4
Material Transfer Agreements	16	11	10	3	17
Invention Disclosures		2	2	3	
New Patent Applications Filed		1	3	3	3
New Patents (patented)	1				1

Finally, as a part of the five-year project plan, ARS scientists identify critical milestones that they will meet during the life of the project, these are reported in annual reports to track project progress. Success of meeting NP106 project milestones is summarized in Table 4 which reports the total number of milestones by year, the number that were fully met or substantially met, or not met.

Table 4. Project Milestone Summary

	2018	2019	2020	2021	2022
# Fully or Substantially Met	104	73	131	111	132
# Not Met	9	9	13	22	27
Total	113	82	144	133	159

The following sections report publication and milestone metrics for each Component and Problem Statement, followed by select accomplishment narratives.

ARS values collaborative research with private, academic, non-governmental organizations and other government agencies. During fiscal years 2018 – 2022, NP106 established 155 outgoing agreements providing funding to over 40 institutions to add aquaculture research expertise and capacity to ARS projects and participate in the training of undergraduate and graduate students and post-doctoral fellows. Information on the agreements underlying some of these partnerships and collaborations is listed in Appendix 8. Similarly, NP106 scientists were successful in obtaining extramural funding for 51 research partnerships with 38 institutions, these are listed in Appendix 9.

Component 1: Improving the Efficiency and Sustainability of Catfish Aquaculture

Table 5. Peer -Reviewed Publications for Component 1 Catfish 2018 – 2022

Problem Statement	2018	2019	2020	2021	2022	Total
Production (A)	13	17	14	4	5	53
Health (B)	19	19	11	9	12	70
Quality (C)	1	2	1		3	7
Total	33	38	26	13	20	130

Problem Statement 1A: Improve Catfish Aquaculture Production Efficiency
Catfish producers, processors, and fingerling suppliers need systems that optimize and maximize production, reduce environmental impact, increase market competitiveness, support sustainable production, and earn consumer confidence. Research in the disciplines of genetics, nutrition, and physiology will support the biological improvement of aquatic animals, while studies in ecology, water quality, engineering, and food science will advance the improvement of systems that minimize environmental impacts and ensure consistent high-quality products that meet consumer demand.

Table 6. Milestones for Problem Statement 1A.

	2018	2019	2020	2021	2022
# Fully or Substantially Met	18	16	19	15	17
# Not Met	0	0	0	7	6
Total	18	16	19	22	23

2018

Genomic selection for growth and carcass yield in the Delta Select strain of channel catfish.
Determining the relative value of an individual fish for breeding has depended on traditional methods that use parentage information and trait measurements. ARS scientists in Stoneville, Mississippi, collaborated with University of Georgia scientists to develop a technology that uses genome information to improve the accuracy of breeding value estimates. This approach led to 30 percent improvement in breeding value accuracy for growth and carcass yield in 2,000 Delta Select strain catfish. The improved breeding value accuracy will result in more rapid genetic gain for growth and carcass yield in the Delta Selects, which will be released to U.S. catfish farmers to improve their production efficiency.

Evaluation of growth and processing yield of blue catfish strains. Blue and channel catfish hybrids now comprise approximately 75 percent of annual U.S. catfish production. As a result, evaluating blue catfish strains for growth and carcass yield has become an important aspect of the breeding program conducted by ARS scientists in Stoneville, Mississippi. In their research, they evaluated six strains of blue catfish and then used the three strains with the best growth and carcass yield in further evaluations. Their results indicated the Rio Grande blue catfish strain was superior to the D&B and Mississippi River strains of blue catfish for growth and carcass yield.

The release of a superior blue catfish strain will improve production efficiency of U.S. catfish farmers and processors.

Assessment of phytase "super-dosing" in catfish diets. ARS scientists at Stoneville, Mississippi, collaborated with Mississippi State University scientists to conduct two experiments to evaluate responses of hybrid catfish (female *Ictalurus punctatus* × male *Ictalurus furcatus*) to "super-dosing" of phytase added to existing commercial catfish feeds. They found that phytase super-dosing in catfish does not appear to have additional benefits beyond the standard dose, at least for promoting growth and preventing anemia, and also did not improve water quality. Ongoing technology transfer efforts recommend 500 phytase units [FTU/kg] phytase, not "super-dosing," to replace inorganic phosphorus in catfish feeds.

Reducing feed cost for catfish fingerling production. Catfish producers are interested in reducing feed expenses because commercial fingerling feeds that are 35 percent protein can cost up to $600-700 per ton. Reducing protein levels or using less expensive alternative feed ingredients can both reduce feed costs. ARS scientists in Stoneville, Mississippi, and their collaborators conducted a feeding trial in pond-raised hybrid catfish fingerlings to evaluate diets containing different levels of protein and protein sourced from either fish meal or pork meat, bone, and blood meal (PMBB). They found no significant production differences associated with the different feeds. However, they determined that using a feed enhanced with 32 percent fish meal could save producers $20/ton; using a feed enhanced with 35 percent PMBB could save producers $69/ton; and using a feed enhanced with 32 percent PMBB could save producers $89/ton. These savings are equivalent to annual savings of $120, $414, and $532 per acre, respectively, assuming a total use of 6 tons of feed per acre in a growing season. The researchers also noted that some savings can be realized using feed enhanced with 32 percent PMBB, because fish meal is much more expensive than PMBB.

Reducing feed cost for catfish fry production. Although catfish fry initially feed on natural foods available in pond ecosystems such as zooplankton, they typically begin consuming commercial feeds as soon as they are transferred to stocking ponds. Pond studies were conducted on both channel catfish and hybrid fry to determine if transferring them to feeding/stocking ponds could be delayed to save on feed costs without compromising production. Results indicated that delaying channel catfish fry feeding for 5 weeks can save $236/acre in feed costs without compromising production. However, hybrid catfish fry production starts to become affected only 2 weeks after feeding is delayed and saves only $15.69/acre

Dissolved oxygen requirements for separated hybrid catfish eggs incubated in vertical tubes. ARS scientists at Stoneville, Mississippi, previously determined that channel catfish eggs incubated as intact egg masses require water with dissolved oxygen at over 95 percent air saturation during the last day of incubation for optimum development. Recent research by the same scientists determined that the maximum dissolved oxygen requirement for separated hybrid catfish eggs during the last days of incubation was only 79 percent. ARS scientists at Stoneville, Mississippi, have begun recommending that farmers maintain the dissolved oxygen in vertical tubes at or above 80 percent air saturation during the last two days of incubation to maximize egg development.

Reducing variability of hybrid catfish growth during year-round pond harvests. Hybrid catfish, which make up 75 percent of U.S. farmed catfish products, exhibit superior growth, better feed conversion, higher survival, availability, and are better suited for intensive production systems. However, their rapid growth, physical characteristics, and behavior have presented some unique production problems, including highly variable fish growth, oversized fish, and increased difficulties with year-round harvests. An ARS scientist at Stoneville, Mississippi, collaborated with Auburn University researchers and catfish producers to conduct extensive field samplings and assess how a number of factors affect size variability at harvest and effective year-round harvest schedules. These factors included culture systems, harvest technology, fingerling size and variability, grading, genetics, stocking rates and schedules, and feeding rates. Their results indicated links between the genetic profiles of purebred parents, stocking fingerlings of different sizes together in production ponds, and variability in hybrid catfish performance. They also found that increasing aeration in production ponds and bar-grading fingerlings before stocking improved the subsequent size uniformity of harvested fish. Models based on economic analyses for specific sets of farm conditions were developed to help catfish farmers improve management practices and increase the profitability of their hybrid catfish production.

Management practices minimize size variation in hybrid catfish food fishponds. Two management practices to reduce food fish size variation and resulting weigh backs have been developed by ARS scientists at Stoneville, Mississippi, and collaborating Mississippi State University scientists. While in some cases economic benefits increase from mid-season partial harvests of larger fish, most farmers have adopted the use of graded fingerlings (recommended by ARS scientists) when processors penalize farmers for out-of-size food fish.

2019

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.

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.

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.

2020

Release of the Delta Select strain of channel catfish. Improved catfish germplasm in aquaculture will reduce production costs and allow U.S. catfish farmers to remain competitive in the global seafood market. ARS researchers in Stoneville, Mississippi, developed the “Delta Select” strain of channel catfish through three generations of genetic selection, leading to a 25 percent increase in growth rate and 0.9 percent increase in carcass yield compared to the non-selected Delta Control line that originated from the same population. Approximately 90,000 head (180,000 pounds) of 2-year-old Delta Select strain catfish were released to industry, providing

U.S. farmers access to improved catfish germplasm that will make them more efficient and profitable.

An alternative aquafeed protein source improves growth and immune responses. Frass is a byproduct of the fly larval meal industry and is composed of larval excrement, shed exoskeletons, and residual feed of the flies. ARS researchers in Auburn, Alabama, evaluated diets containing black soldier fly frass as partial replacements for soybean meal, wheat short (the fine bran particles, germ and particles separated in commercial flour milling), and corn meal. Experimental diets were fed to fingerling catfish and tilapia and the results demonstrated that final weight gain was significantly increased. Additionally, tilapia fed frass diets exhibited increased survival against two important pathogens, *Flavobacterium columnare* and *Streptococcus iniae*. Based on these findings, frass derived from the larvae of black solder flies has potential as an alternative source of protein in aquafeeds or as an ingredient enhancing palatability and growth.

Catfish diets and feeding strategies affect meat yield. During the production and harvest cycle, catfish farmers are sometimes unable to sell market weight catfish for processing if there is a lack of demand. Feeding fish during this time allows the fish to grow larger than the preferred size for processing. If feed is withheld during this time, the fish mobilize muscle tissue for energy, reducing fillet yield. In either case, the price paid to farmers is reduced. ARS researchers in Stoneville, Mississippi, worked in cooperation with Mississippi State University fish nutritionists and determined that hybrid catfish fed once or twice weekly for 4 months remained in the preferred size range for processing but had reduced fillet yield. Thirty days of full feeding were needed to restore fillet yield to normal levels in fish fed once or twice weekly for 4 months. These results allow the catfish farming industry to develop feeding strategies that minimize production costs and maximize profits.

2021

Industry utilization of spawning aid. More than half of U.S. catfish aquaculture produces a hybrid between channel catfish females and blue catfish males. Hybrid production depends on induced ovulation in females, and producers traditionally utilized carp pituitary extract or a synthetic peptide based on a mammalian reproductive hormone to induce ovulation. ARS scientists in Stoneville, Mississippi, developed a synthetic peptide based on the chicken and catfish gonadotropin hormone releasing hormone (GnRH II), and helped producers test it on farms during the 2020 spawning season. The success of the new GnRH II on farms led to its use in 2021 by all eight producers that supply the U.S. catfish industry with hybrid catfish fingerlings, and six of the eight producers used GnRH II exclusively for hybrid production.

2022

New strategy for controlling snails. Trematode infestations on catfish farms have been linked to significant production losses and farm closures. Initially recognized as an emerging pest in the late 1990s, management strategies targeted the trematode life cycle by eradicating the snail intermediate host in the pond environment. Copper sulfate is the most widely used treatment option and is highly effective against snails with a single application of 3 ppm, but this treatment level can result in increased mortality in fish, especially when water temperatures are elevated. ARS researchers in Stoneville, Mississippi, and Mississippi State University researchers

demonstrated that weekly low-dose copper treatments (1.0-1.5 ppm) spread across 4 weeks are as effective in killing snails and treatment rates <0.1 ppm can halt snail reproduction and kill snail embryos. This approach is being combined with a new delivery system to better manage snail populations and reduce trematode populations in catfish ponds. The system utilizes a radar groundspeed sensor and a logic-based control system to distribute granular copper sulfate evenly and accurately along the pond margins in a single pass.

Problem Statement 1B: Reduce the Impacts of Disease in Catfish Aquaculture
Health management strategies, technologies, and biosecurity plans that are safe for the environment and for consumers of aquaculture products are necessary to reduce disease-related losses. Industry growth in catfish aquaculture has been hindered by a lack of validated technologies for early and rapid detection, prevention, and treatment of diseases. Validated diagnostic tools are needed in production systems to quickly detect disease agents. Developing effective control strategies and therapeutants to manage disease is also a priority, since there are currently only a few drugs that have been approved for treating sick fish. New research will support the development of effective vaccines and methods for mass vaccination of aquatic animals.

Table 7. Milestones for Problem Statement 1B.

	2018	2019	2020	2021	2022
# Fully or Substantially Met	19	16	32	25	26
# Not Met	0	0	0	4	3
Total	19	16	32	29	29

2018

Identification of four distinct groups in Flavobacterium columnare with fish host associations. Columnaris disease, which is caused by the bacterium *Flavobacterium columnare*, is one of the most prevalent fish diseases worldwide. An exceptionally high level of genetic diversity among bacterial isolates has long been recognized, but there has been little systematic work on quantifying or characterizing this diversity. ARS researchers at Auburn, Alabama, and collaborators used high resolution methods to characterize the genetic diversity in *F. columnare,* and their results established the existence of four distinct genetic groups within the species and indicated that different bacterial genetic types were associated with different fish species and geographic regions. This research highlights the importance of understanding the genetic diversity in *F. columnare* and has facilitated a standard nomenclature for these groups. The new knowledge gained from this research will aid in identifying which genetic type(s) of *F. columnare* are prevalent in different regions and/or aquaculture industries and will support the development of more targeted control and treatment measures for columnaris disease.

Stress responses in juvenile catfish influence susceptibility to enteric septicemia. Disease is the leading cause of reduced productivity in aquaculture production systems, and intensification of these systems has exacerbated disease susceptibility. Low dissolved oxygen stressors invariably increase the susceptibility of farmed fish to diseases, and concentrations of cortisol, the principal corticosteroid in ray-finned fish, increase rapidly following a stressful event. ARS scientists at Stoneville, Mississippi, classified juvenile catfish based on their cortisol stress response as either ‘high’ or ‘low’ responders to stress. High- and low-responding channel catfish were then exposed to virulent *Edwardsiella ictaluri* bacteria, and the scientists found that catfish mortality rates increased with higher cortisol responsiveness when healthy fish were stressed prior to infection. These results indicate that mitigating stress or stress response may help reduce catfish mortality in aquaculture production systems.

Testing of a recombinant protein vaccine to protect catfish against columnaris disease. *Flavobacterium columnare* is a bacterium that causes columnaris disease, which severely affects

channel catfish production in the United States. ARS researchers at Stuttgart, Arkansas, had previously identified *F. columnare* proteins that activate the adaptive immune response. They followed up this work with the development of a new recombinant protein vaccine that in laboratory tests provides excellent immune protection against columnaris disease.

Channel catfish have little genetic resistance to enteric septicemia of catfish. Enteric septicemia of catfish (ESC) is a major microbially-induced disease that significantly reduces production. ARS scientists at Stoneville, Mississippi, exposed more than 10,000 individual catfish from pedigreed families of the Delta Select catfish line to virulent *Edwardsiella ictaluri* bacteria and found few indications that genetic traits offer promise for breeding robust catfish lines with improved disease resistance. These results indicate that controlling ESC in farmed catfish will more likely be achieved through management rather than selective breeding.

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. Mississippi State University scientists worked with ARS scientists at Stoneville, Mississippi, to develop an effective ESC oral vaccine. To date, approximately 500 million stocked catfish have been vaccinated in field trials or on commercial farms. The net economic benefits at the fingerling production stage for channels and hybrids were determined to be $3,868 and $7,063/hectare (ha), respectively. Results from whole farm mathematical programming models showed additional economic benefits in the range of $397 to $473/ha on farms that integrate fingerling production to their food fish operations.

Immune responses of channel catfish after vaccination with Ichthyophthirius multifiliis. *Ichthyophthirius multifiliis* (Ich) is a parasite of fish and causes severe losses to aquaculture industries worldwide. Treating Ich with chemicals is costly and often ineffective after the parasite penetrates the fish host skin and gill tissue. More information is urgently needed about protective immune responses in fish to develop effective vaccines against the parasite. ARS researchers at Auburn, Alabama, investigated the expression of innate and adaptive immune-related genes in surface and internal tissues of channel catfish following vaccination with live forms of Ich. The research demonstrated significantly higher antibody levels and 95 percent survival in vaccinated fish than non-vaccinated fish. These results reveal new insights into the molecular responses that may govern protective immunity of catfish against Ich infection.

Virulent Flavobacterium columnare degrades catfish mucus. *Flavobacterium columnare* is a bacterium that causes columnaris disease in farmed fish and is a concern for U.S. and international aquaculture producers. Skin mucus is an important defense protecting fish health, but some pathogens have developed adaptions for penetrating this protective layer. ARS researchers at Auburn, Alabama, collaborated with Auburn University scientists and identified the components of catfish mucus that are vulnerable to bacterial damage from the pathogen. They also found that a highly virulent *F. columnare* isolate growing in catfish mucus showed significantly elevated enzyme activity compared to a moderately virulent isolate. This activity may promote greater bacterial virulence by increasing the pathogen's ability to break down the protective mucus layer, which in turns enhances bacterial colonization and disease that may kill that fish host. The data provide new insights on the pathogenic mechanisms of *F. columnare* in columnaris disease that researchers can use in developing strategies for mitigating the disease.

2019

Waterborne exposure to select clay minerals protects catfish against virulent Aeromonas hydrophila infections. *Aeromonas 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 at 1 gram of in 1 liter of water improved 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.

2020

Pyranopyrans are potential bacteriocidal compounds against fish pathogens. Disease losses in catfish aquaculture can cost up to $100 million, so reducing or eliminating bacterial pathogens is critical to the success of the industry and improving fish health and welfare. ARS researchers in Oxford, Mississippi, Stoneville, Mississippi, and Villanova University modified the chemical structures of natural compounds produced by a certain species of fungus to produce novel compounds. *In vitro* experiments with these novel compounds, pyranopyrans, demonstrated that they possess significant antibacterial activities against certain species of fish pathogens. Combatting disease with alternative efficacious natural or natural-based compounds is very acceptable to the catfish industry, which has access to only a few approved therapeutants and is striving to limit the use of antibiotics.

A rapid assay for Flavobacterium columnare. Columnaris disease is caused by the bacterium *Flavobacterium columnare* and affects almost all finfish aquaculture industries in the United States and worldwide. Previous research established the existence of four distinct genetic groups within the species *F. columnare*; however, there were no simple or inexpensive methods to assign an unknown isolate to one of the four groups. Knowing which group is causing outbreak informs decisions on disease treatment strategies. ARS researchers in Auburn, Alabama, developed a molecular assay to quickly assign an isolate to a genetic group, demonstrating the assay is rapid, sensitive, and specific for genotyping *F. columnare*. The assay is inexpensive and can be used by any laboratory with basic molecular capabilities to determine which genetic group(s) are responsible for disease outbreaks. It is currently in use by commercial and academic laboratories.

2021

Using stock rotations to mitigate proliferative gill disease. Proliferative gill disease (PGD) in channel and hybrid catfish is a devastating disease caused by *Henneguya ictaluri*, a parasite present in nearly all catfish ponds during springtime. PGD is associated with catastrophic losses with a mortality rate of more than 90 percent. ARS researchers in Stoneville, Mississippi, found that *H. ictaluri* myxospores (the infective stage of the parasite) matured in channel catfish from 14 to 20 weeks after becoming infected, but hybrid catfish showed no myxospore development after the same period of exposure. This suggests that PGD infection will not persist in hybrid catfish, and shows that strategic rotations between channel and hybrid catfish in ponds could be a viable management strategy to reduce the incidence of PGD in commercial catfish operations.

2022

***Development of an effective oral enteric septicemia of catfish vaccination platform*.** Enteric septicemia of catfish is considered the most problematic bacterial disease affecting catfish fingerling production. Historically, management strategies relied on the use of medicated feed and feed restrictions to limit the oral route of infection. While both strategies can be effective, the overuse of medicated feeds results in the development of antibiotic resistance, rendering the medication useless, and feed restrictions severely limit growth. In efforts to develop more proactive management strategies, ARS researchers in Stoneville, Mississippi, developed a live attenuated vaccine along with a mechanized delivery system enabling in-pond vaccination during the early stages of fingerling production. The oral vaccine is currently available by veterinarian prescription and has dramatically increased survival and profitability of fingerling catfish

production. Currently, more than 90 percent of catfish produced in Mississippi and Alabama are vaccinated with the delivery platform, which is applicable to other live attenuated vaccines as well. The vaccine also provides cross protection against *Edwardsiella piscicida*, an emerging pathogen in hybrid catfish production.

Problem Statement 1C: Improve Catfish Product Quality
The success of the catfish aquaculture industry depends on supplying a consistently high-quality product that meets consumer expectations for flavor, color, texture, and firmness.

Table 8. Milestones for Problem Statement 1C.

	2018	2019	2020	2021	2022
# Fully or Substantially Met	5	2	2	3	3
# Not Met	0	1	2	1	2
Total	5	3	4	4	5

2018

A new method for measuring catfish fillet texture. Catfish texture is important to consumers, especially if textures do not meet consumer expectations for uniform product hardness and other sensory characteristics. ARS scientists in New Orleans, Louisiana, developed a mechanical texture measurement method for sensory characteristics that can be used to quickly evaluate fillet texture. This will support the development of methods catfish processors can use to quickly and conveniently monitor texture quality and evaluate factors that affect fillet texture characteristics; information that in turn can be used to create more marketable catfish products to meet consumer demands.

Managing preharvest off-flavors in catfish raised in split ponds. While conventional earthen ponds have been used in the southeastern United States catfish farming industry for decades, producers are increasingly interested in using partitioned aquaculture systems (PAS). Split-pond systems, one type of PAS, are designed to improve the management of dissolved oxygen levels and fish waste products (e.g., ammonia). Producers have assumed that fish off-flavor incidence will be reduced in split-pond systems because previous research showed that off-flavors were rare in fish grown in PAS, but there have been no studies on the frequency and intensity of common "off-flavor" episodes in split-pond systems. ARS researchers at Oxford and Stoneville, Mississippi, evaluated water and catfish fillet samples collected from commercial and research split pond facilities in west Mississippi and west Alabama for intensities of earthy and musty off-flavor compounds; phytoplankton ecology of the split ponds were also studied. Levels of earthy and musty compounds in fillets and their sensory characteristics were similar to those reported previously for off-flavor catfish from conventional ponds. In addition, the types of phytoplankton and community structures observed in the split ponds were similar to those commonly observed in conventional catfish ponds. These results demonstrate that catfish farmers using split ponds can use the same management approaches and preharvest sampling to monitor for the presence of off-flavors as the strategies used in conventional catfish ponds for dealing with earthy and musty off-flavor problems (e.g., applications of algicides). These determinations are of critical importance to commercial culturists who are considering the benefits and constraints of adopting split-pond technology.

2019

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 do 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 trim the fillet more efficiently.

2022

Texture differences between channel and hybrid catfish. Hybrid catfish are increasingly used in U.S. aquaculture production, so an assessment of product quality and comparison to channel catfish is critical for meeting consumer expectations. Using instrumental texture analysis, ARS scientists in New Orleans, Louisiana, showed differences between the cooked fillets of channel and hybrid catfish, and found that fillet freezing and storage methods (e.g., individually quick-frozen (IQF), fresh, or frozen) affected texture. Firmness, toughness, and chewiness were most associated with the catfish type, and hybrids had lower levels than channels. Other texture attributes were indicative of the cold-storage methods; IQF fillets had higher cohesiveness and lower adhesiveness, and both frozen and IQF fillets had higher springiness attributes. Scientists will need to study genetics, environment, and pond management practices to better understand how these factors affect product quality and to improve attributes that meet consumer expectations.

Component 2: Improving the Efficiency and Sustainability of Salmonid Aquaculture

Table 9. Peer -Reviewed Publications for Component 2 Salmonids 2018 – 2022

	2018	2019	2020	2021	2022	Total
PS A Production and Quality	26	18	23	30	15	112
PS B Health	9	12	9	12	9	51
Total	35	30	32	42	24	163

Problem Statement 2A: Improve Salmonid Aquaculture Production Efficiency and Ensure Product Quality

Salmonid producers need systems that optimize and maximize production, reduce environmental impacts, increase market competitiveness, sustain producers, and earn consumer confidence. Research in the disciplines of genetics, nutrition, and physiology will support the biological improvement of aquatic animals, while studies on water quality and engineering will support system improvement that minimize environmental impacts.

Table 10. Milestones for Problem Statement 2A.

	2018	2019	2020	2021	2022
# Fully or Substantially Met	20	13	26	22	19
# Not Met	3	3	4	1	1
Total	23	16	30	23	20

2018

Gene editing in rainbow trout. Advancements in gene editing technologies have enabled the induction of targeted mutations in genes of interest, allowing for precise manipulation of the genome. ARS researchers at Leetown, West Virginia, have provided the first proof-of-concept for rainbow trout by demonstrating that this technology can produce fish that exhibit a desired trait and that these genetic modifications are transmitted to the next generation via typical reproduction. Gene editing provides a new opportunity to understand gene function and an alternative strategy that can complement other approaches to genetic improvement.

Incubation temperature impacts rainbow trout embryo survival. Incubation temperature is commonly manipulated to control and predict hatch date in salmonids so that suppliers can consistently provide their customers with eyed eggs across the spawning season. However, there is little information on how temperature changes affect embryo survival. ARS scientists in Leetown, West Virginia, found that incubation at 5 degrees Celsius within the first day of fertilization reduced embryo survival around 5 percent compared to incubation at 10 degrees Celsius, and that rapidly transferring embryos between 10 degrees Celsius and 5 degrees Celsius after 100-degree days of incubation did not affect survival. This information suggests that stakeholders should end the practice of initially incubating fertilized eggs at 5 degrees Celsius.

Adding value to broken rice. Broken rice is used worldwide to produce significant amounts of high protein rice flour (HPRF). To increase the value of broken rice and to expand its end uses,

ARS scientists in Aberdeen, Idaho, assessed the feasibility of using physical (dry) and chemical (wet) methods to increase the protein enrichment of commercial HPRF used in fish feed. Results showed that these processes increased HPRF protein levels more than seven-fold and also increased levels of oil, ash, and phytate. However, when the enriched HPRF is fed to animals (such as farmed fish) in large amounts, the high phytate levels resulted in the unwanted excretion of excess phosphorus. Several options were tested to enrich protein and remove phytate, and results indicated an aqueous medium having mild acidic to mild alkaline pH was the most effective.

Benefits of extruded feeds for trout production. While commercial fish feed produced via steam-compressed pelleting has largely been replaced since the late 1980s by dry extruded feeds, most rainbow trout grown in the United States are still fed steam-compressed pellets. ARS scientists in Hagerman, Idaho, determined how feed pellet processing (extrusion versus expansion-steam pelleting) affected feed quality, water quality, fecal durability, and growth in rainbow trout. All feeds had similar chemical compositions, but extruded feeds had a significantly higher degree of starch gelatinization than the expansion-steam pelleted feeds, which led to extruded feeds having much higher water stability, fecal durability, and lower phosphorus discharge. This research is the first to show that extruded feed pellets are more stable in water than pellets made by expansion-steam pelleting, and that they reduce fecal contributions to waste through improved fecal size and durability in water. The use of extruded feeds in commercial rainbow trout culture could improve waste collection and removal and reduce pollution in downstream receiving waters.

Growth and fillet quality in commercially available rainbow trout. Determining if commercially available rainbow trout raised in recirculating aquaculture systems (RAS) exhibit variable growth performance and fillet quality is critical to identifying genetic lines that maximize profitability. ARS funded scientists in Shepherdstown, West Virginia, determined rainbow trout lines contain considerable genetic variation in growth performance; the fastest growing line reached 3 kg while the slowest line lagged 30 percent behind. These findings indicate that farmers interested in maximizing product yields should familiarize themselves with the growth potential of available stocks before purchasing eggs or fish for their systems. In contrast, fillet quality indices, such as nutrient profile, texture, and color, did not differ among commercial genetic lines. These results indicate a producer or processor who values optimal fillet quality above growth-related traits can be more flexible in their genetic stock selection. Researchers also defined changes in processing yields and indices of fillet quality at different harvest weights throughout the production cycle and gathered valuable data that RAS producers can use to predict growth trajectories and fillet characteristics.

Use of woodchip bioreactors to improve water quality in fish farm effluents. As with all intensive agricultural systems, fish farms produce waste that has the potential to impact the surrounding environment. ARS extramural researchers in Shepherdstown, West Virginia, determined that woodchip bioreactors can capture nitrate nitrogen and suspended solids from aquaculture effluent streams to minimize nutrient discharge into surrounding waterways. A cost and engineering assessment demonstrated that the woodchip bioreactor is an affordable, low maintenance technology to treat aquaculture effluent, reduce environmental impacts, and reduce wastewater treatment costs.

Development of a patented method to fractionate small grains for fish feed. Although barley and oats have been domesticated since ancient time, they are not widely used as human food compared to wheat, corn and rice. Nor do they serve as a source for protein feed. Among all the reported studies on wet fractionation of barley and oats, however, almost all dealt with enrichment of one or two nutrients and neglected other possibly valuable co-products. Upon investigating various factors affecting concentration and recovery of nutrients in each fraction, ARS researchers at Aberdeen, Idaho, developed wet method to process barley and oats into fractions enriched with protein, beta-glucan, starch, or other carbohydrates, respectively, therefore, recovering multiple components simultaneously. The method has secured a U.S. patent (No. patent, 10,021,882) and licensed to a U.S. start-up company.

2019

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.

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.

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.

Improved methods for measuring 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.

2020

Genome-enabled breeding tools for Atlantic salmon. The number of U.S. commercial Atlantic salmon farming operations is expected to increase 5-fold over the next 3 years, and demand for genetically improved stocks will increase dramatically. ARS researchers in Franklin, Maine, and Leetown, West Virginia, created an improved genome reference sequence for the North American Atlantic salmon and developed the first DNA chip that enables the use of genomic information in breeding strategies. This DNA chip is publicly available and in use by commercial breeding programs. Genomic enabled breeding is estimated to improve selection accuracy by up to 50 percent, depending on the trait, therefore increasing the pace of genetic improvement and reducing costs of breeding programs.

Improved method to measure starch content and gelatinization. Starch is an important component of various food and feed products. Starch gelatinization is important during processing starch-containing foods or feeds; the degree of starch gelatinization (DSG) affects physiochemical and sensory properties of starchy products and their susceptibility to enzymatic digestion and so affects their nutritional availability for humans and animals. ARS researchers in Hagerman, Idaho, recently developed an improved method for simpler and more accurate measurement of total starch and gelatinized starch *in situ* for wet and dried products. This provides a valuable new tool for food and feed researchers to study the role of starch in food and feed products.

2021

Improved fillet yield and body weight in rainbow trout. The proportion of edible meat (fillet yield) on a carcass is of major economic importance and breeding animals with superior fillet yield can improve production efficiency and profitability. Animals must be slaughtered to measure fillet yield directly, so it cannot be measured in breeding animals. However, developing genetic gains for fillet yield are possible using information, including genome information, from siblings of fish that are potential breeders. ARS researchers in Leetown, West Virginia, compared the accuracy of genetic merit predictions for fillet yield between the family-based selective breeding which used information about family relationships and genomic selection, The genomic selection model increased the accuracy of genetic merit predictions for fillet yield by 50 percent, indicating that the use of genomic selection can enhance genetic improvement for the fillet yield trait and further enhance the efficiency and sustainability of rainbow trout aquaculture.

Reducing early maturation in Atlantic salmon. The development of land-based recirculating aquaculture system (RAS) fish farms for producing market-size Atlantic salmon is becoming a

significant growth area in U.S. aquaculture. However, the RAS environment can support the production of unacceptable levels of early maturing salmon that are considered a downgraded product, resulting in lost revenue. ARS-funded scientists in Shepherdstown, West Virginia, investigated how RAS water temperature affected early maturation in Atlantic salmon post-smolts. Findings indicated that raising salmon in lower temperatures reduced the prevalence of early maturation by 38 percent. These findings will assist farmers in lowering early maturation, thereby increasing the economic viability of their operations.

Development of a new processing method to make new fish feed. The physical property of feed is more important for aquatic animals than for terrestrial animals. For many years, aquafeed is made by thermoplastic extrusion under low moisture conditions. For improving the physical property of the feed, ARS researchers at Aberdeen and Hagerman, Idaho and Bozeman, MT developed a new method to make fish feed, featuring high temperature and high moisture extrusion. The high moisture extruded feed possesses some unique features, including soft texture, high pellet durability and water stability. The significance of the new method lies in its representation of an emerging technology for aquafeed industry, its potential to revolutionize feed processing, and its promising alternative to mitigate water pollution issues associated with aquaculture.

2022

Precision aquaculture technologies for recirculating systems. Although precision agriculture technologies have not been widely applied to U.S. aquaculture, they could eliminate fish stress associated with the traditional, hands-on methods for estimating population biomass. ARS-funded scientists in Shepherdstown, West Virginia, developed an artificial intelligence (AI)-aided computer vision system for real-time fish monitoring of fish size and numbers in recirculating aquaculture systems. Underwater images and videos were acquired to train an AI fish detection model, and the developed vision system detected whole and partial fish in the field of view with more than 85 percent precision. These findings demonstrate the capability for precision technology to assist non-invasive fish condition monitoring and biomass estimation, benefiting fish health, welfare, and production efficiency.

New method detects off-flavor in water and fish tissue. An increase in U.S. land-based aquaculture systems to produce Atlantic salmon and other fish is expected and will require methods to monitor off-flavor to ensure fish products are acceptable to consumers. Off-flavor compounds from bacterial and fungal metabolites can accumulate in fish tissues and result in unpalatable “earthy” or “muddy” flavors. Previous methods to detect off-flavor compounds could only process 10 samples a day and cost $120 per sample. University of Maine researchers and ARS scientists in Franklin, Maine, developed a new method of detecting geosmin and 2-methylisoborneol, two compounds that cause off-flavor in water and fish tissues. The new method can process at least 40 samples per day at a cost of $40 per sample. This lower cost is more affordable for fish farmers, enabling them to provide consumers with consistently high-quality products.

Improved sex reversal in rainbow trout. Most of the rainbow trout industry depends upon production of all-female fish for grow-out. The maintenance of all-female lines depends upon creating genetic “XX” females that produce male gametes, or sperm, a process that involves

supplementing feed with male steroids for 60 days. Drawbacks of this approach include 1) the need to surgically remove testes to access the sperm because the sex-reversed fish seldom develop functional sperm ducts that enable sperm to migrate from the male testes, and 2) the cost of infrastructure to prevent steroid releases into the environment. ARS researchers in Leetown, West Virginia, developed an improved approach to sex reversal by exposing female fry to male steroids through immersion rather than feeding. Treatment consisting of seven 1-hour weekly immersions in the steroid beginning at 4-7 days after hatching greatly reduced the number of fish with sperm duct abnormalities, avoiding the need to euthanize fish to surgically remove testes to harvest sperm, and preventing environmental contamination by enabling the steroid to be easily captured from the immersion bath.

Improving fecal stability and reducing nutrient leaching of rainbow trout feeds. Uneaten feed and fish feces release nutrients that enrich effluent waters from production systems, which can lead to algal blooms and other unintended consequences. Replacing fishmeal (FM) in rainbow trout diets with plant-based protein sources such as soybean meal (SBM) and soy protein concentrate (SPC) has compounded this problem, since these feeds can reduce fecal stability, increase fecal fine particles, and add nutrients to water. ARS researchers in Hagerman, Idaho, and Bozeman, Montana, determined that rainbow trout feeds comprised of a mixture of poultry byproduct meal (PBM), corn protein concentrate (CPC), and SPC with guar gum binder produced more stable feces characterized by larger fecal particles and fewer fine fecal particles, compared to standard fishmeal-based and commercial feeds. Large fecal particles are more easily collected, enabling nutrient capture and minimizing their release to the environment.

Improved growth in North American Atlantic salmon smolt. Commercial salmon farming is rapidly expanding in the United States, so selectively bred North American stocks that can compete with European imports are needed. A higher weight at smolt usually results in a faster time to market and a higher chance of survival. When ARS researchers in Franklin, Maine, began selectively breeding salmon in 2007, the average weight at smolt was 65 grams per fish. After four generations of selecting for growth, the average weight at smolt more than doubled at 167 grams per fish. This improved germplasm has been transferred to industry stakeholders and will have an immediate economic impact on reducing the time to market and improving profitability.

Problem Statement 2B: Reduce the Impacts of Disease in Salmonid Aquaculture
Environmental conditions exacerbate disease outbreaks and increase infectious disease losses from viral, bacterial, and parasitic pathogens, which often occur in mixed combinations. Developing new strategies to control disease requires identifying the host molecular pathways associated with innate and acquired immune responses to common pathogens and understanding how the host immune system evades pathogens and prevents or mitigates the onset of disease. Acquiring the information needed to develop genetic enhancements for disease resistance requires studying aquatic animals with divergent responses to disease challenge and identifying the genetic sources of variation correlated with innate and/or acquired immune status. In addition, more information is needed about how the immune system of aquatic animals responds to vaccination, the variation in vaccine responsiveness, and the mechanisms of protection, all of which will help identify options for deploying multiple lines of protection. Understanding how environmental conditions influence host immunity and pathogen prevalence may reveal new strategies for reducing disease outbreaks in production environments.

Table 11. Milestones for Problem Statement 2B.

	2018	2019	2020	2021	2022
# Fully or Substantially Met	31	16	34	27	29
# Not Met	4	3	4	5	11
Total	35	19	38	32	40

2019

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.

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.

2020

A bacteriophage for preventing disease in rainbow trout. Bacteriophages (phages) are viruses that infect and kill bacteria, self-replicating in high numbers in the process. Used against disease-causing microbes, phages are excellent candidates for the prevention or treatment of bacterial diseases. ARS researchers at Leetown, West Virginia, identified a new phage that kills *Yersinia ruckeri,* the rainbow trout pathogen. This phage is unique; in addition to killing its bacterial host by infection, it also binds to and degrades lipopolysaccharide, a large carbohydrate structure that covers the surface of some bacteria and reduces the effectiveness of the trout immune system. By trimming off this protective layer, the phage renders *Yersinia ruckeri* susceptible to the trout immune system, preventing its survival inside its fish host.

2021

Recycled water use affects rainbow trout disease susceptibility and survival. Fish farmers often re-use water to conserve freshwater resources, but re-use is associated with reduced water quality that in turn is often blamed for disease outbreaks. More information is needed about risk levels associated with short or long-term re-use water exposure, including how risks are associated with genetic traits and vaccine response. ARS researchers at Leetown, West Virginia, collaborated with researchers at Virginia Institute of Marine Science and Virginia Tech to study how two commercial breeds of rainbow trout vaccinated against infectious hematopoietic necrosis virus responded to exposure to re-used water. They found chronic re-use water exposure increased risk of death more than 46-fold and that these risks varied with the genetic makeup of the trout. This research demonstrated the importance of mitigating effects of poor water quality and improving fish genetics to reduce disease loss.

Resistance to infectious hematopoietic necrosis virus in rainbow trout. Infectious hematopoietic necrosis (IHN), a viral disease of salmonid fishes, causes significant mortality and economic losses in aquaculture. Improving resistance to IHN through traditional, family-based selective breeding has shown promise, but is limited since IHN resistance cannot be measured in potential breeders. ARS researchers in Leetown, West Virginia, used disease resistance data from a commercial rainbow trout breeding program to compare the accuracy of genetic merit and family-based selective breeding. They found genome-enabled breeding strategies improved the accuracy of an individual's genetic merit for IHN resistance by 15 percent. These results indicate that genome-enabled breeding can be more effective than traditional, family-based selection in improving the resistance of rainbow trout to the IHN virus.

2022

Detection of a biomarker for bacterial cold-water disease. Fish farmers need rapid methods to assess the health and disease status of their fish. ARS researchers in Leetown, West Virginia, and St. George's University collaborators identified a novel serum biomarker that can distinguish between healthy and diseased fish. The biomarker was increased more than 20-fold in the plasma of fish bred for susceptibility to bacterial cold-water disease when exposed to the causative pathogen *Flavobacterium psychrophilum*. An assay was developed and commercialized that can be completed in under 1 hour, providing a commercially available, rapid method for monitoring population health of rainbow trout and Atlantic salmon during grow-out.

***Marker-assisted selection for resistance to bacterial cold-water disease*.** Bacterial cold-water disease (BCWD) is one of the most devastating diseases in rainbow trout aquaculture. Improving resistance to BCWD using traditional family-based selective breeding or genomic selection with markers spanning the entire genome is promising but limited, because these methods are labor intensive, costly, and the resistance trait cannot be measured directly in potential breeders. For these reasons, marker-assisted selection is advantageous because it can directly and relatively inexpensively predict the genetic merit of potential breeding animals using just a small number of DNA markers. ARS researchers in Leetown, West Virginia, identified a set of six DNA markers that can be used to predict the genetic merit of breeding animals just as accurately or even more accurately than the traditional family-based selective breeding approaches for genomic selection. Using these markers is simpler and less expensive, and the effectiveness of this approach was demonstrated in a commercial breeding population, indicating that it can further improve the efficiency and sustainability of rainbow trout aquaculture in the United States.

Component 3: Improving the Efficiency and Sustainability of Hybrid Striped Bass Aquaculture

Table 12. Peer -Reviewed Publications for Component 3 Hybrid Striped Bass 2018 – 2022

	2018	2019	2020	2021	2022	Total
Component 3 Hybrid Striped Bass	8	2	2	4	4	20

Problem Statement 3A: Enhance Hybrid Striped Bass Aquaculture Production
Hybrid striped bass producers need systems that optimize and maximize production, reduce environmental impacts, increase market competitiveness, sustain producers, and maintain and enhance consumer desirability. Research in the disciplines of genetics, nutrition, microbiology, and immunology will support the biological improvement of aquatic animals, while research in water quality management and production system engineering will support production improvement and reduce potential adverse environmental impacts.

Table 13. Milestones for Problem Statement 3A.

	2018	2019	2020	2021	2022
# Fully or Substantially Met	5	6	6	5	18
# Not Met	1	0	0	3	3
Total	6	6	6	8	21

2021

Sticky fish eggs thwarted by milk. Hybrid striped bass eggs become extremely sticky after they are fertilized. In a hatchery, this results in the eggs clumping together, which limits availability of oxygen and enables fungal infestations. Both problems can destroy an entire batch of eggs. Fish farmers typically use tannic acid treatments to prevent egg adhesion, but it is costly and, if left too long, will form a hard layer on the surface, which can prevent embryos from hatching. ARS researchers in Stuttgart, Arkansas, investigated 12 candidate compounds to prevent stickiness and found that 10 percent whole milk treatment was the most effective strategy. As a result, the largest commercial hybrid striped bass hatchery immediately began using milk for their 2020 production; in 2021, the hatchery exclusively used the milk to prevent clumping and successfully produced 80.9 million larvae using methods developed by ARS.

2022

Zinc: the cheaper, safer alternative to copper for preventing pathogens in aquaculture. Copper has been used as a water treatment to prevent bacterial infections for decades, but the price of copper has risen to $10K per ton and demand has increased. Zinc is a closely related element, but it has a market price of $3K per ton. ARS researchers in Stuttgart, Arkansas, ran a series of toxicity trials with largemouth bass fry to evaluate the possibility of zinc as an alternative to copper as a water decontaminant. Their results indicate that zinc is just as effective as copper in killing fish pathogens, but it is only half as toxic to fish, providing farmers a cheaper, safer treatment for preventing disease outbreaks in aquaculture.

Striped bass are highly susceptible to common aquaculture diseases. ARS researchers established the susceptibility of hybrid striped bass (HSB) and their parental species—white bass

(WB) and striped bass (SB)—to the most common aquaculture diseases identified by the HSB industry, including columnaris disease, motile aeromonad septicemia, and streptococcosis. Following challenge, only 1 percent of SB survived the three diseases and died twice as early as WB and HSB. These results established that WB are the most resistant to all three diseases, SB are most susceptible, and HSB are intermediate to the two parental species. ARS is currently working with North Carolina State University (NCSU) partners to incorporate these results into the ARS – NCSU HSB Selective Breeding Program to incorporate SB disease resistance as a selection trait for potential improvement of HSB disease resistance.

Component 4: Enhancing Shellfish Aquaculture

Table 14. Peer -Reviewed Publications for Component 4 Shellfish 2018 – 2022

	2018	2019	2020	2021	2022	Total
Component 4 Shellfish	4	7	4	7	3	25

Problem Statement 4A: Enhance Shellfish Aquaculture Production
Shellfish producers need systems that optimize and maximize production, reduce environmental impacts, increase market competitiveness, sustain producers, and earn consumer confidence. Research in the disciplines of genetics will support the breeding of aquatic animals, while research in ecology will support the improvement of production systems by characterizing factors that affect environmental conditions and finding strategies to minimize their impacts.

Table 15. Milestones for Problem Statement 4A.

	2018	**2019**	**2020**	**2021**	**2022**
# Fully or Substantially Met	10	4	7	8	11
# Not Met	0	3	0	1	1
Total	10	7	7	9	12

2019

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.

2021

A multi-state oyster herpesvirus detection program. The Ostreid herpesvirus 1 (OsHV-1) has significantly harmed Pacific oyster production around the globe. The first United States detection was in 1995 in Tomales Bay, California, and a more virulent microvariant strain previously detected in other countries was found in San Diego Bay, California, in 2018. Recognizing the risk of regional spread, a multi-state sentinel program was initiated by ARS researchers in Newport, Oregon, and their collaborators to monitor the prevalence and disease development of OsHV-1 in naïve and genetically uniform hybrid oysters planted at commercial farms in California, Oregon, and Washington. A second group of oysters selected for OsHV-1 tolerance was planted for comparison in San Diego and Tomales Bay. Oyster mortalities were almost 100 percent for both families in San Diego Bay where the microvariant was present. Mortalities also occurred in Tomales Bay, but survival was higher for the tolerant family. Mortality events occurred during high seawater temperature spikes and followed peak virus levels. This information enables researchers to establish a consistent protocol for a more extensive OsHV-1 monitoring program.

Component 5: Developing Marine Finfish Seedstocks

Funding for Component 5 was first provided in 2019 after the project plan review was initiated. ARS is in the process of filling vacant scientist positions, work towards this Component has been accomplished through our university Cooperators.

Table 16. Peer -Reviewed Publications for Component 5 Marine Finfish 2018 – 2022

	2020	2021	2022	Total
Component 5 Marine Warmwater Finfish	1	3	3	7

Problem Statement 5A: Develop Warmwater Marine Finfish Seedstocks Optimized for Aquaculture Production Efficiency

Aquaculture producers need access to seedstocks that are available year-round and optimized for the production environment. Research is needed to develop seedstocks that are bred for maximum production efficiency and have minimal impacts on the environment and native populations. Research in the disciplines of genetics, fish health, nutrition, reproductive biology, and physiology will contribute to the development of seedstocks that meet these criteria.

Table 17. Milestones for Problem Statement 5A.

	2020	**2021**	**2022**
# Fully or Substantially Met	5	6	9
# Not Met	3		
Total	8	6	9

2020

A draft genome sequence for Florida pompano. The lack of available genome information is a hurdle in implementing state-of-the-art selective breeding strategies for many aquaculture species, including Florida pompano. ARS funded researchers at Ft. Pierce, Florida, established a complete draft genome of the Florida pompano, using a hybrid sequencing method and a novel bioinformatics workflow. This draft genome will improve farm production and profitability and enhance breeding strategies by allowing researchers to identify genes associated with aquaculture production efficiency and product quality.

Nutritional requirements of Florida pompano broodstock. Quality broodstock diets increase reproductive success and seedstock quality, leading to increased hatchery success, on-farm efficiencies, and profitability. The lack of optimal diets for Florida pompano broodstock (especially during the spawning season) continues to present an obstacle to commercial production, because nutritional status is a powerful determinant of egg quality and the successful development of eggs and larvae. ARS-funded researchers at Ft. Pierce, Florida, employed comprehensive and quantitative lipid analysis to determine different egg and larval lipid compositions and identify lipid requirements for larval development and successful reproduction of Florida pompano. Hatchery managers and marine finfish producers will benefit from efficiencies associated with meeting optimum nutritional needs for reproduction and successful seedstock production.

2021

Algae oil in fish diets is a viable alternative to fish oil. Competition for the limited supplies of long-chain, omega 3 fatty acids, or "fish oil", used in the production of farmed fish feeds has created the need to identify alternative sources of long-chain, omega 3 lipids. Vegetable oils have proven to be insufficient in providing the nutritional requirements for normal growth and well-being of marine finfish and do not provide the heart healthy nutrients valued by U.S. seafood consumers. ARS funded researchers in Fort Pierce, Florida, and their collaborators demonstrated that oils from algae can produce the same long chain omega 3 fatty acids found in fish oils. These findings provide an alternative lipid source that will increase the capacity for raising high-quality marine finfish aquaculture products and meeting the nutritional needs of U.S. seafood consumers.

Nutritional value of new feed ingredients for Florida pompano. The rising costs and ecological impacts of farmed fish feeds have necessitated the search for new and less expensive ingredients to ensure the profitability of U.S. aquaculture farms. ARS funded researchers in Fort Pierce and Tallahassee, Florida, determined that the nutritional needs of Florida pompano can be met by clam byproducts and hemp fibers that are biproducts of the textile industry. Both products are plentiful and provide growth-promoting and healthy nutrients, which indicates they should make very good supplements to marine finfish feeds. These findings have been provided to fish feed manufacturers for the development and marketing of more sustainable fish feeds, and for creating alternative revenue streams for other industries.

2022

Advances in yellowtail amberjack spawning and nutrition. The domestic yellowtail amberjack is a consumer favorite, and there are ongoing efforts to increase production by establishing offshore farms. However, the industry is challenged by the need for year-round production of juveniles to stock these farms and efficient diets for feeding spawning fish to produce efficient, hardy, and robust juveniles. Researchers in Fort Pierce, Florida, and Hubbs Sea World Research Institute (California) collaborators established methods for the successful out of season spawning of yellowtail broodstock. In addition, the team demonstrated that commercially available diets can be used to produce high egg and larval quality and quantities to make the U.S. industry competitive. This accomplishment contributes to the year-round availability of a consistently high-quality product.

Identifying founder stocks for a Florida pompano broodstock program. When establishing a new selective breeding program, it is essential to understand the genetic makeup of the parents to maximize diversity in the gene pool and avoid contaminating the existing gene pool. Researchers at Harbor Branch Oceanographic Institute found that wild populations of Florida pompano off the Atlantic/East Coast of Florida and the Gulf/West Coast of Florida are genetically similar, which suggests they are one population. This provides a greater understanding of the genetic variation found in the wild and informs the collection of broodstock for initiating a selective breeding program to improve production efficiency.

Component 6: Developing Sustainable Aquaponic Production Systems

Aquaponics is a resource-efficient, controlled environment agriculture (CEA) that integrates intensive aquaculture and greenhouse vegetable production systems. Aquaponics can help address food security and safety by contributing to food production in urban, suburban, and rural communities. To be sustainable, aquaponic system managers must optimize inputs and outputs (energy and materials), and internal biological production processes for maximum resource use efficiencies that decrease water usage and maximize nutrient retention while ensuring food safety for the protection of consumers. Aquaponics integrates aquaculture and hydroponic vegetable production, facilitates food production system diversification, creates new sources of employment and economic development, and provides a process for highly intensive and sustainable food production that addresses future food scarcity needs. Developing a model CEA aquaponic system that is scalable and commercially viable is critically important to address current and future sustainable food production and economic opportunity needs.

To this end, in the FY2021 Budget Congress provided the following guidance: "The Committee recognizes the need for improving the development of fresh food production technology to address domestic food security and safety demands." In support of this guidance Congress provided the ARS funding "to coordinate with academic partners and industry to develop a model-controlled agriculture aquaponics system that is scalable and commercially viable with the purpose of advancing increased fresh food production, improved food safety, decreased water usage, improved nutrient utilization, and decreased negative environmental impacts."

Problem Statement 6A: Optimize Aquatic Animal Species Production Systems for Aquaponics
Problem Statement 6B: Optimize Plant Production Systems for Aquaponics
Problem Statement 6C: Optimize the Integration of Fish and Plant Production Systems

Funding for Component 6 was first provided in 2021. ARS is in the process of filling vacant scientist positions, work towards this Component is primarily accomplished through our university Cooperators.

Additional Accomplishments

These accomplishments do not fall directly under the current NP106 Action Plan but were under the previous Action Plan or were the result of ARS scientists using their knowledge, expertise, and research capacity for broader impact.

Table 18. Peer -Reviewed Publications for Additional Accomplishments 2018 – 2022

	2018	2019	2020	2021	2022	Total
Additional Publications	13	10	12	12	13	60

2018

Phytase enzyme substitutes for inorganic phosphorus in diet for tilapia biofloc production. Fish feed typically is supplemented with inorganic phosphorus to ensure adequate dietary phosphorus availability, especially in diets that contain high percentages of plant feedstuffs. Phosphorus is present in plant feedstuffs as phytate, which is not bioavailable to fish because their digestive system lacks sufficient phytase enzyme. As a result, phytate is excreted unmetabolized and high concentrations subsequently accumulate in biofloc technology production systems because of the high quantities of feed fed daily to fish. In a study conducted in an outdoor biofloc production system, ARS researchers at Stuttgart, Arkansas, found that phytase enzyme can substitute completely for inorganic phosphorus in fish feed without negatively affecting tilapia growth and that this substitution reduces phosphorus excretion by tilapia by about 50 percent, resulting in improved water quality. Reducing potential phosphorus excretion will help fish farmers meet National Pollutant Discharge Elimination System permit requirements.

Intensive production of stocker size tilapia in biofloc systems. Market-size fish can be harvested from grow out ponds sooner if larger advanced fingerlings or stockers are stocked but optimizing the overall production cycle requires the efficient production of stockers. Stocking rate is one factor that affects fish growth and can be manipulated to obtain stocker size fish. Much greater numbers of fish are stocked in biofloc technology production systems because microorganisms in the pond water consume fish waste quickly and help maintain suitable water quality. ARS researchers at Stuttgart, Arkansas, quantified the relationship between tilapia stocking rate and growth and yield of stocker-size fish in an outdoor biofloc production system. They found yields of stocker-size tilapia increased linearly with stocking rate, while mean individual weight decreased linearly, but that stocker-size fish was produced at all stocking rates. Farmers can use these findings to optimize their annual production of stocker-size tilapia by manipulating stocking rates and the length of production cycles.

2021

Breeding Nile tilapia for disease resistance does not affect harvest weight. Fish growth is economically important for farmers, so the relationship between growth and other traits that affect performance is paramount. ARS scientists in Auburn, Alabama, and industry stakeholders demonstrated that resistance to *Streptococcus iniae* and *S. agalactiae* is heritable and used this finding to develop improved lines of tilapia with increased resistance to these diseases. They examined data from eight generations of selective tilapia breeding, including survival following *S. iniae*/*S. agalactiae* infection, and did not find any significant associations between harvest

weight and survival. These results demonstrate that selectively breeding for disease resistance will not reduce tilapia harvest weight, and support using multi-trait selection as a potential strategy to balance growth and disease resistance.

Aquaculture Research Related Activities

ARS scientists regularly provide leadership or lend their expertise to their communities through scientific organizations, species-focused commodity groups, local educational institutions, or Federal working groups. This section provides examples of ARS research related activities 2018 – 2022.

Federal Agency Coordination:

- Leadership in the National Science and Technology Council (NSTC) Subcommittee on Aquaculture provided by an ARS Co-Chair and Executive Secretary and the Chair of the Science Planning Task Force developing the National Strategic Plan for Aquaculture Research published in 2022;
- Assistance provided to USDA APHIS towards the development of the National Aquaculture Health Plan and Standards;
- In collaboration with the Office of the Secretary, forming and leading the USDA Working Group on Aquaculture to meet USDA requirements of the President's Executive Order on Seafood Competitiveness and ensure customer service to aquaculture stakeholders;
- Provided consultation to USDA leadership towards including aquaculture in implementation of the Coronavirus Aid, Relief and Economic Security (CARES) Act through Coronavirus Food Assistance Program (CFAP) and CFAP2;
- Partnering with HeroX and NASA to award a prize challenge entitled, "Protecting the Natural Flavor of Catfish". A total of 589 participants from 48 countries formed into 29 teams making 85 submissions, with the top six prizes awarded;
- Contributed to the Theme Teams led by the Office of the Chief Scientist towards implementing the USDA Science Blueprint: A Roadmap for USDA Science from 2020 to 2025;
- Supported the Bureau of Labor and Statistics regarding inclusion of aquaculture products in the Producer Price Index;
- Assembled and led a team of U.S. scientists to develop the aquatic pathogen section of the Agricultural Biorisk Compendium;
- Co-organizing with NOAA the annual United States-Japan Natural Resources Aquaculture Scientific Symposium;
- Partnering with USDA NIFA, National Oceanic and Atmospheric Administration (NOAA) and Harbor Branch Oceanographic Institute to publish a special issue of the Journal of the World Aquaculture Society containing 14 articles that describe the State of the Art for marine finfish aquaculture;
- Partnering with the USDA Office of the Chief Scientist to organize a series of virtual listening sessions titled, "***Aquaculture is Agriculture: USDA's Role in supporting Farmers of Fish, Shellfish and Aquatic Plants***." A white paper having 21 authors from 12 USDA Agencies or Offices details the USDA programs and summaries and input received from stakeholders, available online;
- Forming and leading an interagency Federal Working Group in response to Congressional Guidance that will explore opportunities for reducing ocean acidification through the farming of seaweeds and seagrasses;
- Representing USDA on the OSTP Interagency Council for Advancing Meteorological Services Committee; and

- Partnering with NOAA and USDA NIFA to respond to the Food and Agriculture Organization of the United Nations (FAO) Code of Conduct for Responsible Fisheries Questionnaire.

Outreach, Mentorship and Training
NP106 scientists are active members of their communities, engaging students and general audiences through outreach activities having scientific or aquaculture themes.

Table 19. NP106 advising, mentorship, training and outreach activities 2018 – 2022.

Advising, Mentorship and Outreach	2018	2019	2020	2021	2022
Students and Post-Docs (ARS and Non-ARS)	43	12	29	20	17
Scientists Serving as Advisors	2	5	3	1	
Mentorships	5	4	2		1
Adjunct or Other Appointments	7	9	8	1	5
Student Targeted Outreach	*				
Student related outreach activities - # of activities (Presentations to schools, Science fair participation, Student tours/visits to ARS locations)	*	25	18	5	5
Student related outreach activities - # of student participants (Presentations to schools, Science fair participation, Student tours/visits to ARS locations)	*	1,658	414	30	315
Other Outreach					
Other Outreach Activities - # of activities	81	35	23	12	8
Other Outreach Activities - # of student participants	*		720	31	422
Other Outreach Activities - # of non-student participants	*	1712	3,528	1,937	745

*no data for 2018

Editorships
ARS scientists and funded collaborators serve as Editor, Associate or Guest Editor for the following scientific journals.
- Animal Gene
- Aquacultural Engineering
- Aquaculture Environment Interactions
- Aquaculture Research
- BMC Genomics
- BMC Genomics Data
- Developmental and Comparative Immunology
- Evolutionary Biology of Immunoglobulins

- FEMS Microbiology Letters
- Foods
- Frontiers in Genetics
- Frontiers in Immunology
- Frontiers in Marine Science
- Frontiers in Microbiology
- Frontiers in Veterinary Sciences
- Genes
- Genetics Selection Evolution
- Journal of Animal Feed Science and Technology
- Journal of Applied Aquaculture
- Journal of Applied Microbiology
- Journal of Applied Aquaculture Research and Development
- Journal of Fish Diseases
- Journal of Shellfish Research
- Journal of Fish and Shellfish Immunology
- Journal of the World Aquaculture Society
- Lipids
- Marine Biotechnology
- mBio
- North American Journal of Aquaculture
- Sustainable Food Proteins

Society and Professional Organization Memberships and Offices

- 9th International Symposium on Aquatic Animal Health – Special Session Co-organizer
- Advisory Committee for the NOAA Sea Grant FAO Guideline-U.S. Management Aquaculture Project
- American Association for the Advance of Science
- American Fisheries Society
- American Fisheries Society Fish Health Section
    - Vice-President, President-Elect, President, Past-President
    - Handbook Revision and Oversight Committee
    - Long-Range Planning Committee
    - Chair of the Policy/Position Development Committee
    - Nominating and Balloting Committee
- American Association of Immunologists
- American Oil Chemists Society
- American Society for Microbiology
- Aquaculture Engineering Society, Secretary/Treasurer
- Arkansas Bioinformatics Consortium (AR-BIC)
- Breeding & Genetics panel advisor and Co-Chair, NOAA SeaGrant StriperHub
- European Association of Fish Pathologists
- Functional Annotation of Animal Genomes
- Global Aquaculture Society

- Governing Board of the American Fisheries Society
- Institute of Food Technologists
- International Society for Animal Genetics
- International Society for Developmental and Comparative Immunology
- International Society of Fish and Shellfish Immunology
- National Aquaculture Association
- U.S. Trout Farmers Association
- National Research Support Group – 8 (NRSP-8), National Animal Genome Research Program, Aquaculture Genomics Working Group
- National Shellfisheries Association
- North Carolina Aquaculture Association
- Northeast Regional Aquaculture Center Board of Directors
- Southern Regional Aquaculture Center Technical Advisory Committee
- Striped Bass Growers Association
- United States Aquaculture Society
    - Awards Committee
    - Continuing Education Subcommittee
    - Elections Committee
    - Publications Committee
    - Membership Committee
    - Strategic Planning Committee
    - Student Liaison (ex officio)
    - President and Past President
- World Aquaculture Society
    - Board of Directors

Awards and Recognition

ARS scientists and their funded collaborators have received the following awards or recognition during 2018 - 2022.

2018

Dr. Craig Tucker, Stoneville, Mississippi, received the 2018 Distinguished Alumnus Award from Humboldt State University.

The **National Cold-Water Marine Aquaculture Center,** Franklin, Maine, received the 2018 Mid-Atlantic Regional Excellence in Technology Transfer Award at the Federal Laboratory Consortium Mid-Atlantic Meeting in Rockville, Maryland, on November 14, 2018.

Dr. Benjamin Beck, Dr. Mediha Aksoy, Auburn, Alabama and **Dr. Adam Fuller**, Stuttgart, Arkansas et al., Publications Award from American Fisheries Society; best publication in North American Journal of Aquaculture: "Influence of Kaolin Clay on *Aeromonas hydrophila* Growth, Chemotaxis, and Virulence to Channel Catfish" 2018.

Dr. Benjamin Beck, Auburn, Alabama, Auburn University College of Agriculture Outstanding Alumni Award, Honorees are professionals selected as the most outstanding alumni by their respective academic departments that have had an impact on their profession and on society in general, 2018.

Dr. Marti Riche: an ARS funded scientist in Fort Pierce, Florida, Grand Bassa County Association in the Americas Lifetime Achievement Award for work in Africa in the field of aquaculture development and education.

2020

Dr. Bart Green, Kevin Schrader, Steven Rawles, Carl Webster and Matthew McEntire, Stuttgart, Arkansas, co-authors for an article entitled: *Channel catfish production in biofloc technology systems* was one of the ten most read articles in the first half of 2020, in the publication Global Aquaculture Advocate.

Dr. Keshun Liu, Aberdeen, Idaho, received the American Oil Chemists' Society (AOCS) Protein and Co-Products Division Lifetime Achievement Award.

Dr. David Straus, Stuttgart, Arkansas, received an award for his paper being recognized as one of the most read in *Reviews in Aquaculture*.

Dr. Benjamin LaFrentz, Auburn, Alabama, received a American Fisheries Society Fish Health Section Certificate of Appreciation in recognition of the Achievement of Excellence as the Policy/Position Development Committee Chair, 2019.

Dr. Benjamin LaFrentz, Auburn, Alabama, received a Certificate of Appreciation in recognition of personal contributions to the advancement and improvement of fisheries science, in the professional and public interest, by serving as President of the Fish Health Section of the

American Fisheries Society and as a member of the Governing Board of the American Fisheries Society, 2020.

Dr. John Davidson, **Dr. Steven Summerfelt**, **Dr. Christopher Good,** Shepherdstown, West Virgina, **Dr. John Bland,** New Orleans, Louisiana and Dr. Gregory Fischer Aquacutlural Engineering Society Superior Paper Award for "Depuration system flushing rate affects geosmin removal from market-size Atlantic salmon Salmo salar. 2020. Aquacultural Engineering, 90: 102104.

2021

Dr. Benjamin Beck, Auburn, Alabama, received the USDA-ARS, Research Leadership and Center Directorship Award, "For innovative and visionary leadership promoting scientific integrity, impact, and investment in human capital" 2021.

Dr. Craig Tucker, Stoneville, Mississippi, along with lead author **Dr. Claude Boyd** and their co-authors**,** received the best paper award for 2020 in the category of review article entitled "Achieving sustainable aquaculture: Historical and current perspectives and future needs and challenges" for the Journal of the World Aquaculture Society.

Drs. Kenneth Davis, Phillip H. Klesius, Chorn E. Lim, Yniv Palti, and Craig S. Tucker, were identified in the top 2% of scientists worldwide across 22 scientific disciplines.

Dr. Bart Green, Stuttgart, Arkansas, was invited to give a presentation (virtually) at the Aquaculture Africa Magazine Flagship Webinar: "Biofloc Aquaculture – For Fish Farmers or Wizards?", Johannesburg, South Africa.

Dr. Andrew "Drew" Mitchell, retired ARS scientist from the Stuttgart National Aquaculture Research Center in Stuttgart, Arkansas, was honored with the National Aquaculture Association's 2021 Joseph P. McCraren Award for Distinguished Lifetime Contributions to U.S. Aquaculture.

Governor of Arkansas Asa Hutchinson presenting the award.

Dr. Rakesh Ranjan, Shepherdstown, West Virginia received the Outstanding Reviewer Award from the American Society for Agricultural and Biological Engineers.

2022

Dr. Benjamin Beck, Dr. Benjamin LaFrentz, Dr. Craig Shoemaker, Auburn, Alabama, received the USDA-ARS, Southeast Area Technology Transfer Award, "One fish, two fish,

healthier new fish", for development of fast-growing Nile tilapia with resistance to economically important bacterial pathogens.

Dr. Benjamin LaFrentz, Dr. Craig Shoemaker, Dr. Benjamin Beck, Auburn, Alabama, received a 2023 Impact Award from the Federal Laboratory Consortium for Technology Transfer (FLC), "One fish, two fish, healthier new fish", for development of fast-growing Nile tilapia with resistance to economically important pathogens.

Dr. Keshun Liu, Aberdeen, Idaho received the 2022 Alton E. Bailey Achievement Award from American Oil Chemists Society.

Dr. Keshun Liu, Aberdeen, Idaho received the 2022 Archer Daniel Midlands (ADM) Best Paper Award in protein chemistry and nutrition, by AOCS Proteins and Co-Products Division for a paper "An International Collaborative Study on Trypsin Inhibitor Assay for Legumes, Cereals and Related Products" (Liu et al. 2021, JAOCS, 98:375-390).

Dr. Keshun Liu, Aberdeen, Idaho was selected as a 2022 Fellow of International Academy of Food Science and Technology for outstanding contribution to food science and technology.

International Collaborations

The United States is not currently a leader in aquaculture in terms of production volume or farm gate value, currently ranking 18th and importing far more aquaculture products that we produce. Given foreign investment, experience and expertise in aquaculture, ARS scientists have established collaborations with many countries that enhance research capacity. During 2018 – 2022, ARS scientists collaborated with partners from Belgium, Brazil, Canada, China, Denmark, European Union, Finland, France, Georgia, Germany, Italy, Japan, Malaysia, Norway, Peru, Portugal, Spain, Thailand, Uganda and the Global Seafood Alliance (See Appendix 10).

APPENDICES

Note: Appendices 1-7 include ***all*** accomplishments (not just select accomplishments) and publications from individual project annual reports (written Fiscal Year timeframe). Some accomplishments will be redundant with select revised accomplishments listed in the report narrative.

Appendices 8 and 9 reports information on outgoing and incoming funded agreements, respectively.

Appendix 10 reports details in international collaborations.

Appendix 1 – Accomplishments and Publications for Component 1: Improving the Efficiency and Sustainability of Catfish Aquaculture

Problem Statement 1A: Improve Catfish Aquaculture Production Efficiency

Accomplishments

2018

Management practices for pre-harvest off-flavors of catfish raised in split-ponds. The interest and use of variations of partitioned aquaculture systems (PAS) by the southeastern U.S. catfish farming industry continues to grow, and split-pond systems, one type of PAS, are designed to improve management of dissolved oxygen levels and fish waste products (e.g., ammonia) compared to conventional earthen ponds that have been used for many decades. While many have assumed that fish off-flavor incidence will be reduced in split-ponds because previous research showed that off-flavors were rare in fish grown in PAS, there has not been any assessment of the occurrences and intensities of common off-flavor episodes in split-pond systems. During a multi-year study, ARS researchers at Oxford, Mississippi, and Stoneville, Mississippi, evaluated water and catfish fillet samples collected from commercial and research split-ponds located in west Mississippi and west Alabama for intensities of earthy and musty off-flavor compounds while phytoplankton ecology of the split-ponds was also determined. Concentrations of earthy and musty compounds in fillets and the description and intensities determined via sensory analysis were similar to those reported previously for off-flavor catfish from conventional ponds. In addition, the types of phytoplankton and community structures observed in the split-ponds were not different from those commonly observed in conventional catfish ponds. These results demonstrate that farmers using split-ponds will use the same management approaches and preharvest sampling of catfish to monitor for the presence of off-flavors as those used for dealing with earthy and musty off-flavor problems in catfish raised in conventional ponds (e.g., applications of algicides). These determinations are of critical importance to commercial culturists who are considering the benefits and constraints of adopting split-pond technology.

Development of management practices to minimize size variation in hybrid catfish food fish ponds. Two management practices to reduce food fish size variation and resulting weigh backs have been developed by ARS scientists at Stoneville, Mississippi, in collaboration with Mississippi State University scientists also at Stoneville, Mississippi. While in some cases mid-season partial harvest of larger fish can be used with an economic benefit, most farmers have adopted the use of graded fingerlings as has been recommended by Agricultural Research Service scientists when processors penalize farmers for out-of-size food fish.

Assessment of phytase super-dosing in catfish diets. ARS scientists at Stoneville, Mississippi, in collaboration with Mississippi State University scientists, conducted two experiments to evaluate responses of hybrid catfish, female *Ictalurus punctatus* × male *Ictalurus furcatus*, to super-dosing of 6-phytase added to existing commercial catfish feeds. It was shown that phytase super-dosing does not appear to have additional benefits beyond the standard dose, at least on promoting growth and preventing anemia in catfish, and also had no beneficial effects on water

quality. Ongoing technology transfer efforts recommend 500 phytase units [FTU/kg] phytase and not super-dosing to replace inorganic phosphorus in catfish feeds.

Determination of dissolved oxygen requirements of separated hybrid catfish eggs incubated in vertical tubes. ARS scientists at Stoneville, Mississippi, had previously determined that channel catfish eggs, incubated as intact egg masses, require water with dissolved oxygen at over 95% air saturation during the last day of incubation for optimum development. Recent research by the same scientists determined that the maximum dissolved oxygen requirement for separated hybrid catfish eggs during the last days of incubation was only 79 percent. ARS scientists at Stoneville, Mississippi, have begun recommending that farmers maintain the dissolved oxygen in vertical tubes at or above 80 percent air saturation during the last two days of incubation to maximize egg development.

Genomic selection for growth and carcass yield in the Delta Select strain of channel catfish. Estimation of breeding values in catfish broodstock has depended on traditional methods based only on pedigree information and phenotypic data. ARS scientists in Stoneville, Mississippi, in collaboration with scientists from the University of Georgia, have implemented selection based on genomic estimated breeding values. An array of 55,000 probes for single nucleotide polymorphic loci provided genotypes which were then incorporated into the calculation of estimated breeding values. This approach led to 30 percent improvement in breeding value accuracy for growth and carcass yield in 2,000 Delta Select strain catfish. The improved breeding value accuracy will result in more rapid genetic gain for growth and carcass yield in the Delta Selects which will be released to U.S. catfish farmers to improve their production efficiency.

Evaluation of growth and processing yield of blue catfish strains. Hybrids among blue and channel catfish now comprise approximately 75 percent of annual U.S. catfish production. Therefore, evaluation of blue catfish strains for growth and carcass yield has become an important aspect of the breeding program conducted by ARS scientists in Stoneville, Mississippi. Initially, six strains of blue catfish were obtained and evaluated, then three strains with the best growth and carcass yield were retained for further evaluations. Results indicated that the Rio Grande blue catfish strain was superior to the D&B and Mississippi River strains of blue catfish for growth and carcass yield. Accordingly, we maintain a pure Rio Grande strain because of its superior performance and also maintain a mixed population of all three strains to increase genetic diversity for future selection. Release of a superior blue catfish strain will improve production efficiency of U.S. catfish farmers and processors.

Calcium and magnesium hardness improve the fertility and hatching success of hybrid catfish eggs. Egg quality and water quality influence the hatching success of channel x blue hybrid catfish eggs in hatcheries. Hybrid catfish eggs are incubated in hatching waters with a recommended 60 mg/L of total hardness during embryonic development. The aquifer used for hybrid catfish hatcheries contains 10-70 mg/L of calcium hardness and 1-25 mg/L of magnesium hardness, however, the effect of magnesium hardness in combination with calcium hardness on the hatching success of hybrid catfish eggs is not known. ARS scientists at Stoneville, Mississippi, conducted two hatching trials to determine the effects of varying calcium hardness alone or in combination with magnesium hardness on fertilization and hatching success of hybrid

catfish eggs. Adding graduated levels of magnesium after meeting the calcium hardness requirement had no effect on hatching success of hybrid catfish eggs. However, required water hardness for optimal hatching success of hybrid catfish eggs could be met either through calcium hardness alone or with magnesium hardness replacing up to 50 percent of the total hardness. The results of the study will be useful for designing treatment processes for hatchery water supplies to improve the efficiency of hybrid catfish fry production.

Cortisol responsiveness to stress in juvenile channel catfish influences susceptibility to Enteric Septicemia of Catfish. Disease is considered the greatest cause of reduced productivity in aquaculture production systems and culture intensifications in the catfish industry has exacerbated disease susceptibility. Cortisol is considered the principal corticosteroid in teleosts, and concentrations of cortisol increase rapidly following a stressful event. Low dissolved oxygen stressors invariably escalate susceptibility of fish to diseases in fish farms. ARS scientists at Stoneville, Mississippi, classified juvenile catfish based on their cortisol stress response to a standardized stressor and classified them either as high or low responders to stress. High- and low-responding channel catfish were exposed to virulent *Edwardsiella ictaluri* under controlled conditions. Catfish mortality rates increased with cortisol responsiveness when healthy fish were stressed prior to infection. Mitigation of stress or stress response may lead to improved survival in culture conditions.

Reducing variability of hybrid catfish growth during year-round pond harvests. Hybrid catfish constitute approximately 75 percent of U.S. farm-raised catfish production because of their superior growth, better feed conversion, higher survival, availability, and suitability of intensive production systems. However, rapid growth of hybrids, behavioral differences with channel catfish, and physical characteristics of hybrid catfish have presented some unique problems for production such as highly variable fish growth, oversized fish, and more difficulty for year-round harvest. An ARS scientist at Stoneville, Mississippi, collaborated with Auburn University researchers and catfish producers to develop a holistic approach to identify the causes and provide solutions to this problem through a two-year NIFA Southern Regional Aquaculture Center funded research project. Extensive field samplings were conducted at multiple farms to assess the impact of culture system, harvest technology, fingerling size and variability, grading, genetics, time and rate of stocking, and feeding rates on size variability at harvest and ability to accomplish year-round harvest. Genetics of purebred parents and stocking of multiple sizes of fingerlings in production ponds led to variability in hybrid catfish performance. However, increased aeration in production ponds and bar-grading of fingerlings prior to stocking promoted uniformity in fish size at harvest. Models based on economic analyses for specific sets of farm conditions were developed for potential adoption under field conditions to improve production and profitability of hybrid catfish production.

Reducing feed cost for catfish fingerling production. Prices of commercial 35 percent protein fingerling feeds can sometimes reach $600-700 per ton, so there has been interest among catfish fingerling producers in reducing feed cost. Feed cost can be reduced by lowering protein levels, using less expensive alternative feed ingredients, or both. A feeding trial was conducted to evaluate diets containing 35 or 32 percent protein with fish meal or pork meat, bone, and blood meal (PMBB) for pond-raised hybrid catfish fingerlings. The 35 percent protein diet with fish meal was similar to commercial catfish fingerling feeds in diet composition and was used as the

control. Results show no significant differences in total amount of feed fed, gross yield, feed conversion ratio, or observed mortality in fish fed 35 or 32 percent protein diets containing fish meal or PMBB. Using the 32% protein diet with fish meal, 35% protein diet with PMBB, and 32 percent protein diet with PMBB could save money,reducing feed cost for catfish fry production. Although catfish fry initially subsist on natural food items, they are typically offered commercial diets as soon as they are stocked. Pond studies were conducted on both channel catfish and hybrid fry to determine if feeding ponds could be delayed without compromising production. Delayed feeding of channel catfish fry for 5 weeks can save $236/ac in feed costs without compromising production. However, hybrid catfish fry production starts to be affected at only 2 weeks of delayed feeding. Delayed feeding of hybrid fry for 2 weeks saves only $15.69/ac.

2019

Impact of female brooder on size variation of fry. While size variation in fingerlings has a great impact on food fish size variation, little research has been done on factors resulting in size variation in pond run fingerlings. ARS scientists at Stoneville, Mississippi, conducted a study to determine the variation in fry size resulting from the age of the female brooder and the age of swim-up fry. Ten spawns from each of two, three and four-year-old channel catfish female brooders were collected and incubated in the hatchery. Samples of sac fry, and two-, four-, and six-day-old swim-up fry were collected to determine average wet- and dry-weights, and within-spawn variation based on individual wet weights of 100 fry per spawn. The weight of sac fry and swim-up fry at all ages was less for offspring of two-year-old females, and higher and generally similar for offspring of three- and four-year-old females. The coefficient of variation (CV) in weight increased with fry age among all female-age groups but was greater for all ages of fry for offspring of two-year-old females. Mixing eggs or sac fry from different age females or mixing fry of different ages post swim-up prior to stocking will increase size variation of stocked fry. Any initial variation in size of fry stocked will likely be magnified when fry are stocked in ponds with mixed-size zooplankton populations. It is possible to reduce catfish fingerling size range by stocking only fry of the same age post-hatch and from the same age female brooder in each fry pond. This may reduce the need to grade fingerlings before sale.

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 production costs and remain competitive in the global seafood market. ARS scientists in Stoneville, Mississippi, initiated a selective breeding program to develop a strain of channel catfish (Delta Select) with superior growth rate and meat yield, traits 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 to the Delta Control strain, an unselected strain representative of channel catfish currently being grown by U.S. farmers. The Delta Select strain of channel catfish 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 two-year-old Delta Select strain channel catfish will be available for release to famers during fiscal 2020.

Evaluation and development of 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. Therefore, evaluation and development of improved blue catfish germplasm is warranted to

develop improved catfish germplasm for release to U.S. catfish farmers. 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 four to six-year-old Rio Grande fish, 20,000 2 year-old Rio Grandes, and 100,000 Rio Grande fingerlings will be made available for release to farmers during fiscal 2020, allowing U.S. catfish farmers to be more efficient and profitable.

Development of a blue catfish cryopreserved sperm collection. 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 scientists at the ARS National Animal Germplasm Repository and Louisiana State University, have established the largest collection of cryopreserved blue catfish sperm in existence. 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. Development and release of improved blue catfish germplasm will benefit U.S. catfish producers.

Identification of the sex-determining locus in channel catfish. Channel catfish utilize 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 utilized testosterone to sex-reverse catfish to females, identified XY females using molecular markers, and mated them with normal XY males. The YY male offspring were identified using molecular markers and progeny testing. We then produced a genome assembly from a YY male to obtain the Y chromosome sequence. Comparison with the reference genome X chromosome showed no difference in gene content. However, RNA sequence analysis revealed a transcript from the BCAR1 gene was differentially expressed in males during the critical time of differentiation of gonadal tissues. A gene editing experiment provided functional evidence that disruption of the BCAR1 gene in genetic males led 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 sex determining gene in blue catfish. Culture of only blue catfish males would increase the efficiency of hybrid catfish production.

Effects of catfish diets on meat yield. Meat yield, the percentage of whole fish weight comprised on saleable meat, is an important trait in farm-raised catfish and diet composition and feeding regimes can affect meat yield. Lysine is an important amino acid in catfish diets, and ARS scientists in Stoneville, Mississippi, worked in cooperation with Mississippi State University fish nutritionists to determine that catfish diets supplemented to 1.43 percent available lysine improved meat yield in channel catfish relative to lower rates of lysine supplementation. Ongoing research is designed to determine effects of dietary restrictions on growth and meat yield of hybrid catfish. These results have been provided to catfish producers, processors and feed manufacturers and allow the catfish farming industry to develop feeds and feeding strategies to minimize production costs and maximize profits.

Anesthetization using a portable electrosedation unit reduces catfish handling stress and improves post-spawning survival. Channel x blue hybrid catfish are increasingly raised in commercial catfish ponds in Southeastern United States because of superior production traits. In order to obtain eggs for hybrid production, handling stress on channel catfish is unavoidable 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 at Stoneville, Mississippi, collaborated with scientists at the University of Arkansas, at Pine Bluff, 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. This method could improve broodstock survival to reduce losses due to handling stress.

Novel ghrelin receptor in catfish. The peptide ghrelin is a hormone that regulates feed intake and energy use in animals. Its role in feed intake and its actions on growth hormone are important to researchers working to enhance channel catfish growth and feed efficiency. ARS scientists in Stoneville, Mississippi, in collaboration with scientists at the University of Idaho, identified a ghrelin receptor, GHS-R3a, that is unique in fish. Tissue expression and regulation differ from two previously known ghrelin receptor genes. The GHS-R3a receptor preferentially binds to catfish ghrelin and may be a key regulator of growth and feed intake in catfish. As such, GHS-R3a is being looked at as a possible marker in a selective breeding program for growth and fillet yield.

Reduction of animal protein in catfish production diets. Feed costs represent about one-half of the variable cost of production and in an environment of high grain and energy prices careful consideration to feed formulations is critical to maintaining farm profitability. Since animal protein is one of the most costly feed ingredients, relying in all-plant based diets would represent a considerable reduction in feed costs. Experimental pond studies demonstrated juvenile hybrid catfish could be fed all-plant based diets containing 28 percent protein without reducing total feed fed, final weight gain or survival. However, fish fed the 28 and 32 percent all-plant-protein diets had a slightly, but significantly higher feed conversion ratio than fish fed the 35 percent protein fish meal diet. The 35 percent protein fish meal control diet cost $676 per metric ton, while the 28 and 32 percent all-plant protein diets and the 28 percent protein PMBB diet cost $123, $96, and $112 per metric ton less, respectively. Economic analysis showed switching from a 35 percent protein traditional fingerling diet to alternative diets had an economic benefit, ranging from $1,865 to $2,405/hectare owing to the relatively lower feed ingredient prices. Similar results were demonstrated with food-fish production diets where total feed fed, weight gain and survival was similar among fish fed all plant based diets or diets containing porcine meat and bone meal (PMB). However, fish fed diets containing 10 percent or more PMB had significantly greater net yield and a lower feed conversion ratio than fish fed the control diet without PMB and carcass yield and fillet yield decreased and fillet fat levels increased linearly with increasing PMB levels. Data demonstrates catfish in all stages of production can be raised on less expensive all-plant based diets sparing the use of animal protein in animal production.

Information generated from this study by ARS scientists in Stoneville, Mississippi, is being used by commercial feed mills.

Reduction in feed costs in fingerling production. Fingerling production begins in spring of the year as catfish fry (4-7 days of age post hatch) are transferred from the hatchery to nursery ponds. Based on research generated from the previous Specific Cooperative Agreement Improving Production Strategies in Catfish Farming pond fertilization programs were developed to promote optimal zooplankton communities to serve as a natural food source for newly stocked fry. Channel and hybrid catfish fry had no differences in the timing and extent of zooplankton and feed use in ponds. Both fish types used zooplankton and feed equally to support growth (approximately 50 percent from each source) from the time of pond stocking, and there were no differences in growth rates by length or weight. The fish used feed to support growth before they were visually observed accepting the feed at the surface of ponds 4 weeks after stocking. Zooplankton are clearly important to support desirable growth of channel and hybrid catfish fry in ponds. ARS scientists in Stoneville, Mississippi, recommend managers monitor densities of preferred zooplankton prey in ponds and use inorganic fertilization methods to enhance zooplankton production, as necessary. Although feed partially supported zooplankton production in this study, we caution against overfeeding, because this practice is expensive, inefficient, and risks hypoxia formation in ponds. Finding no differences in diets and growth rates between channel and hybrid catfish, we provisionally recommend managers practice similar pond management strategies for both fish types. Minimizing high protein fry diets during the initial stages of fingerling production can save producers around $500/hectare.

Production efficiency of technology adoption. Adoption of alternative catfish production technologies have resulted in achieving economic efficiencies in the catfish industry, primarily due to the spreading of fixed costs over greater volumes of production. Current trends are indicative of increased adoption of intensively aerated ponds in the catfish industry, primarily due to its relative ease of adoption compared to split ponds. Additionally, the market prices have gone down in recent years. With more favorable and stable market prices, split ponds are preferred over intensively aerated ponds, while the reverse holds true when market prices are more unstable and volatile. Hence, market conditions as well as a producers ability to bear risk determines future trends in adoption of alternative catfish production technologies. This information generated by ARS scientists in Stoneville, Mississippi, is being utilized by integrated and smaller catfish producers to develop information based cost effective production strategies reflecting anticipated market conditions.

2020

Development and release of the Delta Select strain of channel catfish. The Stoneville, Mississippi, location mission includes development of improved catfish germplasm for release to U.S. catfish farmers. Use of improved catfish germplasm will allow U.S. catfish farmers to reduce production costs and remain competitive in the global seafood market. ARS researchers in Stoneville, Mississippi, initiated and continued a selective breeding program to develop a strain of channel catfish (referred to as the Delta Select strain) with superior growth rate and meat yield, traits important to catfish producers and processors. After conducting trials demonstrating improved growth and carcass yield of the Delta Select strain compared to other strains of channel catfish, a release of the Delta Select strain channel catfish to U.S. catfish

farmers was completed in March of 2020. Approximately 90,000 head (180,000 pounds) of 2-year-old Delta Select strain catfish were released to 12 of the 15 commercial catfish hatcheries in the United States with significant channel catfish production. The release provides U.S. farmers access to improved catfish germplasm and will make U.S. catfish farmers more efficient and profitable.

Evaluation, development and release of blue catfish germplasm. Over the last 15 years, U.S. catfish production has shifted from predominant use of purebred channel catfish to the current situation where F1 hybrids between channel catfish and blue catfish accounts for over 60 percent of production. Therefore, evaluation and development of improved blue catfish germplasm has become an important part of the laboratory mission to develop improved catfish germplasm for release to U.S. catfish farmers. ARS researchers in Stoneville, Mississippi, evaluated seven strains of blue catfish and found that the Rio Grande strain produced both purebred and hybrid catfish progeny with superior growth and meat yield relative to other blue catfish strains. ARS reseachers initiated the development of a composite strain of blue catfish, referred to as the Delta Elite strain, by crossing blue catfish from the four best performing strains of blue catfish from the seven strains evaluated.. This composite strain possesses increased genetic variation and will be selected for improved performance along with the Rio Grande Strain. In April of 2020, 60,000 pounds (30,000 head) of Rio Grande and Delta Elite blue catfish were released to seven of the nine commercial hatcheries producing hybrid catfish, providing U.S. catfish farmers with unique and improved blue catfish germplasm.

Effects of catfish diets and feeding strategies on meat yield. Meat yield, the percentage of whole fish weight comprised on saleable meat, is an important trait in farm-raised catfish and diet composition and feeding regimes can affect meat yield. Catfish farmers are sometimes unable to sell market weight catfish for processing due to lack of demand in the supply chain causing issues with production management. If the fish are fed during this time, they grow larger than the preferred size for processing and the price paid to farmers is reduced substantially. If the fish are not fed, they mobilize muscle tissue for energy and fillet yield is decreased and the price paid to farmers is reduced. ARS researchers in Stoneville, Mississippi, worked in cooperation with Mississippi State University fish nutritionists to determine that hybrid catfish fed once or twice weekly for four months remained in the preferred size for processing but had decreased fillet yield. Thirty days of full feeding were needed to restore fillet yield to normal levels in fish that were fed once or twice weekly for four months. Results from these studies are provided to catfish producers and processors and allow the catfish farming industry to develop feeding strategies to minimize production costs and maximize profits.

Release of the GnRH IIa spawning aid to farmers. ARS researchers in Stoneville, Mississippi, have demonstrated the advantages of using a synthetic peptide analog of catfish gonadotropin releasing hormone II (GnRH IIa) compared to a mammalian luteinizing hormone releasing hormone analog for stimulating the final maturation and release of eggs for manual spawning. ARS researchers in Stoneville, Mississippi, developed a Non-funded Cooperative Agreement with DelTaq Fish Health Services in Stoneville, Mississippi, to obtain an Investigational New Animal Drug (INAD) from the U.S. Food and Drug Administration. The new spawning aid became available for purchase in early 2020 and quantities purchased by U.S. producers were estimated to supply half the industry s need for hybrid catfish production during the 2020

spawning season. The success of the new spawning aid in 2020 suggests an increased demand for the peptide in 2021.

Reducing feed costs using alternative feed ingredients for catfish food fish production. Soybean meal has been 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 have reached $500/ton at times. Previous research with channel and hybrid catfish has generally shown up to 50 percent soybean meal could be replaced by cottonseed meal and one of the corn-milling by-products corn gluten feed or corn germ meal, in the diet without negatively affecting fish performance. ARS researchers in Stoneville, Mississippi, and at Mississippi State University examined using combinations of two or three alternative protein sources to replace soybean meal in 28 percent protein diets for channel or hybrid catfish. Results demonstrated it is possible to replace all soybean meal by two or three moderate- and high-protein alternative feedstuffs in the diet without significantly affecting growth performance, processing yield, and fillet proximate composition of both channel and hybrid catfish. These data provide flexibility in formulating cost-effective diets for pond-raised catfish. These alternative diets may be used to feed pond-raised catfish during food fish grow out especially during periods of high soybean meal prices.

Reducing feed cost for catfish fingerling production. Prices of commercial 35 percent protein fingerling feeds can sometimes reach $600-700/ton, so catfish fingerling producers desire reduced feed cost. Feed cost can be reduced by lowering protein levels, using less expensive alternative feed ingredients, or both. ARS researchers in Stoneville, Mississippi, conducted three feeding studies to evaluate diets containing 35, 32, or 28 percent protein with fish meal or pork meat, bone, and blood meal (PMBB), or pork meat and bone meal (PMB) for pond-raised channel or hybrid catfish fingerlings. Results show dietary protein levels can be reduced from 35 to 32 percent without affecting fish growth performance. Although fish meal is a high-quality protein source for catfish, it does not appear to be required dietary ingredient for fingerlings raised in properly fertilized nursery ponds and removing it from the diet will substantially reduce feed cost in fingerling production.

Developing cost-effective feeding strategies to maintain body weight of market-size hybrid catfish during harvest delays. Several circumstances, such as off-flavor, oversupply of fish in the market, or low fish prices, can cause delays in harvesting market-size fish in catfish production. Delays in harvest increases the risk of fish losses and restricts cash flow resulting in diminished production efficiency. Harvest delays can become a serious issue, especially for hybrid catfish, since they feed more aggressively and grow faster than channel catfish. ARS researchers in Stoneville, Mississippi, conducted a pond study and examined effects of no feeding, maintenance feeding (feeding once weekly) and refeeding on production and processing characteristics, and fillet proximate composition of market-size hybrid catfish. Results show feeding once weekly can generally maintain fish body weight, and no feeding or feeding once weekly for two months does not affect survival but significantly reduces fillet yield. These data provide catfish producers with information that can be used to minimize production losses in events of long-term harvest delays.

2021

Industry utilization of spawning aid. ARS researchers in Stoneville, Mississippi, developed a new spawning aid that has been well received by the catfish industry. More than half of U.S. catfish production is in the production of a hybrid between the channel catfish females and blue catfish males, and hybrid production depends on induced ovulation in females. In past years producers utilized a carp pituitary extract or a synthetic peptide based on a mammalian reproductive hormone. Scientists in Stoneville, Mississippi, developed a synthetic peptide based on the chicken and catfish gonadotropin hormone releasing hormone (GnRH II), and tested it on farms to determine the factors affecting catfish aquaculture profitability. Catfish farming practices have changed, resulting in an approximately 50 percent increase in productivity (lb/ac produced) over the 4 years from 2014 to 2018.

Economic evaluation of commercial catfish production strategies provides insights into the costs and profitability structures of the various catfish farming practices observed across the U.S. industry, with a focus on the underlying causes for economic success or failure. Previously profitable practices may no longer be profitable given changes in cost structures and economic conditions. Newer, more intensive farming practices are generally more profitable but require greater attention to cost efficiencies than past extensive practices. Profitability was sensitive to feed efficiency, especially in more intensive systems. Economies of scale is a reality in catfish farming, stemming from two different effects: (a) production intensification in individual ponds creates cost efficiencies by spreading fixed costs over greater volumes of production per acre; and (b) increased farm size reduces costs of production through better capacity utilization of equipment and management. Nevertheless, simply intensifying production and/or increasing farm size is not sufficient to ensure profits. ARS researchers in Stoneville, Mississippi, showed profitability and liquidity risk also varied with choice of stocking density, aeration rates, equity levels, and channel or hybrid catfish. Thus, careful cost control and analysis, including assessment of efficiency of use of capital assets, is essential to make management decisions.

Technology adoption in catfish aquaculture. Technology adoption is critical for the evolution and maintained profitability of U.S. catfish aquaculture. ARS researchers in Stoneville, Mississippi, found that a recent survey monitoring technological progress in the catfish industry suggested the catfish industry is evolving through the increased adoption of intensive production technologies, such as intensively aerated ponds and split-pond systems. More than 33 percent of the catfish production area in 2019 was under intensive production. The average aeration rate in the industry in 2019 was 4.2 hp/acre, a 68 percent increase in just the last decade. More than 96 percent of the surveyed farms had adopted automated oxygen monitoring systems. About 53 percent of the catfish production area was using hybrid catfish. Accurate estimates of the technology progress will provide valuable insights for policymakers in making future industry decisions.

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New treatment strategy for controlling snail populations in catfish production ponds. Trematode infestations on catfish farms have been linked to significant production losses and farm closures. Since recognition as an emergent disease in the late 1990's, management strategies have been developed to break the trematode life cycle by eradicating the snail

intermediate host in the pond environment. Copper sulfate is the most widely used treatment option and is highly effective against snails with a single application of 3 ppm but this treatment level can result in increased mortality in fish, especially when water temperatures are elevated. These losses are associated with direct toxicity to fish and algacidal properties of copper, causing algal bloom die offs that result in oxygen depletion within the pond ecosystem. Recent research by ARS researchers in Stoneville, Mississippi, generated from this project has demonstrated that weekly low-dose copper treatments (1.0-1.5 ppm) spread across four weeks are as effective in killing snails and treatment rates <0.1 ppm can halt snail reproduction and kill snail embryos. This approach is being combined with a new delivery system to better manage snail populations and reduce trematode populations in catfish ponds.

New technology for delivering controlled dosage of copper sulfate to catfish production ponds. Snails are an intermediate host to trematodes that cause significant production losses in catfish production. Copper sulfate pond treatments are used to control snail populations but are inconsistent and unreliable in concentration and distribution which increases potential risks to fish health and production. To improve treatment accuracy and increase application efficiency, ARS researchers in Stoneville, Mississippi, in collaboration with researchers at Mississippi State University, Mississippi, developed a mechanized delivery system to apply copper sulfate crystals consistently and uniformly along the edge of the pond. The system was designed in a three-point hitch configuration, for tractor attachment, and driven in a single pass in a 2 to 5 mph range around pond margins. The system utilizes a radar groundspeed sensor and a logic-based control system to distribute granular copper sulfate evenly and accurately along the pond margins in a single pass. The delivery system is being further evaluated in field trials.

Economics and risk of catfish production. An economic analysis showed that an optimal amount of 4,000-5,000 lbs/acre of channel catfish can be carried over the winter in multiple-batch systems. Relative risk associated with commercial catfish production strategies were estimated by ARS researchers in Stoneville, Mississippi, with multiple batch farming of channel catfish being the least risky production strategy with greater probabilities of lower cost of production. Split ponds and intensively aerated ponds had a greater probability of maximizing returns. Price risk was not a significant contribution to economic risk for any production strategy in the short term (1-year) as fish prices remained relatively high and less fluid. Increased adoption of productivity-enhancing technologies such as intensively aerated ponds, split-ponds, automated oxygen monitors and enteric septicemia of catfish vaccinations were the central drivers of the rise in productivity of the U.S. catfish industry.

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Problem Statement 1B: Reduce the Impacts of Disease in Catfish Aquaculture

Accomplishments

2018

Virulent Flavobacterium (F.) columnare degrades catfish mucus. *Flavobacterium columnare* is an economically important bacteria that causes columnaris disease of farmed fish in the United States and abroad. Skin mucus is known to be an important factor in the early stages of columnaris disease; however, little is known about mucus composition in catfish and how columnaris bacteria respond to mucus. ARS researchers at Auburn, Alabama, in collaboration with Auburn University, determined the terminal glycosylation (sugar) pattern of catfish mucus and showed the sugars alpha-D-mannose/alpha-D-glucose were predominant in mucus and likely important for bacterial binding to the fish. They further demonstrated in multiple experiments that *F. columnare* can replicate and survive for up to 3 months in water containing catfish mucus. A highly virulent *F. columnare* isolate showed significantly elevated extracellular proteolytic activity (enzymes involved in disease) compared to a moderately virulent isolate following growth in catfish mucus. The identification of differential proteolytic ability is likely related to virulence in that some *F. columnare* isolates have a greater ability to break down the protective mucus layer resulting in enhanced colonization, pathology and/or death. The data provide new insights on the pathogenic mechanisms of *F. columnare* in columnaris disease.

Demonstrated molecular immune responses in mucosal and systemic immune tissues of channel catfish after vaccination with Ichthyophthirius multifiliis (Ich). Ich is a parasite of fish and causes severe losses aquaculture industries worldwide. Chemical treatment of Ich is costly and often not effective after the parasite penetrates the fish host skin and gill tissue. There is an urgent need for a better understanding of protective immune responses in fish to develop effective vaccines against the parasite. ARS researchers at Auburn, Alabama, investigated the expression of innate and adaptive immune-related genes in mucosal (skin, gill, intestine) and systemic (kidney, spleen, liver) tissues of channel catfish following vaccination with Ich. The research demonstrated significantly higher antibody levels and survival (95 percent) in vaccinated fish than non-vaccinated control fish. Results of this study revealed the molecular immune responses in vaccinated fish and offers new insights into the molecular responses that may govern protective immunity of catfish against Ich infection.

Characterization of the Flavobacterium (F.) columnare planktonic and biofilm states through transcriptome analyses. Columnaris disease which is caused *by F. columnare* severely impacts the production of freshwater finfish species. Due to the impact on the aquaculture industry, research efforts to better understand the biological processes of *F. columnare* including the formation of biofilms and their contribution to disease are ongoing. ARS researchers at Stuttgart, Arkansas, have identified that catfish mucus activates in vitro biofilm formation of different *F. columnare* isolates. RNA sequencing of free-living bacterial cells or biofilm cells in the presence of catfish mucus has revealed very different gene expression profiles among these different cell populations. Biofilms show an increase in their gene expression for signal transduction, ligand binding and cell homeostasis pathways. Also as observed in other Gram negative biofilms, there is an up-regulation of iron uptake machinery which is required to accommodate for developing biofilm populations. The current studies to explore the effect of catfish mucus on biofilm

development through RNA sequencing will add valuable information about the basic biological processes that occur during the individual planktonic and biofilm states. This work will no doubt serve as a basis for future work on understanding how biofilms are established and how they contribute to disease progression. This will aid in the development of new therapies to treat columnaris disease during the production cycle.

Testing of a recombinant protein vaccine to protect catfish against columnaris disease. *Flavobacterium columnare* is the causative agent of columnaris disease which severely impacts channel catfish production in the United States. ARS researchers at Stuttgart, Arkansas, had previously identified *F. columnare* proteins which activate the adaptive immune response. A novel recombinant protein vaccine has been developed resulting in excellent immune protection against columnaris disease. ARS researchers at Stuttgart, Arkansas, have shown over two consecutive years of experimental vaccine trials that the use of the recombinant protein as an immunogen provides significant protection during laboratory disease challenges. Research on optimization and development of a current vaccine to prevent columnaris disease in catfish is ongoing.

Channel catfish demonstrate low heritability for resistance to Enteric Septicemia of Catfish (ESC). Enteric Septicemia of Catfish is one of three major microbially-induced diseases in catfish, and one approach to reducing mortality and improving farm production is selection for broodstock with improved resistance. ARS scientists at Stoneville, Mississippi, challenged multiple pedigreed families of the Delta Select catfish line by exposure to virulent *Edwardsiella ictaluri.* Two challenges (on 2008 and 2017 year class fish) were conducted on more than 10,000 progeny in 180 full- and half-sibling families from a total of 70 sires. The results indicated a low heritability, 0.1, for this trait which pointed to a low response to selection. Improvement of ESC survival will be more likely through management rather than selective breeding.

A novel vaccine against Enteric Septicemia of catfish. Enteric septicemia of catfish (ESC) is the one of the most problematic bacterial diseases affecting the production of channel catfish fingerlings. Mississippi State University scientists through a cooperative agreement with ARS scientists at Stoneville, Mississippi, have developed an effective vaccine and method of oral delivery against this disease. To date, approximately 500 million stocked catfish have been orally vaccinated using the developed technologies in field trials on commercial farms. The net economic benefit at the fingerling production stage for channels and hybrids were $3,868 and $7,063/hectare (ha), respectively. Results from whole farm mathematical programming models showed additional economic benefits in the range of $397 to $473/ha on farms that integrate fingerling production to their foodfish operations. The model suggested that the improved productivity from vaccinating fingerlings could lead ways of maximizing profits either by intensifying production or by appropriating more of the freed up fingerling ponds to yield maximizing foodfish production strategies.

2019

A killed Aeromonas hydrophila vaccine delivered via immersion 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 highly 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. Due to the ease of manufacture and, ultimately, the licensing of a killed vaccine for farm use, ARS scientists in Auburn, Alabama, designed and evaluated the effectiveness of a simple vAh bacterin (killed vaccine) delivered via immersion (waterborne route) 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.

Waterborne exposure to select clay minerals protects catfish against virulent Aeromonas hydrophila infections. *Aeromonas hydrophila* is one of the most widespread bacterial pathogens affecting freshwater fish and a new strain of *A. hydrophila* 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 0.1 percent led to a significant improvement in survival (66.7 percent) of experimentally infected catfish as compared to untreated fish (28.9 percent). Kaolin treatment did not alter the growth of *A. hydrophila*, but bacterial levels in test suspensions were significantly reduced by kaolin treatment within 15 minutes, indicating the rapid formation of settleable complexes between kaolin and bacteria. These findings suggest that the integration of kaolin-based approaches into some production settings may be beneficial, particularly in scenarios where the large-scale use of antibiotics is not appropriate or advisable, or when it is likely that an *Aeromonas* outbreak is going to occur following stressors such as grading, stocking, or transport of fish.

Elucidation of molecular mechanisms behind catfish resistance to Flavobacterium columnare when treated with copper sulfate. ARS scientists im Stuttgart, Arkansas, and Auburn, Alabama, had previously determined that timing of copper sulfate treatment could either confer protection or make channel catfish more susceptible to the pathogen *Flavobacterium columnare*. Most recently, researchers examined catfish gill tissues of copper sulfate-induced resistance and susceptibility to *F. columnare* using high-throughput RNA sequencing. ARS researchers determined significant gene expression differences as well as functional differences in transcript processing and exon usage were induced over a time-course of infection. These discoveries could aid in the development of new treatments to this problematic bacterial pathogen.

Use of a recombinant Flavobacterium columnare DnaK protein vaccine to protect catfish against columnaris disease. *Flavobacterium columnare* is the causative agent of columnaris disease which severely impacts channel catfish production in the United States. ARS scientists in Stuttgart, Arkansas have identified *F. columnare* proteins which activate the channel catfish adaptive immune response. A recombinant DnaK protein vaccine was developed for testing for immune protection against columnaris disease. To evaluate the immunological effect of bath immunization on catfish with a recombinant *F. columnare* DnaK protein vaccine, ARS scientists inf Stuttgart, Arkansas, performed RNA sequencing on skin explant samples from control and rDnaK immunized catfish at different time intervals. We evaluated the different gene expression patterns between the groups with a focus on identifying differences in innate and adaptive

immune function. Significant differential gene expression was observed between rDnaK vaccinated and control catfish indicates an adaptive immune response was activated in the skin of immunized catfish. The fundamental role of developing a skin cell-mediated immune response and its contribution to overall immune protection against different fish pathogens will need to be studied more thoroughly. We will continue to investigate the adaptive immune response in fish using conventional immunological assays as well as using genomics technologies to determine optimal conditions for long lasting immunization procedures. Research that will further enhance the catfish immune response to *F. columnare* rDnaK is underway; as this protein remains a promising candidate for additional optimization and experimental trials in a production setting.

Cytotoxic T-cell action in a catfish cell line. Cytotoxic T cells are critical elements of the immune response to pathogens or cancers. They function by recognizing and killing abnormal cells, such as malignant cells or those invaded by pathogens. ARS scientists in Stoneville, Mississippi, in collaboration with scientists at the University of Mississippi Medical Center, characterized the reaction of cytotoxic T cell from cell line TS32.15 when exposed to abnormal cells. The small cytotoxic T cells multiplied and changed morphology to become large, granular and able to kill abnormal cell targets. Once the abnormal cells were killed, the cytotoxic cells lost the ability to kill and then died. These experiments added to the understanding of how the catfish immune system eliminates pathogens, the mechanisms involved in disease resistance in fish, and provided clues for the design of better vaccines to protect fish.

Clinical research in fish health management. Catfish aquaculture represents the largest aquaculture industry in the United States. In 2018, U.S. catfish aquaculture produced approximately 340 million pounds from over 60,000 acres, valued at an estimated $350 million (USD). However as catfish production has intensified infectious disease threatens the economic viability of the catfish industry. The development of cost effective disease management strategies are dependent on accurate disease diagnoses and clinical research to understand the pathobiology of specific diseases, identify disease vectors and develop biosecurity measures. The College of Veterinary Medicine Aquatic Research and Diagnostic Laboratory through a cooperative agreement with ARS scientists in Stoneville, Mississippi, offers a comprehensive disease diagnostic service to catfish producers centered in Mississippi and surrounding states, including Alabama, Arkansas, Louisiana, and Texas. These services allow for the construction of an accurate database allowing for the surveillance and mapping of disease and disease trends which is available to other research institutions conducting fish health research. Through this project *Vibrio cholerae, Yersinia ruckeri, Edwardsiella piscicida* and a host of digenetic parasites have been identified as emergent pathogens of catfish. To aid in field monitoring studies quantitative polymerase chain reaction assays have been developed for all important catfish pathogens in tissue and environmental samples. Assays are critical to disease surveillance programs are being used to determine disease vectors, evaluate treatment efficacies and monitor pathogen loading rates in catfish production systems.

Myxozoan parasites associated with catfish aquaculture. *Henneguya ictaluri* is the etiologic agent of proliferative gill disease (PGD) in farm raised channel and hybrid catfish and is attributed to significant annual losses. The previous project investigated the development of *H. ictaluri* in channel catfish, blue catfish, and their hybrid cross. *H. ictaluri* DNA was found in greater abundance throughout multiple organ systems in channel catfish compared to hybrid and

blue catfish. Despite copious myxospores present in channel catfish throughout the study, no myxospores were observed grossly in blue or hybrid catfish. Myxospores in hybrids were only observed histologically and were sparse (<1 percent of examined fish), suggesting significant arrested development in the hybrid host. This data generated through a cooperative agreement with ARS scientists in Stoneville, Mississippi, indicates rotating culture fish between channel and hybrid catfish could be an effective management strategy to reduce parasite loading rates in catfish production systems by breaking the parasites life cycle. This work also lead to the characterization of 9 previously undocumented myxozoan parasites from catfish and other temperate freshwater fish (*H. mississippiensis; H. sutherlandi; H. laseeae; H. bulbosus; Myxobolus (M.) ictiobus; M. minutus; M. lepomis; M. axelrodi; Unicauda fimbrithelae*). The pathobiology and impact on catfish production of these previously uncharacterized parasites is being investigated.

Development of management strategies to control digenetic trematode infestations. The digenetic trematode, *Bolbophorus damnificus*, has become a significant threat to the viability of the commercial catfish industry. The parasite life cycle sequentially involves the Ram s horn snail, *Planorbella trivolvis*, which shed cercarial life stages infectious to fish. The life cycle is complete when the American White Pelican consumes infected fish harboring the adult trematode. Field research and disease monitoring programs through a cooperative agreement with ARS scientists in Stoneville, Mississippi, demonstrated approximately 30 percent of the acreage in catfish production contain trematode infected fish. Economic analysis of this data showed even light infestation decreased incomes with severe infestations resulting in a complete loss of production. This analysis showed even mild infestations can reduce net return land by 61 percent. Sales receipts from pond populations with moderate to severe infestations did not cover the costs of production, resulting in a negative net return to land of $1,929/acre and $2,774/acre respectively. This work led to the development of best management practices for the control of trematode infestations in commercially raised catfish and has been disseminated to catfish producers in Mississippi through extension workshops and direct farmer contacts. To date, all catfish operations in Mississippi have adopted some form of a disease surveillance and treatment program for the control of trematode infestations. Implementation of treatment strategies derived from this work has prevented production losses costing the industry approximately $30.6 million and potential farm closures resulting from lost production. These management practices are also effective for other trematode species that have been recently identified.

Identification Drapanocephalus spathans as a catfish pathogen. Digenetic trematodes have been recognized as a significant threat to catfish production since the discovery of *Bolbophorus damnificus* in the late 1990s. Economic analysis has demonstrated even low infection prevalence of *B. damnificus* can have deleterious effects on the profitability of commercial operations. Through a cooperative agreement with ARS scientists in Stoneville, Mississippi, this work identified another digentic trematode, *Drapanocephalus spathans*, as the cause of mortality in catfish. Developing metacerariae were shown to concentrate in the cranial regions, often occluding blood vessels at the base of the branchial arch is postulated as being the resultant cause of death. While the severe infestations can be the cause of death, the infections appear short lived and resolve within 2 months. Management practices effective for *B. damnificus* infestations are also effective in control *D. spathans* infections since both trematode species utilize planorbid

snails as the first intermediate host. Recognition of *D. spathans* as a cause of catfish mortality is critical to diagnostic evaluations and effective treatment recommendations.

Identification of definitive and intermediate hosts involved in trematode infestations in catfish aquaculture. The Ramshorn snail, *Planorbella trivolvis*, was initially recognized as the only snail species transmitting the deleterious trematode, *Bolbophorus (B.) damnificus*. Research involved with trematode life cycle development through a cooperative agreement with ARS scientists in Stoneville, Mississippi, identified a second planorbid snail species, *Biomphalaria (Bi.) havanensis*, capable of shedding two trematode species, *Bolbophorus (B.) damnificus* and *Drepanocephalus (D.) spathans*. Planrobid species are involved in the development of schistosomiasis in humans but shown refractory to *Schistomsoma mansoni* infection, suggesting they do not pose a risk to human health. These snails were found shedding *D. spathans* and *B. damnificus,* respectively. Infectivity trials of channel catfish exposed to *D. spathans* and *B. damnificus* released from *Bi. havanensis* were successful, providing evidence that naturally occurring *B. damnificus* and *D. spathans* from *Bi. havanensis* are infective to catfish. Furthermore, *Bi. havanensis* snails were also found shedding cercariae of an *Austrodiplostomum* sp. reported from inland silversides inhabiting catfish ponds. Channel catfish fingerlings exposed to these cercariae developed metacercariae in both the eyes and brain, the long-term impacts of which are unknown. In addition, the great egret *Ardea (A.) alba* is often found foraging on commercial catfish operations. During a recent survey, two new species of *Clinostomum (C.)* were described from *A. alba*, known hereafter as *C. album n. sp*. and *C. poteae*, although their effects on catfish production are unknown. Recognition of host species is critical to surveillance and treatment programs effective in controlling digenetic trematode infestations in catfish production system.

Use of iron fortified diets to control catfish anemia. Catfish anemia (CCA) is a disease of unknown etiology that has plagued the catfish industry since its inception. While normal packed cell volumes (PCV) of catfish range from approximately 20-40 percent, anemic catfish often have extremely low PCV of 5 percent or less. Clinically, these fish are often lethargic and show signs of respiratory distress in the face of adequate dissolved oxygen concentrations. Clinical and applied research has led to 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 were validated in field trials and were shown effective in stopping and preventing the development of catfish anemia. As a result of this work through a cooperative agreement with ARS scientists in Stoneville, Mississippi, catfish operations with recurrent anemia have begun using diets fortified with modest ferrous sulfate levels to promote RBC production. To date this practice has resulted in a dramatic decrease in the incidence of CCA which has traditionally cost the industry between $5-10 million annually.

Development of a live attenuated oral vaccine to control Enteric septicemia of catfish. Enteric septicemia of catfish (ESC) is a bacterial disease caused by a gram negative enteric identified as *Edwardsiella ictaluri* and is the most prevalent bacterial disease affecting channel catfish fingerling production, resulting in significant economic losses. In efforts to develop more effective disease management programs through a cooperative agreement with ARS scientists in Stoneville, Mississippi, a live-attenuated ESC vaccine and delivery method facilitating in-pond oral vaccine delivery was developed. The oral vaccination platform has been validated in

experimental and commercial field trials demonstrating significant increases in survival, growth, feed efficiency and yield. Economic analysis of field data showed a net economic benefit of $3,868 and $7,063/hectare (ha) in channel and hybrid catfish, respectively. Whole-farm models showed additional profit ranging from $71,758 to $133,887/400-ha on farms that integrate fingerling production to their production strategies due to the appropriation of more of the otherwise incumbent fingerling production acreage into food fish production. The patented vaccine and patent-pending oral delivery system has been licensed to a locally owned private company for commercialization.

2020

The severity of motile Aeromonas septicemia caused by virulent Aeromonas hydrophila in channel catfish is influenced by nutrients and microbes in water. Outbreaks of motile *Aeromonas* septicemia (MAS) disease, caused by virulent *Aeromonas hydrophila* (vAh), has severely impacted catfish farming in the southeastern United States since 2009. The development of control and prevention strategies has been hindered by a lack of understanding for the conditions that trigger disease in the field. ARS researchers in Auburn, Alabama, assessed the effect of nutrients and selected microbes on the growth of *A. hydrophila* and severity of disease in channel catfish. Laboratory experiments demonstrated that fish feed can serve as a source of nutrients for vAh as indicated by vigorous growth of the bacterium in water containing fish feed; and these bacteria were highly virulent to catfish. The research also found that a potential probiotic bacterium could protect fish against vAh infection, resulting in approximately 54 percent lower fish mortality. This research sheds light on the importance of optimizing feeding practices in intensive catfish farming.

Rapid assay for genotyping flavobacterium columnare. Columnaris disease is caused by the bacterium *Flavobacterium columnare*, which has substantial economic impacts on almost all fin fish aquaculture industries in the United States including catfish, rainbow trout, tilapia, sport fish, baitfish, and ornamental fish. Previous research in our laboratory established the existence of four distinct genetic groups within the species *F. columnare*; however, there were no quick and easy methods to assign an unknown isolate to one of the four groups. ARS researchers in Auburn, Alabama, developed a molecular assay to quickly assign an isolate to genetic group. The results demonstrated that the assay is a rapid, sensitive, and specific molecular tool for genotyping *F. columnare*. It is inexpensive to perform and can be used by any laboratory with polymerase chain reaction capabilities. The assay is being used by stakeholders and university partners to determine which genetic group(s) are responsible for disease losses in different aquaculture industries impacted by columnaris disease. This knowledge is important because our research has indicated biological relevance to the identified genetic diversity, with some genetic groups isolated preferentially from columnaris disease cases in specific fish species. An increased understanding of this will allow for the development of improved targeted control and treatment measures for columnaris disease.

Alternative aquafeed protein source improves growth and immune responses in farmed fish. Frass is a by-product of the black soldier fly larval meal industry and is composed of larval excrement, shed exoskeletons and residual feed ingredients. ARS researchers in Auburn, Alabama, evaluated diets containing frass at levels of 0 to 30 percent as partial replacements for soybean meal, wheat short and corn meal. Experimental diets were fed to both fingerling catfish

and tilapia and the results demonstrated that final weight gain was significantly increased in both fish species when fed feed containing frass. Additionally, catfish fed diets containing frass showed improvements in immune system machinery, while tilapia fed frass diets exhibited increased survival against two important pathogens, *Flavobacterium columnare* and *Streptococcus iniae*. Based on these findings, frass derived from the larvae of black solder flies has the potential as an alternative source of protein in aquafeeds or as an ingredient for enhancing palatability and growth. Further, use of frass in diets may prove beneficial for improving the resistance of catfish and tilapia against bacterial infections.

Omics tools provide practical insights on the host-pathogen-environment interactions triggering disease in the U.S. catfish industry. The Gram-negative bacterium, *Aeromonas hydrophila*, is responsible for nearly $100 million in losses to the catfish industry over the past decade. Usually regarded as a secondary pathogen, a virulent strain (vAh) has emerged with heightened pathogenicity linked to environmental conditions. ARS research has revealed that nutritional cues within the host catfish and water quality parameters in culture ponds together are critical for establishing conditions conducive to disease outbreaks. Research in Auburn, Alabama, has pointed to the importance of iron for vAh replication and survival, such that conditions of iron scarcity (common in ponds in some catfish farming regions), can trigger release of potent vAh virulence factors and lead to significantly elevated catfish mortality. ARS researchers in Auburn, Alabama, and Stuttgart, Arkansas, in collaboration with Auburn University, performed experiments that evaluated growth characteristics and protein expression of vAh following culture in iron-restricted conditions. Using cutting-edge proteomic analyses, they confirmed that iron acquisition is critically linked to virulence in this pathogen. Low iron conditions appear to trigger the release of factors that target catfish red blood cells and initiate the onset of disease. These findings are being applied in the ongoing development of an efficacious vaccine to protect against vAh as well as in practical pond applications/management strategies that seek to stabilize available iron levels.

Fish mucus stimulates Flavobacterium columnare biofilm formation and the upregulation of iron acquisition system pathways. *Flavobacterium columnare* is the bacteria responsible for columnaris disease, an important fish pathogen that causes significant losses to warmwater fish production. ARS researchers in Stuttgart, Arkansas, in collaboration with an ARS researcher in Auburn, Alabama, characterized factors that stimulate the transition of planktonic, or free-living, *Flavobacterium columnare* to the formation of biofilms. Biofilms are a critical stage of bacterial pathogenesis which requires a sustained host-pathogen interaction at mucosal sites including the fish gill and skin. ARS researchers identified that skin mucus from different fish species can stimulate the formation of biofilms to varying degrees. They further found that iron acquisition systems are upregulated in biofilm cells during their transition from being planktonic cells. Understanding biofilm formation and the molecular mechanisms behind this action will benefit the development of new vaccines and novel therapies to treat infectious diseases in aquaculture.

Production of pyranopyrans as potential bacteriocidal compounds against fish pathogens. Loss of commercially raised catfish to disease can result in annual economic losses up to $100 million, and reduction or elimination of bacterial pathogens is critical to the improvement of catfish health. In order to identify novel antibacterial compounds useful against fish pathogens, ARS researchers in Oxford, Mississippi, and in Stoneville, Mississippi, and Villanova University

modified the chemical structures of natural compounds produced by a species of fungus to produce novel compounds. The novel compounds, pyranopyrans, possessed significant antibacterial activities against certain species of fish pathogenic bacteria. The scientists received a U.S. Patent for the production and use of these compounds. The limitations of currently approved therapeutants and the adversity to the use of antibiotic-laden feeds in agriculture make acceptance of alternative efficacious natural or natural-based compounds that are active against common fish pathogenic bacteria very acceptable to the catfish industry, with a high percentage (> 50 percent) of producers expected to utilize the invention. These novel compounds may be useful in mitigating the detrimental effects of common bacterial fish diseases and reduce the economic costs of these diseases which can result in losses of $100 million (U.S.) annually.

2021

Bioeconomics of Flavobacterium columnare vaccine in pond trials with channel catfish. *Flavobacterium columnare* is a well-known fish pathogen that has been studied for over 100 years but, columnaris disease remains poorly controlled in farmed catfish. Outside of therapeutants (chemicals and antibiotics), the disease remains relatively unchanged in the U.S. catfish industry. Attempts to improve the management of this disease have led to the development of a live-attenuated genetic group 2 *F. columnare* vaccine (hereafter 17-23). ARS researchers in Auburn, Alabama, and Auburn University scientists investigated the efficacy of the 17-23 vaccine delivered by immersion to catfish fingerlings that were grown to food size in earthen ponds. Data were collected from the two treatments (vaccinated vs non-vaccinated) to evaluate impacts on survival, growth, feed conversion, antibody development, and economic benefit. There were no obvious natural outbreaks of columnaris disease observed during the trial and no difference in survival of vaccinated versus control fish. The vaccinated fish had significantly elevated antibodies at 4 weeks post-vaccination but not at 12 weeks. However, vaccinated fish were significantly larger at harvest (0.78 ± 0.07 lbs) than control fish (0.64 ± 0.04 lbs), and the feed conversion ratio (FCR) of vaccinated fish (1.35) was significantly better than control fish (2.13). Partial budget analysis demonstrated use of 17-23 vaccinated fingerlings resulted in a net benefit of US $600 per acre. The vaccine showed economic benefit to producers, but the results should be further substantiated under both research and commercial settings.

Selectively breeding Nile tilapia for disease resistance does not negatively impact harvest weight. Fish growth is of high economic importance for farmers; therefore, the relationship between growth and other traits that affect performance is paramount. ARS scientists in Auburn, Alabama, in collaboration with industry stakeholders have demonstrated that resistance to *Streptococcus iniae* and *S. agalactiae* is heritable and improved lines of tilapia have been produced that are more resistant to disease. However, the impact of selective breeding for disease resistance on the harvest weight of tilapia was unknown. Data from eight generations of selective breeding including survival following *S. iniae/S. agalactiae* infection and harvest weight was analyzed. The results demonstrated there were no significant relationships, favorable or unfavorable, between harvest weight and survival to both *Streptococcus* species. This means that selectively breeding for disease resistance will not negatively impact harvest weight of the fish. Thus, multi-trait selection is recommended to balance growth and disease resistance. The goal of this research is to provide fish farmers with a robust stock of tilapia that are resistant to disease and exhibit fast growth to improve the profitability of tilapia aquaculture.

Use of vaccines to control Edwardsiollosis in catfish aquaculture. Catfish aquaculture is plagued by two closely related bacterial species in the genus *Edwardsiella*. *Edwardsiella ictaluri* is the most detrimental bacterial disease affecting catfish aquaculture. The disease is most problematic in the fingerling stage of channel and hybrid catfish production. Historically, disease control has relied on feed restriction and use of medicated feed, both of which have production consequences. With increased adoption of hybrid catfish as a production fish, *E. piscicida* has emerged as a serious threat to hybrid catfish aquaculture. In contrast to *E. ictaluri, E. piscicida* primarily affects market sized fish incurring significant production costs. In efforts to combat *E. ictaluri* infections, ARS researchers in Stoneville, Mississippi, developed a live attenuated vaccine under the previous project agreement as a method of prevention. Experimental and on-farm vaccination trials have shown significant improvements in survival, feeding rates and economic returns. Recent research demonstrated the presence of shared and conserved antigens among *E. piscicida* and *E. ictaluri* conferring protection against cross infections. The vaccine and vaccine delivery system are currently being used to commercially vaccinate channel and hybrid catfish resulting in increased yield and economics returns in fingerling and food fish production systems. To date, over 300 million catfish fingerlings are vaccinated each year representing approximately 90 percent of catfish raised in the southeastern United States.

Potential of stock rotations to mitigate proliferative gill disease in catfish aquaculture. Proliferative gill disease (PGD) in channel and hybrid catfish is a devastating disease associated with myxozoan parasitism. The causative agent is cited as *Henneguya ictaluri*, a myxozoan parasite present in nearly all catfish ponds during the spring of the year and can cause catastrophic losses (>90 percent mortality) in severe outbreaks. The life cycle involves the benthic oligochaete *Dero digitata*, which is ubiquitous in aquatic environments and is often present in very high numbers in the nutrient rich pond sediments of catfish ponds. Previous research has indicated the hybrid catfish may be a dead-end host in the *Henneguya ictaluri* life cycle and submissions to ARS researchers in Stoneville, Mississippi, point to reduced incidence of PGD in hybrid systems. Controlled experimental challenges coupled with newly developed in situ hybridization probes, which target pathogen specific DNA sequences in tissue sections, revealed maturation of *Henneguya ictaluri* myxospores in channel catfish from 14-20 weeks post-infection, but no myxospore development in concurrently exposed hybrid catfish. This supports previous work by our research group demonstrating the suppression of *H. ictaluri* life stages in hybrid pond systems. This research suggests hybrid catfish are a dead-end host in the *H. ictaluri* life cycle, or that development in hybrids is significantly arrested/delayed. This work implies strategic crop rotations between channel and hybrid catfish could be a viable management strategy to minimize incidence of PGD on commercial catfish operations.

2022

Research reveals the fish pathogen Flavobacterium columnare is comprised of four unique bacterial species. *Flavobacterium columnare* is the causative agent of columnaris disease in freshwater fish. ARS researchers in Auburn, Alabama, previously demonstrated four discrete genetic groups exist within the species and reported associated host and virulence differences. The ARS researchers collaboratively determined the taxonomic status, phenotypic and chemotaxonomic traits, and DNA relatedness of the four genetic groups using polyphasic and phylogenomic approaches. Chemotaxonomic, matrix-associated laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) characterization and DNA relatedness afforded

differentiation between the genetic groups, indicating each group is a discrete species. The names *F. covae sp. nov., F. davisii sp. nov*., and *F. oreochromis sp. nov*. were proposed to represent genetic groups 2, 3, and 4, respectively, while genetic group 1 isolates remain recognized as *F. columnare*. Since these pathogens are globally distributed and have significant impacts on wild and cultured fish species, research needs to target the correct bacterial species. Recognition of the four species will advance and improve research to define host-pathogen-environment relationships, epidemiology, and develop effective control and prevention measures to reduce the negative impact of columnaris disease in aquaculture.

Use of vaccine adjuvant reduces time needed for protective immune response in channel catfish. *Flavobacterium covae* is a causative agent of columnaris disease, which is one of the top diseases impacting the channel catfish industry. ARS researchers in Auburn, Alabama, previously developed a recombinant DnaK protein vaccine that provided protection against columnaris disease in laboratory challenges, but further research was needed to improve the effectiveness of the vaccine. ARS researchers evaluated the ability of an immersion adjuvant to boost and prime channel catfish for a more effective immune response. The results demonstrate significant protection of channel catfish at 6 to 8 weeks post vaccination and an overall benefit of using the immersion adjuvant to reduce the time required to stimulate a protective immune response. This research further confirmed the potential for developing an immersion vaccine based on the recombinant protein for the prevention of columnaris disease which is greatly needed for the catfish industry.

Development of an effective oral enteric septicemia of catfish vaccination platform. Enteric septicemia of catfish is considered the most problematic bacterial disease affecting the production of catfish fingerlings. Historically, management strategies relied on the use of medicated feed and feed restrictions to limit the oral route of infection. While both strategies can be effective they are not without limitations. Overuse of medicated results in the development of antibiotic resistance rendering the medication useless and feed restrictions severely limits growth. In efforts to develop more proactive management strategies, ARS researchers in Stoneville, Mississippi, developed a live attenuated vaccine along with a mechanized delivery system allowing for the in-pond vaccination fish during the early stages of fingerling production. The oral vaccine is currently available by veterinarian prescription and has dramatically increased survival and profitability of fingerling catfish production. To date, over 90 percent of catfish produced in Mississippi and Alabama are vaccinated with the delivery platform being applicable to other live attenuated vaccines. The vaccine also provides cross protection against *E. piscicida* which is an emergent pathogen in hybrid catfish production. A patent for the vaccine isolate was granted in 2015 (Patent No. 8,999,319) and the patent for the delivery system granted in 2022 (Patent No. 11,330,833 B2). These technologies were developed and validated with funding from the current and previous appropriated USDA/ARS projects.

Development of technologies for controlling snail populations in catfish production ponds. Trematode infestations have been linked to significant production losses and farm closures. Since recognition as an emergent disease in the late 1990's, management strategies have been developed to break the trematode life cycle by eradicating the snail intermediate host in the pond environment. Copper sulfate is the most widely used treatment option and is highly effective against snails with a single application of 3 ppm but this treatment level can result in increased

mortality in fish, especially when water temperatures are elevated. These losses are associated with direct toxicity to fish and algacidal properties of copper, causing algal bloom die offs that result in oxygen depletion within the pond ecosystem. Recent research by ARS researchers in Stoneville, Mississippi, generated from this this project has demonstrated that weekly low-dose copper treatments (1.0-1.5 ppm) spread across four weeks are as effective in killing snails and treatment rates <0.1 ppm can halt snail reproduction and kill snail embryos. While this approach will greatly improve treatment safety with respect to fish health, it increases the time and labor requirements for treatment application. As a result, a mechanized copper sulfate delivery system was designed to consistently and uniformly apply copper sulfate crystals along the littoral zone of the pond environment. The system was designed in a three-point hitch configuration, for tractor attachment, and driven in a single pass in a 2 to 5 mph range around pond margins. The system utilizes a radar groundspeed sensor and a logic-based control system to evenly and accurately distribute granular copper sulfate along the pond margins in a single pass.

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Problem Statement 1C: Improve Catfish Product Quality

Accomplishments

2018

Method development for measurement of catfish fillet texture. Catfish texture is important to consumers, especially if the texture does not meet consumer expectations for uniform product hardness and mouthfeel characteristics. Therefore, texture of farm-raised catfish has received much interest and ARS researchers in New Orleans, Louisiana, have developed a method for quickly evaluating fillet texture. Two treatments, fresh frozen and individually quick frozen with added phosphate, were evaluated using both sensory evaluation of texture and mechanical methods of texture determination. The two sets of catfish samples (fresh frozen and individually quick frozen) were compared and found to have slightly different texture qualities. A mechanical texture measurement method was developed by ARS researchers to predict the sensory texture measurements. This will lead to methods for catfish processors to quickly and conveniently monitor texture quality and for evaluation of other factors altering fillet texture characteristics for a better value for catfish.

2019

Method development for measurement of catfish fillet texture. The sensory qualities of catfish fillets is of primary importance to the processor and consumer. Textural quality can be equal to taste for subsequent rejection or acceptance by the consumer, and may also create processing difficulties with variable filleting requirements. Therefore, texture of farm-raised catfish has received much interest and ARS researchers in New Orleans, Louisiana, have developed a method for quickly evaluating fillet texture. Differences between male and female catfish of raw and cooked fillets were evaluated using instrumental methods of texture determination. No differences were found between genders. However, gender differences were seen in comparisons of raw and cooked catfish. Two texture attributes were significantly different between raw and cooked for female catfish, but for male catfish, four attributes were significantly different. Since the gender of catfish fillets is not normally known at the processor or the consumer, knowledge of lack of differences between them should have a positive impact on overall product acceptance. Correlations between raw and cooked fillet texture may give guidance to further research of production methods.

Method development for spatial analysis of fat in catfish fillets. The compounds responsible for catfish off-flavor and yellow off-color are very soluble in fat, so the (spatial distribution map) of fat across the catfish fillet is important in understanding and treatment of the flavor and color quality problems. Past methods required large amounts of fillet that prevented a defined spatial mapping. Methods were also limited by the time for analysis. A new method that uses time-domain nuclear magnetic resonance (TD-NMR) was developed by ARS researchers in New Orleans, Louisiana, to overcome these limitations. The TD-NMR method allowed a smaller sample size 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 using the TD-NMR method. In addition, the new method was non-destructive, so samples could be utilized for other studies, such as color and possibly off-flavor analysis. The TD-NMR method can benefit researchers and processors with a rapid analysis (seconds) of fat

content of the fillet. The spatial analysis of fat and its correlation with off-flavor or color can benefit flavor checkers and processors in deciding which fillet region is best for determining quality problems or how to more efficiently trim the fillet.

2020

Determined how the nutritional quality of catfish fillets differ between species and pond type. To ensure there are sufficient vitamins to meet the requirements for normal growth, health, and reproduction, catfish feed manufacturers add vitamin supplements, including most of the B-vitamins. Although, most vitamins may be acquired from algae and other natural sources. To determine the impact of species and pond management practices on catfish fillet vitamins, ARS scientists in New Orleans, Louisiana, have developed an improved method to quickly analyze the B-vitamins. The instrumental method is able to separate, identify, and quantify all B-vitamins (B1, thiamine; B2, riboflavin; B3, nicotinic acid; B5, pantothenic acid; B6, pyridoxine; B7, biotin; B9, folic acid; and B12, cyanocobalamin) 10-times faster than previous methods. This will allow the large number of catfish sample analyses needed in current research on parameters affecting catfish quality.

Improved catfish fat analysis. The major catfish quality issues, such as off-flavors and yellow-colored fillets, are related to the fat in the fillet. Present methods of fat analysis require a relatively large sample size and almost a week to analyze a small number of samples. The sample size limits the ability to determine the specific location or variation of fat in a fillet. To overcome this problem, ARS scientists in New Orleans, Louisiana, developed and validated a new method of fat analysis that uses an instrument similar to a medical MRI. Sample size is reduced in the new method about 5-times, but more importantly, the time for analysis is reduced about 100-fold and could be decreased almost 400-fold, if automated. This will greatly enhance the number of catfish samples that can be analyzed in current research on parameters affecting catfish quality.

New, convenient, ready-to-cook catfish products. ARS scientists in New Orleans, Louisiana, with University of Arkansas and Texas State University collaborators recently developed new catfish products to enhance the marketability of catfish fillets. As an addition to the traditional deep-fried catfish, five, more healthy and convenient products were developed. Three products were a breaded fillet, similar to the fried catfish, but designed to be crunchy when baked. Three sizes were made: strips, standard fillets, and Delacata fillets. Also, two marinated products (ginger and sriracha), using Delacata fillets, were developed. All five were analyzed for nutritional composition and a consumer sensory panel evaluated their appearance, taste, and texture, as well as undergo an experimental auction to determine the price they would be willing to pay for each product. The results showed the baked, breaded Delacata fillet was the most preferred, but varied with socio-economic factors. Consumer preferences of certain visual and taste attributes in addition to their effect on an acceptable price will give guidance to those launching these and similar products in the market.

2022

Composition and nutritional attributes of ready-to-cook catfish products. ARS scientists in New Orleans, Louisiana, developed five new ready-to-cook catfish products, including both breaded and marinated catfish, for use as baked or microwaved meals. Scientists determined the

nutrient content of the samples, including protein, moisture, fat, fiber, ash, carbohydrates, minerals, amino acids, and fatty acids. ARS scientists also determined the triglyceride, cholesterol, saturated fatty acids, and unsaturated fatty acids, which showed differences between the breaded and marinated products. In collaboration with scientists at the University of Arkansas at Pine Bluff and Texas State University, consumers evaluated the products for sensory and other organoleptic properties. Scientists also determined consumer willingness to pay for the products. Results demonstrated that all products were acceptable from a consumer standpoint, with the baked products preferred.

Texture differences were found between channel and hybrid catfish. With the increased use of hybrid catfish in the US, possible quality changes needed to be studied. Using instrumental texture analysis, ARS scientists in New Orleans, Louisiana, have shown that differences exist between the cooked fillets of the two common catfish types produced in the United States, channels and hybrids. Also, the way the fillets are frozen and stored, such as fresh, frozen, or individually quick-frozen (IQF) effected texture. Firmness, toughness, and chewiness were most associated with the catfish type, with hybrids being lower than channels, while other texture attributes were indicative of the cold-storage methods, where IQF fillets had higher cohesiveness and lower adhesiveness and both frozen and IQF had higher springiness. For differences that might cause consumer complaints, scientists will need to study genetics, environment, and pond management practices to better understand the causes and improve catfish quality.

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2018

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Appendix 2 – Accomplishments for Component 2: Improving the Efficiency and Sustainability of Salmonid Aquaculture

Problem Statement 2A: Improve Salmonid Aquaculture Production Efficiency and Ensure Product Quality

Accomplishments

2018

Gene editing in rainbow trout. Advancements in gene editing technologies have enabled inducing targeted mutations in genes of interest, allowing for precision-level manipulation of the genome and the ability to link a specific mutation with a specific phenotypic response. ARS researchers at Leetown, West Virginia, have provided the first proof-of-concept for rainbow trout by showing that this new gene editing technology can produce fish with a complete loss-of-function phenotype. They also confirmed that genetic modifications induced by this technology can be transmitted to the next generation. The genetic modification can even be transmitted into sterile triploids, thereby preventing the unintended propagation of genetically modified germplasm. The researchers also used this technique to target genes important for endocrine regulation of growth and demonstrated that the abundance of proteins encoded by those targeted genes was reduced by more than 95 percent. By optimizing gene editing technology in rainbow trout, researchers now have a tool to study the function of specific proteins and unravel how genetic mechanisms regulate economically important traits.

Growth and fillet quality in commercially available rainbow trout. Determining if commercially available rainbow trout exhibit variable growth performance and fillet quality in recirculating aquaculture systems (RAS) is critical to identifying genetic lines that maximize profitability. ARS extramural researchers in Shepherdstown, West Virginia, determined that there was considerable variation in growth performance between genetic lines of rainbow trout; the fastest growing line reached 3 kg while the slowest line lagged 30 percernt behind. These findings indicate that farmers interested in maximizing product yield on either a fractional (percent yield) or absolute basis (total biomass) should familiarize themselves with the growth potential of available stocks before purchasing eggs or fish for their system. In contrast, indices of fillet quality such as the nutrient profile, texture, and color did not differ among commercial genetic lines. Therefore, a farmer or processor who values optimal fillet quality above growth-related traits can be more flexible in their genetic stock selection. Researchers also defined changes in processing yields and indices of fillet quality at different harvest weights throughout the production cycle, providing valuable data that RAS producers can use to predict growth trajectories and fillet characteristics.

Use of woodchip bioreactors for the removal of nutrients and suspended solids from fish farm effluents. As with all intensive agricultural systems, fish farms produce waste that has the potential to impact the surrounding environment. Certain aquatic environments, such as Chesapeake Bay and the Mississippi River basin, have become significantly impacted by the agricultural release of nutrients and other effluent components into their ecosystems. Woodchip bioreactors are trenches containing woodchips which in turn fuel heterotrophic bacteria that remove nitrogen (N) and total suspended solids (TSS) from passing effluent. ARS extramural

researchers in Shepherdstown, West Virginia, established that woodchip bioreactors can capture the percent of NO3-N and percent of TSS from aquaculture effluent streams to minimize nutrient discharge into surrounding waterways. A cost and engineering assessment shows that the woodchip bioreactor is a relatively low cost, low maintenance technology to treat aquaculture effluent and reduce environmental impacts and wastewater treatment costs.

Adding value to broken rice. A huge amount of biproduct known as high protein rice flour (HPRF) is produced annually worldwide from broken rice. To increase the value of broken rice and to expand its end uses, a study was conducted by ARS scientists in Aberdeen, Idaho, in which a commercial HPRF sample was analyzed and tested for feasibility of further protein enrichment by both physical (dry) and chemical (wet) methods. Results showed that HPRF has more than seven-fold increase over the feedstock, not only in protein, oil, and ash contents, but also in phytate content. However, when HPRF with such high phytate content is fed to animals (such as farmed fish) in large amounts, an undesirable excess of phosphorus is excreted. Several options were tested to enrich protein and remove phytate, with the conclusion that an aqueous medium having mild acidic to mild alkaline pH was most effective.

Benefits of extruded feeds for trout production. While commercial fish feed produced via steam-compressed pelleting has largely been replaced since the late 1980s by dry extruded feeds, most rainbow trout grown in the United States are still fed steam-compressed pellets. ARS scientists located in Hagerman, Idaho, examined the effects of feed pellet processing (extrusion versus expansion-steam pelleting) on feed quality, water quality, fecal durability, and growth in rainbow trout. All feeds had similar chemical composition, but extruded feeds had a significantly higher degree of starch gelatinization than the expansion-steam pelleted feed, which led to extruded feeds having much higher water stability, fecal durability, and lower phosphorus discharge. This work is the first to show that extruded feed pellets are, not only more stable in water than pellets made by expansion-steam pelleting, but they also reduce fecal contributions to waste through improved fecal size and durability in water. The use of extruded feeds in commercial rainbow trout culture could improve waste collection and removal, and reduce pollution in downstream receiving waters.

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 percent of dry matter). Scientists in Aberdeen, Idaho, recently conducted a study to characterize the ash component in algae. The study is among a very few to document that silica-containing materials are important contributors of the ash component for algae, particularly those with high ash content. 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.

Incubation temperature impact on embryo survival. Incubation temperature is commonly used to manipulate hatch date in salmonids but there is little information on the effect of such changes in temperature on embryo survival. Working with a stakeholder, ARS researchers at Leetown, West Virginia found that incubation at 5 degrees Celsius within the first day of fertilization

reduced embryo survival compared to incubation at 10 degrees Celsius. Also, rapidly transferring embryos between 10 degrees Celsius and 5 degrees Celsius after 100 degree days of incubation did not affect survival. This information cautions stakeholders to end the practice of starting incubation of fertilized eggs at 5 degrees Celsius and supports that hatcheries do not need a complicated system to slowly change water temperatures when development needs to be accelerated or slowed to meet production needs.

2019

Improved North American Atlantic salmon germplasm. Commercial salmon farms are expected to increase 5-fold over the next 3 years. There is a need for an Atlantic salmon breeding program to support this industry increase. ARS researchers in Franklin, Maine, have developed a selection index for carcass weight, fillet color, omega-3 fatty acids, and resistance to sea lice in their St. John River strain of Atlantic salmon. 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, processing characteristics, and disease resistance will improve production efficiency and sustainability of the U.S. salmon industry.

Determined effects of peracetic acid on rainbow trout and water quality in recirculating aquaculture systems. Peracetic acid (PAA) is used in industrial settings as a safe and low-cost disinfectant and powerful oxidant to improve water quality, but its potential benefit in recirculating aquaculture systems (RAS) has not been characterized. This led extramural ARS scientists in Shepherdstown, West Virginia, to evaluate the effects of PAA on water quality, growth performance, and off-flavor compounds in rainbow trout grown in RAS. Results of these investigations indicated that application of PAA in RAS did not change water quality, reduce off-flavor compounds in culture tank water, biofilms, and trout fillets, or negatively affect fish growth, survival, and feed conversion ratio. These findings inform the RAS industry of the impact of PAA relative to the traditional use of ozone as a disinfectant, provide a basis for continued research on PAA in RAS at different dosages and application regimens, and aid in the development of standard operating procedures to improve RAS water quality.

Gene expression within eggs can predict reproductive success. Variation in egg quality within a rainbow trout breeding population is quite extensive and unpredictable. As a result of this variation, a single female may produce anywhere from zero to several thousands of offspring. To understand what is contributing to egg quality and reproductive success, ARS researchers in Leetown, West Virginia, compared the gene expression profile in rainbow trout eggs exhibiting good and poor eyeing rate and found over 1,000 differences in gene levels between eggs of varying quality. This new way of predicting the eyeing rate will help producers develop husbandry strategies for improved egg quality and reproductive success.

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 sterile, just like seedless watermelons, 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.

Reduced bottlenecks in capital and operating costs for recirculating aquaculture systems. Land-based recirculating aquaculture systems (RAS) are becoming more common for production of salmon and trout, but high capital costs are a major obstacle for commercial implementation. Extramural ARS scientists in Shepherdstown, West Virginia, performed engineering and cost assessments to identify the major factors that can reduce high capital investments. These analyses indicated that larger RAS facilities, higher fish production, and efficiently designed tank space are significant for reducing costs of fish production. Scientists also determined how capital costs associated with tanks, pipes, and water pumps are affected by facility scale and layout. These findings provide the growing RAS industry with valuable information that reduces capital costs and water requirements, thereby improving energy efficiency and economic viability.

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. The second largest commercial egg retailer in the United States obtained germplasm from ARS trout selected by ARS researchers in Aberdeen, Idaho, for growth and utilization of plant protein feed and is now selling eggs from these lines. The company is expressly marketing eggs from the ARS line as hardier, and have demonstrated their improved growth rate under different environmental conditions, compared to 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 their production farms.

Development of improved methods for measuring anti-nutritional factors from soybeans. Soybeans are an important oilseed source, providing edible oil, defatted protein meals and related products to the food and feed industries. However, soybeans contain trypsin inhibitors (TI), which are antinutritional and 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. The current method approved by American Oil Chemists Society (Method Ba 12-75) and American Association of Cereal Chemists International (Method 22-40.01) has been noted to have problems. Therefore, ARS researchers in Aberdeen, Idaho, developed two improved methods that when compared with the standard method, give more accurate results with less variation and reduced reagent usage. The two methods can be used for measuring TI levels not only in soy products but also in many other TI-containing products.

Extruded trout feeds benefit trout industry. Extruded aquaculture feeds are more economical and improve water quality compared to conventional expanded feeds. Feed costs make up between 60-80% of total production costs in aquaculture production. Some rainbow trout producers use feeds produced with heat expansion technology. ARS researchers in Aberdeen, Idaho, compared expanded versus extruded feeds under conditions that closely mimic commercial trout production conditions. Use of extruded feeds improved feed conversion,

protein retention, and water quality. The use of extruded feeds will greatly benefit the trout industry by increasing productivity and enhancing water quality.

Large scale commercial use of trout selected for plant protein diets. Fishmeal is a finite protein source that is in short supply and is increasing in cost. Sustainable production for aquaculture requires the development of feeds formulated with alternative protein sources, of which plant proteins are the most highly recommended. ARS researchers in Aberdeen, Idaho, have selected rainbow trout for increased growth on aquaculture feeds that have completely replaced fishmeal in the diet with plant protein. Non-selected trout develop an intestinal condition termed enteritis when reared on the plant-based feed. However, the selected fish do not develop enteritis and demonstrate improved performance on the plant-based feed compared to conventional commercial trout strains fed fishmeal 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 on a sustainable feed.

2020

Improved North American Atlantic salmon genetic selection tools. Commercial salmon farms are expected to increase 5-fold over the next three years. There is a need for an Atlantic salmon breeding program to support this industry increase. ARS researchers at Franklin, Maine, and Leetown, West Virginia, have created an improved genome reference for North American Atlantic salmon. The new chip will aid in the discovery of genetic markers for commercially valuable traits in North American Atlantic salmon. The new chip has already attracted interest from commercial breeding companies using salmon of North American origin and from companies that provide biotechnology and diagnostic services to the aquaculture industry.

Feeding strategies to maintain heart-healthy omega-3 fats in rainbow trout fed a sustainable diet containing plant oils. Rainbow trout feeds containing plant-based oils are more sustainable than feeds containing fish oil, but lack the heart-healthy omega-3 fats. Collaborative research between ARS scientists in Leetown, West Virginia, and Grand Forks, North Dakota, developed rainbow trout feed formulations using sustainable plant oils that retained omega-3 fat in the fillet. This work developed feeding strategies for rainbow trout that improves sustainability without sacrificing health benefits for trout consumers.

Quantification of biomass loss during purging and processing. Eliminating off-flavor from farmed fish. Fish reared in recirculation aquaculture systems (RAS) develop an off-flavor that can be eliminated by implementing a purging period before harvest. ARS-funded scientists in Shepherdstown, West Virginia, withheld feed for 6 to 14 days during the purge period, resulting in a weight loss of 2 to 10 percent weight, but removed the off-flavors. These findings provide RAS salmon producers with critical data regarding harvest yield predictions, thus contributing to economic stability by increasing the accuracy of biomass production models.

Use of all-female triploid Atlantic salmon in land-based recirculation aquaculture systems (RAS) eliminates pre-harvest sexual maturation. The use of all-female Atlantic salmon in land-based aquaculture systems (RAS) increases production. The early rate of maturation in male salmon reduces growth performance and down-grades fillet quality. ARS extramurally-funded scientists in Shepherdstown, West Virginia, showed that female salmon grew well in RAS from

1 kg to less than 5 kg harvest weight and that the all-female salmon did not exhibit maturation at harvest. This study showed that rearing all-female Atlantic salmon is an effective approach for land-based Atlantic salmon producers to improve the economic viability of this industry greatly.

Early removal of fish feed does not reduce fish processing weight. Normal production practices for most aquaculture farms consist of continued feeding of the fish until the day before harvest. At this point fish are at their heaviest and are consuming the highest percentage of feed. ARS researchers in Hagerman, Idaho, demonstrated that removal of feed up to eight days prior to harvest does not reduce processing weight, resulting in significant cost saving through reduced feed. This change in management practices will benefit rainbow trout producers and other finfish producers by reducing costs while also reducing nutrient effluent into receiving waters.

Extruded fish feeds are more economical and improve water quality over feeds produced by heat expansion. Feed costs make up between 60-80 percent of total production costs in aquaculture production. Some producers were feeding diets produced using heat expansion technology. ARS researchers in Hagerman, Idaho, tested and provided producers with information regarding expanded and extruded feeds. The scientists tested a commercial mash that was processed using both extruded and heat-expansion technologies and fed it to trout under production conditions in ARS-maintained tanks. Research findings showed a decrease in feed conversion and increase in protein retention with extruded feeds compared to expanded feeds, and an improvement in water quality, which should lead to a reduction in disease. Initial savings were almost $2 million for one producer.

Fecal particle size of rainbow trout influenced by amount and type of soy protein products and gum inclusion in feed. Replacement of fishmeal with alternative plant proteins, especially soybean meal, can cause a diarrhea-like symptom in rainbow trout (RBT), characterized by very fine fecal particles called fines. These fines do not settle out in raceway effluent for collection and contribute to the pollution of receiving waters. A team of ARS scientists in Hagerman, Idaho, conducted experiments to determine the effect of different soy protein sources on fecal quality and then these results were used to refine practical formulations using the soy-based protein sources. All-soy protein produced feces in RBT with high fecal fines and low levels of large particles, while diets containing fishmeal and other plant protein concentrates produced feces having a balanced particle size distribution. Refinement of the plant protein dietary formulations and addition of a binder demonstrated that fecal particles were larger and could be collected prior to escapement into receiving waters. This is important information for trout growers and feed companies because it shows that crude fiber in some plant proteins causes undesirable fecal particle profiles in RBT, and addition of dietary binders could significantly alleviate this negative effect and improve water quality of effluent.

Improved method to measure starch content and gelatinization in wet and dried food and feed products. Starch is an important component of various food and feed products. Starch gelatinization is an important physicochemical process during processing starch-containing foods or feeds. The degree of starch gelatinization (DSG) affects not only physiochemical and sensory properties of starchy products, but also their susceptibility to enzymatic digestion and thus nutritional properties for humans or animals. A simple and reliable method that can accurately measure the total starch content and DSG is important for not only feed quality evaluation but

also for the nutritional and physiological study of fish fed on a feed. ARS researchers at Hagerman, Idaho, recently developed an improved method for simpler and more accurate measurement of both total starch and gelatinized starch in situ for wet and dried products. This provides a valuable new tool for food and feed researchers to study the role of starch in food and feed products.

Large scale commercial utilization of trout selected for plant protein utilization. Fishmeal is a finite protein source that is in short supply and is increasing in cost. Sustainable production for aquaculture requires the development of feeds formulated with alternative protein sources, of which plant proteins are the most highly recommended. ARS researchers in Hagerman, Idaho, have selected rainbow trout for increased growth and utilization on aquaculture feeds that have completely replaced fishmeal in the diet with plant protein. Non-selected trout develop an intestinal condition termed enteritis when reared on the same feed, but the selected fish do not develop enteritis and demonstrate improved performance on the plant-based feed than non-selected commercial trout on fishmeal diets. This year, Pacific Seafoods, the third largest rainbow trout producer in the United States stocked one million of these selected fish in production net pens for commercial production on a sustainable feed.

Method to measure trypsin inhibitors in various protein products. Trypsin inhibitors (TI) are naturally occurring proteinaceous substances that are abundant in legume seeds and cereal grains. These inhibitors are antinutritional and/or bioactive and reduce the utilization of plant proteins in aquaculture feeds. As plant proteins are being used to replace fishmeal as the predominant protein source in aquaculture feeds, it is important to have a standard method to measure trypsin inhibitors in various protein products with high sensitivity and precision. ARS researchers at Hagerman, Idaho, recently carried out a collaborative study involving 12 laboratories in four continents: North America, South America, Asia and Europe, to evaluate the performance of a proposed method for determination of trypsin inhibitor activity in soybeans, pulses, grains and their processed products. The proposed method was a significant improvement over the current AOCS (American Association of Cereal Chemistry) method for TI assessment in soy products and is being evaluated as an approved AOCS method.

Novel volumetric quantification method for fecal particle size classification in rainbow trout. Alternative plant protein sources, such as soybean meal, can interfere with digestion due to the presence of anti-nutritional factors that cause a diarrhea-like condition in rainbow trout, resulting in very fine fecal particles that can adversely affect aquaculture systems and the environment. However, affordable and easy-to-implement methods to measure fecal quality do not exist. A team of ARS scientists in Hagerman, Idaho, have developed a simple, volumetric method which relies on visual measurement of settled fecal samples. The method separates feces into three particle size-classes. Independent observations confirmed that the method is reproducible and without observer bias. Due to the ever-increasing addition of plant protein products into trout diets, this method is of high value to researchers and fish producers to evaluate the impacts on fecal and water quality.

Oysters bred for resistance to oyster herpes virus and acidified seawater. Scientists in Newport, Oregon, conducted breeding experiments to improve stocks of Pacific oysters with desirable performance traits for the U.S. West Coast including resistance to highly pathogenic variants of

the oyster herpes virus (OsHV-1 µVar) and ocean acidification (OA). Seventy-one families of oysters from the Molluscan Broodstock Program (MBP) were screened for survival in a quarantine laboratory in France where they were exposed to the OsHV-1 µVar using a plate assay and also to the original OsHv-1 strain during a single mortality event in Tomales Bay, California. Survival was significantly higher for several of these families in each experiment indicating that selection for resistance to OsHV-1 is possible and results of these first OsHV-1 disease trials were used to produce a second generation by giving equal priority to both OsHV-1 resistance and meat yield. This represented an important first step towards protecting the U.S. West Coast industry from economic impacts due to this potential threat. Scientists also conducted two experiments to examine the effect of acidified seawater on oyster larvae in the hatchery. Larvae from adults selectively bred for yield as part of the MBP, produced on average more and larger oyster spat in ambient and acidified seawater respectively than did those from wild broodstock collected in Willapa Bay, Washington. This suggests there are genetic effects that confer resilience to stress from OA conditions, but breeding for resistance will be more challenging because oysters are most susceptible as larvae and current breeding programs don't examine larval traits separately.

Supplying improved rainbow trout germplasm to the largest producers in the United States. Rainbow trout production is the second largest finfish aquaculture production sector in the United States. ARS researchers in Hagerman, Idaho, have led a rainbow trout selection program for the past 20 years. Through agreements and fish disbursement, the ARS researchers released improved germplasm via eggs, milt, and fry to the three largest rainbow trout producers in the United States with the germplasm to be used for production and incorporation into producer broodstock lines. This germplasm will comprise nearly 70 percent of all rainbow trout produced in the United States.

U.S. producer uses ARS-selected germplasm for rainbow trout egg sales and production. The majority of rainbow trout farmers do not manage their own broodstock and instead purchase their eggs for production from commercial egg producers. ARS researchers in Hagerman, Idaho, have developed trout selected for growth and utilization of plant protein feeds, which is more sustainable than fishmeal feeds. The second-largest commercial egg retailer in the United States is using rainbow trout germplasm developed by the ARS researchers and is now commercially selling eggs from these lines. The company is expressly marketing eggs from this line as hardier and have demonstrated their improved growth rate under different environmental conditions compared to eggs supplied by other, both U.S. and international, egg vendors. This same company uses ARS germplasm almost exclusively in their production farms.

2021

Accuracy of genomic selection for improved fillet yield and body weight in rainbow trout aquaculture. In aquaculture the proportion of edible meat (fillet yield) is of major economic importance and breeding animals of superior genetic merit for this trait can improve efficiency and profitability. Fillet yield is a trait that cannot be measured directly in the potential breeding animals. However, genetic gains for fillet yield are possible via family-based selective breeding using information from siblings of the potential breeders. Genomic selection strategies for selective breeding holds great potential for further improving the accuracy of genetic merit predictions. ARS researchers in Leetown, West Virginia, compared the accuracy of genetic merit

predictions for fillet yield between the genomic selection approach and family-based selective breeding. The genomic selection model increased the accuracy of genetic merit predictions for fillet yield by 50 percent compared to the family-based model, indicating that the use of genomic selection can enhance genetic improvement for the fillet yield trait and further enhance the efficiency and sustainability of rainbow trout aquaculture.

Defining physiological mechanisms through gene editing. Recent advancements in gene-editing technology, specifically CRISPR/Cas9 methodology, increase the efficiency of genomic modification and is helpful to define the molecular regulation of economically important traits. ARS scientists in Leetown, West Virginia, used gene editing to mutate the insulin-like growth factor binding protein-2 (IGFBP2) genes; these genes regulate the action of insulin-like growth factor (IGF), the major growth-promoting hormone in rainbow trout. Findings indicated that other IGFBPs compensated for the loss of IGFBP2, supporting that IGFBPs are coordinately regulated and introducing the novel concept that IGFBPs tightly regulate growth responses through redundant functions. These outcomes define the physiological mechanisms regulating growth in rainbow trout and characterize the genome-to-phenome relationship central for developing novel breeding strategies that improve growth performance.

Identification of novel structural variants in the rainbow trout genome. Genomic structural variants refer to changes in the length or orientation of the DNA sequence at specific locations in the genome. Structural variants were a major source of trait variation in human and plant systems but have not been investigated systematically in rainbow trout. ARS scientists in Leetown, West Virginia, used whole-genome sequence data to identify 13,863 structural variants in the genome of farmed rainbow trout from three U.S. breeding programs. This pioneering study in rainbow trout provides the foundation for studying the role of this important source of variation that is far more abundant in the genome than previously thought. These findings provide a useful resource to investigate further potential associations between structural variants and economically important traits for developing novel breeding strategies in rainbow trout aquaculture.

Reducing early maturation in Atlantic salmon through temperature manipulation. The development of land-based recirculating aquaculture system (RAS) fish farms for producing market-size Atlantic salmon is becoming a significant growth area in U.S. aquaculture. However, the RAS environment is prone to having unacceptable levels of early maturing salmon that are considered a downgraded product and lost revenue. Extramural ARS scientists in Shepherdstown, West Virginia, investigated the impact of water temperature on early maturation in Atlantic salmon post-smolts in RAS. Findings indicated that salmon raised at a lower temperature demonstrated a 38 percent reduction in early maturation prevalence. These findings will assist farmers in lowering early maturation and thereby increasing the economic viability of their operations.

Release of an improved rainbow trout reference genome map. A high-quality reference genome map is vital for facilitating meaningful genetic analyses and enhancing research on the physiology of the organism. ARS scientists from Leetown, West Virginia, used recent improvements in DNA sequencing technology and bioinformatics to generate a new and improved reference genome map for rainbow trout. The number of gaps in the roadmap of chromosome sequences was reduced from over 427,000 in the most recent version of the genome

assembly to only 486 in the current assembly. The importance of the improvement in the genome map contiguity was demonstrated by better annotating the genes in the two complex genome regions that harbor the immunoglobulin heavy chain genes that are important for producing antibodies for the adaptive immune response of the fish. The new rainbow trout genome assembly and chromosome sequences provide significant opportunities for rainbow trout aquaculture genetics research and for all aspects of research aimed at a better understanding of the biology of this economically and scientifically important fish.

Organic acids and essential oils in fish feeds to reduce antibiotic use. In efforts to minimize antibiotic use, alternatives to antibiotics (ATA) have been examined across animal agriculture, including aquaculture. Two promising feed additive classes are organic acids and phytogenic compounds (essential oils or EO). The mode of action of differing additives is not fully described but has shown varied success as antimicrobial agents based on bacterial type. ARS researchers in Stuttgart, Arkansas, and Hagerman, Idaho, along with collaborators at the Bozeman Fish Technology Center, Bozeman, Montana, have performed feeding trials with hybrid striped bass and rainbow trout to test the efficacy of these feed additives on fish performance and health. Results for rainbow trout indicate both organic acids and essential oils improve growth rates and feed efficiency in rainbow trout compared to fish consuming the control diet.

Standardization of trypsin inhibitor activity expression units. Trypsin is a digestive enzyme in humans and animals, breaking down proteins. Trypsin inhibitors are naturally present in legume seeds, such as soybeans, and are antinutritional because of their inhibition on trypsin. Among different methods for measuring trypsin inhibitor activity in various protein products, three units have been used for expressing measured results. This makes comparison of results among studies difficult or impossible. To address the problem, ARS researchers at Aberdeen, Idaho, recently conducted a study using an improved trypsin inhibitor assay method developed earlier in the same USDA lab. The study involved developing new approaches to determine conversion factors between different units and standardize the conversion factors against a reference trypsin. The significance of the study is that when measured trypsin inhibitor activity is expressed in absolute amounts of trypsin inhibited and standardization is applied, comparison of among methods will become possible.

***Delineation of the complex relationship between soybean trypsin inhibitor and urease activities*.** Soybeans are a major source of protein for human and animals. Yet, soybeans contain certain antinutrients, such as trypsin inhibitors, which can only be inactivated by heating for optimal nutrition. In the feed industry, urease activity has historically been measured to indicate if heating has been sufficient to inactivate certain antinutrient factors. However, over the years, controversy has emerged regarding reliability of urease activity as a heating index and surrogate parameter for trypsin inhibitor activity. To shed light on this important issue, ARS researchers in Aberdeen, Idaho, carried out a study to delineate of the complex relationship between soybean trypsin inhibitor and urease activities, and more importantly to offer a new guideline to the feed industry about what heating index should be measured for different types of soy products.

Improved North American Atlantic salmon smolt. Commercial salmon farms are expected to increase 5-fold over the next 3 years and will require a fast-growing fish to compete in a global market. A higher weight at smolt usually results in a faster time to market and a higher chance of survival. When the National Coldwater Marine Aquaculture Center selective breeding program in Franklin, Maine, began in 2007, the average weight at smolt was 65 grams per fish. After four generations of selecting for growth, the average weight at smolt more than doubled at 167 grams per fish. This improved germplasm has been transferred to industry stakeholders and will have an immediate economic impact on reducing the time to market and profitability.

New Method to detect off-flavor in water and fish tissue. An increase in land-based aquaculture systems in the United States to produce Atlantic salmon is expected and will require methods to monitor off-flavor to ensure fish are acceptable to consumers. Previous methods could only process 10 samples a day and cost $120 per sample. University of Maine researchers in collaboration with ARS scientists at Franklin, Maine, developed a new method of detecting geosmin and 2-methylisoborneol, the two compounds that cause off-flavor in water and fish tissues. The new method utilizes a high capacity sorptive extraction with gas chromatography/mass spectrometry detection. The new method can process at least 40 samples per day at a cost of $40 per sample. Industry and stakeholders such as the Institute of Marine and Environmental Technology, the Freshwater Institute, Aquacon, and Superior Fresh are currently sending their water and fish tissue samples to University of Maine. This new technology is reducing the cost to measure off-flavor by two thirds and will save the industry thousands of dollars each year.

Identification of barriers to anaerobic digestion of aquaculture waste. The U.S. aquaculture industry has been moving towards intensive land-based systems to meet the ever-increasing demand for fish protein, but this intensification also leads to the generation of waste streams from these facilities. Anaerobic digestion can be a carbon-neutral biological technique for simultaneous waste treatment and renewable energy generation (heat and electricity). Extramural ARS scientists in Shepherdstown, West Virginia, have identified components of waste streams that negatively affect anaerobic digester effectiveness. These include low solids, high salt concentration, low carbon to nitrogen ratio, high fat content, and high sulfur content. These problems can be mitigated by co-digestion of multiple waste streams to balance the carbon/nitrogen ratio, the use of pre-selected microbial communities adapted to these harsh conditions, and using innovative techniques to simultaneously capture sulfur and boost energy production. These findings will provide farmers with opportunities to troubleshoot and optimize anaerobic digestion performance, thereby improving the sustainability and reducing the environmental impact of land-based aquaculture systems.

Improved procedure for sex-reversal in rainbow trout. Most of the rainbow trout industry depends upon production of all-female fish for grow-out. The maintenance of all-female lines depends upon sex reversal of female fry into sperm-producing fish, a process that involves supplementing feed with 17alpha-methyltestosterone steroid for 60 days. Drawbacks of this approach include the need to surgically remove testes for sperm extraction because the sex-reversed fish seldom develop functional sperm ducts, and release of steroid into the environment through effluent water. ARS researchers in Leetown, West Virginia, developed an improved

approach to sex reversal by exposing female fry to 17alpha-methyltestosterone through immersion rather than feeding. Researchers have shown that immersion treatment consisting of 7 one-hour weekly immersions in the steroid beginning at 4-7 days post-hatching greatly reduced the number of fish with sperm duct abnormalities, avoiding the need to terminate fish to surgically remove testes to harvest sperm, and prevents environmental contamination by allowing for the steroid to be captured from the immersion bath.

Integration of precision agriculture technologies into recirculating aquaculture systems. Application of precision agriculture and precision technologies in the U.S. aquaculture industry is presently minimal. These technologies in aquaculture can eliminate the stress and negative impacts on fish welfare associated with the traditional, hands-on methods for estimating population biomass. ARS scientists in Shepherdstown, West Virginia, have developed an artificial intelligence (AI)-aided computer vision system for real-time fish monitoring in recirculating aquaculture systems (RAS). Underwater images and videos were acquired to train an AI fish detection model, and the developed vision system detected whole and partial fish in the field of view with satisfactory model performance of greater than eighty five percent precision. These findings demonstrate the capability for precision technology to assist non-invasive fish condition monitoring and biomass estimation, benefiting fish health, welfare, and production efficiency.

Alternative protein sources can improve fecal stability and nutrient leeching in rainbow trout. Uneaten feed and fish feces release nutrients that cause enrichment of effluent water of flow-through trout hatchery systems and negatively impact receiving waters. One of the primary effects is algal blooms from an increase in dissolved phosphorus that can lead to oxygen deprivation and fish kills in streams and rivers. The replacement of fishmeal (FM) in diets of rainbow trout with plant-based protein sources, such as soybean meal (SBM) and soy protein concentrate (SPC), has compounded this problem since these feeds can reduce fecal stability, increase fecal fine particles, and add nutrients, such as phosphorus, to water. ARS researchers at Hagerman, Idaho, and Bozeman, Montana, determined that feeds comprised of a mixture of poultry by-product meal (PBM), corn protein concentrate (CPC), and SPC with guar gum binder produced more stable feces characterized by larger fecal particles and less fine fecal particles in rainbow trout compared to standard fishmeal-based and commercial feeds. Additionally, the fine fecal particle fractions contained significantly more phosphorus. Researchers concluded that feeding the alternative protein feeds would produce more large fecal particles that would settle out of the water column and could be collected, while feeding traditional fishmeal-based feeds would result in higher fine particles and additional phosphorus contribution to effluent with negative impacts to the environment.

Developed new and improved methods for measuring acid insoluble ash. In animal nutrition research, markers are used for determining nutrient digestibility of feed and feed ingredients as well as studying digesta kinetics, rumen protein synthesis, herbage intake and species selection. Acid insoluble ash (AIA) is a part of total ash, representing siliceous compounds in herbs, food, feed, and biomasses, due to the natural presence of siliceous compounds and contaminations with dirt and sand. The current method for AIA determination is rudimentary, time-consuming, energy inefficient, prone to errors, and variable in steps and conditions among reports. ARS researchers at Aberdeen, Idaho, systematically investigated effects of various factors at several

functional steps on AIA measurement and developed a new method as well as a significantly improved method that are less time consuming, easier to master, and less prone to analytical errors than previous methods. The improved and standardized methodology should make AIA a much better and more reliable marker for animal nutrition studies. These new and improved AIA measuring methods are important to feed formulators and manufacturers as an economic and reliable assay for evaluation of feeds and feed products.

Development of a climate smart resource utilizing sorghum in aquatic feeds. Sorghum s non-genetically modified organism (GMO) status, as well as its status as a resource-conserving grain, can give sorghum a competitive advantage in the valuable and growing plant-based feed and food production sectors. Through partnering with Virginia Tech University with funds provided by the U.S. Sorghum Check-off Program, ARS researchers in Aberdeen, Idaho, have determined available nutrient content of three sorghum varieties for rainbow trout and hybrid striped bass. These values allowed the researchers to determine appropriate inclusion levels in rainbow trout diets and thereby increase sorghum utilization in aquaculture feeds. Further, evaluation of multiple varieties has led to research aiming to develop methods to produce sorghum protein concentrate products by focusing research on those varieties that are best suited for inclusion in aquaculture feeds. These findings are important to sorghum producers, as it has potential as another lucrative market, and provides aquaculture feed manufacturers another available protein source for feeds.

Investigation of novel distillers products to increase production efficiency of U.S. corn and domestic fuel production. Recent advances in the processing of distillers by-products has dramatically changed the resultant nutrient quality of these products. Modern distillers products thereby may have increased usability and value as alternative feedstuffs for some animals, including fish. Working with stakeholder groups involved in corn production, ethanol production and aquaculture, ARS researchers in Aberdeen, Idaho, have investigated the suitability of commercially available novel fermented corn protein products for their application in rainbow trout feeds. These data demonstrate the potential of these products and provide feed companies with the information necessary to incorporate these products into commercial feed formulations. Identification of a suite of potential feed ingredients increases formulation flexibility and thereby protects aquaculture producers from shifting ingredient prices.

***Developed an optimized method for measuring chymotrypsin inhibitor activity in soybean and other protein products*.** In soybeans and soy products, there are two major types of protease inhibitors: trypsin inhibitors and trypsin and chymotrypsin inhibitors. Historically, trypsin inhibitor activity in legume products has been of primary interest for measurement. However, there is a growing interest in monitoring chymotrypsin inhibitor activity in these products as well. The problem is that at the present reported methods for measuring chymotrypsin inhibitor activity vary greatly and lack details in description. Thus, results cannot be compared among studies. ARS researchers at Aberdeen, Idaho recently developed an optimized method to measure chymotrypsin inhibitor activity in various protein products with accuracy and precision, after investigating the effects of several factors. The robust performance of the proposed method was verified by measuring 11 assorted protein products, paving a way for standardization

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Problem Statement 2B: Reduce the Impacts of Disease in Salmonid Aquaculture

Accomplishments

2018

Identification of four distinct phylogenetic groups in Flavobacterium (F.) columnare with fish host associations. Columnaris disease, caused by the bacterium *F. columnare*, is one of the most prevalent fish diseases worldwide. An exceptionally high level of genetic diversity among isolates has long been recognized; however, little has been done to quantify or characterize this diversity in a systematic fashion. ARS researchers at Auburn, Alabama, and collaborators used high resolution methods to characterize the genetic diversity in *F. columnare*. The results established the existence of four phylogenetically distinct genetic groups within the species. Examination of isolate historical data indicated biological relevance to the identified genetic diversity, with some genetic groups isolated preferentially from specific fish species and/or geographic regions. This research highlights the importance of understanding the genetic diversity in *F. columnare* and has facilitated a standard nomenclature for these groups across the scientific community. The new knowledge gained from this research will aid in identifying which genetic type(s) of *F. columnare* are prevalent in different regions and/or aquaculture industries that will allow for the development of better control and treatment measures for columnaris disease.

Sea lice resistance in Atlantic salmon. Sea lice are the most economically damaging pest to the global and U.S. salmon farming industry. Sea lice are exhibiting resistance to most of the drugs used globally to manage this parasite. ARS researchers in Orono, Maine, have combined selection for resistance to sea lice with selection for improved growth in their St. John River strain of Atlantic salmon. Eggs from the improved strain have been provided to industry stakeholders for integration and propagation on commercial farms. The release of improved genetics for resistance to sea lice represent a new management option for domestic salmon growers in controlling this pest.

2019

Selective breeding improves resistance to bacterial cold water disease and columnaris disease. Bacterial cold water disease and columnaris disease are important diseases that affect rainbow trout aquaculture. Antibiotics are routinely used to control these diseases because there are limited alternative control strategies currently available. ARS researchers in Leetown, West Virginia, evaluated the genetics of resistance to both diseases in two rainbow trout populations. Resistance to both diseases was found to be heritable and favorably genetically correlated, suggesting that a rainbow trout s resistance to both diseases is due, at least in part, to shared genes. Based on these studies, molecular genetic approaches are now being used to identify the actual genes that affect disease resistance. Commercial breeders that select for improved resistance to only one of the diseases can expect to reduce the impacts of both diseases in their population.

The pathogen Yersinia ruckeri can sense its host. Disease-causing bacteria have evolved clever systems to recognize their hosts, and they 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 being recognized, and subsequently killed, 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.

Bacterial secretion products contribute to virulence in Columnaris disease. The pathogen responsible for columnaris disease (*Flavobacterium columnare*) is responsible for large losses in multiple fish species important to the U.S. aquaculture industry. The mechanisms that *F. columnare* bacteria use to cause columnaris disease in freshwater fish are unknown. ARS researchers in Leetown, West Virginia, and researchers from the University of Wisconsin-Milwaukee, St. Norbert College (De Pere, WI) and the Chinese Academy of Sciences (Wuhan, China) identified a component of the *F. columnare* protein secretion system that delivers protein toxins to the outside of the bacterial cell and found this component to be important in the disease process. Deletion of two genes (gldN and porV) that are required for secretion eliminated the ability of the bacterium to cause disease in multiple fish species. While secreted toxins from normal bacterial cells killed fish, the mutants failed to do this. This work helps us understand the virulence mechanisms of *F. columnare* and identifies potential target genes for vaccine development to prevent outbreaks of columnaris disease in commercial aquaculture systems.

The genome is sequenced for a commercially-important bacterial fish pathogen. Columnaris disease in rainbow trout is caused by the bacterial pathogen *Flavobacterium columnare*. ARS researchers in Milwaukee, Wisconsin, and researchers from the University of Wisconsin-Milwaukee and Clear Springs Foods, Inc. report the sequencing and draft assembly of the *Flavobacterium columnare* strain MS-FC-4 genome. This *F. columnare* strain was isolated from a diseased rainbow trout and is currently being used as a reference strain to study host-pathogen interaction in trout. The availability of the genome sequence is aiding in the identification of virulence factors as new targets for vaccine development. Targeted vaccines will show less toxicity, and greater species protection, to limit use of antibiotics and reduce production losses to columnaris disease in the United States.

2020

Newly-identified bacteriophage with unique activities promises to prevent disease in rainbow trout. Bacteriophage (or phage) are viruses that infect and kill bacteria and, in the process, can amplify themselves tremendously. Used against disease-causing microbes, these self-replicating killers are excellent candidates for the prevention or treatment of bacterial diseases and could be safe and sustainable alternatives to traditional antibiotics. ARS researchers at Leetown, West Virginia, have identified a new bacteriophage that kills the rainbow trout pathogen *Yersinia ruckeri*. This phage is unique because, in addition to killing its bacterial host by infection, it also binds to and degrades lipopolysaccharide, a large carbohydrate structure that covers the surface of some bacteria and provides protection from the trout immune system. By trimming off this protective layer the phage renders *Yersinia ruckeri* susceptible to the trout immune system making it unable to survive inside its fish host. This phage therefore has two independent ways to kill bacteria giving it an antimicrobial one-two punch.

2021

Accuracy of genomic selection for resistance to infectious hematopoietic necrosis virus in a commercial rainbow trout breeding population. Infectious hematopoietic necrosis (IHN) is a viral disease of salmonid fish that causes significant mortality and economic losses. Improving resistance to IHN using traditional family-based selective breeding has shown promise but is limited since the IHN resistance cannot be measured in potential breeders. For this reason, genome-enabled breeding strategies are advantageous because they predict the genetic merit of the trait of resistance to IHN directly in the potential breeding animals. ARS researchers in Leetown, West Virginia, compared the accuracy of genetic merit predictions among genome-enabled breeding strategies and the traditional family-based selective breeding approach using disease resistance data from a commercial rainbow trout breeding program. Findings demonstrated that genome-enabled breeding strategies resulted in a 15 percent improvement in prediction accuracy of an individual s genetic merit for IHN resistance. These results indicate that genome-enabled breeding can be more effective than traditional family-based selection in improving the resistance of rainbow trout to the IHN virus.

Aquaculture reuse water exposure affects disease susceptibility and survival of farmed rainbow trout. Fish farmers often reuse water to be efficient stewards of freshwater resources; however, reduced water quality is often blamed for disease outbreaks. The magnitude of risk associated with short- or long-term reuse water exposure and its impact on host genetics and vaccine response is unclear. ARS researchers at Leetown, West Virginia, in collaboration with researchers at Virginia Institute of Marine Science and Virginia Tech, varied the duration of reuse water exposure supplied to two commercial strains of rainbow trout that had either been mock-vaccinated or vaccinated against infectious hematopoietic necrosis virus. Chronic reuse water exposure increased risk of death over 46-fold and interacted with fish genetic background. This research demonstrated the importance of mitigating effects of poor water quality and improving fish genetics to reduce disease loss.

New fish antimicrobial peptides (AMPs) have been identified. AMPs are short peptides/proteins that form key components as the first-line defense of the innate immune system in many species including mammals, lower vertebrates, fish, insects and plants. As such, AMPs exhibit abilities to defend their hosts against broad spectrum of infectious microbial pathogens (bacterial, viral, fungal and parasitic) and are thought to be promising alternatives to the use of antibiotics in human health and agriculture. ARS scientists in Leetown, West Virginia, and Milwaukee, Wisconsin, identified six new AMPs in rainbow trout. These new AMPs show sequence homology to the Nk-lysin family of proteins and their patterns of gene expression were altered following challenge with aquaculture-relevant pathogens as well as by physiological stressors. Overall, these newly characterized AMPs contribute to host innate immunity and understanding their regulation may provide valuable insights into improving animal health in production systems.

2022

Marker assisted selection for resistance to bacterial cold-water disease in rainbow trout. Bacterial cold-water disease (BCWD) is one of the most devastating diseases in rainbow trout aquaculture. Improving resistance to BCWD using traditional family-based selective breeding or genomic selection has shown promise but is limited since these methods are labor intensive,

costly, and the resistance trait cannot be measured directly in potential breeders. For those reasons marker assisted selection (MAS) is advantageous because it can directly predict the genetic merit of potential breeding animals using just a small number of DNA markers. Therefore, MAS is a cost savings approach for improving disease resistance through selective breeding. ARS researchers in Leetown, West Virginia, have identified a set of six DNA markers that can be used to predict the genetic merit of breeding animals with the same or better accuracy than the traditional family-based selective breeding approach. The effectiveness of this cost-reducing approach was demonstrated in a commercial breeding population, indicating that it can further improve the efficiency and sustainability of rainbow trout aquaculture in the United States.

ARS strain of rainbow trout selected for tolerance to plant-based diets displays enhanced non-specific immunity to infectious hematopoietic necrosis virus (IHNV). IHNV infections in trout and salmon can result in high levels of mortality and significant economic losses for aquaculture producers. ARS researchers in Stuttgart, Arkansas, in collaboration with ARS researchers in Hagerman, Idaho, the University of Idaho, and Auburn University examined responses to acute IHNV infection on three major strains of rainbow trout (RBT) used in U.S. aquaculture production: IHNV resistant, viral resistant, and an ARS strain selected for utilization of plant-based feeds. Results demonstrated that strains varied in IHNV resistance, innate immunity and gene expression profiles across multiple tissues and multiple time-points as the disease progressed. These findings highlight the disparity in IHNV resistance in RBT produced in the United States, offered insight into the mechanisms of RBT disease response, and revealed the connection between fish innate immunity and utilization of plant-based diets.

Detection of fish pathogens by high-throughput analysis. The detection of bacteria that cause disease in fish is critical for the U.S. aquaculture industry to reduce mortality and disease. Diseases caused by *Flavobacterium columnare* and *F. psychrophilum* are a major concern because they lead to high mortality and increased use of antibiotics. Researchers at the University of Connecticut developed and validated a high-throughput, next-generation sequencing assay that allows them to detect pathogenic *F. columnare* and *F. psychrophilum* in water and on the surfaces. The researchers were able to detect *F. columnare* in water and on the walls of raceways in a commercial trout farm. Using this assay, researchers and diagnostic labs can identify the source of the flavobacterial pathogens and determine when they increase in number. The identification of pathogen refuge and amplification sites will lead to new intervention strategies that improve the quality and safety of the nation s food supply.

Discovery, validation and commercialization of a novel biomarker for susceptibility to bacterial cold water disease. Fish farmers need rapid methods to assess animal health and disease susceptibility. ARS researchers at Leetown, West Virginia, and St. George's University identified a novel serum biomarker of disease susceptibility. The biomarker was increased over 20-fold in the plasma of susceptible-line fish following exposure to Flavobacterium psychrophilum. A rapid, no-wash assay was developed and commercialized that can be completed in under 1 h total assay time. This assay provides a commercially available, rapid method for farmers of rainbow trout and Atlantic salmon to monitor population health during grow-out.

Early life-stage model of Columnaris disease developed. Rainbow trout susceptibility to Columnaris disease, caused by *Flavobacterium columnare*, is an emerging problem in the U.S. aquaculture industry. ARS researchers at Leetown, West Virginia, developed a pre and post hatch life-stage, disease challenge model and found that host genetic background, pathogen strain and water quality altered disease onset and mortality. This model system allows for large scale evaluations to elucidate virulence mechanisms and screen vaccine candidates that can be used to reduce Columnaris disease on farm.

Identified critical components for potential vaccine against columaris disease in freshwater fish. Columnaris disease, caused by *Flavobacterium columnare*, is a prevalent disease affecting freshwater aquaculture that can cause significant mortality. An understanding of how this bacterium causes disease is lacking, and control measures are inadequate. ARS researchers in Milwaukee, Wisconsin, developed efficient techniques to make targeted mutations in *F. columnare*. Mutation of the type-nine secretion system, or of a combination of secreted proteins, reduced ability to cause disease in rainbow trout and zebrafish. This identifies critical components involved in columnaris disease, and researchers are using these methods to construct attenuated strains for vaccine evaluation.

Determination of improved nonspecific pathogen resistance in ARS select line of rainbow trout. Feed costs and disease losses are the two largest economic components in production aquaculture. ARS researchers in Hagerman, Idaho, have been selecting fish for enhanced capabilities for growth and utilization of an all plant protein-based feed. These fish are currently used in production by the majority of commercial trout producers in the United States. Further evaluation of this strain also shows that the dietary selection has led to an increased resistance to nonspecific pathogens (viral and bacterial) and this increased immunological response has also been recorded by other international research laboratories performing similar research. Unlike other disease-resistant trout strains that have been selected for resistance to one specific pathogen, the Hagerman ARS strain has the capacity to show enhanced pathogen resistance across the ever-changing broad spectrum of devastating pathogens that trout producers face year to year. As pathogens induce reduced health and growth and mortalities are of serious economic concerns for producers, these fish can help mitigate against these losses.

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Appendix 3 – Accomplishments for Component 3: Improving the Efficiency and Sustainability of Hybrid Striped Bass Aquaculture

Problem Statement 3A: Enhance Hybrid Striped Bass Aquaculture Production

Accomplishments

2018

Heritability of growth in hybrid striped bass correlated liver gene expression. Improved growth rate is a highly desirable trait in aquaculture, but growth of hybrid striped bass can vary greatly throughout the production cycle. In a long-term growth study, ARS researchers at Stuttgart, Arkansas, found that hybrid striped bass growth is a heritable trait and thus, likely can be increased through improved genetics. Between the slowest and fastest growing fish, we further identified differentially expressed genes, several of which were correlated with growth hormone and energy expenditure. A first-generation liver gene expression database was released to the public as well as correlation data to further support a growth selection program. The correlation of growth with key energy expenditure genes will enable breeders to develop predictive markers for use in current breeding programs to include only the top performing individuals in each generation. The development and implementation of these markers is ongoing with University and Industry partners.

2019

Commercial protein blends for hybrid striped bass (HSB) diets. In subordinate project 6028-31630-008-02T Minimizing Fishmeal in Hybrid Striped Bass Diets Using Commercial Protein Concentrates for Use in Aquafeeds, ARS scientists at Stuttgart, Arkansas, replaced fish meal with each of four commercially available protein blends (Elite 60", Elite 65", Elite 70", Procision") produced by HJ Baker and Sons and supplemented limiting nutrients based on the latest diet formulation theory. Test diets were extruded by our cooperator at U.S. Fish & Wildlife Service Bozeman Feed Technology Lab to mimic commercial practices and fed to juvenile hybrid striped bass in tanks for 13 weeks. Good growth, composition of growth, and lack of major differences in innate immunity and hematological parameters were found in fish fed test diets containing any of the three Elite" fish meal replacers. Considering the high cost ($2,700/ton), lower feed intake, and lower growth performance of the Procision" test diet, this product does not appear to be a suitable alternative to fish meal in HSB diets. This accomplishment provides science based information to feed and fish producers on cost-effective and sustainable alternatives to fish meal in hybrid striped diets.

Optimum hybrid striped bass (HSB) larval feeding regime using rotifers determined. Scientists in Stuttgart, Arkansas, have been seeking to determine the earliest time that Morone larvae can be converted to a prepared diet by minimizing the time spent on live food items all while maximizing larval growth and survival. In the current study, HSB larvae (4 dph; 3.72±0.13mm SL) were stocked in replicated 110-L recirculating tanks at 75/L, maintained at one of two rearing temperatures (18 degrees Celcius, 26 degrees Celcius) and fed rotifers (*Brachionus* sp.) at increasing two day increments from two days up to twelve days at a set ration of 60 rotifers/mL/d. Following the prescribed period of rotifer feeding, larvae were switched to microcyst Artemia nauplii at a rate of 12 nauplii/mL/d until the end of the study period. Lower

rearing temperatures yielded higher survival across all feeding treatments as compared to the higher temperature treatment ($p < 0.0001$;18 degrees Celcius mean across all treatments 51.8±4.5%; 26 degrees Celcius mean across all treatments 30.2±0.9 percent). After the 12-day rearing period, growth was maximized by feeding 2-4 days of rotifers at 26 degrees Celsius ($p < 0.0001$; 10.97±0.84mm TL). Maximal larval growth at 18 degrees Celcius occurred after 2 days of rotifer feeding (8.44±0.84mm TL), however there was no significant difference amongst any of the treatments ($p > 0.05$). By reducing the time live feeds are needed, the risks and costs to fish farmers involved in hybrid striped bass production are greatly reduced.

2020

Population genetics analyses of domestic and wild white bass (Morone chrysops) and the creation of a new line for selective breeding. White bass is one of the parental species of hybrid striped bass, a major U.S. aquaculture species. Numerous state, federal, and private hatcheries rear these species for stocking as well as food fish. Genetic markers are critical for evaluating population diversity, detecting inbreeding, differentiating unknown individuals by population origin, and, ultimately, selecting for performance traits. ARS researchers in Stuttgart, Arkansas, captured wild white bass along waterways in the states of Arkansas, Alabama and Texas and collected a domesticated strain from North Carolina State University. In collaboration with scientists from Auburn University, genetic markers that could differentiate wild stocks from the only existing domestic stock of white bass were developed. ARS researchers then utilized this information to determine enough genetic diversity was present to create a new line of white bass by single-pair mating wild caught and domestic fish. As white bass reach sexual maturity in approximately 1.5 years, both an even and odd year class of white bass were created. A total of 98 full-sib families were spawned both years for broodstock development and selective breeding.

Off-season spawning of white bass (Morone chrysops). White bass naturally reproduce in the springtime, with the help of natural cues including daylight and temperature. ARS researchers in Stuttgart, Arkansas, have developed and implemented a method to further spawn white bass in the offseason (fall) in an effort to evaluate additional performance traits, including disease-resistance and tolerance to diet reformulations, and to provide for a year-round supply of fingerlings to industry. Both even and odd year-class have been successfully spawned utilizing protocols developed by ARS researchers. Methods are being implemented and amended to continue to improve reproductive success for subsequence generations. This increase in supply of white bass stocks are alleviating the need for wild caught fish in hybrid striped bass hatchery operations.

Disease resistance of white bass, striped bass and hybrid striped bass to Flavobacterium columnare and the mucosal effects on the causative bacteria. Columnaris disease generates substantial losses of many freshwater fish species including hybrid striped bass, a major aquaculture species. ARS researchers in Stuttgart, Arkansas, and Auburn, Alabama, determined the susceptibility of white bass, striped bass, and hybrid striped bass to columnaris disease and investigated the effects of bass mucus on total bacterial growth and biofilm formation. ARS researchers confirmed that white bass are more resistant to columnaris disease than hybrid striped bass and that striped bass were the most susceptible. Results also suggested that one component of variation in moronid disease resistance may be contained in the fish mucus and that survival of resistant fish is likely associated with innate factors such as antimicrobial

effectors present in the mucus. Results will be of use in further elucidation of mechanisms behind disease susceptibility in moronid bass species for therapeutant development and in identifying genetic markers useful in the selection of parental species for the production of improved hybrid striped bass.

2021

High-quality genome sequence of white bass. ARS researchers in Stuttgart, Arkansas, in collaboration with scientists at North Carolina State University mapped and annotated a high-quality genome sequence of the white bass (*Morone chrysops*) for release to the public through the National Center for Biotechnology Information (NCBI). The white bass is a parental species of hybrid striped bass, a fish of increasing commercial importance throughout the United States. Our goal was to advance progress in the genetic improvement of hybrids by building white bass resources to facilitate selective breeding for agriculturally important traits. Toward this goal, we created a white bass genome assembly, which was accomplished using high-throughput Illumina DNA sequencing combined with Chicago® and Dovetail" Hi-C + HiRise" scaffolding. First, available white bass short-read DNA sequences generated from a single female individual on an Illumina GAIIx platform were assembled into 57,533 contigs to build a preliminary genome sequence. Next, DNA from the same female individual was re-sequenced and scaffolded from the initial genome assembly via the Chicago and Dovetail Hi-C + HiRise scaffolding pipeline. This approach produced a 645.14 Mb genome assembly. The largest 24 scaffolds, indicative of the haploid number of chromosomes (n=24) for white bass, ranged in size from 15.4 Mb to 32.2 Mb. The final scaffold N50 was improved from 161 kb to 28.018 Mb and the final scaffold N90 was improved from 24 kb to 21.862 Mb over our initial assembly. Data is available at the NCBI under the accession number JAGRRF00000000.

Determined disease resistance of white bass, striped bass, and hybrid striped bass to Flavobacterium columnare and the role of moronid mucus on bacterial biofilm formation. ARS researchers in Stuttgart, Arkansas, in collaboration with ARS researchers in Auburn, Alabama, determined the survival rate of white bass, striped bass, and hybrid striped bass to *Flavobacterium columnare* and the role of moronid mucus on bacterial biofilm formation. Columnaris disease generates substantial losses of many freshwater fish species, including hybrid striped bass. ARS researchers sought to determine the susceptibility of white bass, striped bass, and hybrid striped bass to infection by *F. columnare* and investigate the effects of moronid mucus on total bacterial growth and biofilm formation. We found that white bass are more resistant to *F. columnare* than hybrid striped bass and that striped bass were the most susceptible of the three. Species and concentration-dependent differences were detected in the total growth of the bacteria to host mucus. Moronid mucus can significantly affect biofilm formation when exposed to *F. columnare*, and that there is a correlation between the bacteria's response of growth and biofilm formation with bass species susceptibility. Results also suggest a component of this disease resistance is contained in the fish mucus and that recovery and survival after *F. columnare* infection may be associated with the presence of mucosal antimicrobial effectors.

Report on the status of striped bass as a commercially ready species for U.S. marine aquaculture. ARS researchers in Stuttgart, Arkansas, in collaboration with the StriperHUB consortium, combined research efforts and expertise to publish a consensus report on the current status of striped bass (*Morone saxatilis*) as an aquaculture species for U.S. farmers. Striped bass

have long been regarded as one of the most important recreational fisheries in the United States. Decades of research have been conducted on striped bass and its hybrid (striped bass x white bass *M. chrysops*). Culture methods have been established, in particular for the hybrid striped bass, which is the fourth largest finfish aquaculture industry in the nation ($50 million USD). Additionally, domestic striped bass have been bred since the 1990's and are available for commercial fry production. In this report, we detail the current status of striped bass aquaculture in the United States, including genetic, nutrition, and production parameters, along with economic opportunities for breeders and producers.

Sticky fish eggs thwarted by common baking ingredient. Hybrid striped bass larvae are produced by strip-spawning, and fertilized eggs become extremely sticky and clump together which limits the amount of oxygen that can reach them and can cause fungal problems. Both issues can destroy an entire batch of eggs. The hybrid striped bass industry typically uses tannic acid treatments to prevent adhesion of eggs, but it is costly and, if left too long, will form a hard layer on the surface of the fertilized eggs which can prevent embryos from hatching. ARS researchers in Stuttgart, Arkansas, investigated 12 candidate compounds to prevent stickiness and found that 10 percent whole milk treatment performed best. To further this research, evaporated milk was explored as an egg de-adhesive as an easier option for remote hatcheries because of the much longer shelf-life as opposed to fresh whole milk. After seeing the success of these experiments and ease of using evaporated and whole milk, the largest commercial hybrid striped bass hatchery decided to start using them immediately for the remainder of their 2020 hybrid striped bass production; in 2021, the hatchery exclusively used the milks to prevent clumping and successfully produced 80.9 million larvae using these methods.

Iron acquisition systems in virulent Aeromonas hydrophila identified as potential target for new therapeutants. ARS researchers in Stuttgart, Arkansas, in collaboration with ARS researchers in Auburn, Alabama, and researchers at Auburn University, characterized additional virulence pathways of the fish pathogen *Aeromonas hydrophila*. This Gram-negative bacterium has been responsible for extensive losses in the aquaculture industry, including catfish, for over a decade. Due to its significance and impact on the aquaculture industry continuing efforts to better understand the basic mechanisms that contribute to virulent *A. hydrophila* (vAh) outbreaks are urgently needed. Recent challenge models demonstrated that vAh cultured in the presence of the iron chelating agent deferoxamine mesylate (DFO), were more virulent to channel catfish (Ictalurus punctatus). Using a global proteome analyses combined with a custom computational pipeline, researchers identified upregulated proteins among the DFO treatment that were enriched for gene ontology groups including iron ion transport, siderophore transport and siderophore uptake transport - all iron acquisition pathways. Protein-protein interactions were also evaluated among the differentially expressed proteins and predicted that many of the upregulated iron acquisition proteins likely form functional physiological networks. Our findings revealed additional virulence factors which can be explored as potential targets for new therapeutants including vaccines.

Use of an immersion adjuvant with a recombinant protein vaccine in channel catfish enhances protection against Flavobacterium Columnare. The Gram-negative bacterium *Flavobacterium columnare*, the causative agent of columnaris disease, generates substantial mortality during the production of freshwater fish species, including catfish. Vaccination remains

a practical alternative that has proven to be effective in the control of different pathogens in the catfish industry. ARS researchers in Stuttgart, Arkansas, in collaboration with ARS researchers in Auburn, Alabama, evaluated the efficacy of a recombinant *F. columnare* DnaK vaccine using different immersion adjuvant strategies. Our studies found that timing of vaccine with adjuvant treatment by bath immersion significantly impacts survival rate after exposure to *F. columnare*. Eight weeks post vaccination, catfish challenged with *F. columnare* had a significantly higher survival rate ($P < 0.05$) among the 5- and 30-min bath immersion rDnaK + adjuvant vaccine groups when compared to the non-vaccinated and adjuvant only control groups. There was also a significant difference in survival between the rDnaK + adjuvant groups ($P < 0.05$). The overall survival rates of the vaccine groups were 37% (5 min) and 17% (30 min) after 5 days of observation, while non-vaccinated and adjuvant only controls had 0% survival after 2.5 days. These results will assist in improving the production and protocols of usage for vaccinations against columnaris disease.

Determined optimum hybrid striped bass larval feeding regimes using live feeds at two rearing temperatures. ARS researchers in Stuttgart, Arkansas, have been seeking to determine the earliest time that Morone larvae can be converted to a prepared diet by minimizing the time spent on live food items while maximizing larval growth and survival by rearing larvae in recirculating aquaculture systems at two temperatures (18 deg C and 26 deg C). Importantly, less infrastructure and expertise is needed to grow and feed Artemia as compared to rotifers and Artemia are less prone to catastrophic losses due to environmental conditions. Therefore, the goals of this study were to determine the earliest time HSB larvae could be weaned off of rotifers and onto Artemia nauplii at an optimum (26 deg C) and suboptimum (18 deg C) culture temperature and compare total length and survival of the resulting larvae (2 - 8 days in two day intervals). There was no difference in survival in HSB larvae based on how many days they were fed rotifers at either 18 deg C or 26 deg C. However, there was a difference in larval survival between the two rearing temperatures. At the end of the study, larvae from the 18 deg C system were 17 percent shorter (8.00 mm) than those reared in the 26 deg C system (9.59 mm), and total length and body depth of larvae varied significantly ($P<0.0001$) among treatments by temperature and the number of days they were fed rotifers. These results demonstrate that minimizing time larvae were fed rotifers maximized growth. This study also showed that Artemia is a suitable diet for six-day-old larval HSB and that these larvae can easily be weaned from rotifers at both optimum (26 deg C) and sub-optimum culture temperature (18 deg C) while maintaining suitable growth and with no significant drop in survival. This will allow tank producers of hybrid striped bass to alter rearing practices to incorporate fewer days of required rotifers which will reduce costs associated with tank production.

Determined the optimum larval feeding regime to maximize white bass larval growth. ARS researchers in Stuttgart, Arkansas, have been seeking to determine the earliest time that several species of Morone larvae can be converted to a prepared diet by minimizing the time spent on live food items while maximizing larval growth and survival by rearing larvae in recirculating aquaculture systems. White bass (WB) Morone chrysops is one of the parental species of the hybrid striped bass (HSB) which represents a valuable sector of the U.S. aquaculture industry. One factor limiting the growth of the HSB industry is the lack of methods to rear larvae in tanks with minimal time spent on live feeds, maximizing growth and survival, including methods targeting the parental species. The goal of this study was to determine the minimum amount of

time WB larvae could be fed live feeds (Artemia) and weaned onto a commercial diet that will maximize growth (eight to 28-days in 4-day increments). At the end of the study, mean length of larvae ranged from 10.91 - 19.12 mm and averaged 14.49 +/- 3.46 mm across all treatments; mean body depth of larvae ranged from 1.86 - 3.40 mm and averaged 2.42 +/- 0.69 mm. Total length and body depth of larvae varied significantly based on the number of days fed Artemia ($P<0.0001$), with both traits significantly increasing with the number of days fed Artemia. The present study demonstrates that maximizing the time WB larvae are fed Artemia before the addition of dry commercial diet results in significantly larger larvae at the conclusion of the larval feeding period. Further refinements of microdiets and feeding strategies with WB larvae could help shorten this requirement. This research is currently ongoing.

2022

Organic acids and essential oils improve growth rates, feed efficiency, and disease resistance in carnivorous fish. ARS researchers in Stuttgart, Arkansas, with ARS colleagues at Hagerman, Idaho, and researchers at the U.S. Fish and Wildlife Service Bozeman Fish Technology Center, Montana performed feeding trials with hybrid striped bass and rainbow trout to test the efficacy of organic acids and essential oils from herbs and spices as alternatives to antibiotics to combat Streptococcus infection in hybrid striped bass and cold-water disease in rainbow trout. Results showed that both organic acids and essential oils improves growth rate and feed efficiency in rainbow trout and hybrid striped bass. The results from phase two studies (disease challenges, gene regulation analyses, and hybrid striped bass taste test panels) convinced a major aquafeed supplier to market a separate line of feed containing essential oils.

Striped bass are highly susceptible to common aquaculture diseases - possible target for selection. ARS researchers previously demonstrated the susceptibility of multiple aquaculture species to several common diseases, but the need to establish the susceptibility of hybrid striped bass (HSB) as well as their parental species (white bass WB and striped bass SB) to the most common aquaculture diseases plaguing the HSB industry was recently identified by producers as an area of high priority. To address this pressing industry need, ARS researchers in Stuttgart, Arkansas, conducted a series of disease challenge studies in these species to the three most common aquaculture diseases identified by the HSB industry: columnaris disease, motile aeromonad septicemia and streptococcosis. Following challenge, only 1 percent of SB survived across the three diseases and died twice as early as WB and HSB. These results established that WB are the most resistant to all three diseases, SB are most susceptible, and HSB are intermediate to the two parental species. We are currently working with our North Carolina State University partners to incorporate these results into the ARS NCSU HSB Selective Breeding Program to incorporate SB disease resistance as a selection trait for potential improvement of HSB disease resistance.

The palmetto bass is almost completely resistant to common aquaculture diseases while the foodfish standard sunshine bass is highly susceptible. Sunshine bass (SSB), a hybrid cross using a female white bass (WB) and a male striped bass (SB), is the dominant fish used in the hybrid striped bass (HSB) foodfish industry while the palmetto bass (PB), another HSB cross, uses a female SB and a male WB; these two hybrids possess different production characteristics. Previous research in our lab demonstrated the disease tolerance in HSB parental species relative to the SSB, however nothing was known about the disease tolerance of PB. ARS researchers in

Stuttgart, Arkansas, conducted a series of foundational experiments to investigate the susceptibility of these hybrid species to two common pathogens plaguing the HBS industry, columnaris disease and motile aeromonad septicemia. Results demonstrated that the hybrids were significantly different in their survival to both diseases, with PB showing near complete resistance to both diseases (> 95 percent survival), whereas SSB was highly affected by both diseases (< 5 percent survival), and that susceptibility to disease for each hybrid appears to mimic the pattern found in their paternal parental species. These studies will aid farmers and researchers with management strategies to combat infectious diseases, aid in the development of new therapeutants, and provide HSB producers additional species for production when faced with diseases in their production environments.

Zinc: the cheaper, safer alternative to copper for preventative pathogen treatments in aquaculture. Copper has been used as a water treatment for prophylaxis against bacterial infections for decades; however, the price of copper has risen to $10K per ton while the demand has increased. Zinc is a closely related element yet has a market price of $3K per ton. ARS researchers in Stuttgart, Arkansas, explored the possibility of zinc as an alternative to copper as a water decontaminant using a series of toxicity trials with largemouth bass fry. These results revealed that zinc is just as effective as copper in its ability to kill fish pathogens yet is only half as toxic to fish, providing farmers a cheaper, safer treatment for the prevention of disease outbreaks in aquaculture.

Finishing diet makes fish fattier. The COVID-19 pandemic disrupted traditional marketing channels for many hybrid striped bass (HSB) farmers, causing them to seek alternative markets for their fish. With much of the domestic HSB crop typically sold either live or as whole fish on ice, these potential new market opportunities for fish larger than 1.5 lbs have farmers concerned that larger fish could have excessive fat content, causing consumer dissatisfaction. In a HSB production experiment, ARS scientists in Stuttgart, Arkansas, found that fish fed a commercial finishing diet used by some farmers to produce leaner, larger market-size fish actually resulted in fattier fish when compared to a standard production diet. This information has been communicated to HSB farmers for use in making management decisions and improving product quality as they develop new markets when producing these larger fish.

Marine fish meal can be replaced by alternative proteins in bass diets. Marine fish meal (FM) is the most nutritious, and most expensive, ingredient in many fish diets, but is a limited resource due to static populations of the fish species used in its production. Thus, replacing FM is essential if aquaculture diets are to be sustainable. ARS researchers in Stuttgart, Arkansas, along with their colleague at Iowa State University, initiated trials comparing diets having varying levels of soybean meal or poultry by-product meal, partially or completely replacing FM, with one test diet consisting exclusively of plant protein sources. All test diets yielded similar growth performance as fish fed a 30 percent FM control diet apart from an all-plant diet and a commercial protein blended product which resulted in reduced performance. This indicates that WB can be fed diets in which some, if not all, FM can be replaced by more sustainable, and cost-effective, ingredients. Results are currently being used in the ARS NCSU HSB Selective Breeding Program to select a WB (to produce HSB) with superior performance on alternative (plant-based) feeds.

Publications

2018

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Appendix 4 – Accomplishments for Component 4: Enhancing Shellfish Aquaculture

Problem Statement 4A: Enhance Shellfish Aquaculture Production

Accomplishments

2019

Eastern oyster genome. Genomic resources are necessary to promote selective breeding practices that can keep pace with industry priorities and consumer demand. 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. The genome and polymorphic markers represent the first step toward precision, genome-enabled selection in the eastern oyster. Implementation of genome-enabled selection methods such as genomic selection has resulted in 20-100 percent increases in selection accuracy and genetic gain in other aquaculture species but has yet to be tested in the eastern oyster. Genome-enabled selection is particularly useful for traits that are difficult or costly to phenotype, such as Dermo resistance in the eastern oyster.

Pacific white shrimp (Litopenaeus vannamei) cultured in onshore tanks in low salinity waters showed that overall performance was acceptable regardless of hatchery source. Inland, low salinity shrimp farmers in west Alabama, that produce the Pacific white shrimp, *Litopenaeus vannamei*, have recently reported abnormally low survivals at harvest. Reduced survival has also been reported by farms in Florida and Texas. Multiple theories exist as to the cause of increased mortality including disease, toxic algae blooms, water quality, shrimp source, and reduced robustness of shrimp in later stages of production. To compare performance of shrimp from different sources, shrimp were obtained from three different hatcheries and stocked on the same day in three different flow-through systems. One tank system (TS) was installed on one farm (Farm 1-TS) and two systems were installed on two different pond banks of another farm (Farm 2-TS1; Farm 2-TS2). Following 107 days of culture on Farm 2-TS1 and Farm 2-TS2 there were no differences in survival (72.8 91.2 percent) or final weight (19.8 24.6 g) among shrimp sourced from three different hatcheries. At Farm 1-TS, following 111 days of culture there were differences in survival from shrimp sourced from one hatchery (40.5 percent) compared to the other two hatcheries (61.0 69.8 percent). A large percentage of the mortality in the trial occurred in the first thirty days for two of the tank systems (Farm 2-TS1 and Farm 1-TS). Results of this trial by ARS scientists in Auburn, Alabama, demonstrate that while hatchery source did influence survival on one farm, overall performance was acceptable from all three sources compared to performance of shrimp reared in the production ponds in which the tank systems were housed and drawing water.

2021

A multi-state oyster herpesvirus detection program was established. The Ostreid herpesvirus 1 (OsHV-1) has severely impacted Pacific oyster production around the globe. Though this disease

was first detected in the United States in 1995 in Tomales Bay, California, a more virulent microvariant strain was detected in San Diego Bay, California, in 2018. Recognizing the risk of regional spread, a multi-state sentinel program was initiated by ARS researchers in Newport, Oregon, and collaborators to monitor the prevalence and disease development of OsHV-1 in naïve and genetically uniform hybrid oysters planted at commercial farms in California, Oregon, and Washington. A second family of oysters selected for OsHV-1 tolerance was planted for comparison in San Diego and Tomales Bay. Oyster mortalities were almost 100% for both families in San Diego Bay where the microvariant was again identified. Mortalities also occurred in Tomales Bay, but survival was higher for the tolerant family. Mortality events occurred during high seawater temperature spikes and followed peaks in the amount of virus present. This was important because it established a consistent protocol for a more extensive OsHV-1 monitoring and research program.

Improved Eastern oyster genomic tools. The Eastern oyster aquaculture industry grows at an annual rate of 5-10 percent, but production is limited by disease and environmental stressors exacerbated by climate change. New approaches and tools are required for genetic improvement to keep pace with the changing environment. ARS researchers at Kingston, Rhode Island, in collaboration with the Eastern Oyster Breeding Consortium (EOBC), conducted a resequencing project to identify single nucleotide polymorphisms distributed throughout the Eastern oyster genome. This collaboration resulted in the identification of over 3 million high-quality single nucleotide polymorphisms (SNPs), which were filtered to create a 600K SNP chip for high throughput genotyping. The 600K SNP chip was screened with 1000 oysters from geographically distinct wild and selected populations to identify high--performing, informative SNPs for designing and manufacturing a publicly available 60K SNP chip. The 60K SNP chip will aid in the discovery of genetic markers for commercially valuable traits and support advanced genome-based selection techniques for the Eastern oyster.

Bioactive and potent recombinant toxins (PirA and PirB) were produced from Vibrio parahaemolyticus. Acute hepatopancreatic necrosis disease (AHPND), caused by emerging strains of *Vibrio parahaemolyticus* (pirA/pirB plasmid positive isolates), in shrimp aquaculture is of concern not only in Asia but also in Central and North America. Toxins produced by the plasmid of *V. parahaemolyticus*, residing in shrimp, are the culprit of AHPND. The two secreted proteins from the *V. parahaemolyticus* culture were subsequently identified and found to be similar with known binary insecticidal toxins, Photorhabdus insect related proteins A and B (PirA and PirB). In this study, recombinant PirA and PirB (rPirA and rPirB) were produced in Escherichia coli and the intra- and inter-molecular interaction between rPirA and rPirB was analyzed. The relative toxicity of rPirA and rPirB was also assessed in shrimp. Results demonstrated bioactive and potent recombinant PirA and PirB were produced. Use of recombinant proteins rPirA and rPirB will enable toxin testing independent of the bacterium. These recombinant proteins may also aid the search for antitoxin strategies against AHPND.

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Appendix 5 – Accomplishments for Component 5: Developing Marine Finfish Seedstocks

Problem Statement 5A: Develop Warmwater Marine Finfish Seedstocks optimized for Aquaculture Production Efficiency

Accomplishments

2020

Completion of a draft genome of Florida pompano. A hurdle in implementing a genetics-informed selective breeding program for many aquaculture species, particularly warm water marine finfish, is the lack of available genetic information. As with most warm water marine finfish, the whole genome of the Florida pompano had not previously been fully sequenced and assembled. Utilizing a hybrid sequencing method and a novel bioinformatics workflow, a complete draft genome of the Florida pompano was established by researchers at Ft. Pierce, Florida. This draft genome will allow ongoing research to identify genes associated with traits of interest in aquaculture, such as increased growth rate, increased disease resistance, and decreased inbreeding. Such traits, along with others can be actively selected for in a breeding program. Implementing a genetics-based selective breeding program allows for development of genetically improved strains of Florida pompano leading to enhanced on-farm production and greater profits for farmers.

Nutritional requirements of Florida pompano broodstock. The lack of optimal diets for broodstock (especially during the spawning season) continues to present an obstacle to commercial production and industry growth. Broodstock nutritional status is a powerful determinant of egg quality and successful development of both eggs and larvae. Quality broodstock diets increase reproductive success and seedstock quality increasing hatchery success, on-farm efficiencies, and farmer profitability. Understanding the nutritional status of the females during spawning overcomes this obstacle. Comprehensive and quantitative lipid analysis (lipidomics) were employed by researchers at Ft. Pierce, Florida, to determine different egg and larval lipid compositions. This baseline dataset was developed to characterize quantitative and qualitative lipid requirements for both larval development and successful reproduction of Florida pompano. Hatchery managers and marine finfish producers will benefit from efficiencies associated with meeting optimum nutritional needs for reproduction and successful seedstock production.

Development of a rapid disease diagnostic tool for marine fish. A principal challenge to the marine finfish aquaculture industry is the inability to detect disease outbreaks and administer an appropriate remedy in a timely manner. Identifying the presence of disease causing organisms prior to a systemic outbreak increases the potential for limiting costly mortality events. Environmental DNA (eDNA) is a noninvasive and highly accurate method for capturing the footprint of an array of disease-causing organisms at very low concentrations. Researchers at Ft. Pierce, Florida, developed a sensitive and specific quantitative PCR (qPCR) test that can detect a single copy of a genetic marker associated with the disease causing organism Amyloodinium ocellatum from the culture water, giving rapid and accurate same-day results. This qPCR test,

currently undergoing validation, will provide a much needed toolbox for routine health monitoring and disease diagnostics on commercial aquaculture farms.

2021

Demonstrated growth, survival, and health of Florida Pompano larvae raised in decreasing salinity. Production of juvenile fish used for stocking marine finfish farms has been restricted to coastal lands, unless large additions of salt are used, which can be costly to both the farmer and the environment. ARS researchers in Fort Pierce, Florida, in collaboration with local fish farmers determined the growth, survival, health and well-being of early-life stage Florida pompano raised in lower salinity than found in the ocean. After evaluating the effects of lower salinity on the bacterial composition, fish health, stress, and well-being, it was determined that the salt in the environment can be reduced by half without any effects on growth or well-being of the fish. This accomplishment demonstrates Florida pompano, and likely other highly prized marine fish can be reared in a lower salinity than originally believed. The benefits include opportunities for a greater number of U.S. marine finfish farmers, and a potential cost savings for producing these juveniles, especially in areas without direct seashore access.

Established high-quality reproductive stocks of high-value warm water marine finfish. To support the expanding warm water marine finfish aquaculture industry, prospective farmers require a steady, consistent supply of high-quality animals. ARS researchers in Fort Pierce, Florida, have established a Florida pompano reproductive stock to produce a steady, high-quality source of fish for these farms by defining and improving the production of high-quality eggs and juveniles to support the industry. A consortium of like-minded researchers from Florida Atlantic University, Fort Pierce, Florida; the University of Miami, Coral Gables, Florida; Hubbs Sea World, San Diego, California; and Mote Marine Laboratory, Sarasota, Florida, established methods for identifying attributes to increase survival, faster growth, and well-being of early-stage Almaco Jack, Florida pompano and other closely related species highly prized by U.S. consumers. This includes understanding the effects of temperature and oxygen availability on the energy needs of the fish during production, under both current and potential climate change conditions. Benefits to marine finfish producers and consumers are better production efficiencies and a better tasting product brought to market safer, faster, and more economically.

Determined nutritional value of new feed ingredients for Florida Pompano. Cost and ecological sustainability of feeds for farm raised fish dictate the success or failure of fish farms, therefore finding new and less expensive ingredients, such as leftover products from making other useful goods, is essential. ARS researchers in both Fort Pierce and Tallahassee, Florida, have determined the availability of nutrients to Florida pompano from the parts of clams not used for human consumption, and from production of hemp fibers for the textile industry. Both products indicate the growth-promoting and healthy nutrients in them are highly available to Florida pompano suggesting they should make very good supplements to marine finfish feeds. These efforts created nutrient availability values provided to fish feed manufacturers to develop and market more sustainable fish feeds. Benefits from this research include information for fish farmers and feed manufacturers to produce more sustainable fish feeds, and potential new revenue streams for two additional industrial manufacturing processes.

Demonstrated algae oil in fish diets as a viable alternative to fish oil. Due to economic and sustainability issues, associated with expensive and diminishing supplies of fish oil used in feeds for farmed fish, alternatives are in high demand. Vegetable oils associated with agriculturally based products (e.g., corn, soy, olive, canola, etc.,) do not contain the right components to meet marine finfish needs (especially those that normally eat fish) for normal growth and well-being or provide the heart healthy nutrients needed by American consumers. ARS researchers in Fort Pierce, Florida, along with other partners demonstrated that oils from algae, processed under the right conditions can fulfil that need. The right algae, produced under the right conditions, can produce the same oil products that result in highly desired marine fish that are fed fish oil diets. Benefits to the American consumer include a safer, better tasting product with all the heart-healthy components consumers seek.

Hosted an industry/stakeholder informational workshop. To ensure U.S. marine finfish aquaculture industry and taxpayer interests are met, it is essential to inform interested parties on programmatic progress and seek input on future programmatic direction. ARS researchers in Fort Pierce, Florida, in coordination with the U.S. Warm Water Marine Finfish Aquaculture Advisory Committee, hosted a workshop to disseminate programmatic progress and seek input for future programmatic direction. The workshop successfully encouraged the ongoing dialogue between project researchers, industry, and other stakeholders. The documented outcomes were productive and instrumental in underscoring ongoing stakeholders needs and refining new industry challenges. The benefits to industry and the U.S. consumer are a very directed and focused effort to increase production efficiencies and a better tasting product brought to market safer, faster, and more economically.

Produced an industry requested publication on the status of U.S. Marine finfish aquaculture. Over 85 percent of the seafood Americans eat is imported, half is produced through foreign aquaculture; therefore, expanding aquaculture in the United States would reduce our $16.8 billion seafood trade deficit. Following a series of workshops, surveys, and presentations by the foremost experts in the United States, a publication on the status of readiness for numerous species and priorities for removing barriers to commercialization were collated, written, and edited by ARS researchers in Fort Pierce, Florida, to spur domestic growth in marine finfish aquaculture. The articles provide up-to-date scientific and technical knowledge needed to sustainably produce safe and nutritious fish that will lead to the creation of new economic opportunities through aquaculture in rural, urban, coastal, and inland communities. The benefits to U.S. Marine finfish aquaculture are innumerable and can be found in the publication at: https://onlinelibrary.wiley.com/toc/17497345/2021/52/3.

2022

Gene expression was altered in cultured Florida pompano following ectoparasite Amyloodinium infection. The disease-causing ectoparasite *Amyloodinium ocellatum* is a nuisance in cultured marine and brackish water fishes worldwide, including the highly desirable pompano, with its high economic value in the United States This parasite infects fish gills and causes tissue damage, increased respiratory rate, and reduced appetite and mortality, especially in recirculating aquaculture systems. ARS researchers in Stuttgart, Arkansas, along with their colleagues at Auburn University, conducted studies which mimicked a natural infection in cultured pompano where they sought to identify fish genomic responses following infection.

Researchers found that genes involved with immune response and inflammation suppression, cellular respiration, tissue repair, and food intake and fat metabolism were all affected. These genes all play an important role in a fish's ability to fight disease and maintain cellular health. This work provides the first genomic report in pompano which is key to understanding this parasitic disease. This will aid in the design of disease monitoring strategies, future selection for disease resistance in pompano, and the development of new drug treatment measures.

Clam meal economical feed component for farm-raised Florida pompano. Clam processing by-product represents expensive and environmentally challenging disposal problems for the clam processing industry. Researchers in Fort Pierce, Florida, in collaboration with Sea Watch International established that clam meal can be used as a suitable substitute for other more expensive and less sustainable fish meal ingredients in fish feed fed to farm-raised Florida pompano. This clam meal promotes growth, health, and well-being in farm raised Florida pompano as well as high quality fish meal. This processing waste-stream could be used at rates as high as 20 percent of the complete diet. This accomplishment represents a more sustainable fish feed, a potential feed cost reduction, in addition to potential new revenue stream for an additional industrial manufacturing process.

Advances in Yellowtail jack spawning and diet promote this U.S. industry. The Yellowtail jack is a U.S. consumer favorite, and there are ongoing efforts to establish offshore net-pen farms for their production in U.S. waters. However, year-round production of juveniles to stock these farms, as well as safe and efficient diets for feeding spawning fish to produce efficient, hardy, and robust juveniles remain challenges to the industry. Researchers in Fort Pierce, Florida, in collaboration with researchers from Hubbs Sea World Research Institute in California have established methods for the successful out of season spawning of Yellowtail broodstock. In addition, it was demonstrated that commercially available diets can be used to produce high egg and larval quality and quantities to make the U.S. industry competitive. This accomplishment benefits consumers and Yellowtail producers alike by providing a more steady, consistent supply of high-quality product year-round.

Increased knowledge of microbiome changes following copper sulfate treatment improve aquaculture production safety. Copper sulfate is used by farmers for the treatment of parasitic infections and excessive algal growth. However, previous indications in other settings suggest that the film of microbes in aquaculture systems can exhibit an increase in the number of microbial metal and antibiotic resistant genes following treatment with copper sulfate. Researchers in Fort Pierce, Florida, evaluated the effects of copper sulfate on microbes within a tank following completion of the treatment regimen and documented an increase in the types and numbers of metal and antibiotic resistant genes over time. This accomplishment provides U.S. fish farmers with improved guidelines for copper sulfate use that will result in a safer and healthier stock, while reducing risk to the environment of developing metal and antibiotic resistant genes.

Quantification of Florida Pompano population genetics promotes improved broodstocks. Selective breeding programs with terrestrial livestock and a limited number of farm-raised fish species indicate it is possible to identify genes encoding beneficial production traits that can be selected for in the parental stock and result in faster and more efficient growth, greater disease

resistance or higher quality products. In selecting the parental broodstock, it is essential to understand the genetic makeup of the parents to maximize the diversity in the gene pool, but not contaminate the existing gene pool. Researchers at Ft. Pierce, Florida, have established that the wild populations of Florida Pompano off the Atlantic/East Coast of Florida and the Gulf/West Coast of Florida are genetically similar suggesting they are one population. This accomplishment provides a greater understanding of the genetic variation found in the wild and is important when collecting individuals for a broodstock that will result in juvenile fish with faster growth and a higher quality product to supply to U.S. consumers.

Publications

2020

Peer Reviewed Article: Watson, A., Napolitano, M., Schock, T., Bowden, J., Frost, J.B., Yost, J., Denson, M. 2020. Evaluation of graded levels of soy oil as a fish oil replacement in high soy protein feeds for juvenile red drum, Sciaenops ocellatus. Aquaculture. 529:735627. https://doi.org/10.1016/j.aquaculture.2020.735627.

2021

Peer Reviewed Article: Weirich, C., Riley, K., Riche, M., Main, K.L., Wills, P.S., Illan, G., Cerino, D.S., Pfeiffer, T.J. 2021. The status of Florida pompano, Trachinotus carolinus as a commercially ready species for U.S. Marine Aquaculture. Journal of the World Aquaculture Society. 52(3):731-763. https://doi.org/10.1111/jwas.12809.

Peer Reviewed Article: Mejri, S., Riche, M., Wills, P., Tremblay, R., Audet, C. 2021. Essential fatty acid requirements in tropical and cold-water marine fish larvae and juveniles. Frontiers in Marine Science. 8:680003. https://doi.org/10.3389/fmars.2021.680003.

Peer Reviewed Article: Rexroad, C.E., Rust, M.B., Riche, M., Wills, P., Davis, M. 2021. Opportunities for US Marine Finfish Aquaculture. Journal of the World Aquaculture Society. 52(3):501-508. https://doi.org/10.1111/jwas.12791.

2022

Peer Reviewed Article: Zhang, D., Mohammed, H., Ye, Z., Rhodes, M., Thongda, W., Zhao, H., Jescovitch, L., Fuller, S.A., Davis, A., Peatman, E. 2022. Transcriptomic profiles of Florida pompano (Trachinotus carolinus) gill following infection by the ectoparasite Amyloodinium ocellatum. Fish and Shellfish Immunology. 125:171-179. https://doi.org/10.1016/j.fsi.2022.05.017.

Peer Reviewed Article: Habte-Tsion, H., Riche, M., Mejri, S., Bradshaw, D., Wills, P.S., Myers, J.J., Perricone, C. 2022. The effects of fish meal substitution by clam meal on the growth and health of Florida pompano (Trachinotus carolinus). Scientific Reports. 12:7696. https://doi.org/10.1038/s41598-022-11675-x.

Peer Reviewed Article: Yamamoto, F.Y., Ellis, M., Bowles, P.R., Suehs, B.A., Carvalho, P.L., Older, C.E., Hume, M.E., Gatlin Iii, D.M. 2022. Dietary supplementation of a commercial prebiotic, probiotic and their combination affected growth performance and transient intestinal microbiota of red drum (Sciaenops ocellatus L.). Aquaculture. 12(19):2629. https://doi.org/10.3390/ani12192629.

Appendix 6 – Accomplishments for Component 6: Developing Sustainable Aquaponic Production Systems

Problem Statement 6A: Optimize Aquatic Animal Species Production Systems for Aquaponics
Problem Statement 6B: Optimize Plant Production Systems for Aquaponics
Problem Statement 6C: Optimize the Integration of Fish and Plant Production Systems

Accomplishment

2020

Teaching aquaponics increases students' interest in science, technology, engineering, and mathematics (STEM) and spurs interest in environmental science. There is a need for secondary schools in the U.S. to get students interested in, and prepared for, jobs that are based in (STEM) as there is a critical shortage and need in these disciplines. However, many students are very reluctant to take STEM courses. Thus, secondary schools need to provide more hands-on, fun ways to teach STEM disciplines and specifically, more project-based investigation (PBI) environments in the classroom to pique student's interests. Aquaponics is a good candidate for hands-on classroom learning since it involves all the STEM disciplines as well as environmental sciences and ecology. ARS researchers in Stuttgart, Arkansas, in collaboration with Kentucky State University investigated how an aquaponics project-based investigation (APBI) unit affected high school students attitudes and interest towards STEM, environmental sciences and ecology, as well as aquaculture and aquaponics. These results demonstrated that a hands-on aquaponics curriculum contributed to students positive attitudes toward STEM and provided meaningful learning of ecological concepts, aquaculture, and aquaponics. These APBI teaching models may promote student learning of scientific concepts, increase students interest in STEM-related disciplines, and should be considered when schools are developing STEM curricula to develop the next generation of scientists in the United States.

Appendix 7 – Additional Accomplishments and Publications

Additional Accomplishments listed below are not directly associated with a Component in the Current Action Plan but may have been incorporated in the previous Action Plan or represent opportunities for ARS scientists to use their research expertise and capacity for broader impact.

Accomplishments

2018

A new, formulated, dry diet improves growth in juvenile lake sturgeon. Sturgeon products (flesh and roe) are among the highest value commodities in global aquaculture. In the Midwest United States, lake sturgeon (*Acipenser fulvescens*) are also a highly priced food fish. However, our understanding of the necessary feeding rates, and access to formulated diets, is limited for this species. Such limitations currently result in feed waste and increased costs to producers. The primary aims of this work were to 1) develop a newly formulated dry diet for juvenile lake sturgeon, and 2) identify and compare optimal feeding rates of juvenile sturgeon fed the newly formulated dry diet against sturgeon fed a commercial semi-moist diet and live feeds (blood worms). ARS scientists in Milwaukee, Wisconsin, in collaboration with researchers at the University of Wisconsin-Milwaukee showed that juvenile sturgeon fed the commercial semi-moist and the newly formulated dry diet grew faster than those fish fed the traditional live feeds. The optimal feeding rate was determined to be approximately 7.0 percent of body weight per day for juvenile (< 8 grams body weight) sturgeon. The use of a formulated dry diet, with an accurate feeding rate, will reduce waste, improve production efficiency and save aquaculture producers money.

Capsular typing of Streptococcus (S.) agalactiae from fish using multiplex PCR and serotyping. *Streptococcus* spp., including *Streptococcus agalactiae* (Lancefield group B streptococci) are considered emerging Gram-positive bacterial pathogens and are responsible for approximately $1 billion USD in annual losses to the global tilapia industry. The capsule is a sugar containing material on the surface of these bacteria that is important for virulence and antigenicity including serotype. There is an urgent need to identify different capsular types of *S. agalactiae* from farmed and wild fish to combat streptococcal disease. ARS researchers at Auburn, Alabama, used multiplex polymerase chain reaction (PCR) capsular typing assay and antiserum to assign capsular type to a total of forty *S. agalactiae* isolates. The multiplex PCR was modified to detect capsular types Ia, Ib, II and III, the common capsular types reported from fish and aquatic animals. Results demonstrated that most isolates from North, Central and South America were capsular type Ib. The modified multiplex PCR assay is used to determine the capsular type of *S. agalactiae* present on a farm and/or region and has assisted with disease management strategies including selective breeding and vaccines.

Ghrelin (a gastric peptide) stimulates neuropeptides that control feed-intake, growth and immunity in fish. In fish, growth is generally related to feed intake. Small proteins, called neuropeptides, which are located in the brain and elsewhere, are known to affect all aspects of vertebrate physiology. Ghrelin is a peptide that is secreted by the stomach during fasting and signals the brain to increase feed intake. In addition, ghrelin has been shown to increase growth hormone levels and stimulate innate immunity in fish. However, our understanding of how

ghrelin interacts within the brain to increase feed intake, growth and immune function is poor. ARS scientists in Milwaukee, Wisconsin, in collaboration with Monash University (Selengor, Malaysia) and Sun Yat-Sen University (Guangzhou, China) scientists mapped specific neurons in the brain of tilapia to determine the effects of centrally-administered (precise injection into the brain) ghrelin on these neurons and subsequent effects on blood hormone levels in this fish. Ghrelin treatment affected levels of growth hormone in the pituitary, insulin-like growth factor-I (a growth-promoting hormone) in blood, and growth hormone releasing hormone levels in the hypothalamus area of the brain. These findings demonstrate that ghrelin controls other hormones related to feed intake, growth and immune function via its effects on brain neuropeptides. This knowledge can enhance feeding practices, diet formulations, and selective breeding approaches in ways that improve growth and immune function in commercially-important finfish species.

Intensive production of stocker size tilapia in the biofloc production system. Market-size fish can be harvested from grow out ponds sooner if larger, advanced fingerlings, or stockers, are stocked. However, optimization of the overall production cycle requires that stockers also be produced efficiently. Stocking rate is one factor that effects fish growth and can be manipulated to obtain stocker size fish. Much greater numbers of fish are stocked in the biofloc technology production system because the fish live within a community of microorganisms in the water the biofloc that consume fish waste quickly, removing it as a threat to the fish. ARS researchers at Stuttgart, Arkansas, quantified the relationship between tilapia stocking rate and growth and yield of stocker-size fish in an outdoor biofloc production system. Yield of stocker-size tilapia increased linearly with stocking rate, whereas mean individual weight decreased linearly. However, stocker-size fish were produced at all stocking rates. Using these relationships farmers can optimize annual production of stocker-size tilapia by manipulating stocking rate and production cycle length.

Phytase enzyme substitutes for inorganic phosphorus in diet for tilapia biofloc production. Fish feed typically is supplemented with inorganic phosphorus to ensure adequate dietary phosphorus availability, especially in diets that contain high percentages of plant feedstuffs, which is the case for diets formulated for tilapia. Phosphorus is present in plant feedstuffs as phytate, which is not bioavailable to fish because their digestive system lacks sufficient phytase enzyme. Thus, phytate is excreted unmetabolized and accumulates to high concentrations in the biofloc technology production system because of the high quantities of feed fed daily to fish. In a study conducted in an outdoor biofloc production system to investigate the impact of dietary inclusion of phytase on phosphorus retention by tilapia and on water quality, ARS researchers at Stuttgart, Arkansas, found that phytase enzyme can substitute completely for inorganic phosphorus in the fish feed without affecting tilapia growth negatively. Additionally, phosphorus excretion by tilapia was reduced by about 50 percent, resulting in improved water quality. Reducing potential phosphorus excretion will help fish farmers meet National Pollutant Discharge Elimination System permit requirements.

Short-term (dietary) exposure of sexually mature yellow perch to environmentally-relevant methyl mercury (MeHg) levels does not affect reproduction. Commercially produced finfish can be inadvertently exposed to MeHg via environmental and dietary (protein and lipids) sources, but our understanding of the sub-lethal impacts of MeHg on fish reproduction is poor. Understanding the impacts of contaminants, and other stressors, is particularly important to the

preservation of genomic resources and crucial to the efficiency and success of breeding and genetic improvement programs. ARS scientists in Milwaukee, Wisconsin, in collaboration with University of Wisconsin at Milwaukee, Michigan State University (East Lansing, MI), McGill University (Quebec, Canada), Idaho State University (Pocatello, Idaho) and U.S. Geological Survey (Seattle, WA) scientists found that dietary exposure of yellow perch and zebrafish to MeHg resulted in accumulation of this metal in the tissues of both fish species. Analyses of ovarian gene expression in zebrafish exposed over their entire life-cycle showed changes in several ovarian genes involved in reproductive processes, but there were no changes observed with measures of ovarian structure and function (hormone levels, egg production or embryo mortality). By contrast, ovarian gene expression and measures of ovarian function were not altered by MeHg exposure in yellow perch. These results indicate that environmentally relevant MeHg exposures do not impact the spawning ability of yellow perch within a single season. However, whole life-cycle exposures, similarly to that conducted in zebrafish, should be performed in yellow perch to ensure that environmentally-relevant doses do not affect life-time reproductive capacity of selectively-bred yellow perch broodstocks.

2019

Microbial profiling of tilapia gut reared in either biofloc or flow-through aquaculture systems reveals dynamic populations associated to each environment. ARS researchers in Stuttgart, Arkansas, in collaboration with ARS researchers in Auburn, Alabama, and Gainesville, Florida, reared juvenile tilapia in each system for 154 days on two test diets and a control diet. Water, feed and gut contents were sampled at multiple time points over the course of the study, including day 0, day 7, day 60, and at the study endpoint. DNA was isolated from a total of 440 tilapia gut, feed, and water samples including biological replicates. This genetic material was used for amplicon sequencing of the 16S ribosomal DNA gene and the Nuclear Large Ribosomal RNA gene to assess the composition of bacteria, fungi, and protozoa in each sample. Data was processed using the QIIME2 software. After statistical analyses between replicates, it was revealed that the prokaryotic and eukaryotic communities were strongly structured by the sample type (gut, feed, and water) and by the environment (outdoor, biofloc systems vs. indoor, flow-through systems). Since diet and the environment affect the intestinal microbiota of fish and microbial communities are highly relevant to fish health, these findings provide for a better understanding of the dynamics in rearing tilapia in biofloc technology.

Algae-based diets have been developed and tested in yellow perch. Alternative protein and lipid sources are required to make aquafeeds sustainable and affordable. As opposed to terrestrial plant protein sources, seaweeds possess dispensable and indispensable amino acids, as well as micronutrients such as vitamins and minerals, which are required for proper animal development and growth. Seaweeds also possess pre-biotic components that may benefit production performance in finfish. ARS researchers in Milwaukee, Wisconsin, along with University of Wisconsin at Milwaukee scientists, investigated the potential of defatted microalgae meal, a co-product from the bio-energy industry, as a feed ingredient for yellow perch. Results of this study indicate that defatted microalgae meal, when blended with soy protein isolate, can be used to replace 25 percent of the fishmeal in a standard test diet while maintaining performance of yellow perch. Digestible algae-based diets can reduce nutrient outputs such as phosphorus and synergize with the bio-energy industry to improve the efficiency, profitability, and sustainability of yellow perch production.

The dose needed for rapid anesthesia has been identified for yellow perch. Anesthetics are an integral part of farmed fish production and research as they allow fish to be easily handled, and transported, in a way that reduces stress. It is well known that the responses to the same anesthetic can vary considerably within and among various species, and it is inappropriate to extrapolate optimal anesthetic doses between different species. For yellow perch, there are no published guidelines. To address this, ARS researchers in Milwaukee, Wisconsin, along with scientists from the University of Wisconsin at Milwaukee, performed studies in juvenile yellow perch and identified an optimal dose of the anesthetic tricaine methanesulfonate that enables rapid anesthesia and recovery for brief periods of time. The research also demonstrated that anesthesia induction and recovery times are affected by the size of the fish. These results give fish handlers an optimal combination for minimizing handling disturbance in juvenile yellow perch which could improve the welfare of animals subjected to frequent handling events in the research and commercial production settings.

2020

Diet development for emerging food fish species. Bluegill, and their hybrids, used to only be grown to supply the sportfish industry. Today, however, they are emerging as a popular food fish in the southern United States, as well as globally. But lack of economical, nutritious diet formulations and winter mortality are widely recognized as major factors that decrease profitability for producers. A uniform strategy for minimizing winter weight loss, reducing winter mortality, and optimizing intensive production-oriented diets that contain sustainable alternative protein ingredients to marine fish meal are high priorities for this industry. Diet composition, as well as feeding strategy, can affect the nutrition and disease resistance of fish. Deficiency of essential fatty acids (EFAs), for example, can result in growth reduction, abnormal physiological stress during cold temperatures, and suppressed immune function. However, the effects of dietary EFAs, protein sources, and feeding strategy on growth performance and winter survivability is unknown. A series of cooperative studies between ARS researchers in Stuttgart, Arkansas, and the University of Arkansas at Pine Bluff Agricultural Extension Service determined that: 1) feeding frequency impacted final fish weight at the end of simulated winter culture periods for some centrarchid species, but not all; 2) bluegill and hybrid bluegill exhibited preferential sequestering of long chain polyunsaturated fatty acids (the "n-3 heart healthy fatty acids") and utilization of monounsaturated fatty acids for energy during winter water temperatures, regardless of feeding frequency; and 3) centrarchids were able to effectively utilize a variety of alternative protein ingredients in place of marine fish meal; however, more work is needed to develop effective all-plant protein diets. These findings were rapidly adopted in the industry and have changed both the feeding practices and philosophy of diet formulation for both centrarchid sportfi

New genomic resources developed for yellow perch, Perca flavescens. Yellow perch is a freshwater fish in high demand for human consumption and, consequently, numbers in the wild have been substantially reduced due to overfishing and other factors. Modern genomic resources are needed for conservation genetics and for genetic improvement programs for the aquaculture industry. To address this, ARS researchers in Milwaukee, Wisconsin, and researchers from The Ohio State University (Piketon, Ohio) sampled yellow perch from eight populations to develop libraries consisting of two types of genomic markers called simple sequence repeats and single nucleotide polymorphisms. This research resulted in the identification of thousands of new

genetic markers distributed throughout the yellow perch genome. These genetic markers are important genomic resources that will enable scientists to increase the rate of genetic gain for traits of interest in yellow perch aquaculture, and will enhance efforts aimed at conserving genetic diversity within and among wild yellow perch populations.

The nonvirion region, is part of the viral hemorrhagic septicemia virus (VHSv) gene, but encodes for this non-structural protein. VHSv has been found to affect the host antiviral response in unexpected ways. VHSv is one of the most deadly infectious fish pathogens posing a serious threat to the aquaculture industry. As with related viruses, the Great Lakes VHSv contains a unique and highly variable nonvirion gene, which is thought to enable infection of host cells and its ability to evade the host immune response. To elucidate function of the nonvirion gene, ARS researchers in Milwaukee, Wisconsin, and researchers from the University of Toledo and Wright State University (Ohio) used a fish cell-line and observed that the native Great Lakes type of VHS virus suppressed the host antiviral response by inhibiting production of most new proteins within the cells, except for production of viral proteins. Infection of the fish cell line with a Great Lakes VHS virus, not containing the nonvirion region, exhibited lower levels of viral protein synthesis despite increased levels of viral expression. These findings show a subtle role for the nonvirion region in Great Lakes type of VHS virus mediated pathogenesis via alteration of host protein shutoff. Information from this work may enable identification of new viral targets that modulate the host-pathogen interaction, and immunogenicity, which could be adopted to design more efficient vaccination strategies.

Publications

2018

Abstract: Lafrentz, B.R., Shoemaker, C.A., Lozano, C.A., Garcia, J.C., Soto, E., Xu, D., Beck, B.H., Rye, M. 2018. Resistance of Nile tilapia (Oreochromis niloticus) to Streptococcus iniae and S. agalactiae Ib is heritable but not correlated. [abstract] Annual Eastern Fish Health Workshop. p. 35.

Abstract: LaFrentz, B.R., Shoemaker, C.A., Lozano, C.A., Garcia, J.C., Soto, E., Xu, D., Beck, B.H., Rye, M. 2018. Resistance of Nile tilapia Oreochromis niloticus to Streptococcus iniae and S. agalactiae is heritable but not correlated [abstract]. International Symposium on Aquatic Animal Health. p. 56.

Abstract: Shelley, J.P., Yanong, R., Hawke, J.P., Griffin, M. 2018. Edwardsiellosis in ornamental fish [abstract]. International Symposium on Aquatic Animal Health. p. 251.

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Abstract: Garcia, J.C., Shoemaker, C.A., Lafrentz, B.R. 2018. Streptococcus agalactiae infection in Nile tilapia (Oreochromis niloticus) and its transmission potential to cohabitated sheepshead minnows (Cyprinodon variegatus)[abstract]. Annual Eastern Fish Health Workshop. p. 34.

Peer Reviewed Article: Nebo, C., Gimbo, R.Y., Kojima, J.T., Overturf, K.E., Dal-Pai-Silva, M., Portella, M.C. 2018. Depletion of stored nutrients during fasting in Nile tilapia (Oreochromis niloticus) juveniles. Journal of Applied Aquaculture. 30(2):157-173. https://doi.org/10.1080/10454438.2017.1420516.

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Appendix 8 – ARS Funded Partnerships

Congress directs ARS to partner with land grant universities and other institutions to conduct research and technology transfer. To this end, ARS develops cooperative agreements that provide funds for research that aligns with the National Program Action Plan and the local project's objectives. Some of these partnerships are Congressionally mandated, and others are developed by scientists at their discretion to expand research capacity for their project. Agreements can last up to five years, the following agreements were initiated in 2018 – 2022.

Component 1

10/1/2017 Component/Problem Statement 1a, Research Support Agreement
Acquisition of Goods and Services, MISSISSIPPI STATE UNIVERSITY

10/1/2017 Component/Problem Statement 1a, Non-Funded Cooperative Agreement
GnRHIIa INAD Obtention and Development, AQUATACTICS

5/1/2018 Component/Problem Statement 1a, Research Support Agreement
Repeatability of Incidence and Time of Ovulation, Fecundity and Fertility in Channel Catfish Females Induced to Ovulate for Production

9/1/2018 Component/Problem Statement 1a, Non-Assistance Cooperative Agreement
Cryopreservation of Catfish Sperm Samples for Use in the Warmwater Aquaculture Research Unit's Breeding Program, LOUISIANA STATE UNIVERSITY

10/1/2018 Component/Problem Statement 1a, Research Support Agreement
Acquisition of Goods and Services, MISSISSIPPI STATE UNIVERSITY

5/1/2019 Component/Problem Statement 1a, Non-Funded Cooperative Agreement
Field Evaluation of 'Portable Electrosedation System' (PES) to Sedate Ovulating Channel Catfish to Produce Hybrid Catfish..., AMERICAS CATCH CATFISH FARM

5/1/2019 Component/Problem Statement 1a, Non-Funded Cooperative Agreement
Field Evaluation of 'Portable Electrosedation System' (PES) to Sedate Ovulating Channel Catfish to Produce Hybrid Catfish... on Baxter Land Company, BAXTER LAND COMPANY, INC.

5/1/2019 Component/Problem Statement 1a, Non-Funded Cooperative Agreement
Field Evaluation of 'Portable Electrosedation System' (PES) to Sedate Ovulating Channel Catfish to Produce Hybrid Catfish... on Jubilee Farms, JUBILEE FARMS INC.

5/1/2019 Component/Problem Statement 1a, Non-Funded Cooperative Agreement
Field Evaluation of 'Portable Electrosedation System' (PES) to Sedate Ovulating Channel Catfish to Produce Hybrid Catfish... on Needmore Fisheries, NEEDMORE FISHERIES

5/1/2019 Component/Problem Statement 1a, Non-Funded Cooperative Agreement

Field Evaluation of 'Portable Electrosedation System' (PES) to Sedate Ovulating Channel Catfish to Produce Hybrid Catfish... on Tackett Farms, TACKETT FISH FARMS

5/1/2019 Component/Problem Statement 1a, Non-Funded Cooperative Agreement
Field Evaluation of 'Portable Electrosedation System' (PES) to Sedate Ovulating Channel Catfish to Produce Hybrid Catfish... on Wright Fish Farms, WRIGHT FISH FARMS

7/1/2019 Component/Problem Statement 1a, Non-Assistance Cooperative Agreement
Development of Active and Passive Acoustic Measurements to Improve the Production and Profitability of U.S. Aquaculture, UNIVERSITY OF MISSISSIPPI

10/1/2019 Component/Problem Statement 1a, Research Support Agreement
Acquisition of Goods and Services, AUBURN UNIVERSITY

4/1/2020 Component/Problem Statement 1a, Non-Assistance Cooperative Agreement
Improving Efficiency in Catfish Aquaculture, MISS AGRI & FORESTRY EXP STATION

5/1/2020 Component/Problem Statement 1a, Non-Assistance Cooperative Agreement
Harmful Algal Blooms: Prevalence and Control Measures for the Domestic Fish and Shellfish Industries, AUBURN UNIVERSITY

5/1/2020 Component/Problem Statement 1a, Non-Assistance Cooperative Agreement
Development of Alternative Feeds, Feed Management Regimes, and Production Systems for Improved Production and Health, AUBURN UNIVERSITY

6/18/2020 Component/Problem Statement 1a, Outgoing Interagency Agreement
ORISE" ARS Research Participation Program", OAK RIDGE NATIONAL LAB

7/1/2020 Component/Problem Statement 1a, Research Support Agreement
Acquisition of Goods and Services, MISSISSIPPI STATE

7/1/2020 Component/Problem Statement 1a, Non-Assistance Cooperative Agreement
Utilization of Soybean Hulls in Animal Feed Binder, AUBURN UNIVERSITY

7/1/2020 Component/Problem Statement 1a, Non-Assistance Cooperative Agreement
Hill Area Aquaculture, MISSISSIPPI STATE UNIVERSITY

9/1/2020 Component/Problem Statement 1a, Non-Assistance Cooperative Agreement
Genomic Selection of Channel, Blue and Hybrid Catfish, UNIVERSITY OF GEORGIA

9/15/2020 Component/Problem Statement 1a, Non-Assistance Cooperative Agreement
Development of Methods to Detect Snails in Catfish Aquaculture Ponds, MISSISSIPPI STATE UNIVERSITY

10/1/2021 Component/Problem Statement 1a, Research Support Agreement
Acquisition of Goods and Services, AUBURN UNIVERSITY

1/3/2022 Component/Problem Statement 1a, Research Support Agreement
Acquisition of Goods and Services, MISSISSIPPI STATE UNIVERSITY

4/1/2022 Component/Problem Statement 1a, Outgoing Interagency Agreement
Nuisance Animal Control at the Aquatic Animal Health Research Unit, USDA, APHIS, WILDLIFE SERVICES

7/1/2022 Component/Problem Statement 1a, Research Support Agreement
Acquisition of Goods and Services, MISSISSIPPI STATE UNIVERSITY

9/1/2022 Component/Problem Statement 1a, Non-Assistance Cooperative Agreement
Effects of Herbicide Exposure on Channel Catfish Biology, Health, and Behavior, AUBURN UNIVERSITY

9/1/2022 Component/Problem Statement 1a, Non-Assistance Cooperative Agreement
Improving Product Quality in Farm-Raised Catfish, LOUISIANA STATE UNIVERSITY

9/15/2022 Component/Problem Statement 1a, Non-Assistance Cooperative Agreement
Manufacturing of Bioactive Materials from Biomass as Functional Feed Additives for Aquatic Animals, AUBURN UNIVERSITY

10/1/2017 Component/Problem Statement 1b, Research Support Agreement
Acquisition of Goods and Services, AUBURN UNIVERSITY

10/1/2017 Component/Problem Statement 1b, Research Support Agreement
Leukocyte Immune-type Receptors: Gene Structure and Function

6/1/2018 Component/Problem Statement 1b, Non-Assistance Cooperative Agreement
Identification and Modification of Pathogenicity-Associated Genes of Virulent Aeromonas hydrophila Using CRISPR-Cas9 System, AUBURN UNIVERSITY

9/1/2018 Component/Problem Statement 1b, Non-Assistance Cooperative Agreement
Disease Resistance and Susceptibility of Blue Catfish Strains, AUBURN UNIVERSITY

10/1/2018 Component/Problem Statement 1b, Research Support Agreement
Acquisition of Goods and Services, AUBURN UNIVERSITY

8/1/2019 Component/Problem Statement 1b, Non-Assistance Cooperative Agreement
Integrated Research to Improve Aquaculture Production and Aquatic Animal Health of Warmwater Aquaculture Species in West Alabama, AUBURN UNIVERSITY

10/1/2019 Component/Problem Statement 1b, Research Support Agreement
Novel Protein-based Therapy For Managing Disease That Stimulates Catfish Antimicrobial Immunity and Tissue Repair Systems

7/1/2020 Component/Problem Statement 1b, Non-Assistance Cooperative Agreement
Assessment of the Immunomodulatory Effect of Probiotics on the Catfish Adaptive Immune Response and Columnaris Disease Resistance, AUBURN UNIVERSITY

7/1/2020 Component/Problem Statement 1b, Non-Assistance Cooperative Agreement
Identification of Natural Compounds to Control Pests in Catfish Ponds, UNIVERSITY OF MISSISSIPPI

7/27/2020 Component/Problem Statement 1b, Material Transfer Research Agreement
Determination of Efficacy of an Inactivated Oral Vaccine for Aeromonas Hydrophila

9/1/2020 Component/Problem Statement 1b, Non-Assistance Cooperative Agreement
Development of Application Technologies for Prescription Delivery of Pond Treatments, MISSISSIPPI STATE UNIVERSITY

6/1/2021 Component/Problem Statement 1b, Non-Assistance Cooperative Agreement
Improving Catfish Health, MISSISSIPPI STATE UNIVERSITY

9/1/2021 Component/Problem Statement 1b, Non-Assistance Cooperative Agreement
Defining the Complex Interactions of Coinfections and Methods for Control, AUBURN UNIVERSITY

6/1/2022 Component/Problem Statement 1b, Research Support Agreement
Phage Endolysins as Alternatives to Antibiotics for Treating Systemic Infections of Streptococcus Iniae in Fish,

7/1/2022 Component/Problem Statement 1b, Research Support Agreement
Leukocyte Immune-type Receptors: Gene Structure and Function (Extension)

9/1/2020 Component/Problem Statement 1c, Outgoing Interagency Agreement
"ORISE" ARS Research Participation Program, OAK RIDGE NATIONAL LAB

8/1/2021 Component/Problem Statement 1c, Research Support Agreement
Acquisition of Goods and Services to Explore Consumer Perceptions of Aquacultural Food Products, LOUISIANA STATE UNIV. AG. CENTER

Component 2

10/1/2017 Component/Problem Statement 2a, Outgoing Interagency Agreement
U.S. Fish and Wildlife, Bozeman, Montana, FISH AND WILDLIFE SERVICE

10/1/2017 Component/Problem Statement 2a, Research Support Agreement
Production of Reproductively Sterile Rainbow Trout for Environmentally-Responsible and Economically-Sustainable U.S. Aquaculture Industry

2/15/2018 Component/Problem Statement 2a, Material Transfer Research Agreement
Improving Trout Production Through Genetic Characterization and Management

7/1/2018 Component/Problem Statement 2a, Material Transfer Research Agreement
Histidine Requirement of Atlantic Salmon (Salmo salar)

9/1/2018 Component/Problem Statement 2a, Research Support Agreement
Improving Trout Production Through Genetic Characterization and Management, UNIVERSITY OF IDAHO

10/1/2018 Component/Problem Statement 2a, Outgoing Interagency Agreement
U.S. Fish and Wildlife, Bozeman, Montana, FISH AND WILDLIFE SERVICE

11/1/2018 Component/Problem Statement 2a, Research Support Agreement
Acquisition of Goods and Services, UNIVERSITY OF IDAHO

2/7/2019 Component/Problem Statement 2a, Grant Agreement
Aquaculture 2019/Physiological Insights Towards Improving Fish Culture V, AMERICAN FISHERIES SOCIETY, INC

8/19/2019 Component/Problem Statement 2a, Non-Assistance Cooperative Agreement
Improving the Competitiveness of Rainbow Trout Production by the Integrated Development of Improved Feedstuffs, Feeds, and Trout, UNIVERSITY OF IDAHO

9/1/2019 Component/Problem Statement 2a, Research Support Agreement
Improving Trout Production Through Genetic Characterization and Management, UNIVERSITY OF IDAHO

9/1/2019 Component/Problem Statement 2a, Outgoing Interagency Agreement
The Use of Organic Acids and Essential Oils in Rainbow Trout and Hybrid Striped Bass Feeds to Alleviate the use of Antibiotics, FISH AND WILDLIFE SERVICE

9/1/2019 Component/Problem Statement 2a, Interagency Reimbursable Agreement
Advancing the use of Alfalfa Leaf Protein Concentrate in Aquafeeds to Enhance Finfish Production.

10/1/2019 Component/Problem Statement 2a, Outgoing Interagency Agreement
U.S. Fish and Wildlife, Bozeman, Montana, FISH AND WILDLIFE SERVICE

10/1/2019 Component/Problem Statement 2a, Outgoing Interagency Agreement
Waste Management and Water Quality Improvement on Commercial Trout Farms Through Nutritional Strategies, FISH AND WILDLIFE SERVICE

10/1/2019 Component/Problem Statement 2a, Cooperative Agreement
Waste Management and Water Quality Improvement on Commercial Trout Farms Through Nutritional Strategies, UNIVERSITY OF IDAHO

10/1/2019 Component/Problem Statement 2a, Interagency Reimbursable Agreement
Waste Management and Water Quality Improvement on Commercial Trout Farms Through Nutritional Strategies

2/15/2020 Component/Problem Statement 2a, Cooperative Agreement

Evaluation of Natural Astaxanthin Produced by Microalgae as a Potential Pigment Source for Atlantic Salmon (Salmo Salar) Feed, DELAWARE STATE UNIVERSITY

4/1/2020 Component/Problem Statement 2a, Cooperative Agreement
Support the Viability and Expansion of Land-Based Closed-Containment Aquaculture, THE CONSERVATION FUND

4/1/2020 Component/Problem Statement 2a, Research Support Agreement
High-quality Reference Assembly And Annotation Of The Rainbow Trout Genome

7/1/2020 Component/Problem Statement 2a, Research Support Agreement
Acquisition of Goods and Service, UNIVERSITY OF MAINE

7/1/2020 Component/Problem Statement 2a, Non-Assistance Cooperative Agreement
Genetic Improvement of North American Atlantic Salmon & the Eastern Oyster for Aquaculture Production, UNIVERSITY OF MAINE

7/15/2020 Component/Problem Statement 2a, Research Support Agreement
Acquisition of Goods and Services, UNIVERSITY OF IDAHO

8/1/2020 Component/Problem Statement 2a, Non-Assistance Cooperative Agreement
Effect of Trout Strain (genotype x diet) on Digestibility of Alfalfa Leaf Meal Protein Concentrate (APC), UNIVERSITY OF IDAHO

10/1/2020 Component/Problem Statement 2a, Outgoing Interagency Agreement
U.S. Fish and Wildlife Service, Bozeman, Montana, FISH AND WILDLIFE SERVICE

12/14/2020 Component/Problem Statement 2a, Non-Assistance Cooperative Agreement
Metabolic and Proteomic Comparison of Salmonid Diet Utilization, UNIV.ONTARIO INST OF TECH

3/15/2021 Component/Problem Statement 2a, Material Transfer Research Agreement
Growth Performance of Atlantic Salmon Smolts Fed Diets Containing a yeast protein at Various Levels

7/1/2021 Component/Problem Statement 2a, Outgoing Interagency Agreement
ORISE" ARS Research Participation Program", OAK RIDGE NATIONAL LAB

7/1/2021 Component/Problem Statement 2a, Research Support Agreement
Acquisition of Goods and Services, UNIVERSITY OF MAINE

8/1/2021 Component/Problem Statement 2a, Research Support Agreement
Acquisition of Goods and Services, UNIVERSITY OF IDAHO

9/1/2021 Component/Problem Statement 2a, Non-Funded Cooperative Agreement

Cooperative Research Program on Fish Nutrition and Diet Development, MONTANA STATE UNIVERSITY

9/1/2021 Component/Problem Statement 2a, Research Support Agreement
Acquisition of Goods and Services, UNIVERSITY OF IDAHO

9/1/2021 Component/Problem Statement 2a, Research Support Agreement
Sustainable Aquaculture Systems Supporting Atlantic Salmon

9/13/2021 Component/Problem Statement 2a, Outgoing Interagency Agreement
ORISE" ARS Research Participation Program, OAK RIDGE NATIONAL LAB

9/30/2021 Component/Problem Statement 2a, Outgoing Interagency Agreement
ORISE" ARS Research Participation Program", OAK RIDGE NATIONAL LAB

10/1/2021 Component/Problem Statement 2a, Outgoing Interagency Agreement
U.S. Fish and Wildlife Service, Bozeman, Montana (FY22), FISH AND WILDLIFE SERVICE

11/29/2021 Component/Problem Statement 2a, Research Support Agreement
Acquisition of Goods and Services, UNIVERSITY OF MAINE

12/1/2021 Component/Problem Statement 2a, Research Support Agreement
Examination of The Nutritional Value of Novel Sorghum Protein Concentrates

1/1/2022 Component/Problem Statement 2a, Grant Agreement
Aquaculture America 2022/Physiological Insights Towards Improving Fish Culture VI, AMERICAN FISHERIES SOCIETY, INC

4/1/2022 Component/Problem Statement 2a, Research Support Agreement
Research toward understanding photoperiod control of salmon development, UNIVERSITY OF MASSACHUSETTS

7/1/2022 Component/Problem Statement 2a, Research Support Agreement
Acquisition of Goods and Services, UNIVERSITY OF MAINE

7/1/2022 Component/Problem Statement 2a, Research Support Agreement
Acquisition of Goods and Services, UNIVERSITY OF MAINE

9/1/2022 Component/Problem Statement 2a, Outgoing Interagency Agreement
ORISE" ARS Research Participation Program", OAK RIDGE NATIONAL LAB

9/15/2022 Component/Problem Statement 2a, Research Support Agreement
Acquisition of Goods & Services, UNIVERSITY OF IDAHO

9/26/2022 Component/Problem Statement 2a, Research Support Agreement

Acquisition of Goods and Services - Improving Fish Management Practices, UNIVERSITY OF IDAHO

12/1/2017 Component/Problem Statement 2b, Outgoing Interagency Agreement
ORISE" ARS Research Participation Program", OAK RIDGE NATIONAL LAB

7/1/2018 Component/Problem Statement 2b, Outgoing Interagency Agreement
ORISE" ARS Research Participation Program", OAK RIDGE NATIONAL LAB

10/1/2018 Component/Problem Statement 2b, Cooperative Agreement
Underlying Mechanisms for Selected Disease Resistance and Enhanced Non-Specific Resistance in Rainbow Trout, UNIVERSITY OF IDAHO

10/1/2018 Component/Problem Statement 2b, Interagency Reimbursable Agreement
Underlying Mechanisms for Selected Disease Resistance and Enhanced Non-Specific Resistance in Rainbow Trout

11/1/2018 Component/Problem Statement 2b, Material Transfer Research Agreement
Use of Dietary Additives to Reduce/Inhibit Sea Lice Infection on Atlantic Salmon (Salmo Salar)

1/1/2019 Component/Problem Statement 2b, Research Support Agreement
Improving Hatchery Techniques of Lumpfish (Cyclopterus lumpus) for Use as a Cleaner Fish to Control Sea Lice

5/1/2020 Component/Problem Statement 2b, Outgoing Interagency Agreement
Improving Hatchery Techniques of Lumpfish for Use as a Cleaner Fish to Control Sea Lice in Atlantic Salmon and Steelhead Trout Net Pens, FISH AND WILDLIFE SERVICE

6/1/2020 Component/Problem Statement 2b, Interagency Reimbursable Agreement
Systematic Sampling of Fish and Systems for the Presence of ISAV HPR0

8/1/2020 Component/Problem Statement 2b, Non-Assistance Cooperative Agreement
Characterization of the Microbiome of Aquaculture Production Systems and Development of Intervention Strategies to Improve Animal Health, UNIVERSITY OF CONNECTICUT

8/1/2020 Component/Problem Statement 2b, Research Support Agreement
Reducing Bottlenecks in the Development of a US Cleaner Fish Program for Sea Lice, UNIVERSITY OF MAINE

4/1/2021 Component/Problem Statement 2b, Research Support Agreement
Acquisition of Goods and Services, UNIVERSITY OF WISCONSIN

6/1/2021 Component/Problem Statement 2b, Non-Funded Cooperative Agreement
Utilizing the University of Wisconsin-Milwaukee Animal Care and Use Committee, UNIVERSITY OF WISCONSIN

7/1/2021 Component/Problem Statement 2b, Non-Assistance Cooperative Agreement ***Identifying Rhabdoviral Virlulence Factors Using In Vitro Approaches,*** UNIVERSITY OF TOLEDO

9/20/2021 Component/Problem Statement 2b, Non-Assistance Cooperative Agreement ***Genetic Analysis of Virulence of Flavobacterium Fish Pathogens,*** UNIVERSITY OF WISCONSIN

4/1/2022 Component/Problem Statement 2b, Research Support Agreement ***Improving Lumpfish Grow-out Production: Optimizing Feed Strategies***

4/1/2022 Component/Problem Statement 2b, Research Support Agreement ***Acquisition of Goods and Services,*** UNIVERSITY OF WISCONSIN

7/1/2022 Component/Problem Statement 2b, Non-Funded Cooperative Agreement ***Institutional Biological Safety Committee (IBC) Agreement - University of Wisconsin Milwaukee,*** UNIVERSITY OF WISCONSIN

8/1/2022 Component/Problem Statement 2b, Non-Assistance Cooperative Agreement ***Genome-Wide Association Analysis of Economically Important Traits in Rainbow Trout,*** UNIVERSITY OF CONNECTICUT

Component 3

7/15/2022 Component/Problem Statement 3a, Memorandum of Understanding ***USDA/ARS/HKDSNARC and UAPB Aquaculture and Fisheries Department Cooperative Aquaculture Research, Technology Transfer, and Stakeholder Engagement,*** UNIVERSITY OF ARKANSAS

9/1/2022 Component/Problem Statement 3a, Non-Assistance Cooperative Agreement ***Enhancing Sustainable Production of Hybrid Striped Bass and White Bass Through Use of Novel Plant and Insect Feed Ingredients,*** UNIVERSITY OF ARKANSAS

9/15/2022 Component/Problem Statement 3a, Outgoing Interagency Agreement ***ORISE" ARS Research Participation Program",*** OAK RIDGE NATIONAL LAB

Component 4

10/1/2017 Component/Problem Statement 4a, Outgoing Interagency Agreement ***Facilities Support: ARS Aquaculture Ecology Research, Newport, Oregon,*** U.S. ENVIRON PROTECT AGENCY HQ

6/1/2018 Component/Problem Statement 4a, Research Support Agreement ***Acquisition of Goods and Services,*** UNIVERSITY OF MARYLAND

7/1/2018 Component/Problem Statement 4a, Research Support Agreement

Acquisition of Goods and Services, OREGON STATE UNIVERSITY

7/1/2018 Component/Problem Statement 4a, Research Support Agreement
Acquisiton of Goods and Services, UNIVERSITY OF RHODE ISLAND

7/1/2018 Component/Problem Statement 4a, Research Support Agreement
Acquisition of Goods and Services, UNIVERSITY OF RHODE ISLAND

9/24/2018 Component/Problem Statement 4a, Non-Assistance Cooperative Agreement
Leveraging the Eastern Oyster Genome to Facilitate Enhanced Germplasm Improvement, UNIVERSITY OF RHODE ISLAND

6/1/2019 Component/Problem Statement 4a, Research Support Agreement
Acquisition of Goods and Services, UNIVERSITY OF MARYLAND

7/1/2019 Component/Problem Statement 4a, Research Support Agreement
Acquisition of Goods and Services, OREGON STATE UNIVERSITY

7/1/2019 Component/Problem Statement 4a, Research Support Agreement
Acquisition of Goods and Services, UNIVERSITY OF RHODE ISLAND

7/1/2019 Component/Problem Statement 4a, Non-Assistance Cooperative Agreement
Penaeid Shrimp Pathogen Surveillance: Critical Support to the Emerging U.S. Shrimp Farming Industry, AUBURN UNIVERSITY

8/1/2019 Component/Problem Statement 4a, Non-Assistance Cooperative Agreement
Identifying Genetic Factors Associated with the Expression and Regulation of Economically Important Traits in Cultured Pacific Oysters, OREGON STATE UNIVERSITY

10/1/2019 Component/Problem Statement 4a, Outgoing Interagency Agreement
Facilities Support: ARS Aquaculture Ecology Research, Newport, Oregon, U.S. ENVIRON PROTECT AGENCY HQ

6/1/2020 Component/Problem Statement 4a, Research Support Agreement
Acquisition of Goods and Services, UNIVERSITY OF MARYLAND

7/1/2020 Component/Problem Statement 4a, Research Support Agreement
Acquisition of Goods and Services, OREGON STATE UNIVERSITY

7/1/2020 Component/Problem Statement 4a, Research Support Agreement
Acquisition of Goods and Services, UNIVERSITY OF RHODE ISLAND

7/1/2020 Component/Problem Statement 4a, Non-Assistance Cooperative Agreement
Genetic Improvement of North American Atlantic Salmon and the Eastern Oyster for Aquaculture Production, AUBURN UNIVERSITY

9/1/2020 Component/Problem Statement 4a, Non-Assistance Cooperative Agreement
Reassembly of the Eastern Oyster Genome with Advanced Assembly Pipelines, UNIVERSITY OF MISSOURI

9/1/2020 Component/Problem Statement 4a, Non-Assistance Cooperative Agreement
Screening Eastern Oyster Populations for Optimal Aquaculture Performance, UNIVERSITY OF RHODE ISLAND

9/1/2020 Component/Problem Statement 4a, Non-Assistance Cooperative Agreement
Production of Pedigreed Families for Research on Disease Resistance and Correlation of Resistance with Field Traits, VA INST MARINE SCI - COLL W&M

10/1/2020 Component/Problem Statement 4a, Research Support Agreement
Acquisition of Goods and Services, AUBURN UNIVERSITY

10/1/2020 Component/Problem Statement 4a, Outgoing Interagency Agreement
Facilities Support: ARS Aquaculture Ecology Research, Newport, Oregon, U.S. ENVIRON PROTECT AGENCY HQ

4/1/2021 Component/Problem Statement 4a, Research Support Agreement
Acquisition of Goods and Services, DELAWARE STATE UNIVERSITY

6/30/2021 Component/Problem Statement 4a, Research Support Agreement
Acquisition of Goods and Services, UNIVERSITY OF RHODE ISLAND

7/1/2021 Component/Problem Statement 4a, Research Support Agreement
Acquisition of Goods and Services, OREGON STATE UNIVERSITY

9/1/2021 Component/Problem Statement 4a, Research Support Agreement
Acquisition of Goods and Services, UNIVERSITY OF CALIFORNIA

9/1/2021 Component/Problem Statement 4a, Non-Assistance Cooperative Agreement
Population Genomic Evaluation of Wild and Cultured Eastern Oyster Populations from the Northeast Region, UNIVERSITY OF RHODE ISLAND

10/1/2021 Component/Problem Statement 4a, Outgoing Interagency Agreement
Facilities Support: ARS Aquaculture Ecology Research, Newport, Oregon, FY22, U.S. ENVIRON PROTECT AGENCY HQ

5/1/2022 Component/Problem Statement 4a, Research Support Agreement
Acquisition of Goods and Services, OREGON STATE UNIVERSITY

5/1/2022 Component/Problem Statement 4a, Research Support Agreement
Acquisition of Goods and Services, OREGON STATE UNIVERSITY

5/1/2022 Component/Problem Statement 4a, Research Support Agreement

Acquisition of Goods and Services, OREGON STATE UNIVERSITY

6/1/2022 Component/Problem Statement 4a, Research Support Agreement
Acquisition of Goods and Services, UNIVERSITY OF MARYLAND

8/1/2022 Component/Problem Statement 4a, Non-Funded Cooperative Agreement
Establishment and Operation of the Regional Northeast Oyster Breeding Center (NOBC), NOAA, NATL OCEAN SERVICE

9/1/2022 Component/Problem Statement 4a, Research Support Agreement
Acquisition of Goods and Services, UNIVERSITY OF CALIFORNIA

9/1/2022 Component/Problem Statement 4a, Research Support Agreement
Acquisition of Goods & Services, DELAWARE STATE UNIVERSITY

9/15/2022 Component/Problem Statement 4a, Research Support Agreement
Acquisition of Goods and Services, UNIVERSITY OF RHODE ISLAND

Component 5

6/1/2019 Component/Problem Statement 5a, Cooperative Agreement
Ensuring A Steady Supply of Warm Water Marine Finfish Seed Stock to Support the Development of the U.S. Aquaculture Industry, FLORIDA ATLANTIC UNIVERSITY

Component 6

7/1/2021 Component/Problem Statement 6a, Non-Assistance Cooperative Agreement
Developing Sustainable Aquaponic Production Systems, AUBURN UNIVERSITY

9/1/2021 Component/Problem Statement 6a, Non-Assistance Cooperative Agreement
Development of Marine Aquaponics for Saline Tolerant Plant Production and High-Value Fish Production, AUBURN UNIVERSITY

9/1/2021 Component/Problem Statement 6a, Non-Assistance Cooperative Agreement
Saltwater Aquaponics in Puerto Rico: Queen Conch and Sea Vegetable Aquaculture, HARBOR BRANCH OCEANOGRAPHIC INST

9/1/2021 Component/Problem Statement 6a, Non-Assistance Cooperative Agreement
Low Salinity Aquaponics with Halophytes and Pacific White Shrimp, AUBURN UNIVERSITY

9/1/2022 Component/Problem Statement 6a, Non-Assistance Cooperative Agreement
Development of Freshwater Aquaponics for Plant (Herbs, Vegetables, and Flowers) and High-Value Fish Production, UNIVERSITY OF ARKANSAS

Additional Outgoing Agreements

5/1/2019 Component/Problem Statement 7a, Non-Funded Cooperative Agreement
Characterizing the Growth Performance and Disease Resistance of Black Bass (Micropterus),
AUBURN UNIVERSITY

9/13/2021 Component/Problem Statement 7a, Material Transfer Research Agreement
Selective Breeding of Nile Tilapia for Disease Resistance and Investigation of Genomic Selection

10/1/2021 Component/Problem Statement 7a, Reimbursable Cooperative Agreement
Mixing up an Optimal Diet for White Sturgeon Grow-out

Appendix 9 – Partnerships Funded by Non-ARS Sources

ARS scientists are also able to enter research, development and technology transfer agreements that are funded by non-ARS sources, these must align with and enhance their project's objectives. Names of cooperators are omitted. The following agreements were initiated in 2018 – 2022

Component 1

8/1/2019 Component/Problem Statement 1a, (CRADA) COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENT
Evaluation of Probiotics to Enhance Growth Performance of Catfish

3/2/2020 Component/Problem Statement 1a, (MTRA) MATERIAL TRANSFER RESEARCH AGREEMENT
Release of Delta Select Channel Catfish to Commercial Producers

3/2/2020 Component/Problem Statement 1a, (MTRA) MATERIAL TRANSFER RESEARCH AGREEMENT
Release of Delta Select Channel Catfish to Commercial Producers

3/2/2020 Component/Problem Statement 1a, (MTRA) MATERIAL TRANSFER RESEARCH AGREEMENT
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3/2/2020 Component/Problem Statement 1a, (MTRA) MATERIAL TRANSFER RESEARCH AGREEMENT
Release of Delta Select Channel Catfish to Commercial Producers

3/2/2020 Component/Problem Statement 1a, (MTRA) MATERIAL TRANSFER RESEARCH AGREEMENT

Release of Delta Select Channel Catfish to Commercial Producers

3/10/2020 Component/Problem Statement 1a, (MTRA) MATERIAL TRANSFER RESEARCH AGREEMENT
Release of Rio Grande and Delta Elite Strains of Blue Catfish

3/10/2020 Component/Problem Statement 1a, (MTRA) MATERIAL TRANSFER RESEARCH AGREEMENT
Release of Rio Grande and Delta Elite Strains of Blue Catfish

3/10/2020 Component/Problem Statement 1a, (MTRA) MATERIAL TRANSFER RESEARCH AGREEMENT
Release of Rio Grande and Delta Elite Strains of Blue Catfish

3/10/2020 Component/Problem Statement 1a, (MTRA) MATERIAL TRANSFER RESEARCH AGREEMENT
Release of Rio Grande and Delta Elite Strains of Blue Catfish

3/10/2020 Component/Problem Statement 1a, (MTRA) MATERIAL TRANSFER RESEARCH AGREEMENT
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3/10/2020 Component/Problem Statement 1a, (MTRA) MATERIAL TRANSFER RESEARCH AGREEMENT
Release of Rio Grande and Delta Elite Strains of Blue Catfish

3/10/2020 Component/Problem Statement 1a, (MTRA) MATERIAL TRANSFER RESEARCH AGREEMENT
Release of Rio Grande and Delta Elite Strains of Blue Catfish

3/10/2020 Component/Problem Statement 1a, (MTRA) MATERIAL TRANSFER RESEARCH AGREEMENT
Release of Rio Grande and Delta Elite Strains of Blue Catfish

3/10/2020 Component/Problem Statement 1a, (MTRA) MATERIAL TRANSFER RESEARCH AGREEMENT
Release of Rio Grande and Delta Elite Strains of Blue Catfish

8/1/2018 Component/Problem Statement 1b, (RCA) REIMBURSABLE COOPERATIVE AGREEMENT
Novel Protein-based Therapy for Managing Disease that Stimulates Catfish Antimicrobial Immunity and Tissue Repair Systems

Component 2

11/15/2017 Component/Problem Statement 2a, (MTRA) MATERIAL TRANSFER RESEARCH AGREEMENT
Digestibility of Several Alternative Protein Ingredients Developed for Atlantic Salmon

1/1/2018 Component/Problem Statement 2a, (CRADA) COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENT
Evaluation of Single-Cell Protein Sources on Growth and Health of Rainbow Trout

2/1/2018 Component/Problem Statement 2a, (RCA) REIMBURSABLE COOPERATIVE AGREEMENT
Improving Rainbow Trout Growth Performance Through Optimization of Diet-Epigenetic Interactions

10/1/2018 Component/Problem Statement 2a, (IRA) INTERAGENCY REIMBURSABLE AGREEMENT
Underlying Mechanisms for Selected Disease Resistnce and Enhanced Non-specific Resistance in Rainbow Trout

1/1/2019 Component/Problem Statement 2a, (RCA) REIMBURSABLE COOPERATIVE AGREEMENT
Evaluation of Natural Astaxanthin Produced by Microalgae as a Potential Pigment Source for Atlantic Salmon (Salmosalar) Feed

6/1/2019 Component/Problem Statement 2a, (MTRA) MATERIAL TRANSFER RESEARCH AGREEMENT
Retrospective Evaluation of the Accuracy of Genomic Selection (GS) Models Predictions and Refinement of QTL Regions for Egg Quality Traits I.

2/1/2020 Component/Problem Statement 2a, (MTRA) MATERIAL TRANSFER RESEARCH AGREEMENT
Testing of ProSaf as a Feed Additive for Improving Growth and Health in Rainbow Trout Fed Diets Containing

2/1/2021 Component/Problem Statement 2a, (MTRA) MATERIAL TRANSFER RESEARCH AGREEMENT
Atlantic Salmon Strain Comparison in a Recirculating Aquaculture System,

2/1/2021 Component/Problem Statement 2a, (MTRA) MATERIAL TRANSFER RESEARCH AGREEMENT
Atlantic Salmon Strain Comparison in a Recirculating Aquaculture System

2/1/2021 Component/Problem Statement 2a, (RCA) REIMBURSABLE COOPERATIVE AGREEMENT
Whole-Genome Analysis/Selection to Increase Muscle Yield and Reduce Fillet Downgrading in Rainbow Trout

5/1/2021 Component/Problem Statement 2a, (MTRA) MATERIAL TRANSFER RESEARCH AGREEMENT
Evaluation of Atlantic salmon grown in a recirculating aquaculture system for potential release in a newly established fishery

4/15/2022 Component/Problem Statement 2a, (MTRA) MATERIAL TRANSFER RESEARCH AGREEMENT
Salmon Smolt Growth and Performance

4/15/2022 Component/Problem Statement 2a, (MTRA) MATERIAL TRANSFER RESEARCH AGREEMENT
Atlantic Salmon Strain Growth and Performance

6/1/2019 Component/Problem Statement 2b, (MTRA) MATERIAL TRANSFER RESEARCH AGREEMENT
Evaluation and Identification of Positional Candidate Genes and Favorable SNP Marker Haplotypes From QTL Regions Associated With BCWD

6/1/2019 Component/Problem Statement 2b, (MTRA) MATERIAL TRANSFER RESEARCH AGREEMENT
Evaluation of the Genetic Architecture of Resistance to Infectious Hematopoietic Necrosis Virus (IHNV) In Rainbow Trout

10/1/2020 Component/Problem Statement 2b, (RCA) REIMBURSABLE COOPERATIVE AGREEMENT
Development of a Decision Support System for Sea Lice Management in Salmon Aquaculture

5/9/2022 Component/Problem Statement 2b, (MTRA) MATERIAL TRANSFER RESEARCH AGREEMENT
Improving Lumpfish Breeding and Grow-out Production

Component 3

1/1/2018 Component/Problem Statement 3a, (RCA) REIMBURSABLE COOPERATIVE AGREEMENT
De Novo Sequencing and Assembly of the White Bass Genome

7/1/2018 Component/Problem Statement 3a, TFCA) TRUST FUND COOPERATIVE AGREEMENT
Mapping Sex-Linked Genes in Temperate Bassess for Improved Hybrid Striped Bass Culture

1/1/2019 Component/Problem Statement 3a, (RCA) REIMBURSABLE COOPERATIVE AGREEMENT
Practical, Integrated Agriculture to Supply Year-round Farmer Income and Fresh, Nutritious Food to Underserved Communities

12/4/2019 Component/Problem Statement 3a, (MTRA) MATERIAL TRANSFER RESEARCH AGREEMENT
Development of A Select Strain of White Bass For Creation of Improved Hybrid Striped Bass

7/1/2020 Component/Problem Statement 3a, (MTRA) MATERIAL TRANSFER RESEARCH AGREEMENT
Development of A Select Strain of White Bass For Creation of Improved Hybrid Striped Bass on Farm Evaluation

8/21/2020 Component/Problem Statement 3a, (MTRA) MATERIAL TRANSFER RESEARCH AGREEMENT
Screening Phage Based Endolysins Against Isolates of Streptococcus Iniae

10/1/2021 Component/Problem Statement 3a, (RCA) REIMBURSABLE COOPERATIVE AGREEMENT
Use of Phage Endolysins as Alternative to Antimicrobials for the Zoonotic Fish Pathogen Streptococcus Iniae

6/1/2022 Component/Problem Statement 3a, (MTRA) MATERIAL TRANSFER RESEARCH AGREEMENT
Hybrid Striped Bass Finishing Diet Study 2022

Component 4

5/1/2018 Component/Problem Statement 4a, (MTRA) MATERIAL TRANSFER RESEARCH AGREEMENT
Transfer of Pacific White Shrimp for Disease Phenotyping

5/29/2018 Component/Problem Statement 4a, (MTRA) MATERIAL TRANSFER RESEARCH AGREEMENT
Characterizing Phenotypes Associated with Dermo Resistance in the Eastern Oyster, Crassostrea virginica

5/20/2019 Component/Problem Statement 4a, (RCA) REIMBURSABLE COOPERATIVE AGREEMENT
Do Staghorn Sculpins and Nematode Parasites Control Burrowing Shrimp Populations on Shellfish Aquaculture

12/1/2021 Component/Problem Statement 4a, (RCA) REIMBURSABLE COOPERATIVE AGREEMENT
Rapid Dynamics of Burrowing Shrimp and Relationship to Oyster Performance

Component 5

1/10/2018 Component/Problem Statement 5a, (MTRA) MATERIAL TRANSFER RESEARCH AGREEMENT

Comparison of Extruded Moist Feed or Commercially Extruded Dry Feed for Growth and Health in Yellowtail and Tuna

10/1/2020 Component/Problem Statement 5a, (MTRA) MATERIAL TRANSFER RESEARCH AGREEMENT

Evaluation of Clam Meal as an Alternative Protein Ingredient and Palatant in Aquaculture Diets

Appendix 10 – International Collaborations

2018

BELGIUM:

- Identifying the host-derived factors that govern the susceptibility or resistance to *Flavobacterium columnnare* infection to better understand how this bacterium infects an array of aquaculture species.
- Studying how biofilms are formed by different clinical isolates of *F. columnare* to better understand how this bacterium infects an array of aquaculture relevant species.

CANADA:

- Providing assistance to Wild-West Steelhead, a commercial trout producer in Canada, with a *Weissella ceti* (Wc) outbreak occurring at their facility in southwest Saskatchewan.
- Working with the Atlantic Salmon Federation in Canada to organize the 2018 Aquaculture Innovation Workshop to be held in Miami, Florida, December 4-6, 2018.

CHINA:

- Evaluating and developing effective parasiticides for controlling and preventing parasite *Ichthyophthirius multifiliis* in cultured fish and study fish immune responses against parasites.

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.
- Testing Pacific oyster for resistance to OsHV-1

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 *Saprolegni*a spp. on fish.

ITALY:

- Evaluating and discovering natural compounds with toxicity towards common disease-producing bacteria of pond-raised channel catfish.

NORWAY:

- Determining the feasibility of selectively breeding Nile tilapia for resistance to *Streptococcus* species other tilapia pathogens.
- Working to make closed-containment aquaculture systems a reliable and economically viable technology and identify biological solutions for producing Atlantic salmon.

SPAIN:

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

2019

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.

2020

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.

BRAZIL:

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

CANADA:

- Collaborating with the Aquatic Omics Laboratory at the Ontario Technology University, to process and analyze tissue samples taken from five strains of rainbow trout raised on a

fishmeal protein control diet and a diet where fishmeal is completely replaced with sustainable protein derived from soybeans.

CHINA:

- Collaborating with researchers at Nanchang University to analyze metatranscriptomics and proteomic interaction related to the intestinal microbiome of selected and non-selected fish reared on plant and fishmeal-based feeds.
- Working with scientist from Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Sciences to characterize gender-specific regulation of hepcidin in yellow perch.
- 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:

- Conducting collaborative research with Technical University of Denmark to provide information on peracetic acid used in aquaculture.

FINLAND:

- Working with scientist from the University of Jyväskylä on genetic techniques to manipulate *Flavobacterium columnare*.

FRANCE:

- Working with scientists from INRA (National Institute for Agricultural Research), on genetic techniques to manipulate *Flavobacterium columnare*.

GERMANY:

- Collaborating with researchers 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:

- Collaborating with researchers at the Nord University to utilize commercial and ARS selected strains of trout to characterize intestinal microbial populations and correlate these populations with host intestinal immunological cell populations, both qualitatively and quantitatively.
- 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.

- Conducting collaborative research with Benchmark Genetics Norway AS to determine the feasibility of selectively breeding Nile tilapia for resistance to Streptococcus species and other tilapia pathogens.
- Collaborating with researchers in the Fish Health Department of Nofima to establish the importance of the potent disinfectant peracetic acid to the global aquaculture industry.
- 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.

PERU:
- Collaborating with researchers at the Universidad Nacional Agraria and the aquaculture egg producing company Ovaseed to evaluate rainbow trout stocks reared in different environmental settings and determine if lines of fish can be developed for improved growth under these conditions.

PORTUGAL:
- Collaborating with researchers at the University of Coimbra to analyze metabolomics and proteomic interaction related to the intestinal microbiome of selected and non-selected fish reared on plant and fishmeal-based feeds.
- Collaborating with researchers at the University of Coimbra located to analyze the metabolome of tissues from selected and non-selected rainbow trout at early and late stages of development.

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 the National Agricultural Research Organisation, Aquaculture Research & Development Centre to assist in formulating feeds and setting up a selection program for tilapia in eastern Africa using localized resources

2021

CANADA:
- Collaborating with researchers at the University of Ontario Institute of Technology to determine the metabolic and physiologic changes in a selected line of ARS rainbow trout to metabolize plant proteins.

DENMARK:
- Conducting collaborative research with Technical University of Denmark to provide information on peracetic acid used in aquaculture.

GERMANY:

- Collaborating with researchers 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 pathogens on fish.

NORWAY:

- Collaborating with researchers at Benchmark Genetics to determine the feasibility of selectively breeding Nile tilapia for resistance to *Streptococcus* species and other tilapia pathogens.
- Collaborating with researchers in the Fish Health Department of Nofima to establish the importance of the potent disinfectant peracetic acid to the global aquaculture industry.
- 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 at the University of Coimbra on non-invasive methods for the detection of enteritis caused by dietary plan proteins.

EUROPEAN UNION:

- To foster future collaboration, the Conservation Fund Freshwater Institute Research Director, affiliated with the National Center for Cool and Cold Water Aquaculture Research, joined the advisory panel of iFishIENCi (Intelligent Fish Feeding Through Integration of Enabling Technologies and Circular Principles, a European Union Horizon 2020 project involving numerous diverse scientists and industry partners.

2022

CANADA:

- Collaborating with a researcher at Ontario Tech University(OTU) in Ontario, Canada to utilize proteomics to determine physiological differences between selected ARS lines and unselected lines when reared on plant-based feeds.

DENMARK:

- ARS researcher in Stuttgart, Arkansas, continue collaborating with scientists at the Technical University of Denmark in Hirtshals on research designed to provide information on peracetic acid used in aquaculture. Collaboration takes place by phone, email exchanges and formal meetings at conferences over many years.

FRANCE:

- University of Wisconsin-Milwaukee cooperator collaborated with from the Pasteur Institute, Paris, France to analyze *F. columnare* mutants constructed for virulence against larval zebrafish to expand the significance our work by comparing the effects of mutations on different zebrafish life stages (larval and adult).

GEORGIA:

- The Conservation Fund Freshwater Institute (TCFFI) is working closely with the USDA's Foreign Agriculture Service (USDA-FAS) on the project "Improving Disease Management in Georgian Trout Farms". USDA-FAS has been working within the Republic of Georgia in a multi-year program to improve agricultural extension services to foster economic growth and rural employment in the agricultural sector. TCFFI personnel will provide expertise to investigate the cause(s) of fish mortalities, provide recommendations to reduce mortalities, and work with USDA-FAS, local extensionists, and diagnostic laboratories to develop training curricula for on-site disease sampling methods and an effective pathogen surveillance program.

GERMANY:

- An ARS researcher in Stuttgart, Arkansas, continues collaboration with scientists at the Leibniz-Institute of Freshwater Ecology and Inland Fisheries in Berlin on research designed to study the toxicity/effectiveness of peracetic acid to fish and the effectiveness of this compound to control pathogens on fish. Collaboration takes place by email exchanges, video conferencing, formal meetings at conferences and reciprocal visits to labs over many years.

MALAYSIA:

- ARS scientists in Hagerman, Idaho, and Bozeman, Montana, are collaborating with feed companies and the U.S. Grains council based in Kuala Lumpur, Malaysia, and Ohio Corn in Delaware, Ohio. The objective is to investigate and increase incorporation of corn distiller's dried grains with solubles (DDGS), a byproduct of ethanol production, in tilapia feeds to expand use in domestic and Asian tilapia aquaculture markets.

NORWAY:

- ARS researchers in Auburn, Alabama, are conducting collaborative research with Benchmark GeneticsNorway AS under a formal agreement. The goal of the research is to determine the feasibility of selectively breeding Nile tilapia for resistance to *Streptococcus* species and other tilapia pathogens.
- ARS researcher in Stuttgart, Arkansas, continue collaborating with scientists in the Fish Health Department of Nofima (Norwegian Institute of Food, Fisheries and Aquaculture Research) in Ås on research designed to establish the importance of the potent disinfectant peracetic acid to the global aquaculture industry. Collaboration takes place by email exchanges, video conferencing and formal meetings at conferences over the last several years.
- The Conservation Fund Freshwater Institute personnel have been collaborating in the CtrlAQUA project, 7-year research initiative by Nofima, funded by the Norwegian Research Council and industry partners. The project aims to make closed-containment aquaculture systems reliable and economically viable through identifying biological solutions for producing Atlantic salmon.
- The Conservation Fund Freshwater Institute personnel have entered into a collaborative agreement with The Arctic University of Norway (UiT) to contribute RAS expertise and research towards "CandRAS", a 7-year project funded by the Norwegian Research Council. Among other things, the work will involve collaboration on design, execution, evaluation,

and reporting of experiments in RAS salmon production, the specific areas of which will be based on challenges identified by RAS salmon producers.

PERU:

- ARS scientists in Aberdeen, Idaho, are collaborating with researchers from the trout company Ovaseed and the University of Lima in Lima, Peru, to determine the physiological factors related to improved health and growth for rainbow trout when reared at high elevation, low oxygen conditions.

EUROPEAN UNION:

- The Conservation Fund Freshwater Institute Research Director joined the advisory panel of iFishIENCi (Intelligent Fish feeding through Integration of Enabling technologies and Circular principles), a European Union Horizon 2020 project involving numerous diverse scientist and industry partners in pursuit of improving aquaculture management and practices through innovation and advanced digital information technology.

Global Seafood Alliance:

- The Conservation Fund Freshwater Institute personnel worked with Global Seafood Alliance (GSA) to create a standard for the certification of RAS farms under their Best Aquaculture Practices certification program. Work included organizing and chairing a Technical Committee (TC) with industry representatives, academics, and conservation group representatives, developing the standard framework and text, creating consensus among the TC and GSA staff, and making the draft standard available for public comment.