National Program 106
Aquaculture

ACCOMPLISHMENT REPORT 2008-2012

United States Department of Agriculture

Research, Education, and Economics

AGRICULTURAL RESEARCH SERVICE

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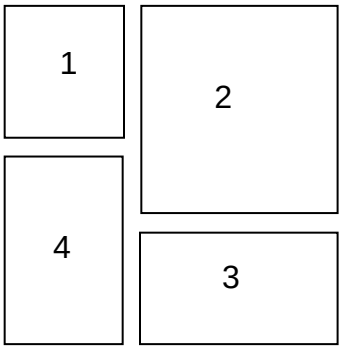
Captions of front page photos, clockwise from upper left:



1. ARS oyster research is conducted primarily in the Pacific Northwest.
2. Catfish hatchery, Stoneville Mississippi.
3. Atlantic salmon is a major species in aquaculture and an important target for alternative feeds development.
4. Juvenile rainbow trout being raised in a tank at the ARS National Center for Cool and Cold Water Aquaculture in Leetown, West Virginia.

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CONTENTS

ACRONYMS AND ABBREVIATIONS

ARS	Agricultural Research Service
BT2	Biotype 2 of *Yersinia ruckeri*
CART	Cocaine and amphetamine-regulated transcript
CCK	Cholecystokinin
cDNA	Complementary DNA
DDGS	Dried distillers grains with solubles
DO	Dissolved oxygen
ESC	Enteric septicemia of catfish
FE	Feed-efficiency
GH	Growth Hormone
GHRH	Growth Hormone–Releasing Hormone
GnRHa	Gonadotrophin-releasing hormone analogues
gpm	Gallon per minute
GRIN	Germplasm Resources Information Network
IGF	Insulin-like growth factor
IGFBP	Insulin-like growth factor binding protein
MAS	Motile aeromonad septicemia
MIB	2-Methylisoborneol
mRNA	Messenger RNA
NAGP	National Animal Germplasm Program
NCCCWA	National Center of Cool and Cold Water Aquaculture
NP	National Program
NPL	National Program Leader
NPY	Neuropeptide Y
PGD	Proliferative gill disease
PTA	Power Tube Airlift
QTL	Quantitative trait locus (loci)
RAS	Recirculating aquaculture system
SAE	Standard aeration efficiency
SNP	Single nucleotide polymorphism
SOTR	Standard oxygen transfer rate
SPC	Soy protein concentrate
TCFFI	The Conservation Fund’s Freshwater Institute
VHS	Viral Hemorrhagic Septicemia

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BACKGROUND AND GENERAL INFORMATION

The Aquaculture National Program (NP 106) encompasses all aspects of research associated with the production and protection of aquatic animal species. The research also encompasses food products and waste management aspects of aquaculture. The aim of the Agricultural Research Service (ARS) aquaculture research and technology transfer efforts is to support a thriving domestic industry with better genetic stocks and scientific information on biotechnologies and management practices. The goal of the program is to ensure the Nation has a high-quality, safe supply of healthful seafood and aquatic products.

NP 106 consists of 22 active projects in 10 locations and associated worksites. The 51 scientists working in NP 106 form a multidisciplinary group that includes biologists, ecologists, chemists, physiologists, geneticists, food technologists, plant pathologists, and microbiologists.

This report of accomplishments for the period 2008–2012 is meant to serve as a 5-year snapshot of the long-term, ongoing goals and objectives of NP 106. In the context of measuring and assessing the extent of accomplishments within the national program, this report examines the progress toward meeting the goals established by the problem statements and research needs in the current Action Plan for NP 106.

The Aquaculture National Program has seen some major accomplishments over the past 5 years. Genomic tools have been developed for catfish and trout, and have brought full reference sequences within reach for both species. These tools are helping researchers to identify genomic regions and even specific genes critical for improved performance. In the selective breeding

realm, projects that are having effects at the industry level are Atlantic salmon and yellow perch with improved growth, and rainbow trout bred for resistance to bacterial coldwater disease and improved ability to use plant-based feeds. Work in alternative ingredients and diet formulation has led to wide use of an ARS plant-based diet formulation. Additionally, a nutrient digestibility database posted on the World Wide Web and updated regularly has been widely distributed and used in commercial diet production. The improved productivity of catfish is one outcome that has linked together decades of research in the past few years so that improvements in aeration and small changes to aeration strategy bettered feeding and growth rates, which improved the feed conversion ratios and decreased both time to harvest and losses to mortality. Furthermore the split-pond technology described herein has also been shown experimentally and in farm trials to consistently yield more than 10,000 pounds of catfish per acre. These are a few of the highlights described in this report.

PLANNING AND COORDINATION FOR THE NP 106 FIVE-YEAR CYCLE

The current Action Plan for NP 106, which was drafted by the National Program Leader and revised with input by NP 106 scientists and stakeholders, serves as a guide for the research within the Aquaculture National Program. It incorporated input from customer/stakeholder interactions, the NPL's knowledge of the science subject matter, input from other ARS scientists, and frequent input from key research partners in other Federal laboratories, industry, and universities. In writing the Action Plan, the writing team identified those research priorities that could be realistically addressed with ARS resources and base funding. These individual research needs were aggregated into problem statements under each of the five research components. The final Action Plan guided development of new individual NP 106 research projects that began the current 5-year research cycle in FY 2010.

The individual project plans in NP 106 were evaluated at the beginning of the 5-year cycle (in 2009) for scientific quality and feasibility by external peer review panels. This ARS peer review process, through the Office of Scientific Quality Review, is an essential part of the 5-year ARS research program cycle. This process is mandated by the Agricultural Research, Extension, and Education Reform Act of 1998, which requires successful completion of peer review as a prerequisite to the performance of research. Each research project in every national program includes statements of the agricultural problem being addressed, anticipated outputs or information to be generated by the project, how the planned research contributes to mitigating or solving the larger national program problem statements, and time lines and milestones for measuring progress toward achieving project objectives. Project plans were revised in response to review panel recommendations as needed, and were implemented in 2010.

Coordination and planning for NP 106 are the tasks of the NPL. The NPL also coordinates NP 106 activities with other ARS national programs and with other agencies and departments. Some of the interagency research coordination associated with NP 106 is conducted through Federal interagency committees such as the Joint Subcommittee on Aquaculture and the USDA/NOAA Alternative Feeds Initiative. The NP 106 NPL also confers and coordinates with colleagues from other USDA agencies, such as the National Institute for Food and Agriculture (NIFA), the Animal and Plant Health Inspection Service, and the Foreign Agricultural Service; and with

other Federal agencies such as the U.S. Agency for International Development and the National Oceanic and Atmospheric Administration (NOAA) within the Department of Commerce.

Customer and stakeholder interaction and research coordination continue to play important roles in helping the NPL guide NP 106 research and to maintain the relevance of aquatic animal research in U.S. agriculture. The NP 106 NPL attended or organized numerous research planning, coordination, and stakeholder workshops during 2009–2013. These workshops addressed specific challenges, emerging high-priority issues, and new scientific developments in the fields of aquaculture production and protection, and frequently include input from other scientific collaborators with universities, industry, or other Federal laboratories. One good example is the U.S. Catfish Regional Collaborative Research and Extension Forum, co-organized with Gary Jensen from NIFA to coincide with the Catfish Farmers of America annual meeting in Natchez, Mississippi, in 2009, and followed up with farmer meetings in 2009 in Indianola, Mississippi. Participants from universities across the catfish-producing states were involved. The NPL co-organized technical sessions around alternative feeds with NOAA and Plant Products in Aquafeeds (PPA) partners at Aquaculture America annual meetings for several years. Along with NOAA and PPA, USDA organized a workshop of Federal, university, and industry scientists from a variety of scientific disciplines in 2011. The workshop was titled “Improving Productivity, Efficiency, Product Quality and Environmental Sustainability of Aquaculture Operations through Systematic Integration of Information and Knowledge” with the goals of meeting and discussing the definition of important performance measures, develop defined vocabularies, and develop databases to store and organize data from a variety of species and experimental paradigms.

STRUCTURE OF NATIONAL PROGRAM 106

The current NP 106 Action Plan is composed of five research components.

COMPONENT 1: Understanding, Improving, and Effectively Using Animal Genetic and Genomic Resources

To meet preferences and needs of consumers for aquatic animal products while effectively utilizing the natural resource base, fish and shellfish are produced in a wide array of environments and management systems. Existing genetic resources may provide producers with options that can be tailored to meet these demands, but several of these resources are at risk of being lost even before they are adequately characterized. Greater emphasis is being placed on germplasm conservation, which requires a wide range of information and analytical tools.

Moreover, insufficient quantitative and genomic characterization of existing genetic resources limits efficiency of production, efficient use of feed resources, and response to disease threats across the United States. We are gaining production efficiency by exploiting genotype by environment and gene-by-gene interactions.

Genetic improvement of fish and shellfish is one of the keys to increasing efficient production in a sustainable manner. These improvements, however, are hindered by a lack of data on some economically important traits, inadequate understanding of quantitative,

molecular, and genetic controls of component traits and interrelationships among traits, less than optimal methods for evaluating candidates for selection, inefficient strategies for incorporating genomic data into breeding programs, and an inability to move novel forms (alleles) of genes between populations. To facilitate genetic improvement, we are developing new knowledge of the genome and its interactions with environmental factors within a comprehensive framework that encompasses animal adaptation and well-being, reproductive efficiency, and nutrient utilization and conversion to animal products and product quality.

These efforts are significantly enhanced by the development of appropriate genomic tools and reagents. Public involvement in the construction of these resources is critical to ensure the development of economically feasible management tools for aquaculture producers and to provide all researchers access to these tools and reagents to spur the development of technology and its transfer to industry producers and breeders as quickly as possible.

COMPONENT 2: Enhancing Animal Performance, Well-being, and Efficiency in Diverse Production Systems

Proper matching of animal genotypes to the production system and to consumer requirements is critical for sustainable and profitable production. This requires a comprehensive understanding of factors that affect animal growth and efficiency, adaptability, and well-being.

Feeding for maintenance and growth are the most expensive inputs in animal production. Achieving the desired partitioning of nutrients into the competing systems of muscle development, health maintenance, and reproductive development within animals are critical needs for improving productivity. Likewise, the development of scientific measures of well-being and an enhanced ability to interpret such measures are critical to maximizing animal growth, efficiency, health, and product quality. Stressors caused by social, nutritional, and environmental factors and their interactions need to be understood to limit negative effects on animal health, production efficiency, and product quality.

Equally essential to ensuring reliable production is the successful and efficient reproduction of aquatic animals. Numerous environmental factors affect reproductive and larval development success, including inconsistent hatching rates, incompatibility of valuable hybrid gametes, and difficulty identifying sexually mature and ripe individuals. Control of these factors is needed to achieve year-round spawning and fingerling production.

COMPONENT 3: Defining Nutrient Requirements and Nutrient Composition of Feedstuffs and Expanding Alternative Ingredients

Feed is the greatest cost associated with most aquatic animal production. Small changes (less than 1 percent) in nutrient requirement definitions can greatly affect the cost of diets. Therefore, a thorough knowledge of the nutrient requirements for optimum growth and performance at specific life stages is essential for efficient production of aquatic animals. Additionally, development and evaluation of novel feed ingredients is vitally important to feed manufacturers because it gives them the flexibility to improve their formulations when

some commodities increase in cost. As the chemical composition and nutrient bioavailability of feedstuffs are determined, least-cost feeds can be formulated on the basis of nutrient requirements and the relative costs of different ingredients.

COMPONENT 4: Improving the Health and Welfare of Aquatic Animals

The U.S. aquaculture industries need to control endemic, emerging, and catastrophic diseases that result in large aquatic animal production losses. Despite the progress that has been made in aquatic animal health, significant loss of animals to disease still occurs, and at great cost to producers. Loss of more than 30 percent of the population from the juvenile stage to harvest is not rare for aquaculture species and represents a huge economic loss. Health management strategies, technologies, and bio-security plans that are safe for the environment and consumers alike are necessary to reduce disease-related losses.

We are developing technologies to rapidly detect, prevent, and treat diseases in production systems. These discoveries and technology developments will help the growth and competitiveness of the U.S. aquaculture industry. In addition, given that only a few drugs are currently approved for treating sick fish, U.S. producers need diagnostic mechanisms, effective control strategies, and therapeutics to properly manage disease outbreaks. We have a strong program to create new, effective vaccines and methods to mass vaccinate aquatic animals. Such new vaccines would serve to supplement the *Edwardsiella ictaluri*, and *Flavobacterium columnare* vaccinations that are currently available and in use by aquatic animal farmers and hatchery managers.

This vaccine work is benefitting from new molecular tools. For example, genetic sequencing to identify regions of similarity among many strains of pathogens may aid in producing vaccines that offer broad protection. Additionally, molecular tools enable researchers to examine thousands of genes simultaneously, and genetic maps enable them to localize genomic regions associated with innate and acquired immunity.

COMPONENT 5: Improving Production Systems, Developing New Products, and Enhancing Product Quality

Aquatic animal producers are continually challenged to produce fish, shellfish, and crustaceans efficiently and economically. Producers must have the necessary information and technology to meet consumer demands. To that end, we are developing production technologies to culture new aquatic species and to optimally culture existing species in existing and new environments. The performance of aquatic animal production systems can also be improved through the development and application of innovative biological and engineering approaches.

Aquatic animal production systems range from low-energy/trophic production to super-intensive systems. Although production intensity varies widely among systems, optimal production efficiency is required for profitability. Optimal utilization of production inputs, including water, feed, and mechanical energy, and minimization of waste outputs, requires

knowledge of the interactions among inputs, culture species, the production environment, and economics; however, these interactions are not understood fully.

The marketplace for foods from animal muscle is competitive, requiring new and improved aquaculture products to meet consumer demands and expectations, maintain market share for aquatic animal products, and sustain the growth of "aquatic products as food" industry. In addition, there are great opportunities to enhance the use of lower-valued materials that result from fish processing as feed and food ingredients.

There have been a number of changes to the Aquaculture National Program over the past 5 years. We have had changes in the research leader position at three of our major centers: Leetown, West Virginia (new leader, Caird Rexroad, III); Stoneville, Mississippi (new leader, Craig Tucker); and Stuttgart, Arkansas (new leader, Carl Webster). Interaction and collaboration with industry has always been a high priority, but the number of cooperative research and development agreements and material transfer agreements indicates an increased effort to make an impact on aquaculture production through increased public-private partnerships. Diet development with selectively bred soybeans from Schillinger Genetics is one example of this trend, as well as the germplasm transfer agreement with the Maine Aquaculture Association to distribute ARS improved Atlantic salmon germplasm quickly.

The aquaculture national program has decreased in size. One metric is the number of scientists; down to 51 from 61 over the past 5 years. This has necessitated changes to the extent of the program. During the period documented by this retrospective report, a number of funding reductions to NP 106 resulted in less activity and fewer accomplishments in some areas than anticipated at the time the Action Plan was developed. For example, in 2010 and 2011, funding that supported joint research by the Aquatic Animal Health Research Unit in Auburn, Alabama, and Auburn University scientists in aquaculture health and genomics was terminated by Congress. This reduced progress on milestones concerning identification and sequencing of host and pathogen genes involved in host immunity and pathogen virulence. Additionally, programs in Florida, addressing low-salinity recirculating aquaculture systems for pompano and other marine fish; and in Alaska, working with fish processing co-products, were cut due to budgetary shortfalls. These projects, while both very productive, were unable to fulfill the entire suite of anticipated accomplishments. In all cases, the cuts to the ARS program were accompanied by equally disruptive cuts to university partners, who contributed tremendously in many of the accomplishments cited.

RELATIONSHIP OF NP 106 TO OTHER NATIONAL PROGRAMS

ARS research is organized into four national program areas: Animal Production and Protection (APP); Nutrition, Food Safety, and Quality; Natural Resources and Sustainable Agricultural Systems; and Crop Production and Protection. NP 106 is one of four national programs within the APP program area, and research conducted under the program area is often integrated with that of other national programs. For example, ARS plant scientists have tested new plant cultivars for use in aquatic animal feeds, and aquaculture scientists are working with human nutrition scientists to understand the effect of seafood consumption on human health. Food safety

activities addressing aquaculture commodities have also created linkages among the aquaculture and food safety programs.

HOW THIS REPORT WAS CONSTRUCTED AND WHAT IT REFLECTS

Information about NP 106 achievements in this report is organized according to the current Action Plan, Research Components, and their constituent Problem Statements. Selected accomplishments are presented for each of the Problem Statements to illustrate progress ARS scientists made in meeting the goals of that section of the Action Plan. These accomplishments include the impact or potential anticipated benefits those achievements have had on solving the problems and meeting the needs identified by customer/stakeholders in the NP 106 Action Plan.

For the most part, the content of this report is derived from responses of the NP 106 scientists to requests that they summarize their major accomplishments and provide key references to document those accomplishments. Consequently, this report does not include all accomplishments achieved in the national program. As a result, the scope of this report encompasses a subset of the total spectrum of NP 106 accomplishments, which were chosen to illustrate and exemplify the total progress and significant achievements of the national program.

Just as only selected accomplishments are reported, the details of those accomplishments are selectively documented to illustrate the overall variety of products and knowledge generated by NP 106. The references following each accomplishment provide greater details, and additional references can be found in Appendix 2.

NP 106 encompasses 22 appropriated research projects. The titles of the individual projects and scientific staffing are listed in Appendix 1, which is organized according to the geographic location of the research unit.

Appendix 2 is a complete bibliography of peer-reviewed publications generated by the scientists in NP 106 during the 2008–2012 time frame organized by project. Additionally, Appendix 3 provides the same bibliography organized alphabetically by the name of the lead author.

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COMPONENT 1. UNDERSTANDING, IMPROVING, AND EFFECTIVELY USING ANIMAL GENETIC AND GENOMIC RESOURCES

The preferences and needs of consumers for aquatic animal products require that fish and shellfish be produced in a wide array of environments and management systems in ways that effectively use the natural resource base. Existing genetic resources may give producers options that can be tailored to meet these demands, but several of these resources are at risk of being lost even before they are adequately characterized. ARS research includes germplasm conservation, which requires a wide range of information and analytical tools. Insufficient quantitative and genomic characterization of existing genetic resources is limiting the efficiency of production, use of feed resources, and response to disease threats across the United States. ARS research envisions opportunities to gain production efficiency by exploiting genotypes via the environment and gene-by-gene interactions.

Genetically improving fish and shellfish populations is a key strategy for increasing efficient production in a sustainable manner. The rate of improvement, however, is hindered by a lack of data on some economically important traits; inadequate understanding of quantitative, molecular, and genetic controls of component traits and interrelationships among traits; less than optimal methods for evaluating candidates for selection; inefficient strategies to incorporate genomic data into breeding programs; and an inability to move novel forms (alleles) of genes between populations. To facilitate genetic improvement, new knowledge of the genome of various fish and shellfish species and their interactions with environmental factors must be acquired in a comprehensive framework that encompasses animal adaptation and well-being, reproductive efficiency, nutrient utilization, and conversion to animal products and product quality.

These efforts would be significantly enhanced with appropriate genomic tools and reagents. Public involvement in the construction of these resources is critical to ensuring that aquaculture producers have economically feasible management tools and to provide all researchers with access to these tools and reagents to spur forward technology development for transfer to industry producers and breeders as quickly as possible.

PROBLEM STATEMENT 1A. MAINTAIN AND ENHANCE GENETIC DIVERSITY

Many species of aquatic animals offer high potential economic and nutritional value. Research is occurring to characterize genetic diversity in domesticated and wild species, develop the means to identify and protect improved stocks, and conserve valuable germplasm. To support this work, sperm and other forms of DNA of relevant germplasm are being stored and used by researchers in genomic characterization and analysis.

Research needs to encompass the characterization of genetic diversity using microsatellite and single nucleotide polymorphism (SNP) panels to ensure that the appropriate range and degree of variation is preserved. Collections of germplasm are being selected, catalogued, and curated for research and germplasm conservation purposes. Research in storing and using cryopreserved genetic material, including gametes and embryos of various aquatic animals, is being done in coordination with the National Animal Germplasm Program (NAGP). Researchers are taking

advantage of the Germplasm Resources Information Network (GRIN), a bioinformatic platform that provides an interface to the genetic resources held by the NAGP.

Selected Accomplishments

Genetic diversity and population genetic structure of yellow perch on the East coast and Midwestern United States

The decline in natural yellow perch populations and its high demand as food has been an impetus for the development of yellow perch aquaculture. The existing wild stocks will supply the source of germplasm to develop a captive and selectively bred population. The population genetic structure of yellow perch on the East coast and upper Midwest was unknown, and knowledge of its genetic diversity and variation is needed to design a broad-based genetic improvement and breeding program. First, wild yellow perch, *Perca flavescens,* from 17 sampling areas were collected and analyzed. These findings showed significant divergence between samples from both areas, indicating that inclusion of fish from both geographic regions would increase the diversity in the base generations for captive breeding.

Grzybowski, M., Sepulveda-Villet, O.J., Stepien, C.A., Rosauer, D., Binkowski, F., Klaper, R., Shepherd, B.S., and Goetz, F. 2010. Genetic variation of 17 wild yellow perch populations from the Midwest and East Coast analyzed via microsatellites. Transactions of the American Fisheries Society 139:270-287. http://hdl.handle.net/10113/55574

Initiating a yellow perch breeding program with diverse sources of germplasm

On the basis of results from the survey of wild populations, pair crosses of wild perch were produced from three divergent populations: the Perquimans River (North Carolina), Choptank River (Maryland), and Lake Winnebago (Wisconsin). The progeny of these single pair matings were evaluated for growth for a 12-month period. Perquimans River fish attained a final average weight of 138.12 g; Choptank River fish, 126.70 g; and Winnebago fish, 52.08 g. Final lengths, weights, and absolute growth rates were not significantly different between Choptank and Perquimans fish, but both groups were significantly larger than the Winnebago fish. Condition factors also differed between the strains and at the end of the performance, the Choptank fish had the highest condition factor followed by Perquimans, and then Winnebago fish. The top 25–35 percent performers for each strain were individually pit-tagged, genotyped, and held for spawning. Discussions with several commercial entities are ongoing to include fish from this ARS-sponsored breeding program with their independent breeding efforts to increase the genetic diversity of yellow perch and improve production performance.

Rosauer, D.R., Biga, P.R., Lindell, S., Binkowski, F., Shepherd, B.S., Palmquist, D.E., Simchick, C., and Goetz, F.W. 2011. Development of yellow perch (*Perca flavescens*) broodstocks: initial characterization of growth and quality traits following grow-out of different stocks. Aquaculture 317: 58-66. http://hdl.handle.net/10113/55550

Restoring the native Olympia oyster

There is intense interest in restoring the native Olympia oyster to Pacific Northwest estuaries, using hatchery-produced seed oysters to restock depleted populations. However, without

knowledge of the existing genetic structure among remnant populations, these well-intended efforts could inadvertently alter their genetic composition in ways that compromise population or even the long-term viability of the entire species. ARS scientists with the Shellfish Genetics Program in Newport, Oregon, developed new, species-specific genetic markers and used them to analyze samples from populations ranging from British Columbia to San Francisco Bay. They found that remnant Olympia oyster populations show higher levels of genetic differentiation than expected on the basis of studies of other oyster species, probably as a consequence of the shorter duration of the free-swimming larval stage, and potentially as a consequence of human activities such as harvesting and habit degradation. These results provide valuable baseline knowledge for resource managers and restoration practitioners who are designing and implementing restoration efforts.

Camara, M.D. and Vadapolas, B. 2009. Genetic aspects of restoring Olympia oysters and other native bivalves: Balancing the need for action, good intentions, and the risks of making things worse. Journal of Shellfish Research 28(1): 121-145. http://hdl.handle.net/10113/40803

Stick, D.A., Langdon, C.J., Banks, M.A., and Camara, M.D. 2009. Nineteen novel microsatellite markers for the Olympia oyster, *Ostrea conchaphila/lurida*. Molecular Ecology Resources 9:153-155. http://hdl.handle.net/10113/55640

PROBLEM STATEMENT 1B. DEVELOP AND IMPLEMENT GENETIC IMPROVEMENT PROGRAMS

Genetic improvement in aquatic animals has lagged behind that of terrestrial livestock species. This has been hindered by a combination of the small numbers of families that have thus far been evaluated and lack of phenotypic information. ARS researchers are taking advantage of the increased scope and power of computing platforms and the large sizes of many aquatic families available for study and apply these to large-scale pedigreed phenotypic data sets. Genetic evaluation and improvement programs are reaching new levels through the elucidation of genetic parameters and interrelationships among traits, and the development of objective, multitrait breeding goals.

Thus, research is describing genetic parameters for a suite of economically important traits, including growth, efficiency of nutrient utilization, reproductive capacity, animal behavior, stress tolerance and resistance to disease, fillet dress-out in finfish and meat, shell ratios in shellfish, flesh composition, and product quality. Research is yielding new diagnostics and genetic evaluation tools regarding specific genes and gene complexes, and this new molecular information will be incorporated to maximize genetic improvement.

Selected Accomplishments

Atlantic salmon, improved for farming, released to industry

Commercial salmon producers in the eastern United States are legally required to culture stocks certified to be of North American origin. Therefore, they use stocks that are only a few generations removed from wild, unselected stocks with relatively poor performance under farming conditions. ARS researchers at the National Cold Water Marine Aquaculture Center in Franklin, Maine, selectively bred salmon of North American origin for faster growth and evaluated their growth from the breeding program in commercial sea cages in collaboration with

industry. A salmon line selected for increased growth, which is more than 50 percent larger than unselected control fish (Figure 1), has been generated and germplasm was released for commercial production. Genetic gain has been estimated to be 7–10 percent per generation. Utilization of improved germplasm will increase the profitability and sustainability of coldwater marine aquaculture in the United States while providing a quality seafood product to U.S. consumers. Each year, eggs are distributed to producers through an agreement with the Maine Aquaculture Association, speeding the movement of the improved lines into commercial production. Improved germplasm has been transferred to industry for the past 7 years, and fish are grown predominantly by the salmon farming industry in Maine. Some germplasm from Maine has been transferred into New Brunswick by commercial companies and is being used in salmon production there.

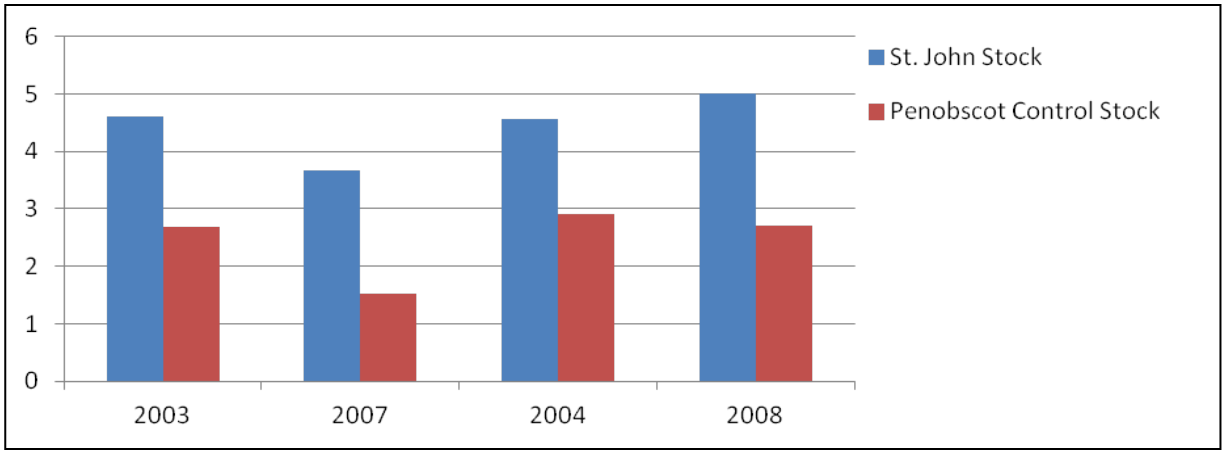

Figure 1. Carcass weight of two Atlantic salmon year classes over two generations (2003–2007 and 2004–2008) compared with unselected control stock.

Wolters, W.R. 2010. Sources of phenotypic and genetic variation for seawater growth in five north american Atlantic salmon, *Salmo salar*, stocks. Journal of the World Aquaculture Society 41:421-429. http://hdl.handle.net/10113/55767

Wolters, W.R., Barrows, F., Burr, G.S., and Hardy, R.W. 2009. Growth parameter of wild and selected strains of Atlantic salmon (*Salmo salar*) on two experimental diets. Aquaculture 297:136-140. http://hdl.handle.net/10113/36771

Production and release of disease-resistant germplasm to the rainbow trout industry

Bacterial cold-water disease caused by *Flavobacterium psychrophilum*, is a widely distributed and economically important disease that results in elevated mortality and deformity rates in rainbow trout aquaculture. Currently, only limited chemotherapeutics, and no commercial vaccines, are available to control or prevent the disease. ARS researchers at the National Center for Cool and Cold Water Aquaculture (NCCWA) in Leetown, West Virginia, have developed the ARS-Fp-R rainbow trout line through multiple generations of genetic selection for improved disease resistance. The ARS-Fp-R line has been selected for improved survival (i.e., resistance) following laboratory challenge with *F. psychrophilum*, and selection response for survival has been estimated at 22.3 percentage points per generation (see Figure 2). Researchers have demonstrated that the resistance phenotype remained stable as the fish size increased more than 300-fold, and in collaboration with researchers at Clear Springs Foods Inc. (Buhl, Idaho),

demonstrated that laboratory challenge results are reproducible. To date, approximately 175,000 embryos from the ARS-Fp-R line have been distributed to farms in Idaho and Utah as part of designed field trials to evaluate the line in production settings where fish are naturally exposed to the pathogen. In all field trials (n = 5) with confirmed *F. psychrophilum* exposure, survival of the ARS-Fp-R line has been ≥95.5 percent through a minimum of 80 days post initial feeding. This is significantly greater than reference control and hatchery populations. In addition to greater survival, the ARS-Fp-R line has also generally exhibited a smaller percentage of fish that yield a positive culture for *F. psychrophilum.* Ongoing research is being conducted to evaluate whether the improved resistance is broad-based (i.e., whether it is effective against other *F. psychrophilum* variants and other bacterial and viral pathogens), and to better understand the biological mechanisms of disease resistance.

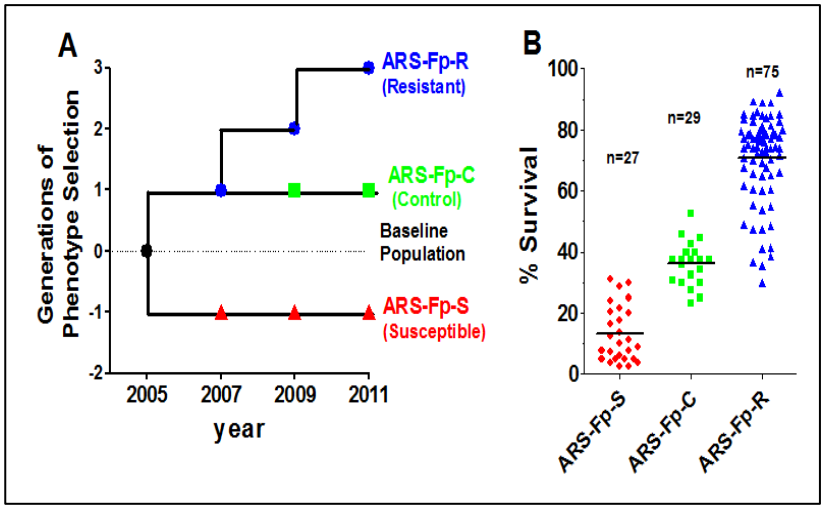

Figure 2. ***A:*** Schematic showing the direction and generations of selection (y-axis) applied to rainbow trout lines in comparison to the original baseline population. ***B:*** Phenotype of genetic lines measured in 2011. Percent family survival after 21 days challenge. n, number of families evaluated per line. Each point represents a separate family (ARS-Fp-R and ARS-Fp-S lines), while for the ARS-Fp-C line, each point represents a challenge tank containing pooled families.

As a result of its documented superiority in laboratory challenges and field trials, commercial producers of trout with genetic improvement programs and state hatcheries have requested the ARS-Fp-R line. As of 2012, 80,000 embryos from 52 pedigreed families have been released to Clear Springs Foods and to Troutlodge Inc. (Sumner, Washington) for on-site evaluation as broodstock. Pending results, these fish will allow industry stakeholders to propagate or introgress the line and produce future generations of fish with improved disease resistance that will be available for commercial production and sale. Additionally, the Utah Division of Wildlife Resources received approximately 50,000 embryos from 43 trout families, and plans to multiply and distribute the germplasm as a pure line to state hatcheries within Utah for recreational fishing purposes.

Leeds, T.D., Silverstein, J., Weber, G.M., Vallejo, R.L., Palti, Y., Rexroad III, C.E., Evenhuis, J., Hadidi, S., Welch, T.J., and Wiens, G.D. 2010. Response to selection for bacterial cold water disease resistance in rainbow trout. Journal of Animal Science 88:1936-1946. http://hdl.handle.net/10113/45794

Hadidi, S., Glenney, G.W., Welch, T.J., Silverstein, J.T., and Wiens, G.D. 2008. Spleen size predicts resistance of rainbow trout to *Flavobacterium psyhchrophilum* challenge. Journal of Immunology 180:4156–4165. http://www.jimmunol.org/content/180/6/4156.short

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Identification of genetic variation for plant protein tolerance and fatty acid elongation in rainbow trout

Fishmeal is an expensive component of many trout diets. To reduce the reliance on fishmeal-based feed, scientists with the Small Grains and Potato Germplasm Research Unit in Aberdeen, Idaho, have selectively bred a strain of rainbow trout that grows faster and more efficiently when fed a fishmeal-free, plant-based diet. The intestinal tract of the improved fish have become adapted and are now more able to more effectively utilize a plant-based diet without developing the intestinal inflammation observed with conventional strains of rainbow trout fed such a diet. Increasing our understanding of how trout adapt to being fed plant-based diets is aiding in the continued development of improved strains of trout. Additionally, a major commercial producer of rainbow trout, Clear Springs Foods, Inc. (Buhl, Idaho), has incorporated the plant protein–tolerant fish into its breeding program.

Within the same lines of trout, efforts to reduce reliance on fish oil are also occurring. Fish oil is one of the most expensive feed ingredients and is the primary source of the healthy omega-3 fatty acids, EPA and DHA, which are found in trout fillets. Scientists evaluated the ability of families of rainbow trout to convert plant oils to long-chain omega-3 fatty acids and store them in their tissues. The study clearly demonstrated that genetic variation exists between families for this ability. Rearing rainbow trout that have been bred for this trait should maintain the beneficial fatty acids currently found in trout fillets while reducing feed costs and the industry's current reliance on fish oil. These findings are recent, and the ability to select for improved ability to convert omega-6 vegetable oils to omega-3 fish oils is currently being further evaluated with the goal of generating fish for the industry that will maintain healthy levels of omega-3 fatty acids while reducing the industry's dependence on fish oil.

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Improving the hybrid catfish

Hybrid catfish (female channel catfish crossed with a male blue catfish) have been produced experimentally for more than 50 years; however, new advances in reproduction techniques have led to tremendous expansion in the United States over the past 5 years (see Figure 3**)**. Whereas channel catfish strains have been well characterized over several decades, little information is available on blue catfish germplasm with respect to production traits, especially in channel × blue hybrid catfish offspring. ARS scientists with the Catfish Genetics Research Unit in Stoneville, Mississippi, have obtained blue catfish from several geographic sources (strains) and initiated a program to determine the effects of blue catfish strain and individual within-strain differences on hybrid offspring growth and fillet yield. Significant effects of individual male and female parent on offspring performance were identified and, importantly, these effects were additive. This result means that the effect of parent on offspring performance is predictable and improvements will be cumulative. On the basis of results of these trials, blue catfish germplasm that produces superior performing hybrid offspring will be released to the industry.

Figure 3. ***Top:*** blue catfish (*Ictalurus furcatus*); ***middle:*** hybrid catfish (*Ictalurus furcatus* sire × *Ictalurus punctatus* dam); ***bottom:*** channel catfish (*Ictalurus punctatus*).

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Li, M.H., Robinson, E.H., Tucker, C.S., Oberle, D.F., and Bosworth, B.G. 2009. Comparison of channel and blue catfish fed diets containing various levels of protein. Journal of the World Aquaculture Society 39:646-655. http://hdl.handle.net/10113/55438

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PROBLEM STATEMENT 1C. DEVELOP AND IMPLEMENT GENOMIC TOOLS IN GENETIC IMPROVEMENT PROGRAMS

Significant public resources have been devoted to developing genomic and bioinformatic infrastructure for catfish, rainbow trout, Atlantic salmon, and Atlantic oysters over the past 5–10 years. These efforts have culminated in significant improvement in the genomic information on these species available in the public domain, yet considerable work remains to bring these aquaculture genomes to a level of information that would be necessary for optimal "mining" through functional genomic and proteomic approaches. ARS remains committed to devoting the necessary resources to fill these research gaps.

ARS research is endeavoring to develop physical genetic maps of catfish and rainbow trout, and genetic linkage maps for other aquatic species. In particular, the identification of genomic regions associated with specific traits, quantitative trait loci (QTL), has been a major effort over the past 5 years.

Selected Accomplishments

Development of genetic mapping and functional genomic resources for identifying genes affecting stress response in rainbow trout

The use of molecular and genetic technologies is broadly anticipated to enhance the ability to selectively breed traits associated with production efficiency. ARS scientists at the NCCCWA in Leetown, West Virginia, are developing and using molecular-genetic technologies to identify genes that affect stress response with the goal of using these to selectively breed rainbow trout. This requires the development of species-specific tools such as genetic maps that characterize the order and distance between genetic markers, and physical maps that represent the genome through overlapping DNA fragments anchored to chromosomes. A comprehensive map that integrates genetic and physical maps was constructed by connecting 274 reference points throughout the 29 chromosomes of the trout genome. This integrated mapping resource will be

used to reveal associations in the inheritance of superior production traits with the genes that underlie them. An example of this is the work on understanding the stress response in rainbow trout.

At this time, the maps are maintained locally and accessed through public Web sites (e.g. www.genome.clemson.edu/rainbowTrout3). These resources are used primarily by researchers, not farmers. However, farmers such as those with Troutlodge, Inc., have used markers from the maps to conduct population genetic analyses in their GxE study, which evaluated the performance of multiple families in different environments. Researchers use the maps more routinely in seeking a functional association between a particular performance trait and the genome (see Sanchez et al. 2011 and Rexroad et al. 2012).

Under fish farming conditions, fish are routinely exposed to stressors of the aquaculture production environment. How fish respond to such stressors affects their growth, feed efficiency, immune response, and reproductive development. Through genetic mapping scientists have identified 15 chromosomal regions in rainbow trout that are linked to stress response and are measuring genetic variation for this trait within and between populations. In parallel, a functional genomics approach that takes advantage of advanced DNA sequencing technologies has enabled the identification of genes that are expressed in response to high salinity, high or low temperatures, crowding, and reduced water quality. This research will improve our understanding of the physiology of the stress response and provide a more rapid and accurate way to identify rainbow trout that perform well under routine farming conditions. Although stress responses are of great interest to our stakeholders, this information is new and not yet being applied.

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Developing genomic tools for yellow perch research

Yellow perch is relatively new to aquaculture production and few molecular tools exist to develop genetic maps to accelerate its genetic improvement and to enhance selective breeding with genomic information.

A cell culture system for head kidney granulocytic phagocytes (a key immune cell type) of yellow perch was developed and used to study the effects of various pathogens on the perch immune system. We have also characterized about 9,000 new gene sequences from the head kidney cells using this culture system. This new gene sequence information has enabled us to characterize and identify *1)* a suite of perch-specific immune genes, *2)* the molecular pathways involved following infection by the viral hemorrhagic septicemia virus (VHS) and the effects of infection in yellow perch, and *3)* new molecular markers (called single nucleotide polymorphisms—SNPs) for genotyping. In other studies, genomic resources were developed by preparing samples of liver, brain, and ovary from controls and fish treated with estradiol. These samples yielded more than 24,000 new sequences, and many microsatellite and SNP markers. These new molecular markers will be used for pedigree tracking to further improve selective breeding efforts in our yellow perch broodstock. These sequences and tools are available through public databases such as www.ncbi.nlm.nih.gov/, and have been referenced in publications by the ARS and University of Wisconsin, Milwaukee team. Improved lines of perch have been developed and are being distributed to interested commercial producers.

Goetz, F.W., Rise, M.L., Rise, M., Goetz, G.W., Binkowski, F.P., and Shepherd, B.S. 2009. Stimulation of growth and changes in the hepatic transcriptome by estradiol-17-beta in the yellow perch (*Perca flavescens*). Physiological Genomics 38:3 261-280. http://physiolgenomics.physiology.org/cgi/content/abstract/00069.2009v1.

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Rosauer, D.R., Biga, P.R., Lindell, S., Binkowski, F., Shepherd, B.S., Palmquist, D.E., Simchick, C., and Goetz, F.W. 2011. Development of yellow perch (*Perca flavescens*) broodstocks: initial characterization of growth and quality traits following grow-out of different stocks. Aquaculture 317: 58-66. http://hdl.handle.net/10113/55550

Molecular tests for stress tolerance genes in Pacific oysters

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

Lang, R.P., Langdon, C.J., Taris, N.G., and Camara, M.D. 2010. Use of laboratory assays to predict subsequent growth and survival of Pacific oyster (*Crassostrea gigas*) families planted in coastal waters. Aquaculture 306:68-79. http://hdl.handle.net/10113/45763

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Development of highly inbred channel catfish for genome sequencing and large-scale development of single-nucleotide polymorphisms

The use of molecular-genetic technologies to develop genomic sequences of aquaculture species is expected to accelerate the incorporation of genomic data into selective breeding programs and the rate of genetic improvement for aquaculture production traits. To facilitate generation of the catfish genomic sequence, use of DNA from a highly inbred individual is advantageous. There is greatly reduced variation between homologous chromosomes within the individual, thus simplifying assembly of the sequenced fragments. Scientists at the Catfish Genetic Research Unit in Stoneville, Mississippi, fertilized catfish eggs with sperm that had been exposed to ultraviolet light and its DNA destroyed. Because the sperm genome was not viable, the resulting one-celled eggs were haploid. These haploid eggs went through DNA replication and were treated with pressure or high temperature to inhibit the first mitotic division, so the resulting offspring contained a doubled haploid genome. Molecular analysis of gene loci verified the offspring were homozygous at all loci with no paternal genetic contribution. These homozygous, doubled-haploid catfish are completely inbred and can be used to produce completely inbred populations for genetic analyses. The genomic DNA from doubled-haploid catfish is also valuable for establishing a reference sequence of the channel catfish genome.

Once a reference sequence is established, a key tool for identifying the genetic causes of phenotypic variation are genetic markers distributed throughout the genome. A large number of DNA sequence variants is needed to identify functional variants throughout the genome. In a separate project, high-throughput sequencing of DNA fragments from multiple catfish was conducted, and the DNA sequences were aligned to permit efficient detection of single-nucleotide variations between individuals. Interrogated were 37,764 genomic locations, which yielded 9,674 high-likelihood DNA sequence variants that will be useful for catfish identification, population structure, and selective breeding.

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Waldbieser, G.C., Bosworth, B.G., and Quiniou, S. 2010. Production of viable homozygous, doubled haploid channel catfish (*Ictalurus Punctatus*). Marine Biotechnology 12:380–385. http://hdl.handle.net/10113/55420

Wang, S., Peatman, E., Abernathy, J., Waldbieser, G.C., Lindquist, E., Richardson, P., Murdock, C.A., Small, B.C., Quiniou, S., and Liu, Z. 2010. Assembly of 500,000 inter-specific catfish expressed sequence tags and large scale gene-associated marker development for whole genome association studies. Genome Biology 11:R8. http://hdl.handle.net/10113/40309

COMPONENT 2. ENHANCING ANIMAL PERFORMANCE, WELL-BEING, AND EFFICIENCY IN DIVERSE PRODUCTION SYSTEMS

Proper matching of animal genotypes to the production system and to consumer requirements is critical for sustainable and profitable production. This requires a comprehensive understanding of factors affecting animal growth and efficiency, adaptability, and well-being.

Feeding for maintenance and growth are the most economically demanding inputs in animal production. Achieving the desired partitioning of nutrients into the competing systems of muscle development, health maintenance, and reproductive development within animals is critical to improving productivity. Likewise, the development of scientific measures of well-being and an enhanced ability to interpret them are critical to maximizing animal growth, efficiency, health, and product quality. Stressors caused by social, nutritional, and environmental factors, and their interactions need to be understood to limit negative effects on animal health, production efficiency, and product quality.

Equally essential to ensuring reliable production is the successful and efficient reproduction of aquatic animals. Numerous environmental factors affect reproductive and larval development success, including inconsistent hatching rates, incompatibility of valuable hybrid gametes, and difficulty identifying sexually mature and ripe individuals. Control of these factors is needed to achieve year-round spawning and fingerling production.

PROBLEM STATEMENT 2A. IMPROVE GROWTH, NUTRIENT UTILIZATION, AND WELL-BEING

Growth is a key trait in all animal production. As production systems focus on weight gain and gains in product or protein mass, efficient conversion of nutrients into muscle has become a priority. Identifying the genes and pathways that lead to better growth and optimal nutrient utilization are important in our ability to maximally improve these traits in fish production. Knowledge of behavioral hierarchies and appropriate rearing densities are also essential to maximizing the health and growth performance of many aquatic species. Additionally, new technologies for transporting aquatic animals that result in less stress and improved survival are critical.

Thus, ARS scientists have worked to expand their knowledge of nutrient utilization in aquatic animals. Specifically, the genetic and physiological bases for variation in growth and feed efficiency are not well defined and required further study. Scientists are developing a better definition of feed efficiency that improves protein retention rather than generating fatter animals and additional waste material. Understanding the genes and metabolic pathways in nutrient assimilation and how nutrient balance affects these pathways is also required.

Research in these areas has led to a better understanding of the nutritional value of feedstuffs and the physiology of nutrient retention and conversion. This in turn is enabling the targeting of genes and pathways to improve feed consumption and nutrient utilization by fish. It is also enabling producers to increase the amount of nutrients deposited in the edible portion of animals, lower feed costs, and reduce the amount of waste generated. Meanwhile, research on animal well-being is benefiting animals, producers, and ultimately, consumers, by reducing costs of

animal health maintenance and improving production efficiencies. Achievement of these goals will maintain and increase the demand for farmed aquatic products in domestic and international markets.

Selected Accomplishments

Feed efficiency—the efficiency with which consumed feed is converted into body mass—is a critical determinant of profitability in animal production, so improving this measure is a prime research objective in all animal production, including fish farming.

Identification of pathways regulating protein turnover in rainbow trout

Improvements in growth and feed efficiency in rainbow trout can occur by increasing the efficiency of their ability to retain dietary protein. Protein retention is determined by the balance of rates of protein synthesis and protein degradation. Through a series of studies, ARS scientists at the NCCCWA, in Leetown, West Virginia, have determined how protein metabolism is affected by genetic variation, hormones, and nutrients. More specifically, they determined that genetic variation affects how fish respond to insulin-like growth factor I (IGF-I), a hormone that increases protein synthesis and reduces protein degradation. They were also able to determine that insulin and leucine also increase protein retention in cell cultures. This better understanding of protein degradation regulation is essential to the development of better aquatic feed formulations, feeding strategies, and husbandry practices.

In related research, ARS researchers in Leetown also discovered that higher rates of muscle protein degradation occur in sexually maturing rainbow trout with high sex steroid levels, even when feed consumption is maintained. Subsequent studies indicated that estrogens, but not androgens, act directly on muscle to reduce protein retention by both increasing rates of protein degradation and decreasing rates of protein synthesis. These data demonstrate that harvesting before sexual maturation and sex steroid exposure should result in the most efficient production of high-quality fillets. We know that sexual maturity affects growth and meat quality in most terrestrial livestock, but until recently, little has been known about its action in fish. Thus, this information is important in our ability to understand how to counteract the negative effects of sexual maturity on muscle building. Understanding how sex steroids affect muscle growth and quality will enable the development of strategies for more efficient fillet production.

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Cleveland, B.M. and Weber, G.M. 2010. Effects of insulin-like growth factor-I, insulin, and leucine on protein turnover and pathways that regulate ubiquitin ligase expression in rainbow trout primary myocytes. American Journal of Physiology - Regulatory Integrative and Comparative Physiology 298:R341-350. http://hdl.handle.net/10113/43948

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Feed efficiency in catfish

Growth hormone (GH) is a key regulator of growth in all animals and the GH–target tissue axis in fish is not well described. In mammals, GH release into the bloodstream is driven by the GH–releasing hormone (GHRH). A similar hormone is known in fish, named GHRH-like peptide, but it has poor GH-releasing activity. Scientists at the Catfish Genetics Research Unit in Stoneville, Mississippi, have identified novel genes encoding what appears to be a fish GHRH in channel catfish using genomic mapping techniques. Other recently discovered genes in catfish that are part of the GH axis include ghrelin and its receptors, which were found to be highly expressed in pancreatic tissue. Additional studies have identified genes and assays to measure gene transcription/protein activity of the catfish GH axis. Catfish IGF-I and IGF-II are key regulators of growth and nutrition, and assays have been developed to allow their measurement, their binding proteins (IGFBPs), and the messenger RNAs (mRNAs) of somatostatins SS-14 and SS-22. Studies with fish that are fed or unfed demonstrated that IGF-I, IGF-II, IGFBP-1, IGFBP-3, and SS-14 play important roles in growth. The regulation of these genes during fed and fasted conditions support the existence of a growth hormone axis in catfish, similar to what has been reported for mammals. Nevertheless, a novel finding in this research was that IGF-II plays an important role in postnatal growth in catfish. In mammals, the primary role of IGF-II occurs during embryonic development.

Ghrelin, cocaine and amphetamine-regulated transcript (CART), neuropeptide Y (NPY), and cholecystokinin (CCK) are neuropeptides involved in the regulation of appetite and feeding in channel catfish. ARS scientists have identified two separate complementary DNAs (cDNAs) for CCK (CCKa and CCKb) that provide new insights into the neural and gastroenteric mechanisms that regulate appetite in channel catfish. For example, scientists in the ARS laboratory in Stoneville have examined mRNA levels of genes that regulate growth and appetite in fast- and slow-growing families of channel catfish. The data are currently being analyzed, although they do show that CART mRNA is lower in faster-growing fish. These genes could be used as selective markers to improve growth or appetite.

Another avenue of feed efficiency involves research on mitochondria, the energy conversion organelles in every cell. Scientists in Stoneville are investigating mitochondrial respiratory chain enzyme activities and gene expression levels in low- and high-feed-efficient (FE) families of catfish. Mitochondrial enzyme activities in liver, muscle, and intestine were reduced in low-FE channel catfish. Also, some mitochondrial genes (e.g., NADH dehydrogenase subunit 1)

involved in controlling oxidative phosphorylation were up-regulated in liver and down-regulated in muscle and intestine of the high-FE family. These differences in gene expression and enzyme activities provide insight into the cellular mechanisms of fish with differences in growth rate and feed efficiency, and are being examined as selection markers for fish that grow more efficiently. Other genes and enzyme activities that regulate FE are being examined in families of fish with high and low FE. Before these genes and enzymes can be utilized as selection markers, we need to first understand how they are regulated.

These genes are expected to be useful in DNA marker-assisted selection of catfish broodstock because the mechanisms that regulate their physiological functions seem to be similar to what is known for mammals. We envision the use of a set of genetic markers that contains growth and disease indicators that will be used to accelerate selection of catfish broodstock.

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Peterson, B.C., Bilodeau-Bourgeois, A.L., and Small, B.C. 2009. Response of the somatotropic axis to alterations in feed intake of channel catfish (*Ictalurus punctatus*). Comparative Biochemistry and Physiology 153:457-463. http://hdl.handle.net/10113/55424

Peterson, B.C. and Waldbieser, G.C. 2009. Effects of fasting on IGF-I, IGF-II, and IGF-binding protein MRNA levels in channel catfish (*Ictalurus punctatus*). Domestic Animal Endocrinology 37:74-83. http://hdl.handle.net/10113/31933

Peterson, B.C., Waldbieser, G.C., Riley, Jr, L.G., Upton, K.R., Kobayashi, Y., and Small, B.C. 2012. Pre and postprandial changes in orexigenic and anorexigenic factors in channel catfish *Ictalurus punctatus*. General and Comparative Endocrinology 176:231-239. http://handle.nal.usda.gov/10113/55390

Small, B.C., Quiniou, S., and Kaiya, H. 2009. Sequence, genomic organization and expression of two channel catfish, *Ictalurus punctatus*, Ghrelin receptors. Comparative Biochemistry and Physiology 154(4):451-464. http://hdl.handle.net/10113/55422

Performance of salmonids in recirculating aquaculture systems

Water availability and cost of water use are two key variables for fish production in recirculating aquaculture systems (RAS), so one research goal is to minimize the rates of water exchange while maintaining excellent conditions for fish (Figure 4). Rainbow trout and Atlantic salmon were reared by The Conservation Fund's Freshwater Institute (TCFFI) researchers in Shepherdstown, West Virginia, for 6 months in replicated water-recirculating systems operated with either high water exchange (97.5 percent of system flow recirculated, with total system water volume exchanged 1.43 times each day) or low water exchange (99.75 percent of system flow recirculated, with total system water volume exchanged every 7 days). Fish performance, health, and welfare outcomes were measured.

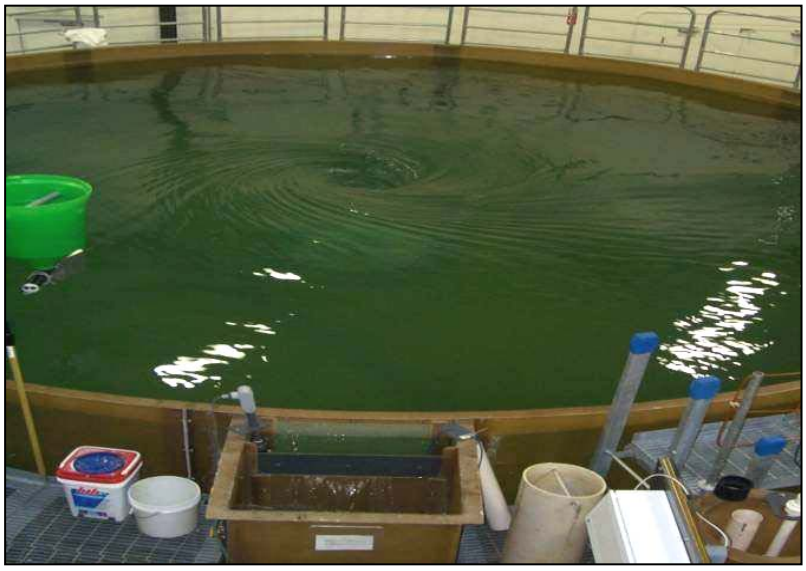

Figure 4. Dual drains, on tank bottom and sides, improve management of rotation (e.g., velocity) and provide for solids separation at the tank.

By study's end, no significant differences were determined in growth, survival, and other fish health outcomes even though the low water flushing treatment operated with 10 times less water use. Though significant differences in a variety of water quality parameters were noted, none of these parameters was outside acceptable ranges for raising salmonids. This study illustrates that both rainbow trout and Atlantic salmon perform well in recirculating systems with low water exchange and demonstrate that farmers with limited water resources can compete in salmon production. (*This accomplishment also appears on page 50 to illustrate research outcomes also contributing to Problem Statement 5A.*)

The use of RAS enables tight control of culture tank parameters affecting health and well-being of the fish, such as dissolved oxygen levels and rotational velocity/swimming speed. The relationships of fish growth to dissolved oxygen levels and swimming speeds have been characterized individually, but not in combination. Two factorial studies (the first for rainbow trout; the second for Atlantic salmon) were carried out at TCFFI to identify whether swimming speed (two body lengths per second vs. 0.5 body length per second) or dissolved oxygen levels (100 percent saturation vs. 70 percent saturation), or both of these factors affect various important performance and health outcomes in these fish species. For rainbow trout, at 338 days post hatch, mean weight was significantly greater in the high-oxygen treatment groups, although swimming speed did not significantly affect mean final weight. Greater swimming speed, however, did significantly increase the mean fish cardiosomatic index, which has important implications in animal fitness and the ability to withstand periods of increased stress. There were no significant differences between mortality rates, fish length, feed conversion, or butterfly fillet yields among treatment groups. For Atlantic salmon, at 440 days post hatch, greater dissolved oxygen and swimming speed were both significantly associated with better growth performance; additionally, faster swimming speed was associated with a significant reduction in precociously mature males. No differences in survival were determined among the treatment groups. These findings indicate that circular tanks and oxygenation systems can be used to optimize salmonid growth and fitness by maintaining 100 percent oxygen saturation concentrations and culture tank water rotational velocities to provide swimming speeds of two body lengths per second. Greater swimming speed appears to have the additional benefit of lowering early sexual maturation in pre- and post-smolt Atlantic salmon.

The high/low makeup studies, in particular the water quality data gained from this research, have influenced system design and operational recommendations for producers aiming to raise Atlantic salmon, rainbow trout, arctic charr, yellow perch, and walleye. These results have been disseminated at numerous research meetings and producers' workshops, and through publication in peer-reviewed journals.

Davidson, J., Good, C., Welsh, C., Brazil, B.L., and Summerfelt, S. 2009. Heavy metal and waste metabolite accumulation and their affect on rainbow trout performance in a replicated water reuse system operated at low or high system flushing rates. Aquacultural Engineering 41:136-145. http://hdl.handle.net/10113/34640

Davidson III, J.W., Good, C.M., Welsh, C., and Summerfelt, S.T. 2011. Abnormal swimming behavior and increased deformities in rainbow trout *Oncorhynchus mykiss* cultured in low exchange water recirculation aquaculture systems. Aquacultural Engineering 45(3):109-117. http://hdl.handle.net/10113/55677

Davidson, J., Good, C., Welsh, C., and Summerfelt, S.T. 2011. The effects of ozone and water exchange rates on water quality and rainbow trout *Oncorhynchus mykiss* performance in replicated water recirculating systems. Aquacultural Engineering 3:44:80-96. http://hdl.handle.net/10113/55681

Good, C., Davidson, J., Welsh, C., Brazil, B., Snekvik, K., and Summerfelt, S. 2009. The impact of water exchange rate on the health and performance of rainbow trout *Oncorhynchus mykiss* in water recirculation aquaculture systems. Aquaculture 294:80-85. http://handle.nal.usda.gov/10113/55792

Good, C., Davidson, J., Welsh, C., Snekvik, K., and Summerfelt, S. 2010. The effects of carbon dioxide on performance and histopathology of rainbow trout *Oncorhynchus mykiss* in water recirculation aquaculture systems. Aquacultural Engineering 42:51-56. http://handle.nal.usda.gov/10113/55787

Good, C., Davidson, J., Welch, C., Snekvik, K., and Summerfelt, S. 2011. The effects of ozonation on performance, health and welfare of rainbow trout *Oncorhynchus mykiss* in low-exchange water recirculation aquaculture systems. Aquacultural Engineering 44(3): 97-102. DOI: 10.1016/j.aquaeng.2011.04.003. http://handle.nal.usda.gov/10113/55769

Wolters, W.R., Master, A., Vinci, B., and Summerfelt, S. 2009. Design, loading, and water quality in recirculating systems for Atlantic salmon (*Salmo salar*) at the USDA ARS National Cold Water Marine Aquaculture Center (Franklin, ME). Journal of Aquaculture Engineering 41:60-70. http://hdl.handle.net/10113/34644

PROBLEM STATEMENT 2B. ENHANCE REPRODUCTIVE PERFORMANCE AND REDUCE REPRODUCTIVE LOSSES

Improving reproductive success is critical in the production of many aquatic species. Managing this process requires basic research to better understand the neuroendocrine regulatory mechanisms that coordinate functions of the hypothalamo-pituitary-gonadal axis. Likewise, the effects of handling and other management interactions on reproduction are being better understood to minimize problems arising from animal management. Research is occurring to develop strategies to improve suboptimal fertilization rates and poor development during early life stages. Basic research on the physiology of the larval stages of aquatic species is improving larval survival. Additional research is occurring to improve reproductive performance, including devising methods to control timing of availability of juveniles for grow-out and to produce animals of the gender that are more desirable for food production. By improving reproductive

success in these ways, research can significantly increase aquatic animal production efficiency and profitability.

Selected Accomplishments

Controlling sexual maturation in farmed fish

The long period of time required for most farmed fish to reach sexual maturity significantly slows genetic improvement and impedes fingerling production. Kisspeptins are newly recognized proteins that naturally control when animals enter puberty. Scientists at the Stuttgart National Aquaculture Research Center, in Stuttgart, Arkansas, showed that sexually immature male white bass entered puberty more quickly, and that adult female white bass had larger ovaries with more mature eggs following administration of kisspeptins. Together, these findings highlight the potential of kisspeptin treatment in fish to accelerate and help control reproductive timing. This will speed breeding and improve year-round reproductive potential.

Beck, B.H., Fuller, S.A., Peatman, E., McEntire, M.E., Darwish, A.M., and Freeman, D.W. 2012. Chronic exogenous kisspeptin administration accelerates gonadal development in basses of the genus Morone. Comparative Biochemistry and Physiology 162A(3):265-273. http://handle.nal.usda.gov/10113/55372

Year-round spawning achieved with pompano

Lack of sustained year-round production of juveniles for grow-out operations is one of the foremost bottlenecks of marine finfish aquaculture. Spawning induction protocols were developed and tested. Spawning performance of Florida pompano broodstock, measured as number of eggs, fertilization, egg quality, and hatch rate was quantified over a 12-month period (Figure 5). Spawning was achieved in 10 months of the year with an average production of 1.9 million eggs/year, with no discernible diminishment in egg quality over time. This work demonstrates that Florida pompano seedstock can be produced year-round from a small population of broodstock, thus overcoming one of the key bottlenecks to marine finfish aquaculture.

Figure 5. Fish nutritionist Marty Riche and technician Patrick Tracy harvest market-size Florida pompano from grow-out tanks at the Harbor Branch Oceanographic Institute to determine growth rates during feed nutrition studies.

Cavalin, F.G. and Weirich, C.R. 2009. Larval performance of aquacultured Florida pompano, *Trachinotus carolinus* L. fed rotifers enriched with selected commercial diets. Aquaculture 292:67-73. http://hdl.handle.net/10113/29922

Riley, K.L., Weirich, C.R., and Cerino, D.S. 2009. Development and growth of hatchery-reared larval Florida pompano *Trachinotus carolinus*. Fishery Bulletin 107:318-328. http://hdl.handle.net/10113/55544

Weirich, C.R., Wills, P.S., Baptiste, R.M., and Riche, M.A. 2010. Production characteristics and body composition of juvenile cobia fed three different commercial diets in recirculating aquaculture systems. North American Journal of Aquaculture 72:43-49. http://hdl.handle.net/10113/42084

A nonlethal and rapid method for reliable identification of gender in yellow perch

Yellow perch females grow faster than males, so aquaculture producers would like to separate yellow perch by gender. Despite the difference in size, it is difficult to distinguish the sex of immature animals without causing harm or death to the animal. To address this, ARS researchers in Milwaukee, Wisconsin, developed criteria that enable gender identification in yellow perch on the basis the coloring and shape of the external reproductive openings, with an accuracy exceeding 97 percent. This nonlethal method provides a useful and practical tool that will enable aquaculture producers and researchers to sort sexes for *1)* development and management of broodstocks (breeding stock) prior to and during breeding; *2)* conducting multitank replicate experiments aimed at studying gender-specific differences in yellow perch; and *3)* identifying the fastest growing individuals of each sex in yellow perch broodstock genetic-selection programs dedicated to developing improved growth performance in this species. The criteria developed for gender identification in yellow perch are easy and inexpensive.

Shepherd, B.S., Rees, C.B., Sepulveda-Villet, O.J., and Palmquist, D.E. 2013. Identification of gender in yellow perch (*Perca flavescens*) by external morphology: validation in four geographic strains and effects of estradiol. *N Am J Aquacult.* In press.

Improving hybrid catfish production

Hybrid catfish, the offspring of a channel catfish female crossed with a blue catfish male, are in great demand for catfish farming. They are resistant to several of the common diseases, have improved feed intake and growth under production conditions, tolerate lower oxygen concentrations without going off feed, and generally return a higher yield over a growing season than pure-bred channel catfish. Nevertheless, hybrid juvenile production has been a severe bottleneck for decades. Over the past 5 years, a number of studies conducted at the Catfish Genetics Research Unit, in Stoneville, Mississippi, have led to improved hybrid production.

A synthetic spawning hormone, salmon gonadotrophin-releasing hormone analogues (GnRHa), has been tested and found to perform as well as the mammalian hormone analog that is currently used in commercial hybrid catfish production. This synthetic hormone improved synchronization of ovulation, and number of eggs ovulated by each female, and fry production per unit female weight was comparable to the mammalian form of the hormone. A low dosage of salmon GnRHa can reduce the hormone costs associated with strip spawning of channel catfish in hybrid embryo production. The results of this research were field tested in four commercial hatcheries. Hormone costs were reduced to 50 percent by administering salmon GnRHa in all the hatcheries.

Additional techniques and technologies developed include holding hormone induced channel catfish suspended in individual mesh bags and monitoring the pH of stripped eggs. Holding

individual females in mesh bags after hormone injection during latency until ovulation is a considerable improvement over previous methods of repeatedly crowding and handling stress during hormone injection. Stress (plasma cortisol) response was significantly lower in fish that were suspended in mesh bags compared with that in communally held fish. Ovulated eggs from females held in bags had a 5.6 percent higher hatching success compared with females held communally in tanks. This method of holding fish has improved the efficiency of hybrid catfish production (regardless of the ovulating hormone administered) and has been adopted by six of the eight commercial hybrid catfish hatcheries. Also, in stripped eggs, monitoring has shown that low pH (<7.0) eggs were suggestive of low quality and poor hatchability, whereas eggs with a pH of 7.6 or greater resulted in consistently higher hatch of hybrid catfish. This procedure will be field tested in two hatcheries and for possible inclusion in the spawning protocols to produce hybrid catfish fry.

Increased production efficiency of hybrid fry have lowered the cost of hybrid fry production in the U.S. catfish industry. Over the past 5 years the numbers of hybrid juveniles being produced has risen from approximately 41 million to more than 150 million per year, at similar hatchery costs. This tremendous improvement in production without a considerable rise in costs reveal greatly improved hatchery efficiency.

According to a report by the National Agriculture Statistics Service, hybrid catfish production was attributed to 21.2 percent of total catfish processed in 2011. The demand for hybrid fingerlings remained high despite higher cost of hybrid fingerlings in 2012. We can expect hybrid catfish may contribute to 30 percent of the total catfish processed in 2012 because of higher hybrid fry production numbers in 2011 compared with 2010.

Chatakondi, N.G. and Torrans, E.L. 2012. The inluence of ovarian fluid ph of stripped unfertilized channel catfish *Ictalurus punctatus* eggs on the hatching success of channel catfish x blue catfish *I. furcatus* hybrid catfish eggs. Journal of the World Aquaculture Society 43(4):585-593. http://handle.nal.usda.gov/10113/55386

Chatakondi, N.G., Yant, R.D., Kritanto, A., Umali-Maceina, G.M., and Dunham, R.A. 2011. The effect of luteinizing hormone releasing hormone analog regime and stage of oocyte maturity for induced ovulation of channel catfish, *Ictalurus punctatus*. Journal of the World Aquaculture Society 42(6):845-853. http://handle.nal.usda.gov/10113/55394

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COMPONENT 3. DEFINING NUTRIENT REQUIREMENTS AND NUTRIENT COMPOSITION OF FEEDSTUFFS AND EXPANDING ALTERNATIVE INGREDIENTS

For most aquatic species the largest cost of animal production lies in feed inputs. Small changes (less than 1 percent) in nutrient requirement definitions can greatly affect the cost of diets. Therefore, a thorough knowledge of the nutrient requirements for optimum growth and performance at specific life stages is essential for efficient production of aquatic animals. Additionally, development and evaluation of novel feed ingredients is vitally important to provide feed manufacturers flexibility to improve formulations when some commodities increase in cost. As the chemical composition and nutrient bioavailability of feedstuffs are determined, feeds with the least cost can be formulated on the basis of nutrient requirements and the relative costs of different ingredients.

PROBLEM STATEMENT 3A. DETERMINE NUTRIENT REQUIREMENTS

Specific nutrient requirements must be met to realize the performance potential of farmed aquatic animals. However, for many species of aquatic animals, the nutrient requirements are not well defined, and where they are defined, the information is not relevant to the fast-growing strains and high-performance feeds available today. There is also a need for feeding practices and strategies that deliver nutrients to meet those requirements to optimize production and also to minimize nutrient losses to the environment.

To meet these needs, ARS is conducting research on techniques to determine nutrient requirements. Researchers are determining whether genetic improvement in production traits such as growth may affect nutrient requirements. Other research efforts include the development of methods to utilize genomic tools to evaluate metabolic responses to varied levels of nutrients.

Selected Accomplishments

Classification and quantification of phospholipids and their dietary effect on lipid composition in shrimp (Litopenaeus vannamei)

It is important to identify and quantify the types of phospholipids that are essential nutrients for shrimp growth and survival. Through liquid chromatography analysis, the Oceanic Institute's ARS researchers were able to identify and quantify 11 types of phospholipids in shrimp. Researchers found that the majority of the total lipid fractions found in shrimp tail muscle consisted of phospholipids. This research has been central to establishing the nutritional requirement of phospholipids for shrimp and demonstrating the high nutrition value and health benefit of this product for consumers.

Forster, I.P., Bechtel, P., Dominy, W.G., Lane, S., Avena, R., Ju, Z.Y., and Conquest L. 2011. Use of fish hydrolysates and fish meal byproducts of the Alaskan fishing industry in diets for pacific white shrimp *Litopenaeus vannamei*. North American Journal of Aquaculture 73:288–295. http://dx.doi.org/10.1080/15222055.2011.598371

Forster, I.P., Dominy, W.G., Obaldo, L.G., Hartnell, G.F., Sanders, E.F., Hickman, T.C., Ruebelt, M.C. 2011. The effect of soybean oil containing stearidonic acid on growth performance, n-3 fatty acid deposition and sensory characteristics of pacific white shrimp (*Litopenaeus vannamei*). Aquaculture Nutrition 17:200–213. http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2095.2009.00726.x/abstract

Forster, I.P., Dominy, W.G., and Obaldo, L.G. 2010. Effect of diet manipulation on cholesterol and docosahexaenoic acid levels in shrimp, *Litopenaeus vannamei*. Journal of the World Aquaculture Society 41:240–249. http://dx.doi.org/10.1111/j.1749-7345.2010.00361.x

Ju, Z., Forster, I., Dominy, W., and Lawrence, A. 2012. Classification and quantification of phospholipids and dietary effects on lipid composition in pacific white shrimp *Litopenaeus vannamei*. North American Journal of Aquaculture 74:221–229. http://dx.doi.org/10.1080/15222055.2011.579035

Summer diets for hybrid striped bass

High summertime feeding rates can result in concentrations of ammonia in pond water that are toxic to hybrid striped bass. In response, farmers may curtail feeding or switch to a lower protein feed, both of which reduce production. With input from Mississippi-based hybrid striped bass producer Nature's Catch, ARS scientists at the Stuttgart National Aquaculture Research Center demonstrated that a diet with higher digestible protein and fat that was supplemented with deficient amino acids maximized growth and nutrient retention and reduced ammonia waste production (see Figures 6 and 7). Fish feed manufacturers are using new digestibility coefficients, also recently developed at the Stuttgart laboratory, in their diet formulations for hybrid striped bass, feeding the new summer diet formulation, and improving summer production.

Figure 6. Biological science technician Rebecca Jacobs preparing to feed experimental diets to hybrid striped bass in circular tanks.

Figure 7. Hybrid striped bass consuming the experimental feeds.

Rawles, S.D., Gibson Gaylord, T., Scott Snyder, G., and Freeman, D.W. 2010. The influence of protein and energy density in commercial diets on growth, body composition, and nutrient retention of sunshine bass, *Morone chrysops* ♀ × *Morone saxatilis* ♂, reared at extreme temperature. Journal of the World Aquaculture Society 41(Suppl 2):165–178. http://hdl.handle.net/10113/44328

Rawles, S.D., Green, B.W., Gaylord, T.G., Barrows, F.T., McEntire, M.E., and Freeman, D.W. 2012. Response of sunshine bass (*Morone chrysops* × *M. saxatilis*) to digestible protein/dietary lipid density and ration size at summer culture temperatures in the southern United States. Aquaculture 356-357:80–90. http://hdl.handle.net/10113/55623

A critical component to reducing reliance on fish meal in aquafeeds

As the cost of fish meal increases and its inclusion in the diet of carnivorous fish such as rainbow trout decreases, several minerals become limiting. ARS scientists in Aberdeen, Idaho, conducted a study that demonstrated that rainbow trout fed plant-based, fish meal–free diets, required supplementation with potassium chloride, sodium chloride, and magnesium oxide and myoinositol to improve feed efficiency and fish health. These minerals and vitamins are abundant in fish meal, but limited in plant-based proteins meals. Supplementation of these nutrients now occurs in all fish meal–free trout feeds for both research and commercial production of ARS formulations (Figure 8). In addition, feed processing and nutritional research have led to many improvements in fish meal over the past 2 decades; however, the last open-formula vitamin premix for trout was released 20 years ago. ARS scientists in Aberdeen, Idaho, have developed a vitamin premix, ARS 702, which was adjusted for losses during extrusion processing and was designed for use in alternative feeds being manufactured today. The vitamin premix formulation is now being commercially produced, fed to a variety of species, and used by at least three commercial feed manufacturers in feeds for a variety of species, including the ARS plant-based feed for trout with production of hundreds of tons of feed per year. These key improvements in understanding nutrient requirements of aquatic animals have led to greater profitability in aquaculture because ingredient choice has expanded as essential nutrients were recognized and included in the premix.

Figure 8. Nutritionist Rick Barrows preparing vitamin premixes for rainbow trout feed.

Barrows, F.T., Gaylord, T.G., Sealey, W.M., Porter, L., and Smith, C.E. 2009. Supplementation of plant-based diets for rainbow trout, *Oncorhynchus mykiss* with macro-minerals and inositol. Aquaculture Nutrition on-line doi:10.1111/j.1365-2095.2009.00717.x http://hdl.handle.net/10113/55447

Barrows, F.T., Gaylord, T.G., Sealey, W.M., Porter, L., Smith, C.E. 2008. The effect of vitamin premix in extruded plant-based and fish meal based diets on growth efficiency and health of rainbow trout, *Oncorhynchus mykiss*. Aquaculture 283:148–155. http://dx.doi.org/10.1016/j.aquaculture.2008.07.014

Gaylord, T.G., Barrows, F.T., and Rawles, S.D. 2008. Apparent digestibility of gross nutrients from feedstuffs in extruded feeds for rainbow trout, *Oncorhynchus mykiss*. Journal of the World Aquaculture Society 39:827–834. http://hdl.handle.net/10113/30491

PROBLEM STATEMENT 3B. EVALUATE THE NUTRITIONAL VALUE OF ALTERNATIVE SOURCES OF PROTEIN AND LIPID

Presently, aquaculture feeds, especially those used for carnivorous species, are heavily dependent on fish meal and fish oils to meet their critical protein, lipid, and energy requirements. However, the global supply of fish meal will likely remain static or decline because capture fisheries have reached or exceeded maximum sustainable yields. Adding to this supply limitation is the increased competition among consumers for these products. Thus, for the U.S. aquaculture feed manufacturing, animal production, and processing industries to expand and remain competitive and cost-effective, sustainable sources of protein and oil must be identified and developed.

The nutritional value of feedstuffs depends on the bioavailability of nutrients they contain, the presence of antinutritional factors, and interactions among the ingredients. Thus, researchers are improving the nutritional value of alternative protein sources through the use of better processing methods such as heat treatment, removal of antinutritional factors, and use of additives (enzymes, essential amino acids, and palatability enhancers).

Selected Accomplishments

Development of a standardized nutrient digestibility database for traditional and alternative feed ingredients

To develop new ingredients for fish feed diets, the availability of the nutrients in the ingredients, known as digestibility, need to be determined. This information must be generated empirically by testing the ingredients on fish; there are no laboratory methods with commercial processing to determine digestibility. ARS scientists in Hagerman, Idaho, and Stuttgart, Arkansas, compiled a first-of-its-kind database containing digestibility coefficients for macronutrients, amino acids, and minerals for 80 ingredients for rainbow trout and 26 ingredients for hybrid bass. This information has been requested by commercial aquafeed companies and ingredient suppliers both nationally and internationally because it promotes more efficient ingredient substitution, and, ultimately, will lead to less nutrient waste and lower-cost feeds that meet the highest nutrient requirement standards for the aquaculture industry.

Gaylord, T.G. and Barrows, F. 2009. Multiple amino acid supplementations to reduce dietary protein in plant-based rainbow trout, *Oncorhynchus mykiss*, feeds. Aquaculture 287:180-184. http://hdl.handle.net/10113/28815

Gaylord, T.G., Barrows, F., and Rawles, S.D. 2009. Apparent amino acid availability from feedstuffs in extruded diets for rainbow trout *Oncorhynchus mykiss*. Aquaculture Nutrition 16: 400–406. http://hdl.handle.net/10113/43859

Gaylord, T.G., Barrows, F., Rawles, S.D., Liu, K., Bregitzer, P.P., Hang, A., Obert, D.E., and Morris, C.F. 2009. Apparent digestibility of nutrients in extruded diets from cultivars of barley and wheat selected for nutritional quality in rainbow trout *Oncorhynchus mykiss*. Aquaculture Nutrition 15:306-312. http://hdl.handle.net/10113/30491

Metts, L.S., Rawles, S.D., Brady, Y.J., Thompson, K.R., Gannam, A.L., Twibell, R.G., and Webster, C.D. 2011. Amino acid availability from selected animal- and plant- derived feedstuffs for market-size sunshine bass (*Morone chrysops* × *Morone saxatilis*). Aquaculture Nutrition 17:e123–e131. http://hdl.handle.net/10113/55629

Thompson, K.R., Rawles, S.D., Metts, L.S., Smith, R., Wimsatt, A., Gannam, A.L., Twibell, R.G., Johnson, R.B., Brady, Y.J., and Webster, C.D. 2008. Digestibility of dry matter, protein, lipid, and organic matter of two fish meals, two poultry by-product meals, soybean meal, and distiller's dried grains with solubles in practical diets for sunshine bass, *Morone chrysops* × *M. saxatilis.* Journal of the World Aquaculture Society 39:352–363. http://dx.doi.org/10.1111/j.1749-7345.2008.00174.x

Evaluation of alternative ingredients to meet nutritional needs of aquatic species

For aquaculture to expand, alternate sources of protein and oil must be developed and production efficiencies must be increased. New diet formulations in which fish meal and fish oil are replaced with alternative ingredients can reduce fish feed cost and variability, and move the aquafeed industry to a more sustainable foundation. However, feeding some alternative ingredients can lead to reduced feed intake and lower feed digestibility, which can cause metabolic alterations, health problems, and reduced growth rates. ARS scientists have developed a process to thoroughly evaluate each alternative ingredient to determine its potential effect on fish performance. One part of this program has been the development of a database of nutrient digestibilities of commonly used and alternative feed ingredients, which is now available to all interested groups (www.ars.usda.gov/Main/docs.htm?docid=21897). Some ingredients won't support good fish performance in their current form, so they must be modified to improve nutrient profile, reduce antinutrients, and meet specifications for aquafeeds by genetic selection of the plant, postharvest processing, and feed formulation and processing. The extensive work by ARS aquaculture laboratories in Alabama, Arkansas, Idaho, and Maine, along with top national and international collaborators, has made this an area of unique strength within USDA. The incorporation of new ingredients has accelerated, partly due to the methods and evaluations being conducted. Furthermore, the ingredient testing by commercial start-ups and potential start-ups has grown steadily over the past 5 years; ingredients include feed grade sources of grain and oil seed concentrates (i.e., soy, barley, oat), insect larvae, single-celled fermentation products, fisheries processing co-products, and co-products from biofuel manufacturing and human food processing. ARS has licensed one protein concentrate process to an industry partner.

Burr, G.S., Wolters, W.R., Barrows, F.T., and Hardy, R.W. 2012. Replacing fishmeal with blends of alternative proteins on growth performance of rainbow trout (*Oncorhynchus mykiss*), and early or late stage juvenile Atlantic salmon (*Salmo salar*). Aquaculture 334–337:110–116. http://hdl.handle.net/10113/55765

Snyder, G.S., Gaylord, T.G., Barrows, F.T., Overturf, K., Cain, K.D., Hill, R.A., and Hardy, R.W. 2012. Effects of carnosine supplementation to an all-plant protein diet for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 338–341:72–81. http://handle.nal.usda.gov/10113/55852

Sealey, W.M., Gaylord, T.G., Barrows, F.T., Tomberlin, J.K., McGuire, M.A., Ross, C., and St-Hilaire, S. 2011. Sensory analysis of rainbow trout, *Oncorhynchus mykiss,* fed enriched black soldier fly prepupae, *Hermetia illucens.* Journal of the World Aquaculture Society 42:34–45. http://hdl.handle.net/10113/55458

Sealey, W.M., Hardy, R.W., Barrows, F.T., Pan, Q., and Stone, D.A.J. 2011. Evaluation of 100% fish meal substitution with chicken concentrate, protein poultry by-product blend, and chicken and egg concentrate on growth and disease resistance of juvenile rainbow trout, *Oncorhynchus mykiss*. Journal of the World Aquaculture Society 42:46–55. http://dx.doi.org/10.1111/j.1749-7345.2010.00442.x

Lee, K.J., Powell, M.S., Barrows, F.T., Smiley, S., Bechtel, P., and Hardy, R.W. 2010. Evaluation of supplemental fish bone meal made from Alaska seafood processing byproducts and dicalcium phosphate in plant protein based diets for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 302:248–255. http://hdl.handle.net/10113/40981

Rawles, S.D., Gaylord, T.G., McEntire, M.E., and Freeman, D.W. 2009. Evaluation of poultry by-product meal in commercial diets for hybrid striped bass, *Morone chrysops* ♀ × *Morone saxatilis* ♂, in pond production. Journal of the World Aquaculture Society 40:141–156. http://hdl.handle.net/10113/30490

Sealey, W.M., Barrows, F.T., Smith, C.E., Overturf, K., and LaPatra, S.E. 2009. Soybean meal level and probiotics in first feeding fry diets alter the ability of rainbow trout *Oncorhynchus mykiss* to utilize high levels of soybean meal during grow-out. Aquaculture 293:195–203. http://hdl.handle.net/10113/33872

Sealey, W.M., Barrows, F.T., Hang, A., Johansen, K.A., Overturf, K,. LaPatra, S.E., and Hardy, R.W. 2008. Evaluation of the ability of barley genotypes containing different amounts of β-glucan to alter growth and disease resistance of rainbow trout *Oncorhynchus mykiss*. Animal Feed Science Technology 141:115–128. http://dx.doi.org/10.1016/j.anifeedsci.2007.05.022

Distillers dry grains with solubles and their use in fish feed and affects on immunity

A plentiful co-product of ethanol biofuel production, dried distillers grains with solubles (DDGS) are very palatable to fish, and a level of 20 percent can be included in catfish or tilapia diets as replacement for soybean meal and corn in a diet containing 8 percent fish meal (Figure 9). With lysine supplementation, DDGS levels can be increased up to 40 percent. Specific immune responses against fish pathogens, measured as lysozyme activity and hemolytic complement activity, were enhanced by 10 to 40 percent in catfish and tilapia fed diets containing DDGS. The Auburn Aquatic Animal Health Research Unit, in Auburn, Alabama, has led the examination of both the nutritional value of new ingredients and the effects on fish health and immunity. This is a unique expertise that has provided deep scientific information on diet by immune system interactions.

Figure 9. Agricultural engineer Kurt Rosentrater and technician Sharon Nichols examine the quality of pelleted animal feed made from dried distiller's grains with solubles (DDGS).

Hu, B., Ferrell, M., Lim, C.E., and Davis, D.A. 2012. Evaluation of traditional diet and corn gluten feed substituted alternative diet for pond-raised hybrid catfish on production and xanthophyll level. Aquaculture 354–355:22–26. http://handle.nal.usda.gov/10113/55867

Li, E., Lim, C., Klesius, P., and Cai, C. 2012. Enhancement effects of dietary wheat distiller's dried grains with solubles on growth, immunity, and resistance to *Edwardsiella ictaluri* challenge of channel catfish, *Ictalurus punctatus*. Journal of the World Aquaculture Society 43:814–827. http://dx.doi.org/10.1111/j.1749-7345.2012.00605.x

Vechklang, K., Lim, C., Boonanuntanasarn, S., Welker, T., Ponchunchuwong, S., Klesius, P.H., and Wanapu, C. 2012. Growth performance and resistance to *Streptococcus iniae* of juvenile Nile tilapia (*Oreochromis niloticus*) fed diets supplemented with GroBiotic-A and brewtech dried brewers yeast. Journal of Applied Aquaculture 24:183–198. http://dx.doi.org/10.1080/10454438.2012.678786

Welker, T.L., Lim, C., Yildirim-Aksoy, M., and Klesius, P.H. 2012. Use of diet crossover to determine the effects of β-glucan supplementation on immunity and growth of Nile tilapia, *Oreochromis niloticus*. Journal of the World Aquaculture Society 43:335–348. http://handle.nal.usda.gov/10113/55857

Li, E., Lim, C., Cai, C., and Klesius, P.H. 2011. Growth response and resistance to *Streptococcus iniae* of Nile tilapia, *Oreochromis niloticus*, fed diets containing different levels of wheat distiller's dried grains with solubles with or without lysine supplementation. Animal Feed Science Technology 170:246–255. http://dx.doi.org/10.1016/j.anifeedsci.2011.09.002

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Welker, T.L., Lim, C., Yildirim-Aksoy, M., and Klesius, P.H. 2011. Effect of short-term feeding duration of diets containing commercial whole-cell yeast or yeast subcomponents on immune function and disease resistance in channel catfish, *Ictalurus punctatus*. Journal of Animal Physiology and Animal Nutrition 96:159–171. http://hdl.handle.net/10113/55444

Evans, J.J., Pasnik, D.J., Aksoy, M., Lim, C.E., and Klesius, P.H. 2010. Histologic changes in channel catfish, *Ictalurus punctatus* Rafinesque, fed diets containing graded levels of gossypol-acetic acid. Aquaculture Nutrition 16:385-391.http://hdl.handle.net/10113/55434

Lim, C.E., Aksoy, M., and Klesius, P.H. 2009. Growth response and resistance to *Edwardsiella ictaluri* of channel catfish (*Ictalurus punctatus*) fed diets containing distiller's dried grains with solubles. Journal of the World Aquaculture Society 40:182-192. http://hdl.handle.net/10113/32030

Shelby, R.A., Lim, C., Yildirim-Aksoy, M., Welker, T.L., and Klesius, P.H. 2009. Effects of yeast oligosaccharide diet supplements on growth and disease resistance in juvenile Nile tilapia, *Oreochromis niloticus.* Journal of Applied Aquaculture 21:61–71. http://hdl.handle.net/10113/28091

Improved soy concentrate for aquafeeds

One emerging area in the global feed industry is development of alternative feed sources for aquafeed. Among all the alternative sources for feed protein, soybean is first considered because it is abundant and has a high protein content.

Although defatted soy meal is the most widely used ingredient for animal feeds, soy protein concentrate (SPC), due to its higher protein level and removal of antinutrients, can be used at higher inclusion levels. Yet, a major constraint limiting soy protein concentrate use in fish diets has been its high production cost. ARS researchers in Aberdeen, Idaho, have developed a modified method for producing feed-grade SPC at a significantly reduced cost (estimated at 40 percent reduction compared with current production of food-grade SPC) while maintaining the same nutritional quality as food-grade SPC, as evidenced by comparable composition profile and performance of fish feeding trials (Figure 10).

The researchers, working with a partner through a Cooperative Research and Development Agreement, have successfully completed laboratory testing, and may license the technology for commercialization. Availability of a feed-grade SPC will make fish meal–free diets more cost-effective and the aquaculture industry more sustainable. This is an example of ingredient modification research that is increasing both the variety and quality of ingredients available for aquafeeds. Another example of a product currently being investigated is a palatable barley protein concentrate.

Figure 10. Food-grade soy concentrate (left) compared with ARS feed-grade soybean concentrate (right).

Han, J. and Liu, K. 2010. Changes in composition and amino acid profile during dry grind ethanol processing from corn and estimation of yeast contribution toward DDGS proteins. Journal of Agricultural and Food Chemistry 58:3430–3437. http://dx.doi.org/10.1021/jf9034833

Liu, K., and Han, J. 2011. Changes in mineral concentrations and phosphorus profile during dry-grind processing of corn into ethanol. Bioresource Technology 102:3110–3118. http://hdl.handle.net/10113/55460

Moreau, R.A., Liu, K., Winkler-Moser, J.K., and Singh, V. 2011. Changes in lipid composition during dry grind ethanol processing of corn. Journal of the American Oil Chemists' Society 88:435–442. http://dx.doi.org/10.1007/s11746-010-1674-y

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Liu, K., Barrows, F.T., and Obert, D. 2009. Dry fractionation methods to produce barley meals varying in protein, beta-glucan, and starch contents. Journal of Food Science 74:C487–C499. http://hdl.handle.net/10113/34575

Liu, K. 2008. Particle size distribution of distillers dried grains with solubles (DDGS) and relationships to compositional and color properties. Bioresource Technology 99:8421–8428. http://dx.doi.org/10.1016/j.biortech.2008.02.060

COMPONENT 4: IMPROVING HEALTH AND WELFARE OF AQUATIC ANIMALS

The United States aquaculture industries need to control endemic, emerging, and catastrophic diseases that result in aquatic animal production losses and restrict the movement of aquatic animals out of affected regions. Although progress in aquatic animal health has been made, significant animal loss to disease still occurs, at great cost to producers. Loss of more than 30 percent of a population from the juvenile stage to harvest is not rare for aquaculture species and represents a huge economic impact. Health management strategies, technologies, and biosecurity plans that are safe for the environment and consumers are necessary to reduce disease-related losses.

The lack of validated technologies for early and rapid detection, prevention, and treatment of diseases in production systems has hindered the growth and competitiveness of the U.S. aquaculture industry. Validated diagnostic tools that can be used in production systems to rapidly detect disease agents are required. Effective control strategies and therapeutics are required to manage outbreaks, given that only a few drugs are currently approved for treating sick fish. Further research is also needed to provide new, effective vaccines and methods for mass vaccination of aquatic animals and to supplement the vaccines against *Edwardsiella ictaluri*, and *Flavobacterium columnare* that are currently available and in use by aquatic animal farmers and hatchery managers.

This vaccine work is benefiting greatly from new molecular tools. For example, sequencing the pathogens to identify regions of similarity among many strains of the pathogen is aiding in producing vaccines that offer broad protection. Additionally, molecular tools are enabling researchers to examine thousands of genes simultaneously and use genetic maps to localize genomic regions associated with innate and acquired immunity.

PROBLEM STATEMENT 4A. IDENTIFY GENES INVOLVED IN IMMUNITY AND ANIMALS WITH DISEASE-RESISTANT PHENOTYPES

ARS is identifying molecular pathways of the host that are involved in innate and acquired immune responses and of the pathogens involved in transmission, virulence, and recognition by the host. Additionally, experiments on animals with divergent response to disease challenge are revealing the genetic sources of variation that correlate with innate and/or acquired immune status.

To identify these molecular pathways, researchers are using microarrays, quantitative polymerase chain reaction, and massively parallel sequencing technologies to evaluate immune responses of the skin, gut, and other epithelial tissues to economically important pathogens. Additionally, animals with resistant/susceptible phenotypes are being investigated to understand the mechanisms and location of genes (quantitative trait locus, or QTL) leading to reduced mortality.

Selected Accomplishments

Assessment of catfish susceptibility to Flavobacterium columnare *and virulence of genetic types in aquaculture species*

Critical information is lacking on the nature of the immune response to and virulence of *F. columnare* in aquaculture fish species (Figure 11). Genetic variation in resistance to *F. columnare* was demonstrated in channel catfish, and this may allow for identification of immune genes correlated with resistance. Isolates of *F. columnare* recovered from diseased fish were typed using a genetic typing method. Isolates recovered from channel catfish were shown to belong to genotypes I, II, and III, and isolates from rainbow trout only belonged to genotype I.

Figure 11. Yellow rhizoid colonies of *Flavobacterium columnare*.

An association between virulence and genotype was demonstrated in challenge studies with both fish species, with the genotype II isolates exhibiting increased virulence compared with those of genotype I. *F. columnare* was shown to be attracted to skin mucus via chemotaxis, and results suggested that genotype II isolates had a greater chemotactic response than genotype I isolates. The chemotactic response by *F. columnare* may be an important first step in the colonization of susceptible fish. Using this knowledge, ARS scientists established differential susceptibility in channel catfish families to *F. columnare* and demonstrated the genetic type of the pathogen is important in assessing virulence to aquaculture species. The introduction of genotype II *F. columnare* isolates to rainbow trout production facilities may negatively affect production by increasing mortality due to *F. columnare*.

LaFrentz, B.R., Shoemaker, C.A., Booth, N.J., Peterson, B.C., and Ourth, D.D. 2012. Spleen index and mannose-binding lectin levels in four channel catfish families exhibiting different susceptibilities to *Flavobacterium columnare* and *Edwardsiella ictaluri*. Journal of Aquatic Animal Health 24:141–147. http://hdl.handle.net/10113/55500

LaFrentz, B.R., LaPatra, S.E., Shoemaker, C.A., and Klesius, P.H. 2012. Reproducible challenge model to investigate the virulence of *Flavobacterium columnare* genomovars in rainbow trout *Oncorhynchus mykiss.* Diseases of Aquatic Organisms 101:115–122. http://dx.doi.org/10.3354/dao02522

Shoemaker, C.A., Klesius, P.H., Drennan, J.D., and Evans, J.J. 2011. Efficacy of a modified live *Flavobacterium columnare* vaccine in fish. Fish and Shellfish Immunology 30:304–308. http://hdl.handle.net/10113/55545

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Shoemaker, C.A., Olivares-Fuster, O., Arias, C.R., and Klesius, P.H. 2008. *Flavobacterium columnare* genomovar influences mortality in channel catfish (*Ictalurus punctatus*). Veterinary Microbiology 127:353–359. http://dx.doi.org/10.1016/j.vetmic.2007.09.003

Klesius, P.H., Shoemaker, C.A., and Evans, J.J. 2008. *Flavobacterium columnare* chemotaxis to channel catfish mucus. Federation of European Microbiological Societies. Microbiological Letters 288:216-220. http://hdl.handle.net/10113/43713

Characterization of the genetic response of catfish to vaccination

Catfish immunized with AquaVac COL vaccine against *F. columnare* have effective protection against this economically important bacterial pathogen. The nature of the genetic response of fish immunized with vaccine is important to the future development of new fish vaccines. A total of 32 genes were isolated from a channel catfish 10 minutes post vaccination. Of those, 28 were found to be up-regulated in at least one vaccinated fish. Of the 28 up-regulated genes, 12 were consistently up-regulated at least twofold in vaccinated fish compared with unvaccinated control fish. The 28 induced genes have putative functions in the following five major categories: *1)* immune response (46 percent); *2)* signal transduction (21 percent); *3)* transcriptional regulation (11 percent); *4)* cell maintenance (11 percent); and *5)* unknown (11 percent). In a related study on the genetic response of channel catfish to AquaVac ESC vaccine, global gene expression was elucidated in channel catfish after immersion vaccination with the AquaVac ESC vaccine developed by ARS scientists.

ESC (enteric septicemia of catfish) is the most prevalent disease in farm-raised channel catfish. ESC is caused by *Edwardsiella ictaluri*, a Gram negative bacterium. The vaccine protects channel catfish against ESC with a single bath immersion. ARS researchers in Auburn, Alabama, performed microarray analysis and found that 52 genes were up-regulated by the vaccine at 48 hours post vaccination, whereas 129 genes were down-regulated.

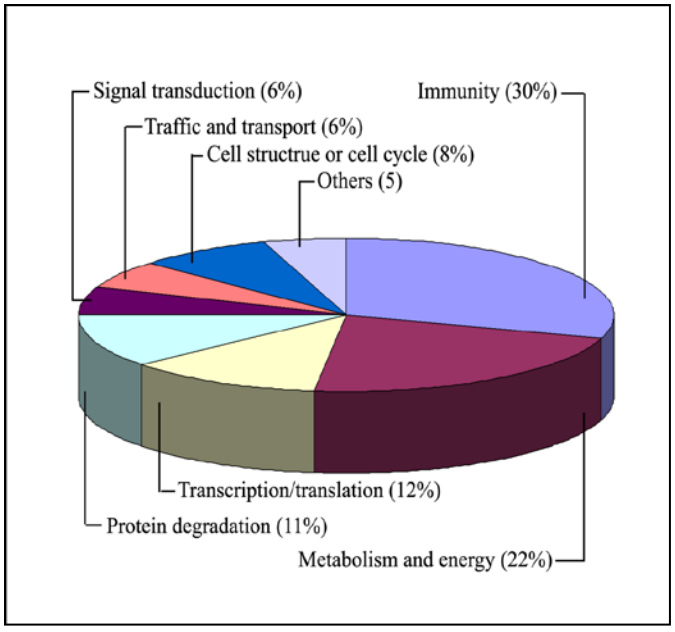

Figure 12. Pie chart based on the classification of functionally known genes up-regulated by the challenge. The pie chart represents the distribution of the 167 up-regulated genes according to their putative biological function on the basis of similarity with known genes.

When vaccinated fish were challenged with *E. ictaluri*, a total of 167 unique transcripts were found to be up-regulated, whereas 40 were down-regulated. The 167 up-regulated transcripts (Figure 12) represent genes with putative functions in the following eight major categories: *1)* immunity (30 percent); *2)* metabolism and energy production (22 percent); *3)* transcription or translation (12 percent); *4)* protein degradation (11 percent); *5)* signal transduction (6 percent); *6)* traffic and transport (6 percent); *7)* cell structure or cell cycle (8 percent); and *8)* others (5 percent). The 40 down-regulated transcripts represent genes with putative functions in the following six major categories: *1)* metabolism (27.5 percent);

2) immunity (17.5 percent); *3)* cell structure (17.5 percent); *4)* cell motility (10 percent); *5)* signal transduction (15 percent); and *6)* others (12.5 percent). Microarray analysis revealed that lysozyme C was up-regulated the most (70-fold) in vaccinated fish at 48 hours post challenge with virulent *E. ictaluri*. These studies have resulted in the identification of genes that play important roles in fish immunity against diseases, which will provide novel strategies to develop efficacious vaccines to benefit the aquaculture industry.

Bebak, J., Matthews, M., and Shoemaker, C. 2009. Survival of vaccinated, feed-trained largemouth bass fry (*Micropterus salmoides floridanus*) during natural exposure to *Flavobacterium columnare*. Vaccine 27:4297–4301. http://hdl.handle.net/10113/33732

Bebak, J. and Wagner, B. 2012. Use of vaccination against enteric septicemia of catfish and columnaris disease by the U.S. catfish industry. Journal of Aquatic Animal Health 24:30–36. http://handle.nal.usda.gov/10113/55878

LaFrentz, B.S., LaPatra, S.E., Shoemaker, C.A., and Klesius, P.H. 2012. Reproducible challenge model to investigate the virulence of *Flavobacterium columnare* genomovars in rainbow trout *Oncorhynchus mykiss*. Diseases of Aquatic Organisms 101:115–122. http://dx.doi.org/10.3354/dao02522

Pridgeon, J.W. and Klesius, P.H. 2010. Identification and expression profile of multiple genes in channel catfish fry 10 min after modified live *Flavobacterium columnare* vaccination. Veterinary Immunology and Immunopathology 138:25–33. http://hdl.handle.net/10113/55440

Pridgeon, J.W., Mu, X., and Klesius, P.H. 2012. Expression profiles of seven channel catfish antimicrobial peptides in response to *Edwardsiella ictaluri* infection. Journal of Fish Diseases 35:227–237. http://hdl.handle.net/10113/55455

Pridgeon, J.W., Russo, R., Shoemaker, C.A., and Klesius, P.H. 2010. Identification of in-vitro upregulated genes in a modified live vaccine strain of *Edwardsiella ictaluri* compared to a virulent parent strain. Comparative Immunology Microbiology and Infectious Diseases 33:e31–e40. http://hdl.handle.net/10113/55440

Pridgeon, J.W., Yeh, H., Shoemaker, C.A., Klesius, P.H. 2012. Global transcription analysis of vaccinated channel catfish following challenge with virulent *Edwardsiella ictaluri. Vet Immunol Immunopathol.* 146:53–61. http://hdl.handle.net/10113/55456

Pridgeon, J.W., Yeh, H., Shoemaker, C.A., Mu, X., and Klesius, P.H. 2012. Global gene expression in channel catfish after vaccination with an attenuated Edwardsiella ictaluri. Fish and Shellfish Immunology 32:524–533. http://hdl.handle.net/10113/55457

Shoemaker, C.A., Klesius, P.H, Drennan, J.D., and Evans, J.J. 2011. Efficacy of a modified live *Flavobacterium columnare* vaccine in fish. Fish and Shellfish Immunology 30:304–308. http://hdl.handle.net/10113/55545

The genetic basis for Biotype 2 disease caused by Yersinia ruckeri

A new variant of the bacterium *Yersinia ruckeri*, the causative agent of enteric redmouth (Figure 13), is an emerging disease problem in U.S. and European finfish aquaculture. The new variant is known as biotype 2 (BT2) and its emergence has been associated with failure of *Y. ruckeri* vaccination.

Scientists at the National Center for Cool and Cold Water Aquaculture identified mutations across four different strains of the variant that cause the loss of motility and enzyme (lipase) secretion, which defines BT2 *Y. ruckeri* strains. All four mutations were in genes that encode essential components of the flagellar apparatus, which allows the bacterium to swim and secrete certain enzymes. These results demonstrate that the BT2 phenotype has emerged independently

several times in the US and throughout Europe resulting in distinct bacterial lineages. This research is critical for understanding *Y. ruckeri* vaccine failure and for developing and evaluating improved vaccines or other management practices aimed at controlling this emerging problem and has led to change in the formulation of the enteric redmouth vaccine used in the North Carolina trout farming industry. This work also provides a powerful experimental paradigm for understanding pathogen evolution and its impact on vaccine failure.

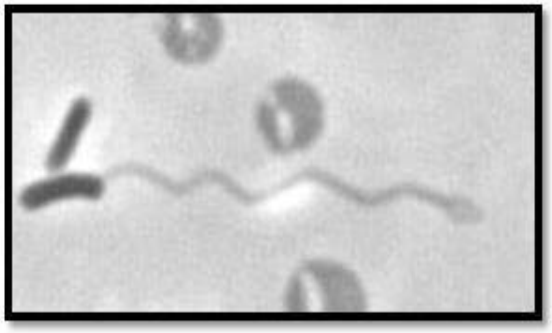

Figure 13. Flagellar stained micrograph of *Y. ruckeri* cells. The cell at bottom displays the flagellar swimming apparatus indicative of the Biotype 1 phenotype; the cell above displays the nonmotile, Biotype 2 phenotype.

Evenhuis, J.P., LaPatra, S.E., Verner-Jeffreys, D.W., Dalsgaard, I., and Welch, T.J. 2009. Identification of flagellar motility genes in Yersinia ruckeri by transposon mutagenesis. Applied and Environmental Microbiology 75:6630–6633. http://hdl.handle.net/10113/55558

Haig, S.J., Davies, R.L., Welch, T.J., Reese, R.A., and Verner-Jeffreys, D.W. 2011. Comparative susceptibility of Atlantic salmon and rainbow trout to *Yersinia ruckeri:* relationship to O antigen serotype and resistance to serum killing. Veterinary Microbiology 147:155–161. http://hdl.handle.net/10113/55552

Verner-Jeffreys, D.W., Haig, S.J., Welch, T.J., Pond, M.J., Stone, D., Davies, R.L., and Gardiner, R. 2011. Characterisation of a serotype O1 *Yersinia ruckeri* isolate from the Isle of Man: further evidence that O antigen serotype is not a reliable indicator of virulence. Bulletin of the European Association of Fish Pathologists 31:86–91. http://handle.nal.usda.gov/10113/55805

Welch, T.J. 2011. Rapid genotyping assays for the identification and differentiation of *Yersinia ruckeri* biotype 2 strains. Letters in Applied Microbiology 53:383–385. http://onlinelibrary.wiley.com/doi/10.1111/j.1472-765X.2011.03114.x/pdf

Welch, T.J., Verner-Jeffreys, D.W., Dalsgaard, I., Wiklund, T., Evenhuis, J., Garcia Cabrera, J.A., Hinshaw, J.M., Drenna, J.D., and Lapatra, S.E. 2011. Independent emergence of *Yersinia ruckeri* biotype 2 in the United States and Europe. Applied and Environmental Microbiology 77:3493–3499. http://handle.nal.usda.gov/10113/55803

Wheeler, R.W., Davies, R.L., Dalsgaard, I., Garcia, J. Welch, T.J., Waqley, S., Bateman, K.S., and Verner-Jeffrey, D.W. 2009. *Yersinia ruckeri* biotype 2 isolates from mainland Europe and the UK likely represent different clonal groups. Diseases of Aquatic Organisms 84:25–33. http://handle.nal.usda.gov/10113/55804

PROBLEM STATEMENT 4B: DETECTION AND CONTROL OF PATHOGENS

To prevent the introduction and spread of harmful diseases, the domestic and international trade of aquatic animals needs rapid, automated, and accurate tests to demonstrate that animals, seed stocks, and products are free from harmful pathogens. Having an accurate determination of preclinical infections (i.e., prior to disease) will enhance our ability to determine disease risk potential and develop earlier preventive measures to reduce the impact of emerging or catastrophic diseases in the United States.

Molecular tools, including pathogen genome sequencing, are being used to develop validated, rapid and automated microbiological and immunological methods to detect infectious and noninfectious disease agents and toxins in aquatic animals. Researchers are working to identify

compounds that can be used as therapeutics, create programs to obtain regulatory approval of potential therapeutics, and develop methods of mass delivery.

Selected Accomplishments

Laboratory challenge models to assess pathogenesis of Viral Hemorrhagic Septicemia (VHS) disease

VHS emerged as a new disease threat in the Midwest in 2003. A lack of models and methods hampered development of countermeasures against this disease. Over the past several years, collaborations among scientists from ARS, University of Wisconsin-Milwaukee, and U.S. Geological Survey led to development of a standard challenge model for the strain of VHS found using yellow perch. Standardized exposure route, viral dose range, and exposure time for

subsequent studies to evaluate differences in yellow perch broodstock VHS susceptibility have been determined. These methods will enable systematic testing of vaccines and other countermeasures.

Olson, W., Emmenegger, E., Glenn, J., Winton, J., and Goetz, F. 2013. Comparative susceptibility among three stocks of yellow perch, *Perca flavescens* (Mitchill), to viral haemorrhagic septicaemia virus strain IVb from the great lakes. Journal of Fish Diseases http://dx.doi.org/10.1111/jfd.12068

Identification of a novel and significant disease outbreak in farmed rainbow trout and development of an autogenous vaccine

Pathogens emerge in farmed aquatic systems much more frequently than they do in terrestrial systems, and emerging pathogens are a significant threat to U.S. aquaculture. An ARS researcher at the NCCCWA in Leetown, West Virginia, isolated a new Gram positive bacterial pathogen that is causing significant loss of rainbow trout in North Carolina. The pathogen was found to belong to the *Weissella* species and is associated with recent disease outbreaks in farmed rainbow trout in both Brazil and China. This is the first report of this pathogen in the United States. At the request of trout farmers in North Carolina, the NCCCWA developed and validated an effective autogenous vaccine that is now in commercial production and in use in some North Carolina farms. Early pathogen detection and the rapid development and implementation of a vaccine are aiding control efforts and reducing the likelihood of further pathogen dissemination in the United States.

Hydrogen peroxide treatments for control of fungus on catfish eggs

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

Marchand, P., Phan, T., Straus, D.L., Farmer, B.D., Stüber, A., and Meinelt, T. 2012. Reduction of in vitro growth in *Flavobacterium columnare* and *Saprolegnia parasitica* by products containing peracetic acid. Aquaculture Research 43:1861–1866. http://dx.doi.org/10.1111/j.1365-2109-2011-02995.x

Mitchell, A.J., Radomski, A.A., Straus, D.L., and Carter, R. 2009. The effect of hydrogen peroxide on the hatch rate and *Saprolegnia* spp. infestation of channel catfish eggs. North American Journal of Aquaculture 71:276–280. http://hdl.handle.net/10113/49386

Pharmacokinetics of florfenicol in channel catfish

Researchers at Mississippi State University College of Veterinary Medicine in Stoneville, conducted pharmacokinetic studies of florfenicol in catfish. These studies, along with laboratory and field efficacy studies, were pivotal in obtaining Food and Drug Administration approval for the use of florfenicol-medicated feed to treat *Flavobacterium columnare* and *Edwardsiella ictaluri* infection in catfish. In addition, these studies, along with one conducted by the U.S. Fish and Wildlife Service, in Bozeman, Montana, cleared the way for approval of florfenicol to treat *F. columnaris* infections in all freshwater-reared warm-water fish. The drug is prescribed under a veterinary feed directive and has been shown to be extremely effective in controlling bacterial infections in warm-water fish.

Gaunt, P.S., Endris, R., McGinnis, A., Baumgartner, W., Camus, A., Steadman, J., Sweeney, D., and Sun, F. 2010. Determination of florfenicol dose rate in feed for control of mortality in Nile tilapia infected with *Streptococcus iniae.* Journal of Aquatic Animal Health 22:158–166. http://hdl.handle.net/10113/55484

Gaunt, P.S., Gao, D., Sun, F., and Endris, R. 2010. Efficacy of florfenicol for control of mortality caused by *Flavobacterium columnare* infection in channel catfish. Journal of Aquatic Animal Health 22:115–122. http://handle.nal.usda.gov/10113/55866

Gaunt, P.S., Langston, C., Wrzesinski, C., Gao, D., Adams, P., Crouch, L., Sweeney, D., and Endris, R. 2012. Multidose pharmacokinetics of orally administered florfenicol in the channel catfish (*Ictalurus punctatus*). Journal of Veterinary Pharmacology and Therapeutics DOI: 10.1111/j.1365-2885.2012.01426.x. http://dx.doi.org/10.1111/j.1365-2885.2012.01426.x

Gaunt, P.S., Langston, C., Wrzesinski, C., Gao, D., Adams, P., Couch, L.S. 2011. Single intravenous and oral dose pharmacokinetics of florfenicol in the channel catfish *(Ictalurus punctatus). J Vet Pharmacol Therapeut.* 35:503–507. http://hdl.handle.net/10113/55419

Straus, D.L., Bowker, J.D., Bowman, M.P., Carty, D., Mitchell, A.J.,and Farmer, B.D. 2012. Safety of aquaflor-medicated feed to sunshine bass. North American Journal of Aquaculture 74:1–7. http://hdl.handle.net/10113/55635

Development of a molecular test to detect significant catfish pathogens in environmental and tissue samples

Disease is one of the leading causes of fish loss and poor yields in catfish production. Researchers with the Mississippi Agricultural and Forestry Experiment Station and College of Veterinary Medicine, working with ARS, have developed and validated assays for the detection and quantification of proliferative gill disease (PGD) and motile aeromonad septicemia (MAS), diseases that cause serious losses in channel catfish. These assays provide reliable methods for the detection and quantification of pathogens in ponds and are being used to evaluate rapid diagnostics and treatment efficacies against recommended disease management strategies and research. Using this technology, risk assessment models for PGD have been developed and are

being used by diagnostic services to mitigate losses during catfish pond stocking and restocking programs. Additionally, a bacterial assay for the causative agent of MAS is currently in use to confirm the presence or absence of the emerging virulent strain of *Aeromonas hydrophila* in suspect cases. In addition to their diagnostic benefits, these assays provide a means for conducting epidemiological and environmental studies and evaluating how management strategies alter pathogen loading rates in commercial catfish ponds. This information will be used to optimize disease management practices aimed at maximizing production efficiencies and economic returns.

Griffin, M.J., Khoo, L.H., Quiniou, S.M., O'Hear, M.M., Pote, L.M., Greenway, T.E., and Wise, D.J. 2012. Genetic sequence data identifies the cercaria of *Drepanocephalus spathans* (Digenea: Echinostomatidae), a parasite of the double-crested cormorant (*Phalacrocorax auritus*), with notes on its pathology in juvenile channel catfish (*Ictalurus punctatus*). Journal of Parasitology 98:967–972. http://dx.doi.org/10.1645/GE-3039.1

Griffin, M.J., Mauel, M.J., Greenway, T.E., Khoo, L.H., and Wise, D.J. 2011. A real time polymerase chain reaction assay for quantification of *Edwardsiella ictaluri* in catfish pond water and genetic homogeneity of diagnostic case isolates from Mississippi. Journal of Aquatic Animal Health 23(4):178-188. http://handle.nal.usda.gov/10113/55863

Griffin, M.J., Pote, L.M., Camus, A.C., Mauel, M.J., Greenway, T.E., and Wise, D.J. 2009. Application of a real-time PCR assay for the detection of *Henneguya ictaluri* In commercial channel catfish ponds. Diseases of Aquatic Organisms 86:223-233. http://dx.doi.org/10.3354/dao02079

Griffin, M.J., Pote, L.M., Wise, D.J., Greenway, T.E., Mauel, M.J., Camus, A.C. 2008. A novel *Henneguya* species from channel catfish described by morphological, histological, and molecular characterization. Journal of Aquatic Animal Health 20:127–135. http://dx.doi.org/10.1577/H07-001.1

Griffin, M.J., Wise, D.J., Camus, A.C., Mauel, M.J., Greenway, T.E., and Pote, L.M. 2008. A real-time polymerase chain reaction assay for the detection of the myxozoan parasite *Henneguya ictaluri* in channel catfish. Journal of Veterinary Diagnostic Investigation 20:559–566. http://dx.doi.org/10.1177/104063870802000504

Griffin, M.H., Wise, D.J., and Pote, L.M. 2009. Morphology and small-subunit ribosomal DNA sequence of *Henneguya adiposa* (Myxosporea) from *Ictalurus punctatus* (Siluriformes). Journal of Parasitology 95:1076-1085. http://dx.doi.org/10.1645/GE-2012.1

Griffin, M.J., Wise, D.J., Yost, M.C., Doffitt, C.M., Pote, L.M., Greenway, T.E., and Khoo, L.H. 2010. A duplex real-time polymerase chain reaction assay differentiates between *Bolbphorus damnificus* and *Bolbophorus* type II sp. Journal of Veterinary Diagnostic Investigation 22:615-622. http://hdl.handle.net/10113/55483

Mischke, C.C. and Griffin, M.J. 2011. Laboratory mass culture of the freshwater oligochaete *Dero digitata*. North American Journal of Aquaculture 73:13-16. http://hdl.handle.net/10113/55534

Wise, D.J., Griffin, M.J., Terhune, J.S., Pote, L.M., and Khoo, L.H. 2008. Induction and evaluation of proliferative gill disease in channel catfish fingerlings. Journal of Aquatic Animal Health 20:236–244. http://dx.doi.org/10.1577/H08-023.1

PROBLEM STATEMENT 4C. PREVENTION OF DISEASE

Aquatic animal farmers are experiencing a lack of available vaccines to prevent infectious disease agents. Presently, some vaccines are available but are effective only when administered by injection or with adjuvant, a strategy that may be impractical and not always economically feasible. Ultimately, vaccine research must result in products that are safe, easy to administer, and effective on the farm. The development of new vaccines will require techniques such as killed, modified live, DNA, and recombinant technologies. However, new and novel approaches for the development of vaccines may be employed with information obtained from microbial genomics and proteomics. Additionally, in order for vaccination to be feasible for many fish species, strategies for mass vaccination are needed, such as through immersion as juveniles or eggs, or through feed.

Vaccines are thus being developed and tested in the laboratory for safety and effectiveness. Researchers are evaluating many strains of recognized pathogens through genetic screening and microbial sequencing and are endeavoring to develop vaccines that offer broad protection against pathogen classes. Techniques are being investigated to apply vaccines and medicines using mass delivery strategies in on-farm trials. Other basic and applied research to enhance health management in production systems being conducted include studies on behavior and environmental factors affecting animal welfare and the use of probiotics, immunostimulants, and some nutrients to improve fish health.

Selected Accomplishments

Development of a live attenuated vaccine and in-pond vaccination platform to protect catfish against enteric septicemia

Although a vaccine to prevent enteric septicemia of catfish (ESC) is available, a perception of undependable performance has limited its penetration in the catfish farming industry. A new vaccine and vaccine delivery platform have been developed that provide exceptional protection against ESC in small-pond–based studies. On the basis of experimental pond studies, vaccination increased survival, fish size, and feed consumption while improving the feed conversion ratio by approximately 40.4 percent. The improved feed conversion could represent a 30 percent reduction in feed costs, whereas improved production efficiencies in vaccinated fish increased gross sales more than 100 percent. Similar results were obtained over 2 years of field testing. Field and laboratory research is being conducted as part of the USDA licensing process for live attenuated vaccines. Declining survival and yield in catfish production over the past several years has been a major impediment to catfish production profitability; this new vaccine will help to reverse that trend.

Development of modified live Edwardsiella tarda *and* Aeromonas hydrophila *vaccines for prevention of diseases in aquaculture*

Losses to *E. tarda* and *A. hydrophila* bacterial pathogens result in severe economic losses to farmers worldwide, and both these pathogens are reemerging strongly in the United States. Currently, no vaccines have been successfully developed to thwart these pathogens. ARS

researchers in Auburn, Alabama, developed and patented these vaccines under a Cooperative Research and Development Agreement with a vaccine manufacturer. These modified live vaccines can be effectively used by a bath immersion method to cost-effectively immunize large numbers of fish. The new *A. hydrophila* vaccine may be especially useful in preventing the huge losses of food-size catfish (more than 1 million pounds in 2009) that have caused considerable economic hardship to Alabama catfish producers. The safety of the modified live vaccines has been confirmed by back-passage studies. Currently, the small market for aquaculture vaccines and unpromising financial returns for these vaccines are holding up commercialization of the products.

Mu, X., Pridgeon, J.W., and Klesius, P.H. 2011. Transcriptional profiles of multiple genes in the anterior kidney of channel catfish vaccinated with an attenuated *Aeromonas hydrophila*. Fish and Shellfish Immunology 31:1162-1172. http://dx.doi.org/10.1016/j.fsi.2011.10.011

Pridgeon, J.W. and Klesius, P.H. 2011. Development and efficacy of novobiocin and rifampicin-resistant *Aeromonas hydrophila* as novel vaccines in channel catfish and Nile tilapia. Vaccine 29:7896–7904. http://hdl.handle.net/10113/55582

Pridgeon, J.W. and Klesius, P.H. 2011. Molecular identification and virulence of three *Aeromonas hydrophila* isolates cultured from infected channel catfish during a disease outbreak in west Alabama (USA) in 2009. Diseases of Aquatic Organisms 94:249–253. http://handle.nal.usda.gov/10113/55876

Pridgeon, J.W. and Klesius, P.H. 2011. Virulence of *Aeromonas hydrophila* to channel catfish *Ictaluras punctatus* fingerlings in the presence and absence of bacterial extracellular products. Diseases of Aquatic Organisms 95:209–215. http://handle.nal.usda.gov/10113/55877

Pridgeon, J.W., Klesius, P.H, and Mu, X. 2011. Evaluation of an in-vitro cell assay to select attenuated bacterial mutants of *Aeromonas hydrophila* and *Edwardsiella tarda* to channel catfish. Journal of Applied Microbiology 111:1310–1318. http://hdl.handle.net/10113/55453

Pridgeon, J.W., Klesius, P.H., Mu, X., Carter, D., Fleming, K., and Xu, D. 2011. Identification of unique DNA sequences present in highly virulent 2009 Alabama isolates of *Aeromonas hydrophila*. Veterinary Microbiology 152:117-125. http://hdl.handle.net/10113/55614

Pridgeon, J.W., Yildirim-Aksoy, M., Klesius, P.H, Srivastava, K.K., and Reddy, P.G. 2012. Attenuation of a virulent *Aeromonas hydrophila* with novobiocin and pathogenic characterization of the novobiocin-resistant strain. Journal of Applied Microbiology 113:1319–1328. http://dx.doi.org/10.1111/j.1365-2672.2012.05430.x

Wiedenmayer, A.A., Klesius, P.H, Evans, J.J., and Shoemaker, C.A. 2008. The macrophage chemotactic activity of *Edwardsiella tarda* extracellular products. Journal of Fish Diseases. 31:331–342. http://dx.doi.org/10.1111/j.1365-2761.2008.00905.x

Yeh, H. and Klesius, P.H. 2012. Construction, expression and characterization of 11 putative flagellar apparatus genes of *Aeromonas hydrophila* AL09-73. Journal of Fish Diseases 35:853–860. http://dx.doi.org/10.1111/j.1365-2761.2012.01438.x

Commercialization of Streptococcus iniae *vaccine for disease prevention in farm-raised tilapia*

According to the Food and Agriculture Organization of the United Nations, production of tilapia had increased worldwide to more than 2 million metric tons in the decade before 2010. *Streptococcus iniae,* a Gram positive bacterium, is a significant pathogen with an estimated economic impact reaching at least $100 million globally in tilapia and other aquaculture products. ARS researchers in Auburn, Alabama, determined that an ARS-developed *S. iniae* vaccine was protective in tilapia challenged with different *S. iniae* isolates from diverse geographic locations. The results show that the *S. iniae* vaccine has broad-ranging protection

against the majority of *S. iniae* isolates that infect fish. The vaccine was developed under a Cooperative Research and Development Agreement, and has been patented and commercialized for use in U.S. aquaculture. The vaccine received a technology transfer award for excellence from the National Federal Laboratory Consortium.

LaFrentz, B.S., Shoemaker, C.A., and Klesius, P.H. 2011. Immunoproteomic analysis of the antibody response obtained in Nile tilapia following vaccination with a *Streptococcus iniae* vaccine. Veterinary Microbiology 152:346–352. http://hdl.handle.net/10113/55525

Pasnik, D.J., Evans, J.J., and Klesius, P.H. 2011. Specific serum antibody responses in channel catfish (*Ictalurus punctatus*) provide limited protection against *Streptococcus ictaluri* challenge. Veterinary Immunology and Immunopathology 144:144–146. http://hdl.handle.net/10113/55493

Pretto-Giordano, L.G., Müller, E.E., Klesius, P., and da Silva, V.G. 2010. Efficacy of an experimentally inactivated *Streptococcus agalactiae* vaccine in Nile tilapia (*Oreochromis niloticus*) reared in Brazil. Aquaculture Research 41:1539–1544. http://hdl.handle.net/10113/44682

Pridgeon, J.W., Aksoy, M., Klesius, P.H., Li, Y., Mu, S., Srivastava, K., and Reddy, G. 2011. Identification and expression profiles of multiple genes in Nile tilapia in response to bacterial infections. Veterinary Immunology and Immunopathology 144:111-119. http://handle.nal.usda.gov/10113/55870

Pridgeon, J.W. and Klesius, P.H. 2011. Development and efficacy of a novobiocin-resistant *Streptococcus iniae* as a novel vaccine in Nile tilapia (*Oreochromis niloticus*). Vaccine 29:5986–5993. http://hdl.handle.net/10113/55582

Pridgeon, J.W. and Klesius, P.H. 2011. Identification and expression profile of multiple genes in Nile tilapia in response to formalin killed *Streptococcus iniae* vaccination. Veterinary Immunology and Immunopathology 142:201–206. http://hdl.handle.net/10113/55598

Shoemaker, C.A., Lafrentz, B.R., and Klesius, P.H. 2011. Vaccination of sex reversed hybrid tilapia (*Oreochromis niloticus* X *O. aureus*) with an inactivated *Vibrio vulnificus* vaccine. Biologicals 39:424-429. http://hdl.handle.net/10113/55660

Shoemaker, C.A., Lafrentz, B.R., Klesius, P.H., and Evans, J.J. 2010. Protection against heterologous *Streptococcus iniae* isolates using a modified bacterin vaccine in Nile tilapia, *Oreochromis niloticus* (L.). Journal of Fish Diseases 33:537-544. http://hdl.handle.net/10113/55433

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COMPONENT 5. IMPROVING PRODUCTION SYSTEMS, DEVELOPING NEW PRODUCTS, AND ENHANCING PRODUCT QUALITY

Aquatic animal producers are continually challenged to meet consumer demand for fish and shellfish products in ways that are efficient and economical. To that end, they need production information and technologies to optimally culture existing species in existing and new environments, and to culture new aquatic species. The performance of aquatic animal production systems can also be improved through the development and application of innovative biological and engineering approaches.

Aquatic animal production systems range from low-energy/trophic to super-intensive systems. Although production intensity varies widely among systems, optimal production efficiency is required for profitability. Optimal utilization of production inputs, including water, feed, mechanical energy, and minimization of waste outputs requires knowledge of the interactions among inputs, culture species, production environment, and economics; however, these interactions are not understood fully.

The marketplace for foods from animal muscle is competitive, requiring new and better aquaculture products to meet consumer demands and expectations, maintain market share for aquatic animal products, and sustain the growth of the aquatic-products-as-food industry. New methods need to be developed to enhance the sensory and nutritional qualities of aquaculture products. In addition, great opportunities exist to enhance the utilization and value of lower valued materials that result from fish processing as feed and food ingredients.

PROBLEM STATEMENT 5A. IMPROVE TECHNOLOGIES FOR RECIRCULATING PRODUCTION SYSTEMS

Recirculating aquaculture systems are dependent on large energy inputs and thus have high operation costs. In return for high energy expenditures, however, the water quality within recirculating systems is constant, waste is contained and highly managed for optimal fish production and health, barriers are in place to prevent escape of fish and entry of pathogens, and water use is greatly minimized. Strategies for reducing energy and water requirements are important for these systems.

For flow-through systems, ARS is improving aeration, water quality related to off-flavor, continuous water quality monitoring systems, dynamic process control systems, and automation technologies to increase aquaculture production system reliability, efficiency, and cost-effectiveness. Additionally, research is occurring to maximize waste removal and production per unit of water used. For recirculation systems, research is ongoing to improve energy efficiency; to identify the critical parameters for fish performance and welfare when reducing water exchange, and to develop methods of rearing marine fish in low salinity water.

Selected Accomplishments

Lower water exchange rates save money without negative effects on health and performance of Atlantic salmon and rainbow trout

Water availability and cost of water use are two key variables for fish production in recirculating aquaculture systems, so one research goal is to minimize the rates of water exchange while maintaining excellent conditions for fish. In a series of trials, Atlantic salmon and rainbow trout were reared by scientists at the Freshwater Institute in Shepherdstown, West Virginia, for 6 months in replicated water recirculating systems. The treatments were either high water exchange (97.5 percent of system flow recirculated; that is, 2.5 percent new water added each day) or low water exchange (99.75 percent of system flow recirculated; 0.25 percent new water added each day). Fish performance, health, and welfare were measured. In every case, at study's end, no significant differences were determined in growth, survival, and other fish health outcomes even though the low water flushing treatment operated with 10 times less water use. Though significant differences in a variety of water quality parameters were noted for both Atlantic salmon and rainbow trout, none of these parameters was outside acceptable ranges for raising salmonids. This study illustrates that Atlantic salmon and rainbow trout perform well in recirculating systems with low water exchange and demonstrate that farmers with limited water resources can compete in salmonid production.

In addition to research to identify critical water exchange rates, addressing the question of how much water is required. Scientists at the Freshwater Institute examined the tolerance of rainbow trout to carbon dioxide. (*This accomplishment also appears on page 24 to illustrate research outcomes also contributing to Problem Statement 2A.*)

Effects of carbon dioxide concentration on rainbow trout health and performance

Elevated carbon dioxide levels (12.5 mg/L) in aquaculture settings have been linked to poor growth, poor feed conversion, and reduced survival. The management response necessary to reduce CO_2 levels is to pump more water; however, pumping water to increase water exchange and hold down CO_2 levels adds significantly both to energy costs and fixed costs for equipment. ARS-funded scientists at the Freshwater Institute raised rainbow trout in recirculating aquaculture systems for 6 months in either high (25 mg/L) or low (10 mg/L) CO_2 concentrations. Performance, survival, and other fish health outcomes were unaffected by the high CO_2 treatment. The findings of this study demonstrate that rainbow trout can be raised to market size at CO_2 concentrations of 25 mg/L without detrimental consequences, and therefore, water pumping costs can be substantially lowered. These results contrasted with findings from those by a Norwegian scientists who found that elevated levels of CO_2 significantly impaired performance. The high alkalinity levels in the systems in West Virginia were a pivotal difference compared with the systems studied in Norway.

These studies on water exchange rates and CO_2 tolerance identify critical operating parameters that will help determine and reduce the cost of recirculating aquaculture systems.

Davidson, J., Good, C., Welsh, C., Brazil, B.L., Summerfelt, S. 2009. Heavy metal and waste metabolite accumulation and their affect on rainbow trout performance in a replicated water reuse system operated at low or high system flushing rates. *Aquacult Eng.* 41:136–145. http://hdl.handle.net/10113/34640

Davidson III, J.W., Good, C.M., Welsh, C., and Summerfelt, S.T. 2011. Abnormal swimming behavior and increased deformities in rainbow trout *Oncorhynchus mykiss* cultured in low exchange water recirculation aquaculture systems. Aquacultural Engineering 45(3):109-117. http://hdl.handle.net/10113/55677

Davidson, J., Good, C., Welsh, C., and Summerfelt, S.T. 2011. The effects of ozone and water exchange rates on water quality and rainbow trout *Oncorhynchus mykiss* performance in replicated water recirculating systems. Aquacultural Engineering 3:44:80-96. http://hdl.handle.net/10113/55681

Good, C., Davidson, J., Welsh, C., Brazil, B., Snekvik, K., and Summerfelt, S. 2009. The impact of water exchange rate on the health and performance of rainbow trout *Oncorhynchus mykiss* in recirculation aquaculture systems. Aquaculture 294:80–85. http://handle.nal.usda.gov/10113/55792

Good, C., Davidson, J., Welsh, C., Snekvik K, and Summerfelt, S. 2010. The effects of carbon dioxide on performance and histopathology of rainbow trout *Oncorhynchus mykiss* in water recirculation aquaculture systems. Aquacultural Engineering 42:51–56. http://handle.nal.usda.gov/10113/55787

Good, C., Davidson, J., Welsh, C., Snekvik, K., Summerfelt, S. 2011. The effects of ozonation on performance, health and welfare of rainbow trout *Oncorhynchus mykiss* in low-exchange water recirculation aquaculture systems. Aquacultural Engineering 44:97–102. http://handle.nal.usda.gov/10113/55769

Pfeiffer, T.J., Summerfelt, S.T, and Watten, B.J. 2011. Comparative performance of CO_2 measuring methods: marine aquaculture recirculation system application. Aquacultural Engineering 44:1–9. http://hdl.handle.net/10113/55606

Wolters, W., Masters, A., Vinci, B., and Summerfelt, S. 2009. Design, loading, and water quality in recirculating systems for Atlantic salmon (*Salmo salar*) at the USDA ARS National Cold Water Marine Aquaculture Center (Franklin, Maine). Aquacultural Engineering 41:60–70. http://hdl.handle.net/10113/34644

Alternative low-salinity marine recirculating aquaculture systems outperforms traditional recirculating system

Although recirculating aquaculture technologies allow intensive fish production, increased biosecurity, and reduction in water use and effluent discharge, traditional systems are expensive to operate, and capital and energy intensive. Therefore, improvements in cost and energy efficiencies are needed to maximize profits per unit of volume. ARS researchers in Fort Pierce, Florida, in collaboration with Florida Atlantic University, demonstrated greater production and economic efficiencies using a low-energy (energy costs about 33 percent of conventional system), low-pressure recirculating system in a direct side-by-side comparison with traditional recirculating systems for the production of market-ready Florida pompano in low-salinity environments. By nearly doubling the number of pompano produced per unit volume while also establishing greater cost and production efficiency, this technology provides marine fish producers an option that can be readily implemented to offset some of the $10 billion annual U.S. seafood trade deficit.

Pfeiffer, T.J. and Riche, M.A. 2011. Evaluation of a low-head recirculating aquaculture system used for rearing Florida pompano to market size. Journal of the World Aquaculture Society 42:198-208. http://hdl.handle.net/10113/55605

Pfeiffer, T. 2010. Recirculation technology:The future of aquaculture. American Society of Agricultural and Biological Engineers, 17(3), 7-9. www.thefreelibrary.com/Recirculation technology: the future of aquaculture.-a0229303302

Pfeiffer, T.J. and Wills, P.S. 2010. Low-head saltwater recirculating aquaculture systems utilized for juvenile red drum production. International Journal of Recirculating Aquaculture 10:1-29. http://devel.w-m-t.com/library/pdf/Pfeiffer_and_Wills_Roanoke_08.pdf

New cascade column and low-head pump combination reduces the energy requirement for producing fish in water reuse systems

Construction and operation costs of recirculating aquaculture systems is a major impediment to adoption of these technologies. Scientists at the Conservation Fund Freshwater Institute, working with ARS researchers in Leetown, West Virginia, developed a sidewall box that contains a forced-ventilated cascade column and a low-head axial flow pump to provide a new high-water flow and low-lift method of gas exchange just outside of the culture tank. Water and air flow rates, energy consumption, and dissolved oxygen and carbon dioxide transfer rates and efficiencies were measured. The novel application was found to reduce electric and oxygen costs by as much as 50 percent compared with more conventional, partial–water reuse technology. The sidewall box cascade column and low-head pump can be integrated into the design of much larger recirculating aquaculture systems to improve gas control, provide culture tank rotation, and reduce total power requirements and the carbon footprint of these systems.

PROBLEM STATEMENT 5B. ENHANCE CONTROL OF POND-BASED ECOSYSTEMS TO MAXIMIZE PRODUCTION AND PRODUCT QUALITY

Pond-based culture systems are common for catfish and striped bass. For these large-pond systems, often greater than 10 acres, the magnitude and scale of treatments is large, so there is a critical need for research in smaller systems that can be verified in large, commercial scales. To promote the profitability of the fish production industry, there is also a need to manipulate pond microbial and phytoplankton communities to control product flavor, predation of larval or juvenile fish, and predation of larger fish.

Research has thus occurred to improve existing pond systems or develop new ones for aquatic animal production using innovative, nontraditional approaches that result in optimal production, greater economic competitiveness, and less environmental impact. Studies have occurred to determine combinations of production inputs (e.g., feed and aeration) that optimize product quality within economic, engineering, and biological constraints to identify bottlenecks and opportunities for greater efficiencies. Research has occurred to improve aeration, water quality monitoring systems, dynamic process control systems, and automation technologies. To enhance and improve pond management, research is ongoing to control the phytoplankton species making up the photosynthesizing biomass in the pond, and thereby reduce off-flavor in fish.

Selected Accomplishments

Development and validation of split pond production systems to increase production efficiency of catfish

Most U.S. aquaculture production comes from large earthen ponds. Disadvantages of traditional pond production include the need for continuous management of pond oxygen concentrations, sporadic algae-related fish off-flavors, losses to avian predators, disease control, inefficient fish harvesting, and lack of tight control of water quality. Scientists with Mississippi State University in Stoneville have addressed these constraints by modifying ponds to physically separate the fish-holding function from the life-support and waste-treatment functions. A commercial-scale system, called the split pond, has been developed and validated at Stoneville (Figure 14).

Figure 14. Aerial view of split pond for catfish production. The larger left side of the pond is where waste treatment occurs, and the smaller right side, is where fish are grown. The levee between the two sides of the pond is breached by a paddlewheel, to drive water into the fish side, and a sluiceway to allow the water return to the waste side. Aerators are also positioned along the levee on the fish side.

The split pond is constructed by dividing an existing earthen pond into two unequal sections with an earthen levee, and then linking the two sections with water flow. Validation studies indicate that the split pond is easy to manage and that fish production can be tripled compared with production in traditional ponds. On the basis of research in this project, split ponds have been widely adopted by the catfish farming industry. More than 1,000 acres of commercial ponds have been built with at least an additional 1,000 acres under construction or planned.

Brown, T.W., and Tucker, C.S. 2012. Pumping performance of a slow-rotating paddlewheel for split-pond aquaculture systems. North American Journal of Aquaculture 75:153–158. http://dx.doi.org/10.1080/15222055.2012.743935

Tucker, C.S. and Kingsbury, S.K. 2010. High density split pond systems offer high output, low maintenance. Global Aquaculture Advocate 13:64–65. http://pdf.gaalliance.org/pdf/GAA-Tucker-March10.pdf

Equipment inventions for catfish production

Two noteworthy engineering inventions have been developed in the water quality laboratory in Stoneville, Mississippi. The first is a novel incubator that has undergone several years of hatchery trial and is being commercially produced and deployed in Mississippi. The second, more recent invention, is an improved device for pond aeration. Both inventions represent complete departures from conventional designs.

Development of improved aerator.

Paddlewheel aerators (Figure 15) have been used for aeration in aquaculture for more than 25 years. They add a lot of oxygen to water but also distribute a large volume of water, which dilutes the aeration effort over the entire pond volume. Thus, a great deal of equipment and a large amount of power is required to prevent low dissolved oxygen (DO) conditions. ARS researchers in Stoneville have developed a new aerator, the Power Tube Airlift (PTA), which can concentrate DO into a small zone of water in a pond using less energy than traditional methods. Aeration efficiency tests were conducted on the device at a variety of water depths and electric motor speeds (rpm) to determine standard oxygen transfer rate (SOTR) and standard aeration efficiency (SAE). Water velocity was also measured to determine water flow rate and pumping efficiency. At an air injection point (sparger water depth) of 6 m, the ranges of SOTR and SAE were 4.8–9.6 kg O_2/h (10.6–21.1 lb O_2/h) and 1.3–1.9 kg O_2/kW·h (2.1–3.1 lb O_2/hp·h), respectively.

Figure 15. Aeration and oxygen management is now the key to efficient catfish production.

The ranges for water flow rate and pumping efficiency were 25.2–40.3 m^3/min (6,660–10,658 gallons per minute) and 5.5–9.3 m^3/min/kW (1,075–1,831 gallons per minute/hp), respectively. Additionally, when initial DO concentrations were lowered to 0.0 mg/L to simulate a total bloom die-off, the outflow water from the PTA produced DO concentrations of approximately 2.70 mg/L. Two commercial-scale PTAs have been installed in an 8-acre catfish production pond for on-site field testing, and field trials on commercial farms will begin in FY 2013. A patent application for this invention currently pending with the U.S. Patent and Trademark Office. By concentrating the aeration effort into a smaller area of the pond, emergency aeration efficiency would likely exceed that of a paddlewheel aerator. Fewer moving parts and improved efficiency would reduce the costs associated with repair and maintenance, and lower power (electricity) consumption, respectively.

Development of the see-saw catfish egg incubator

Traditional paddle-type catfish egg incubators have served the industry well for nearly a century, but they require a high water exchange rate and lose efficiency at egg loading rates above 15 lbs (6.8 kg) per trough. ARS researchers in Stoneville, in collaboration with industry partners in Watson, Arkansas, and Glen Allan, Mississippi, have developed a new incubator (the see-saw), which can incubate more eggs using less water and labor. In a direct comparison at a similar egg-loading rate (26 lbs per trough), the see-saw produced 2.3 times as many viable swim-up fry as the traditional incubator. Additional studies indicate that 45 lbs of eggs/trough can be hatched in

the see-saw using only one gallon per minute of water with no negative effect on hatch rate, saving considerable labor, space, ground water, and energy for pumps and heaters. In commercial field trials thus far, more than 150 million eggs have been hatched. Field trials have now been completed, and technology transfer is underway with expected rapid adoption by the catfish industry and by public-sector hatcheries, which are typically space-limited.

Torrans, L., Ott, B., Jones, R.S., Jones Jr., R., Baxter, J., Mccollum, B., Wargo III, A., and Donley, J. 2009. A vertical-lift incubator (the "seesaw") designed for channel catfish egg masses. North American Journal of Aquaculture 71:354–359. http://hdl.handle.net/10113/55616

Eliminating off-flavor compounds in catfish ponds

Bioremediation, in which bacteria are employed to treat and degrade pollutant chemicals, has long been viewed as a valuable way to remove these unwanted chemicals from the environment. Bioremediation is being used to remove the off-flavor chemicals geosmin and 2-methylisoborneol (MIB) from catfish ponds as soon as they are produced. Many bacteria that act on geosmin or MIB have now been characterized in detail and placed into three groups according to their activities: *1)* ring oxidation of geosmin, *2)* ring oxidation of MIB, or 3) dehydration of MIB. Each activity eliminates the musty, earthy taste and odor of the parent compound. Advancing this work, scientists at the Southern Regional Research Center in New Orleans, Louisiana, in collaboration with scientists at the University of Louisiana at Lafayette, identified a strain of *Rhodococcus wratislaviensis* that may be useful in reducing off-flavor problems in aquaculture farms. This strain has constitutive biotransformation activities for MIB and geosmin, is nonpathogenic to fish, and can be grown under conditions found in static and recirculating aquaculture systems. Once a delivery system is established, this bacterial strain may control off-flavor problems.

Eaton, R.W. and Sandusky, P. 2009. Biotransformation of geosmin by terpene-degrading bacteria. Applied and Environmental Microbiology 21:71-79. http://hdl.handle.net/10113/43889

Hurlburt, B.K., Lloyd, S.W., and Grimm, C.C. 2009. Comparison of analytical techniques for detection of geosmin and 2-methylisoborneol in aqueous samples. Journal of Chromatographic Science 47:3-6. http://hdl.handle.net/10113/42640

Improved catfish feed conversion through pond oxygen management

Dissolved oxygen (DO) is assumed to be the most critical water quality parameter in warmwater aquaculture, but controlled studies of the effect of this diurnally fluctuating parameter on channel catfish have been lacking. Lack of this research was at least partially due to the faulty strategy of maintaining oxygen at levels adequate for survival but not for growth. ARS researchers in Stoneville, Mississippi, are concluding a 10-year research program examining the effect of pond DO concentrations on blue, channel, and blue × channel catfish growth, production, food consumption, and food conversion (Figure 16). These studies were made possible via the use of a computer-controlled pond oxygen monitoring system that could initiate aeration at precise DO set points and continuously record DO, temperature, and aerator usage. Results with all three species show that higher DO concentrations than previously believed (best performance at minimum daily DO concentrations of 2.5–3.0 mg/L) are required for optimum food intake and

Figure 16. Catfish feed aggressively, grow quickly, and convert feed best when they are not stressed by low dissolved oxygen concentrations in the morning.

growth, and this improved growth will significantly shorten the production cycle (currently 3 to 5 years) down to 2 years from egg to food fish. Whereas food conversion is not directly affected by DO except at extremely low DO concentrations, a shorter production cycle reduces fish losses from all causes, thus significantly improving food conversion. Feed is the greatest single cost in catfish production. Increased growth resulting from improved DO management can reduce food conversion ratios from an estimated industry-wide 2.5–3.0:1 to 2.0:1, and thus greatly improve the profitability of catfish farming by reducing production costsby $0.10–0.20/lb. Technology transfer efforts have been ongoing and commercial farm managers have begun to implement the techniques of increasing both the available per-acre aeration and the minimum DO concentrations.

Arnold, M.B., Torrans, E.L., and Allen, P.J. 2012. Influences of cyclic, high temperatures on juvenile channel catfish growth and feeding. North American Journal of Aquaculture 75:77–84. http://dx.doi.org/10.1080/15222055.2012.732674

Torrans, E.L. 2008. Production responses of channel catfish to minimum daily dissolved oxygen concentrations in earthen ponds. North American Journal of Aquaculture 70:371–381. http://dx.doi.org/10.1577/A07-102.1

Torrans, E.L. 2005. Effect of oxygen management on culture performance of channel catfish in earthen ponds. North American Journal of Aquaculture. 67:275–288. http://www.tandfonline.com/doi/full/10.1577/A04-071.1

PROBLEM STATEMENT 5C. DEVELOP SHELLFISH SYSTEMS TO MAXIMIZE PRODUCTIVITY AND BIOEFFECTIVENESS

Bivalve shellfish are a major production commodity in the United States. Although larvae are often reared in a hatchery, most juveniles and adults are raised directly in multiuse public waters. Survival at various stages of rearing is very low, resulting in harvest rates as low as 50 percent from field deployment. Production is also constrained by recent regulatory actions that have resulted in the need for a better understanding of the interaction between shellfish aquaculture production and the environment.

Research is thus occurring to determine, test, and validate methods to reduce mortality due to disease, predators, pests, and bio-fouling organisms. Research includes identifying and quantifying the interactions of aquaculture practices and natural resources in a way that both benefits the shellfish production industry and satisfies regulatory constraints. In addition, due to recent summer and winter mortality events, ARS has worked to determine the effects and limits

imposed on shellfish by temperature, other environmental factors, and the interactions of these stresses with pathogens.

Selected Accomplishments

Bivalve shellfish aquaculture has local and short-term impacts in estuaries where it is conducted

The effects of bivalve aquaculture on estuarine ecology have been little studied, especially at the landscape scale where relationships between shellfish production and specific habitats are not available. A review by ARS scientists in Corvallis, Oregon, and collaborative studies with researchers at Oregon State University (OSU) and the University of Washington determined that bivalve culture is unlikely to influence material processes (food consumption and waste production) except at the local farm scale or in areas with poor water circulation. A geographic information system was created by ARS scientists based at the OSU Hatfield Marine Science Center, using results from a habitat survey of Willapa Bay, Washington (Figure 17). A hovercraft was used to conduct a grid survey in the field using accurate GPS instruments and a data dictionary. Then, using a mapping protocol, information was collected on burrowing shrimp, eelgrass, oyster culture, sediment characteristics, and bathymetry. A shellfish aquaculture layer delineating production areas was created using results from in-person interviews with shellfish growers. This accomplishment produced a standard base map, which ARS and cooperating scientists are using to design detailed studies and spatial analyses of interactions between culture operations and other fish and invertebrates that inhabit Pacific Northwest estuaries. An underwater video system was developed to observe fish and invertebrate behavior in shellfish culture areas and other habitats, and to implement a broader survey of habitat use.

Figure 17. Long-line oyster culture in Willapa Bay, Washington.

These studies determined that shellfish and structures on which they are grown, and disturbances due to harvest practices, clearly affect the estuarine community at both local and landscape scales. Studies to date suggest that structures provided via aquaculture are functionally similar to those provided by submerged aquatic vegetation for small benthic fauna, but effects are more species-specific for larger, more mobile fish and invertebrates. Bivalve shellfish aquaculture causes disturbances at relatively small spatial and short temporal scales, but does not remove a productive area from the estuary nor degrade water quality like other anthropogenic influences, and thus is not expected to cause changes in the overall ecology of most U.S. estuaries. Results are being used to inform the shellfish aquaculture industry and decision-makers about how environmentally sustainable practices can be conducted on a landscape scale and are informing a more consistent regulatory regime and permitting process for both existing and new aquaculture facilities. For example, these data informed the

Army Corps of Engineers in the renewal of Nationwide Permit 48, and have been referenced in biological opinions rendered by other agencies such as the National Marine Fisheries Service.

Dumbauld, B.R., Ruesink, J.L., and Rumrill, S.S. 2009. The ecological role of bivalve shellfish aquaculture in the estuarine environment: a review with application to oyster and clam culture in West Coast (USA) estuaries. Aquaculture 290:196–223. http://hdl.handle.net/10113/29264

Coen, L.D., Dumbauld, B.R., and Judge, M.L. 2011. Expanding shellfish aquaculture: a review of the ecological services provided by and impacts of native and cultured bivalves in shellfish dominated ecosystems. In: Shumway, S.E. (ed.), *Shellfish Aquaculture and the Environment*. West Sussex, U.K.: Wiley-Blackwell, p. 239–296. http://dx.doi.org/10.1002/9780470960967.ch9

Tallis, H.M., Ruesink, J.L., Dumbauld, B.R., Hacker, S., and Wisehart, L.M. 2009. Oysters and aquaculture practices affect eelgrass density and productivity in a Pacific Northwest estuary. Journal of Shellfish Research 28:251–261. http://hdl.handle.net/10113/55679

Trimble, A.C., Ruesink, J.L., and Dumbauld, B.R. 2009. Factors preventing the recovery of a historically overexploited shellfish species, *Ostrea lurida* Carpenter 1864. Journal of Shellfish Research 28:97–106. http://handle.nal.usda.gov/10113/55821

Wagner, E., Dumbauld, B.R., Hacker, S.D., Trimble, A.C., Wisehart, L.M., and Ruesink, J.L. 2012. Density-dependent effects of an introduced oyster, *Crassostrea gigas,* on a native intertidal seagrass, *Zostera marina.* Marine Ecology Progress Series 468:149–160. http://hdl.handle.net/1957/35785

Wisehart, L.M., Dumbauld, B.R., Ruesink, J.L., and Hacker, S.D. 2007. Importance of eelgrass early life history stages in response to oyster aquaculture disturbance. Marine Ecology Progress Series 344:71–80. http://dx.doi.org/10.3354/meps06942

Burrowing shrimp population assessments

Two species of burrowing shrimp cause significant problems for the U.S. West coast shellfish aquaculture industry, and growers in Washington State have used a pesticide to control them for 60 years. The industry is currently seeking alternative control measures as part of an integrated pest management program (Figure 18). ARS researchers in Newport, Oregon, implemented an annual monitoring program in several estuaries in 2005, and mapped the distribution of these shrimp in large portions of two estuaries; Willapa Bay, Washington, and Yaquina Bay, Oregon, from 2006 to 2012. Results indicate that populations of both species had declined markedly over this 6-year period, and that these declines are tied to interannual fluctuations in larval recruitment. ARS researchers are collaborating with colleagues at Oregon State University to further define mortality sources and to potentially predict population trends. They also are working with industry to incorporate this information directly into integrated pest management plans to sustain aquaculture activities in these estuaries.

Figure 18. Bioscience technician Lee McCoy collecting data to estimate burrowing shrimp abundance. Hovercraft in the background.

Bosley, K.M. and Dumbauld, B.R. 2011. Use of extractable lipofuscin to estimate age structure of ghost shrimp populations in west coast estuaries of the USA. Marine Ecology Progress Series 428:161–176. http://dx.doi.org/10.3354/meps09055

Chapman, J.W., Dumbauld, B.R., Itani, G., and Markham, J.C. 2012. An introduced asian parasite threatens northeastern pacific estuarine ecosystems. Biological Invasions 14(6), 1221-1236. doi: 10.1007/s10530-011-0151-3 http://hdl.handle.net/1957/31346

Dumbauld, B.R., Chapman, J.W., Torchin, M.E., and Kuris, A.M. 2011. Is the collapse of mud shrimp (*Upogebia pugettensis*) populations along the Pacific coast of North America caused by outbreaks of a previously unknown bopyrid isopod parasite (*Orthione griffenis*)? Estuaries and Coasts 34:336–350. http://link.springer.com/article/10.1007%2Fs12237-010-9316-z

Dumbauld, B.R. and Harlan, L. 2009. The potential use of electricity to control burrowing shrimp in oyster aquaculture beds. North American Journal of Aquaculture 71:178-188. http://hdl.handle.net/10113/55492

Dumbauld, B.R., Holden, D.L., and Langness, O.P. 2008. Do sturgeon limit burrowing shrimp populations in Pacific northwest estuaries? Environmental Biology of Fishes 83:283–296. http://dx.doi.org/10.1007/s10641-008-9333-y

Smith, A.E., Chapman, J.W., and Dumbauld, B.R. 2008. Population structure and energetics of the bopyrid isopod parasite *Orthione griffenis* in mud shrimp *Upogebia pugettensis*. Journal of Crustacean Biology 28:228–233. http://dx.doi.org/10.1651/0278-0372(2008)028[0228:PSAEOT]2.0.CO;2

PROBLEM STATEMENT 5D. IMPROVE PRODUCT QUALITY AND DEVELOP NEW PRODUCTS

Opportunities exist to enhance the use of secondary products from aquatic animal production, such as making aquaculture and farm animal feed ingredients from viscera components, frames, skin, and heads.

Economical methods are being developed to collect, preserve, and store valuable co-products from processing fish waste material, including the tremendous quantities of material generated in Alaska alone, until they can be further processed. Research is occurring to develop new product forms, such as protein- and oil-based feed and food ingredients from fish processing byproducts and designer products with enhanced human health benefits, highly unsaturated fatty acids, antioxidants, and vitamins.

Selected Accomplishments

Gels made from cold water fish skin

Dehydrating pollock skins prior to shipment. Pollock skins are an excellent source of gelatin for several commercial products. Pollock skins intended for gelatin production currently must be transported to processing facilities outside of Alaska. Untreated, the skins contain roughly 80 percent water, making transport expensive. ARS researchers in Alaska and California have determined that dehydrating fish skins using chemical desiccants prior to transport can stabilize the material and reduce shipping weight. Results show that dehydration does not harm the functional properties of gelatin, including gel strength, gelling temperature, and viscosity. This research suggests that fish skins can be economically stabilized for transport through the use of reusable desiccants commonly employed in the food industry.

UV-B light and fish skin gelatin properties. Coldwater fish skin gelatins have characteristic physical properties that are significantly different from either warmwater fish skin or mammalian gelatins; however, some applications require improvement of physical properties to resemble those of other types of gelatins. Collaborating scientists at ARS laboratories in Albany, California, and Fairbanks, Alaska, embarked on studies to alter the physical properties of dried, coldwater fish skin gelatins using UV-B light. Results indicated that UV-B light can induce protein chain modifications to the dried and milled cold water fish skin gelatins. The modifications can increase gel strength, gel set temperature, and aqueous viscosity, and affect the mechanical properties of gelatin films. UV-B–modified coldwater fish skin gelatins can be used for specific applications as food thickeners and emulsifiers at different temperature ranges.

Nanofibers from fish gelatin. The potential of using gelatin from fish skin to make nanofibers was studied by ARS researchers in Fairbanks, Alaska, and California. Pollock gelatin was blended with a variety of biodegradable polymers, and these combinations were evaluated for their ability to be electrospun into nanofiber. Characteristics of the nanofiber parameters affecting the spinning process were evaluated. The fibers had diameters of several hundred nanometers, providing high surface areas. Pollock gelatin/poly nanofibers have the potential to be made into many products, including those for medical use, such as wound dressings.

Barrier properties of films made from Alaskan fish skin gelatins. There is increasing interest in making biodegradable and edible films from gelatins; however, gelatin films from coldwater fish skin have physical properties that are different from films made with the more common bovine and porcine gelatins. The effects of drying temperature on barrier and mechanical properties of pollock and salmon gelatin films were determined by ARS scientists in California and Alaska. Results indicate that the films dried below gelation temperature had higher helical content levels, resulting in greater strength, but worse water vapor barrier properties. The research identified new methods of altering the gelatin films physical properties.

Avena-Bustillos, R.J., Chiou, B., Olsen, C.W., Bechtel, P.J., Olson, D.A., and Mchugh, T.H. 2011. Gelation, oxygen permeability, and mechanical properties of mammalian and fish gelatin films. Journal of Food Science 76:E519–E524. http://dx.doi.org/10.1111/j.1750-3841.2011.02312.x

Bower, C.K., Avena-Bustillos, R.J., Hietala, K.A., Bilbao-Sainz, C., Olsen, C.W., and McHugh, T.H. 2010. Dehydration of pollock skin prior to gelatin production. Journal of Food Science 75(3):C317–C321. http://hdl.handle.net/10113/43162

Chiou, B-S., Avena-Bustillos, R.J., Bechtel, P.J., Imam, S.H., Glenn, G.M., and Orts, W.J. 2009. Effects of drying temperature on barrier and mechanical properties of cold-water fish gelatin films. Journal of Food Engineering 95:327–331. http://dx.doi.org/10.1016/j.jfoodeng.2009.05.011

Chiou, B-S, Avena-Bustillos, R.J., Bechtel, P.J., Jafri, H., Narayan, R., Imam, S.H., Glenn, G.M., and Orts, W.J. 2008. Cold water fish gelatin films: effects of cross-linking on thermal, mechanical, barrier, and biodegradation properties. European Polymer Journal 44:3748–3753. http://dx.doi.org/1016/j.eurpolymj.2008.08.011

Otoni, C.G., Avena-Bustillos, R.J., Chiou, B-S., Bilbao-Sainz, C., Bechtel, P.J., and McHugh, T.H. 2012. Ultraviolet-B radiation induced cross-linking improves physical properties of cold- and warm-water fish gelatin gels and films. Journal of Food Engineering 77:E215–E223. http://onlinelibrary.wiley.com/doi/10.1111/j.1750-3841.2012.02839.x/pdf

Chiou, B-S., Jafri, H., Avena-Bustillos, R., Gregorski, K.S., Bechtel, P.J., Imam, S.H., Glenn, G.M., and Orts, W.J. 2013. Properties of electrospun pollock gelatin/poly(vinyl alcohol) and pollock gelatin/poly(lactic acid) fibers. International Journal of Biological Macromolecules 55:214–220. http://dx.doi.org/10.1016/j.ijbiomac.2013.01.010

Snack foods from salmon byproduct powders

Many Alaskan products are at a disadvantage in competing in international markets due in part to high transportation costs. One way to reduce transportation costs and avoid refrigeration is to develop new products from dried fish byproduct powders. Scientists from ARS laboratories in Albany, California, and Fairbanks, Alaska, made dried salmon flakes using an infrared dryer that were subsequently milled to powder. The powders were formulated with starch, water, and seasonings, and then extrusion-molded for deep frying and infrared drying. These shelf-stable fish snacks produced from salmon byproduct powders can be incorporated into shelf-stable ethnic fish snacks and pet foods.

Crapo, C., Oliveira, A.C., Nguyen, D., Bechtel, P.J., and Fong, Q. 2010. Development of a method to produce freeze-dried cubes from 3 Pacific salmon species. Journal of Food Science 75:E269–E275. http://hdl.handle.net/10113/55647

Oil co-products from salmon processing

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

Assessment of salmon head parts for nutrient and oil levels. Many rural Alaskan processors merely grind and dump their processing waste, a process allowed by State and Federal regulators with certain restrictions; however, the potential exists to create value-added products from portions of salmon heads, large volume co-product. A study conducted by ARS scientists in Kodiak, Alaska, to examine red salmon (*Oncorhynchus nerka*) head parts to better understand their lipid composition and the distribution of nutrients in braincases, soft tissue, gills, and face halves. Braincases had the highest lipid and lowest protein content, gills had the lowest lipid content, and soft tissue had the highest protein content, whereas amino acid analysis showed small concentration differences in methionine, phenylalanine, and arginine. The fatty acid profiles of all four parts were not markedly different from each other and were similar to oils extracted from

other salmon or salmon byproducts, suggesting that red salmon heads could be used as a low-cost raw material for both food and feed ingredients.

Stabilizing heart-healthy, long-chain omega-3 oils from salmon co-products. Valuable salmon oils can be extracted from fish processing wastes, but they must be stabilized immediately to prevent oxidative damage to long-chain polyunsaturated fatty acids. ARS scientists in Fairbanks, Alaska, in cooperation with University of Alaska scientists, evaluated smoke-processing as a technology to reduce oxidation of salmon oil. Salmon heads exposed to hot smoking produced oils with decreased oxidation and superior antioxidant potential, with higher levels of antioxidant tocopherols, than their nonsmoked counterparts. Smoking byproducts prior to oil extraction will extend the time frame for oil removal and allow nonrefrigerated transportation of oils without addition of costly antioxidants.

Bower, C.. Hietala, K. 2010. Stabilizing smoked salmon (*Oncorhynchus gorbuscha*) tissue after extraction of oil. Journal of Food Science 75:C241–C245. http://hdl.handle.net/10113/42622

Bower, C.K, Hietala, L.A., Oliveira, A.C.M., and Wu, T.H. 2009. Stabilizing oils from smoked pink salmon (*Oncorhynchus gorbuscha*). Journal of Food Science 74:C248–C257. http://hdl.handle.net/10113/28479

Bower, C.K,, Malemute, C.L., and Oliveira, A.C.M. 2007. Preservation methods for retaining N-3 polyunsaturated fatty acids in Alaska coho salmon (*Oncorhynchus kisutch*) products. Journal of Aquatic Food Product Technology 16 45–54. http://www.tandfonline.com/doi/abs/10.1300/J030v16n04_05

Stone, D.A., Oliveira, A.C., Plante, S., Smiley, S., Bechtel, P.J., and Hardy, R.W. 2011. Enhancing highly unsaturated omega-3 fatty acids in phase-fed rainbow trout (Oncorhynchus mykiss) using Alaskan fish oils. Aquaculture Nutrition 17(2):e501-e510. http://hdl.handle.net/10113/55652

Wan, Y., Li, J., Mis Solval, K., Stine, J.J., Bechtel, P., and Sathivel, S. 2012. Physicochemical properties of red salmon oil (Oncorhynchus nerka) and microencapsulated red salmon oil added to baby food. Journal of the American Oil Chemists' Society 89(4):727-734. http://handle.nal.usda.gov/10113/55865

Wu T.H. and Bechtel P.J. 2008. Salmon by-product storage and oil extraction. Food Chemistry 111:868–871. http://dx.doi.org/10.1016/j.foodchem.2008.04.064

Wu, T.H., Nigg, J.D., Stine, J.J., and Bechtel, P.J. 2011. Nutritional and chemical composition of by-product fractions produced from wet reduction of individual red salmon (*Oncorhynchus nerka*) heads and viscera. Journal of Aquatic Food Product Technology 20(2):183-195. http://handle.nal.usda.gov/10113/55819

Yin, H., Wan, Y., Huang, J., Sathivel, S., and Bechtel, P.J. 2011. Effects of oil extraction methods on physical and chemical properties of red salmon oils (Oncorhynchus nerka). Journal of the American Oil Chemists' Society 88(10):1641-1648. DOI 10.1007/s11746-011-1824-x. http://handle.nal.usda.gov/10113/55813

APPENDIX 1

National Program 106, Aquaculture

ACCOMPLISHMENT REPORT 2008–2012

Research Projects in NP 106[1]

Project Number	Project Title
1915-31000-003-00D	GENETIC IMPROVEMENT OF COLD WATER MARINE FINFISH – William Wolters (P); Orono, Maine.
1930-31000-009-00D	UTILIZATION OF GENOMICS FOR IMPROVING PRODUCTION TRAITS IN COOL AND COLD WATER AQUACULTURE – Yniv Palti (P), Caird Rexroad III, Roger Vallejo, and Sixin Liu; Leetown, West Virginia.
1930-31000-010-00D	UTILIZING GENETICS AND PHYSIOLOGY FOR ENHANCING COOL AND COLD WATER AQUACULTURE PRODUCTION – Gregory Weber (P), Timothy Leeds, and Beth Cleveland; Leetown, West Virginia.
1930-31000-011-00D	IDENTIFYING BIOCHEMICAL PATHWAYS USING GENETICALLY MODIFIED TROUT (UNIVERSITY OF CONNECTICUT) – Caird Rexroad III (P); Leetown, West Virginia.
1930-31320-001-00D	IMPROVING THE SUSTAINABILITY OF LAND-BASED CLOSED-CONTAINMENT SYSTEMS FOR SALMONID FOOD FISH PRODUCTION– Caird Rexroad III (P); Leetown, West Virginia.
1930-32000-005-00D	INTEGRATED APPROACHES FOR IMPROVING AQUATIC ANIMAL HEALTH IN COOL AND COLD WATER AQUACULTURE – Gregory Wiens (P), Timothy Welsh, Jason Evenhuis, and Timothy Leeds; Leetown, West Virginia.
3655-31320-002-00D	COMBATING VIRAL HEMORRHAGIC SEPTICEMIA AND IMPROVING YELLOW PERCH AQUACULTURE FOR THE GREAT LAKES REGION– Brian Sheperd (P); Madison, Wisconsin.
5341-31410-004-00D	ALASKA FISH PROCESSING BYPRODUCTS (Terminated in January 2012) – Peter Bechtel (P); Fairbanks, Alaska.
5358-31000-003-00D	DETERMINE GENETIC DIVERSITY AND DEVELOP TOOLS FOR GENETIC IMPROVEMENT OF OYSTER STOCKS FOR THE PACIFIC NORTHWEST – Gary Banowetz (P); Corvallis, Oregon.

[1] For the sake of consistency, projects are listed and organized in Appendices 1 and 2 according to the ARS project number used to track projects in the Agency's internal database. A (P) after a scientist's name indicates the project's principal investigator.

Project Number	Project Title
5358-63000-003-00D	QUANTIFYING HABITAT UTILIZATION AND REDUCING JUVENILE OYSTER MORTALITY IN PACIFIC SHELLFISH PRODUCTION– Brett Dumbauld (P); Corvallis Oregon.
5366-21310-004-00D	IMPROVING SUSTAINABILITY OF RAINBOW TROUT PRODUCTION BY INTEGRATED DEVELOPMENT OF IMPROVED GRAINS, FEEDS, AND TROUT– Frederic Barrows (P), Keshun Liu, Kenneth Overturf, John Bonman, and Thomas Welker; Aberdeen, Idaho.
6225-31630-006-00D	INTEGRATED APPROACHES FOR IMPROVING THE EFFICIENCY AND SUSTAINABILITY OF MORONE AND OTHER WARM WATER FISH PRODUCTION – Bartholomew Green (P), Steven Rawles, Sidney Fuller, Benjamin Beck, and Martin Riche; Stuttgart, Arkansas.
6225-32000-005-00D	STRATEGIES FOR FISH DISEASE CONTROL AND PREVENTION – David Strauss (P) and Benjamin Beck; Stuttgart, Arkansas.
6225-63000-008-00D	INTEGRATED APPROACHES FOR IMPROVING EFFICIENCY AND SUSTAINABILITY OF LOW-SALINITY MARINE AQUACULTURE PRODUCTION (TERMINATED IN 2011); Stuttgart, Arkansas.
6402-13320-004-00D	IMPROVING PRODUCTION EFFICIENCY IN WARM WATER AQUACULTURE THROUGH WATER QUALITY MANAGEMENT– Eugene Torrans (P) and Craig Tucker; Stoneville, Mississippi.
6402-31000-009-00D	GENETICS, PHYSIOLOGY, AND HEALTH RESEARCH TO IMPROVE CATFISH PRODUCTION– Geoffrey Waldbieser (P), Natha Booth, Sylvie Quiniou, Brian Peterson, Brian Bosworth, Nagarj Chatakondi, and Craig Tucker; Stoneville, Mississippi
6402-31320-001-00D	IMPROVING PRODUCTION STRATEGIES IN CHANNEL CATFISH FARMING – Eugene Torrans (P); Stoneville, Mississippi.
6402-31320-002-00D	DEVELOPMENT OF APPROACHES TO PREVENT AND AMELIORATE DISEASES OF CATFISH – Brian Peterson (P); Stoneville, Mississippi.
6408-41000-009-00D	MANAGEMENT OF OFF-FLAVORS AND UNDESIRABLE BACTERIA IN AQUACULTURE – Kevin Schrader (P); University of Mississippi, Oxford, Mississippi.
6420-31320-001-00D	NUTRITION AND FEED DEVELOPMENT FOR WARM WATER AQUACULTURE – Chhorn Lim (P); Auburn, Alabama.
6420-32000-024-00D	INTEGRATED AQUATIC ANIMAL HEALTH STRATEGIES – J.W. Pridgeon (P), Phillip Klesius, Dehai Xu, Benjamin LaFrentz, Craig Shoemaker, and Dunhua Zhang; Auburn, Alabama.
6435-43440-042-00D	BIOREMEDIATION OF GEOSMIN AND MIB – Barry Hurlburt (P) and Richard Eaton; New Orleans, Louisiana. (Project terminated in December 2012.)
6435-43440-045-00D	POSTHARVEST PROCESSING TO ADD VALUE TO CATFISH – Peter Bechtel and John Bland; New Orleans, Louisiana. (Project established in December 2012.)

APPENDIX 2

National Program 106, Aquaculture

ACCOMPLISHMENT REPORT 2008–2012

Publications by Research Projects[1]

1915-31000-003-00D
GENETIC IMPROVEMENT OF COLD WATER MARINE FINFISH – William Wolters (P); Orono, Maine.

Burr, G.S., Wolters, W.R., Barrows, F., and Hardy, R.W. 2012. Replacing fishmeal with blends of alternative proteins on growth performance of rainbow trout (*Oncorhynchus mykiss*), and early or late stage juvenile Atlantic salmon (*Salmo salar*). Aquaculture 334-337:110-116. http://hdl.handle.net/10113/55765

Burr, G.S., Wolters, W.R., Schrader, K., and Summerfelt, S. 2012. Impact of depuration of earthy-musty off-flavors on fillet quality of Atlantic salmon, *Salmo salar*, cultured in a recirculating system. Aquacultural Engineering 50:28-36. http://hdl.handle.net/10113/55346

Wolters, W.R. 2010. Sources of phenotypic and genetic variation for seawater growth in five north american Atlantic salmon, *Salmo salar*, stocks. Journal of the World Aquaculture Society 41:421-429. http://hdl.handle.net/10113/55767

Wolters, W.R., Barrows, F., Burr, G.S., and Hardy, R.W. 2009. Growth parameter of wild and selected strains of Atlantic salmon (*Salmo salar*) on two experimental diets. Aquaculture 297:136-140. http://hdl.handle.net/10113/36771

Wolters, W.R., Master, A., Vinci, B., and Summerfelt, S. 2009. Design, loading, and water quality in recirculating systems for Atlantic salmon (*Salmo salar*) at the USDA ARS National Cold Water Marine Aquaculture Center (Franklin, ME). Journal of Aquaculture Engineering 41:60-70. http://hdl.handle.net/10113/34644

1930-31000-009-00D
UTILIZATION OF GENOMICS FOR IMPROVING PRODUCTION TRAITS IN COOL AND COLD WATER AQUACULTURE; Yniv Palti (P), Caird Rexroad III, Roger Vallejo, and Sixin Liu; Leetown, West Virginia.

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IDENTIFYING BIOCHEMICAL PATHWAYS USING GENETICALLY MODIFIED TROUT (UNIVERSITY OF CONNECTICUT) – Caird Rexroad III; Leetown, West Virginia.

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INTEGRATED APPROACHES FOR IMPROVING AQUATIC ANIMAL HEALTH IN COOL AND COLD WATER AQUACULTURE – Gregory Wiens (P), Timothy Welsh, Jason Evenhuis, and Timothy Leeds; Leetown, West Virginia.

Wiens, G.D, LaPatra, S,E., Welch, T.J., Evenhuis, J.P., Rexroad III, C.E., Leeds, T.D. 2013 On-farm performance of rainbow trout (Oncorhynchus mykiss) selectively bred for resistance to bacterial cold water disease: effect of rearing environment on survival phenotype. Aquaculture. http://dx.doi.org/10.1016/j.aquaculture.2013.01.018.

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COMBATING VIRAL HEMORRHAGIC SEPTICEMIA AND IMPROVING YELLOW PERCH AQUACULTURE FOR THE GREAT LAKES REGION – Brian Sheperd (P); Madison, Wisconsin.

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DETERMINE GENETIC DIVERSITY AND DEVELOP TOOLS FOR GENETIC IMPROVEMENT OF OYSTER STOCKS FOR THE PACIFIC NORTHWEST – Gary Banowetz (P); Corvallis, Oregon.

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QUANTIFYING HABITAT UTILIZATION AND REDUCING JUVENILE OYSTER MORTALITY IN PACIFIC SHELLFISH PRODUCTION – Brett Dumbauld (P); Corvallis Oregon.

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IMPROVING SUSTAINABILITY OF RAINBOW TROUT PRODUCTION BY INTEGRATED DEVELOPMENT OF IMPROVED GRAINS, FEEDS, AND TROUT – Frederic Barrows (P), Keshun Liu, Kenneth Overturf, John Bonman, and Thomas Welker; Aberdeen, Idaho.

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GENETICS, PHYSIOLOGY, AND HEALTH RESEARCH TO IMPROVE CATFISH PRODUCTION – Geoffrey Waldbieser (P), Natha Booth, Sylvie Quiniou, Brian Peterson, Brian Bosworth, Nagarj Chatakondi, and Craig Tucker; Stoneville, Mississippi

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IMPROVING PRODUCTION STRATEGIES IN CHANNEL CATFISH FARMING – Eugene Torrans (P); Stoneville, Mississippi.

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DEVELOPMENT OF APPROACHES TO PREVENT AND AMELIORATE DISEASES OF CATFISH – Brian Peterson (P); Stoneville, Mississippi.

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APPENDIX 3

National Program 106 – Aquaculture

ACCOMPLISHMENT REPORT 2008-2012

Publications in Alphabetical Order by Lead Author

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