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National Program 106-Aquaculture
Action Plan (2010-2014)

Goal: The U.S. aquaculture industries face formidable challenges. The demand for seafood is increasing worldwide, yet the ability for U.S. aquaculture producers to meet that demand requires development of technologies to reduce the cost of production while maintaining and improving product quality. Producers, processors and breeders are in need of systems that successfully identify, promote, and harness the aquatic animal improvements to maximize profits, secure supply, reduce environmental impacts, increase market competitiveness, sustain small and mid-sized scale producers, and earn consumer confidence. Research in the disciplines of genetics, nutrition, health, and physiology will support the biological improvement of animals, while ecology, engineering and economics will support the improvement of systems and help to ensure sustainability.

The vision for ARS aquaculture research and technology transfer is *to support a thriving domestic industry based on improved genetic stocks and scientific information on biotechnologies and management practices to ensure a high quality, safe supply of healthful seafood and aquatic products.*

Our research components strive to develop and ensure an abundant, safe, and affordable supply of seafood products for the 300 million U.S. consumers produced in a healthy, competitive, and sustainable aquaculture sector, a sector supported by over 4,300 aquaculture farmers that produced in excess of $1 billion dollars worth of goods in 2005 (NASS, 2005 Census of Aquaculture).

Aquaculture Program Components

- Understanding, Improving, and Effectively Using Animal Genetic and Genomic Resources
- Enhancing Animal Performance, Well-being and Efficiency in Diverse Production Systems
- Defining Nutrient Requirements, Nutrient Composition of Feedstuffs and Expanding Alternative Ingredients
- Improving Health and Welfare of Aquatic Animals
- Improving Production Systems, Developing New Products and Enhancing Product Quality.

This National Program mission follows from the USDA/ARS Strategic Plan (see http://www.ars.usda.gov/SP2UserFiles/Place/00000000/ARSStrategicPlan2006-2011.pdf), which is directed toward achieving goals mandated by the USDA Research, Education, and Economics Mission Area Strategic Plan (see http://www.ocfo.usda.gov/usdasp/usdasp.htm).

Relationship of this National Program to the ARS Strategic Plan: The products of research conducted in National Program 106 contribute toward broader goals associated with six specific Performance measures from the ARS Strategic Plan.

Performance Measure 2.1.2: Develop cost effective, functional industrial and consumer products, including higher quality, healthy foods, that satisfy consumer demand in the United States and abroad. **Target:** Provide food crops and products with higher quality and extended shelf life; convenient and acceptable healthy foods; non-food, non-fuel bio-based products with cost and performance features comparable or superior to petroleum-based products; and valuable co-products from agricultural residues and processing wastes.

Performance Measure 2.2.1: Develop systems and technologies to reduce production costs and risks while enhancing natural resource quality. **Target**: Reduce the cost and increase profitability, improve the efficiency, or increase the yield, and increase the economic and environmental sustainability of production.

Performance Measure 2.2.2: Develop new technologies, tools, and information contributing to improved precision animal production systems to meet current and future food animal production needs of diversified consumers, while simultaneously minimizing the environmental footprint of production systems and enhancing animal well-being. **Target**: Increase production efficiency and enhance the economic value and well-being of U.S. food animal production while decreasing the environmental footprint of production systems.

Performance Measure 4.2.1: Provide scientific information to protect animals, humans, and property from the negative effects of pests, infectious diseases, and other disease-causing entities. **Target**: Development of technologies for the integration of prevention and treatment strategies to manage top priority endemic and exotic threats to livestock, humans and property.

Performance Measure 4.2.2: Develop and transfer tools to the agricultural community, commercial partners, and government agencies to control or eradicate domestic and exotic diseases and pests that affect animal and human health. **Target**: Transfer technologies to the commercial, public and government sectors.

Performance Measure 4.2.5: Provide environmentally sound fundamental and applied scientific information and technologies to action agencies, producers, exporters and importers of commercially important plant and animal products in support of exclusion, early detection and eradication of quarantine pests and pathogens that can impede foreign trade. **Target:** Mitigate risk of pests and pathogens resulting in expanded export markets while protecting the safety and security of American agriculture.

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 on germplasm conservation is needed, which will require 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. There may be opportunities to gain in production efficiency by exploiting genotype by environment and gene by gene interactions.

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

These efforts will be significantly enhanced with appropriate genomic tools and reagents. Public involvement in the construction of these resources is critical to ensure development of economically feasible management tools for aquaculture producers and to provide all researchers 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

There are many species of aquatic animals with high potential value for aquaculture that are in early stages of development. Maintaining genetic diversity will be essential for providing a genetic basis for current and future breeding programs that will develop stocks with efficient performance in different climates and production systems. Specifically, research is needed 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 should be stored and provided to researchers for genomic characterization and analysis. In addition to improving the present value of aquaculture production, this conservation and characterization work is also needed to prepare for the repopulation of aquaculture systems with improved animals in case of widespread disease outbreak or other catastrophe.

Research Needs:

Selection of specific germplasm to be preserved requires phenotypic and genetic characterization for a wide array of phenotypic characters measured within appropriate production systems. Characterization of genetic diversity should be

performed using microsatellite and single nucleotide polymorphism (SNP) panels to ensure that the appropriate range and degree of variation is preserved. There is also a need to select, catalogue, and curate collections of germplasm for research and germplasm conservation purposes. Research is needed to store and use cryopreserved genetic material, including gametes and/or embryos of various aquatic animals, in coordination with the National Animal Germplasm Program (NAGP). Researchers should take advantage of the Germplasm Resources Information Network (GRIN), the bioinformatics platform that provides an interface to the genetic resources held by the NAGP.

Anticipated Products:

- Conservation of a broad spectrum of genetic diversity in the form of viable and well documented aquatic animal germplasm. Material will be available through the National Animal Germplasm Program.
- Genomic diversity in conserved populations characterized through the use of molecular technologies
- Successful and efficient sperm and/or gamete cryopreservation technologies and methodologies available for aquatic species.

Potential Benefits:

Careful strategic planning and implementation of genetic resource preservation will provide industry producers and breeders with dependable and diverse sources of genetic material. For example, the repository provided by the NAGP will become a source of resources for long term use by the aquaculture breeders and natural resource conservators, and the ability to store and move gametes will enhance the ability to transfer genetic gains geographically and use sperm from elite males throughout the year. Ultimately, species with threatened natural populations will be better preserved and more secure.

Problem Statement 1B: Develop and Implement Genetic Improvement Programs

The application of quantitative genetics theory to animal populations of livestock and poultry has produced significant genetic changes in particular components of performance, such as the doubling of milk production per cow over the past 25 years. Meanwhile, aquatic animal genetic improvement has lagged behind, hindered by a combination of the small numbers of families evaluated and lack of phenotypic information. Researchers should take advantage of the increased scope and power of computing platforms and the large family sizes available for many aquatic animals by applying them to large-scale pedigreed phenotypic data sets. Genetic evaluation and improvement programs are poised to reach new levels through the elucidation of genetic parameters and interrelationships among traits, development of objective multi-trait breeding goals, and the availability of genomic resources for characterization at the individual gene and gene sets level. These genetic improvement programs will focus on animal production and well-being and emphasize the use of functionality driven strategies.

Research Needs:

The traits included in genetic evaluation programs of aquatic animals need to be expanded beyond growth and survival. Specifically, research is needed to describe genetic parameters for a suite of economically-important traits, including 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. These parameters include additive and dominance effects; heritabilities; and genetic, phenotypic, and environmental correlations. As new traits continue to be defined and added to the genetic evaluation pipeline(s), work will be needed to define breeding objectives incorporating information from multiple traits under specific production environments. Research in functional genomics and proteomics will yield new diagnostics and genetic evaluation tools regarding specific genes and gene complexes, and this new molecular information will be incorporated to maximize genetic improvement. Additionally, new approaches to holistically use molecular information from the entire genome at the haplotype map level must be investigated and validated to determine if and how they can be employed in genetic selection and mating programs. The complexity of the new types of information becoming available for genetic evaluation and improvement will require significant advances in statistical methodology and software to effectively employ such data in practical genetic improvement.

Anticipated Products:

- Genetic prediction for a suite of new traits in aquatic animals related to adaptability and functionality in multiple production environments.
- Enhancement of genetic evaluation for economically-important production traits in aquatic animals. Results will be communicated through regional and commodity specific meetings (e.g. Pacific Coast Shellfish Growers Association, Catfish Farmers of America).
- Development of technologies allowing breeders to identify genetic inputs fitting breeding objectives defined within their specific production environment. Results will be communicated through regional and commodity specific meetings (e.g. US Trout Farmers Assoc. meetings, Striped Bass Growers Assoc. meetings)
- Statistical tools and methodology for incorporating molecular genetic data into genetic evaluation and prediction programs, resulting in “genome-enabled” genetic improvement.

Potential Benefits:

Genomic-level information will allow complex traits previously excluded from genetic improvement to be evaluated; these traits include many of the most important to enterprise profitability. Moreover, identification of elite genetic seed stock at younger ages with higher levels of inherent accuracy will allow the rate of genetic change to accelerate to previously unattainable levels. The enhancement of genetic improvement programs by adding traits that enable producers to better match genetic potential to the production resource base and consumer and societal demands will ultimately lead to more profitable and sustainable aquaculture.

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, and Atlantic salmon over the past 5-10 years, with shellfish (oysters) lagging behind. 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 content necessary for optimal "mining" through functional genomic and proteomic approaches. ARS remains committed to devoting the necessary resources to fill these research gaps.

Research Needs:

ARS research will continue 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), will be a major effort over the next 5 years. Scientists will also develop a full and comprehensive array of full-length cDNAs for functionally important tissues under differing environmental conditions and developmental stages, which will be useful for ascertaining alternative splicing of genes and fully exploring gene function and regulation. In addition, there is need for further development of gene expression tools to a commercially available level, such as RNA interference (gene silencing) and targeted gene transfer. On a related note, ARS should further develop research/resource populations and specialized cell lines for aquaculture species, which will provide an optimal framework for mining genomic information and studying gene expression. Fundamental research is also needed to further develop methodology to transfer genes within and across species. Using these and other tools, ARS will work to fill in the significant gaps that exist in the bioinformatic infrastructure and provide the scientific community with the genomic and proteomic data needed to support further research.

Anticipated Products:

- Sequenced genomes for catfish, rainbow trout, oysters, and Atlantic salmon.
- Improved physical and genetic linkage maps of the catfish, rainbow trout, oyster, and Atlantic salmon genomes.
- Identification of QTL for economically important traits and linking of genotype and DNA sequence variation to phenotype and production performance.
- Full-length cDNA libraries and gene expression tools spanning important tissues, environments, and developmental stages for important aquatic animal species.
- Well-characterized and deeply phenotyped resource populations of catfish, rainbow trout, Atlantic salmon, striped bass, yellow perch, and oysters.
- Specialized cell lines enabling the study of gene expression.
- Improved techniques and methodologies for intra- and inter-species gene transfer.
- Improved bioinformatic tools for rapid, routine access to and visualization of genomic information.

Potential Benefits:

The completion of these genome-enabling tools and reagents will facilitate the continued genetic improvement of aquatic animal production systems. These tools will be used to develop new genetic selection methodologies, identify the functional role and interactions of gene products in the animal, and ultimately to develop "precision genetic management systems" for aquatic animals. These

tools will not only be used for traditional animal production research applications (reproduction, growth and development, nutrient intake and utilization, product quality), but will also be used to decrease the environmental footprint of aquatic animal production; improve animal health, well-being, and resistance to disease; and enhance food safety.

Component 1 Resources

The following ARS locations have research projects addressing the problem statements identified under Component 1:

- **Franklin, ME**
- **Hagerman, ID**
- **Leetown, WV**
- **Milwaukee, WI**
- **Newport, OR**
- **Stoneville, MS**
- **Stuttgart, AR**

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 the 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 impacts 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. Furthermore, as production systems focus not only on weight gain but also on gains in product or protein mass, efficient conversion of nutrients into muscle has become a priority. Identification of the genes and pathways leading to improved growth and nutrient utilization efficiency and product quality are important for maximizing our ability to improve these traits in the production environment.

Knowledge of behavioral hierarchies and the appropriate rearing densities is also key 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.

Research Needs:

ARS scientists should work to expand our knowledge of the factors affecting nutrient utilization in aquatic animals. Specifically, the genetic basis for variation in feed efficiency is not well defined and needs further study. Scientists also need to develop an improved definition of feed efficiency that improves protein retention rather generating fatter animals and additional waste material. Understanding of the genes and metabolic pathways involved in nutrient assimilation and how nutrient balance affects these pathways is also required. Further areas of research will include the development of production techniques to reduce animal stresses and decrease losses from aggressive interactions among cultured animals in aquatic animal production systems and technologies to improve animal condition associated with transport.

Anticipated Products:

- Determination of the degree of genetic control of feed efficiency in aquatic animal species.
- Evaluations of protein and fat retention, rather than gross feed efficiency, in aquatic animals.
- Mapping of the gene pathways involved in feed intake and metabolism.
- Development of production techniques that reduce stress and disease outbreaks in aquatic animals. Results will be communicated through regional and commodity specific meetings.
- Development of transport technologies that result in less stress and improved animal welfare and better product quality. Results will be communicated through regional and commodity specific meetings.

Potential Benefits:

Research in these areas will lead to an improved understanding of the nutritional value of feedstuffs and the physiology of nutrient retention and conversion, which will enable the targeting of genes and pathways for improved feed consumption and nutrient utilization. This work, in turn, will enable 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 will benefit 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.

Problem Statement 2B: Enhance Reproductive Performance and Reduce Reproductive Losses

Improving reproductive success is a critical need for the production of many aquatic species. Managing this process requires basic research to better understand the the basic neuroendocrine regulatory mechanisms that coordinate functions of the hypothalamo-pituitary-gonadal axis. Likewise, the effects of handling and other

management interactions on reproduction need to be better understood to minimize problems arising from animal management. In addition to these efforts, research is needed 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 will improve larval survival. Other opportunities for improving reproductive performance include devising methods to control timing of availability of juveniles for grow-out and to produce animals of the gender more desirable for food production. By improving reproductive success in these ways, research can significantly increase aquatic animal production efficiency and profitability.

Research Needs:

Research will seek to define and enhance understanding of basic neurendocrine regulatory mechanisms and gonadal development and function, as well as to understand the impacts of environmental stressors on successful gamete production, fertilization, hatch, and survival, for aquatic species. Furthermore, researchers must identify intervention strategies, including the use of hormones, to enhance reproductive output and quality, particularly since optimal or even minimal requirements are not always met for fish species in captivity. ARS will also work to develop methods to control the sex of aquatic animals. Other research will include work to improve egg quality, which requires understanding basic processes involved in the assembly of the egg and needs of the developing embryo, developing methods to provide the broodstock with the environmental conditioning and nutritional requirements to produce quality gametes (egg and sperm), and devising appropriate strategies for spawning and fertilization.

Anticipated Products:

- Identification of critical developmental stages and environmental requirements for improving reproductive performance in aquatic animals.
- Identification of hormonal treatment strategies to enhance reproductive output and quality.
- Methods to control the sex of animals produced.
- Methods to manipulate reproduction and control timing of spawning. Results will be presented at regional and commodity specific meetings.

Potential Benefits:

Increased reproductive success will lead to more stable and economical production of animals. Producing monosex populations should improve farm productivity and may also increase the uniformity of the product. Meanwhile, the spreading juvenile availability throughout the year will lead to better use of the production space.

Component 2 Resources

The following ARS locations have research projects addressing the problem statements identified under Component 2:

- **Ft. Pierce, FL**
- **Hagerman, ID**
- **Leetown, WV**
- **Milwaukee, WI**
- **Newport, OR**

- **Stoneville, MS**
- **Stuttgart, AR**

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

The largest cost of aquatic animal production, for most species, 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, least cost feeds can be formulated based on 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. Additionally, past data is generally for a single stage of production and not partitioned into nutrient requirements for various stages of production, such as larval development, grow-out, and reproductive stages. 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. Further, while research in this area has generally focused on growth performance and product quality, the effect of nutrition on reproductive performance is of growing concern, requiring research on nutrient requirements for reproductive performance as well.

Research Needs:

ARS will conduct research on techniques for determining nutrient requirements, which is critical for species where traditional measures of feed intake are difficult (for example, shrimp and shellfish do not quickly ingest feeds whole, but ingest small particles from the diet provided), and use those technologies to quantify nutrient requirements. Researchers will also work to determine whether genetic improvement for production traits such as growth may affect nutrient requirements. Other efforts will include developing methods to utilize genomic tools for evaluating metabolic responses to varied levels of nutrients.

Anticipated Products:

- Definition of nutrient and energy requirements of commercially important aquaculture species at various life stages reared in different culture systems.
- Quantification of requirements and effects of nutrients such as vitamins, minerals, fatty acids, and amino acids on reproductive efficiency and larval quality.
- Improved understanding of functional genomics associated with nutrition, growth, and development.
- Knowledge of effects of specific nutrients on metabolism and endocrine pathways.

Potential Benefits:

Research in this area will support the formulation of cost-effective diets that promote optimal performance for growth and development, improved product quality, and production efficiency. Also, by maximizing nutrient retention, environmental impacts of nutrient release will be minimized.

Problem Statement 3B: Evaluate the Nutritional Value of Alternative Sources of Protein and Lipid

Changes in dietary nutrient intake is the primary factor affecting animal performance measures such as growth and development, reproductive success, carcass quality, disease resistance, and waste production. Individual feed ingredients, whether traditional, novel, and/or genetically-modified, greatly impact these characteristics, though few ingredients have been thoroughly evaluated for their potential nutrient bioavailability. 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 captured fisheries have reached or exceeded maximum sustainable yields. Adding to this limitation on supply is the increased competition among consumer segments 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/or developed. Possible sources of these nutrients meriting investigation include co-products from bio-fuel production, which provide an attractive source of nutrients with a potentially large supply, as well as co-products from various meat processing industries. Additionally, the effect of feed manufacturing on nutrient content and availability needs to be optimized. Another rising need is for increased knowledge of feeding strategies to improve nutrient utilization and assimilation and decrease waste output. Furthermore, the impact of diet alteration on product quality and how this relates to consumer preferences must be evaluated.

Research Needs:

The nutritional value of feedstuffs depends on the bioavailability of nutrients they contain, the presence of anti-nutritional factors, and interactions among the ingredients. In addition to determining the digestibility of traditional feed materials used in livestock diets, co-products from bio-fuels provide a new abundant source of material that will be evaluated for their nutritional value in feeds for a variety of aquatic species. Other potential feed ingredients to be evaluated include co-products from fish processing and plant oil as a substitute for fish oil, especially in marine carnivorous fish diets. Researchers will also work toward improving the nutritional value of alternative protein sources through the use of better processing methods, such as heat treatment, removal of anti-nutritional factors, and use of additives (enzymes, essential amino acids, and palatability enhancers).

Anticipated Products:

- Database of digestible/available nutrients from traditional and alternative ingredients for specific species, available in a web-based interface.
- Indices of nutritional value of ingredients from many new sources for a variety of aquatic animals.

- Diets based on ingredient nutrient bioavailability that conform to the nutrient and energy requirements of commercially important aquaculture species at different life stages reared in different culture systems.
- New sustainable sources of ingredients as replacements for fish meal and oil in aquatic animal diets.
- Enhanced methods for ingredient processing to improve nutritional value for aquatic species.
- Feed processing criteria necessary to optimize nutritional value of alternative and traditional feed ingredients. Results will be presented at feed manufacturing specific meetings.
- High inclusion levels of alternative protein and lipid sources.

Potential Benefits:
Reduced reliance on fish meal and fish oil from pelagic capture fisheries will enable increased production of fish feeds for sustainable aquaculture production. Furthermore, increasing the number of high quality alternative ingredients will provide flexibility in formulating least cost diets.

Component 3 Resources
The following ARS locations have research projects addressing the problem statements identified under Component 3:

- **Auburn, AL**
- **Fairbanks, AK**
- **Ft. Pierce, FL**
- **Hagerman, ID**
- **Hilo, HI**
- **Stoneville, MS**
- **Stuttgart, AR**
- **Waimanalo, HI**

Component 4: Improving Health and Welfare of Aquatic Animals
The United States aquaculture industries, including producers growing animals in high densities, and germplasm and fingerling and recreational fish producers dependent on moving animals need to control endemic, emerging, and catastrophic diseases that result in losses of aquatic animal production and restrict movement of aquatic animals out of affected regions. Despite the progress that has been made in aquatic animal health, significant loss of animals to disease still occurs, at great cost to producers. Loss of over 30 percent of the population from the juvenile stage to harvest is not rare for aquaculture species and represents a huge economic impact. Health management strategies, technologies, and bio-security plans that are safe for the environment and for consumers of aquaculture products are necessary to reduce disease-related losses.

However, there is presently a lack of validated technologies for early and rapid detection, prevention, and treatment of diseases in production systems, which has hindered the growth and competitiveness of the U.S. aquaculture industry. Validated diagnostic tools that can be used in production systems to detect the disease agents in a rapid fashion are required. In addition to the need for diagnostics, effective control strategies and therapeutants 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 to supplement

the *Edwardsiaella ictaluri*, and *Flavobacterium columnare* that are currently available and in use by aquatic animal farmers and hatchery managers.

This vaccine work will benefit greatly from new molecular tools. For example, sequencing the pathogens to identify regions of similarity among many strains of the pathogen may aid in producing vaccines that offer broad protection. Additionally, molecular tools enable 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

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

Research Needs:

Molecular tools will be used to identify cells and regulatory substances (cytokines) important in natural resistance and acquired immunity in aquatic animals. Tools such as microarrays, quantitative PCR, and new massively parallel sequencing technologies will be used to evaluate the immune responses of the skin, gut, and other epithelial tissues to economically important pathogens. Additionally, animals with resistant/susceptible phenotypes will be investigated to understand the mechanisms and location of genes (quantitative trait locus, or QTL) leading to reduced mortality. Using genetic variations in response to vaccination, researchers will select for good responders and identify gene correlates for vaccine efficacy. This work will require close collaboration with genetics and breeding programs.

Anticipated Products:

- Characterization of the cells/tissues and specific proteins such as cytokines important in natural resistance and acquired immunity in aquatic animals.
- Determination of the immune response following infection and vaccination that results in protective immunity against economically important pathogens.
- Collaborations using animals in well-structured populations with good genetic and phenotypic records.
- Quantification of population responses to vaccines and the effects of genetic variation on the response and selection of good vaccine responders.

Potential Benefits:

Information on immune system components will provide targets for intervention to enhance immune system responsiveness. Animals with positive response to vaccination will be identified, forming an animal resource for locating the immune system pathways or components correlated with enhanced responsiveness.

Additionally, animals with enhanced resistance will be identified and will form the basis of select disease resistant lines.

Problem Statement 4B: Detection and Control of Pathogens

To prevent the introduction and spread of harmful diseases, domestic and international trade of aquatic animals needs rapid, automated, and accurate tests to demonstrate that aquatic animals, seed stocks, and products are free from harmful pathogens. In addition, the accurate determination of preclinical infections (*i.e.*, prior to disease) will enhance opportunities to determine the potential risk of disease and allow for earlier application of preventative measures to reduce or eliminate the impact of emerging or catastrophic diseases in the United States. However, methods and reagents to rapidly detect pathogens and diagnose diseases in aquatic species are still unavailable or have not been applied at the farm. Microbial genomic sequences, or particular diagnostic regions of the genome, will be important tools for pathogen identification and understanding pathogenesis. Once pathogens have been identified, strategies are needed to identify effective treatments, understand their pharmacokinetics, and apply therapeutants to control them.

Research Needs:

Molecular tools, including pathogen genome sequencing, will be used to develop validated rapid and automated methods, both microbiological and immunological, to detect infectious and non-infectious disease agents and toxins in aquatic animals. Studies on the factors contributing to how, when, and where disease outbreaks occur are also needed. As for control, researchers will work to identify compounds that can be used as therapeutants, create programs for obtaining regulatory approval of potential therapeutants, and develop methods of mass delivery.

Anticipated Products:

- Rapid and automated PCR-based and specific immunological test methods to detect infectious and non-infectious disease and toxins in aquatic animals.
- Genome sequences of pathogens that will allow identification of pathogen strain and source prior to overt disease.
- Identification of microbial genes and pathways critical for pathogenesis.
- Safe and effective therapeutants for control of economically important pathogens.
- Methods for delivery of therapeutants.
- Approval of new aquatic animal therapeutants.

Potential Benefits:

Sequence information on the microbial genomes will permit better identification of the pathogens and improve our understanding of pathogenesis and virulence factors. The capability to rapidly identify disease and/or disease-causing organisms will enable swift intervention through the application of the proper therapeutic or treatment and reduce the severity of disease outbreaks and economic loss. The ability to survey for pathogens prior to a disease outbreak would also presumably lead to reduced mortality. Meanwhile, more effective compounds and improved delivery systems will enhance disease control through

the use of therapeutics, and the approval of new aquatic animal therapeutants will extend the capability to reduce animal and economic losses.

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 only effective when administered by injection or with adjuvant, a strategy that may be impractical and not always economically feasible. Ultimately, vaccine research must result in a product that is 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 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 vaccination through immersion as juveniles or eggs or through feed. Another important aspect of disease prevention involves addressing animal welfare concerns through physiology and stress research. When environmental conditions deteriorate due to poor water quality or some other stressor, disease outbreaks are more likely to occur.

Research Needs:

Vaccines will be developed and tested in the laboratory for safety and effectiveness. Researchers will evaluate many strains of recognized pathogens through genetic screening and microbial sequencing and endeavor to develop strategic vaccines offering broad protection against pathogen classes. Techniques will also be investigated to apply vaccines and medicines using mass delivery strategies in on farm trials. Other basic and applied research to enhance health management and increase animal health in production systems will be conducted, including studies on behavior and environmental factors affecting animal welfare and the use of probiotics, immunostimulants, and some nutrients to improve fish health. Additionally, research to improve bio-security to prevent and control infectious and non-infectious diseases will be performed.

Anticipated Products:

- Safe and effective vaccines with broad effectiveness for prevention and control of economically important pathogens of aquatic animals.
- Multivalent vaccines against multiple (and sometimes concurrent) pathogens of aquatic animals.
- Identification of effective mass delivery strategies for aquatic animal vaccines.
- Research and development to support approval and licensing of new aquatic animal vaccines.
- Determination of the inclusion levels and efficacy of probiotic and immunostimulant compounds to prevent disease and improve production.
- Cost-effective health management plans and employment of these plans by aquaculturists. Results will be presented at regional and commodity specific meetings.
- Identification of factors that affect animal well-being and performance.

Potential Benefits:

Vaccines hold the potential to greatly reduce the need for other therapeutants (*e.g.*, antibiotics), improve the economic returns of aquatic animal production by requiring less reactive drug use, and reduce the environmental impact by reducing use of antibiotics and other antimicrobial compounds. Use of probiotics could also improve disease prophylaxis and reduce the reliance on antibiotics. Meanwhile, improved biosecurity should lessen the frequency and dispersion of disease problems, improve animal well being, and increase system productivity and reliability.

Component 4 Resources

The following ARS locations have research projects addressing the problem statements identified under Component 4:

- Auburn, AL
- Ft. Pierce, FL
- Hagerman, ID
- Leetown, WV
- Newport, OR
- Oxford, MS
- Stoneville, MS
- Stuttgart, AR

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 be provided with the necessary information and technology to meet consumer needs for desired fish and shellfish products. To that end, production technologies must be developed to culture new aquatic species and to optimally culture existing species in existing and new environments. 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, requires knowledge of the interactions among inputs, culture species, production environment, and economics; however, these interactions are not understood fully.

The marketplace for foods coming 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 “aquatic products as food” industry growth. New methods need to be developed to enhance the sensory and nutritional qualities of aquaculture products. In addition, there are great opportunities 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 and Flow-through Production Systems

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

On the other hand, flow-through systems, such as those typically used in rainbow trout production, have the advantage of low energy inputs and abundant water supplies at the cost of reduced water quality and water quality control. These quality and control disadvantages are the result of water in raceway systems being serially used as it passes down an elevation gradient on its way through several raceway steps. For flow-throw systems, the challenge is to improve water quality and contain waste production while maximizing energy efficiency.

Depending on the system employed, the production of (live) feeds for first feeding stages within many culture systems is either a critical gap or a high cost. There is a significant need to address this bottleneck by improving each of these systems to optimize production of feed organisms for larval fish and shellfish.

Research Needs:

For flow-through systems, research is needed to improve 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 needed to maximize waste removal and production per unit of water used. For recirculation systems, sensitivity analysis to model the inputs and outputs is needed to determine the factors with highest leverage. Further, research is needed to develop improved systems for growing and delivering live feed for fish with small larvae and larvae that do not accept prepared feeds. Work is also needed to develop methods of rearing marine fish in low salinity water

Anticipated Products:

- Increased application of water moving, water quality monitoring, and process control technology to aquatic animal production.
- New tools and methods to analyze aquatic animal production systems and manage off-flavors. Results will be presented at regional, national and commodity specific meetings.
- Methods for production of high value marine species in low salinity recirculating systems. Results will be presented at commodity specific meetings.
- Systems for live feed production.

Potential Benefits:

Increasing levels of automation will lower the cost of labor and improve the cost competitiveness of domestic aquaculture products, and improved efficiencies in production and waste removal will maximize production per unit of volume. The ability to grow marine fish in low salinity water will reduce aquaculture's reliance on freshwater, as will improved waste capture and water remediation. Finally, developments in larval feed production can support the growth of new species that require live feeds as first feeding animals.

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 to make any change is large, so there is a critical need for research in smaller systems that can be verified in large commercial scales. To promote fish production industry profitability, there is also a need for manipulation of pond microbial and phytoplankton communities to control product flavor, predation of larval or juvenile fish, and predation of larger fish and shellfish by birds. Nutrient removal from the pond waters during draining is another increasing concern.

Research Needs:

To enhance pond management and reduce off-flavor in fish, research is needed to control the phytoplankton species making up the photosynthesizing biomass in the pond. Control or avoidance strategies are also necessary to combat insects and insect larvae that prey on larval and juvenile stages of farmed aquatic animals. In addition, novel approaches are needed to solve the ongoing problem of depredation of fish and shrimp by birds. Other research should develop methods to minimize introduction of nutrients into receiving water at the end of a growth cycle or whenever ponds are drained .

Anticipated Products:

- Strategies and/or compounds for favoring the beneficial or neutral phytoplankton species or selectively reducing the abundance of harmful and/or undesirable phytoplankton to reduce levels of “off-flavor” compounds.
- Strategies to avoid or kill predacious insect and insect larvae.
- Strategies to reduce losses due to birds.
- Methods to reduce effluent nutrient levels.

Potential Benefits:

Reducing the negative impacts of undesirable phytoplankton blooms to decrease off-flavor episodes and reduce oxygen demand during the night in ponds would greatly improve the efficiency of pond production. Additionally, strategies to avoid or kill predacious insects and reduce bird predation would be a direct benefit to producers by extending the production of juveniles and minimizing product losses. Meanwhile, capturing nutrients from the effluents would not only improve effluent quality, but also provide material for soil fertilization.

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 multi-use 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 regarding siting resulting in the need for better understanding of the interaction between shellfish aquaculture production systems and the environment.

Research Needs:
Research is needed to determine and validate test methods to reduce mortality due to disease, predators, pests, and bio-fouling organisms. Research is also necessary to identify and quantify interactions of aquaculture practices with natural resources to benefit the shellfish production industry and satisfy regulatory constraints. In addition, due to recent summer and winter mortality events, it is clear that the effects and limits imposed on shellfish by temperature, other environmental factors, and the interactions of these stresses with pathogens need to be defined.

Anticipated Products:
- Environmentally-compatible practices to combat predators and pests and treatments that selectively impede predators and bio-fouling organisms.
- Published science that the shellfish industry can use to complete environmental management plans and policy makers can use to develop science-based policy that sustains the marine environment.
- Identification of genes with altered regulation under high and low temperature challenges and/or exposure to pathogens.

Potential Benefits:
Production efficiency can be improved through efforts to reduce the loss of juveniles and adults in open production systems. In addition, authoritative scientific reports that can support environmentally-compatible best management practices will reduce regulatory uncertainty for the shellfish aquaculture industry. Further, identifying the genes affected by thermal stress and pathogens and identifying individuals that are more and less susceptible to these challenges will suggest a methodology to understand the progression of the disease and potential remediation strategies.

Problem Statement 5D: Improve Product Quality and Develop New Products
Lack of product consistency is a major concern for some aquatic animal species. The consumer demands consistent high quality products, and when defects are detected, corrective actions must be taken. For example, non-invasive, non-destructive testing procedures must be created to identify product problems and measure product yield and quality characteristics. In addition to improving product quality, there are opportunities to create new products and add value to existing ones to meet consumer needs for improved economic viability of aquaculture industries. There are also opportunities 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.

Research Needs:
For many of the edible aquaculture products, methods will be developed to measure attributes such as color, firmness, and taste, and to optimize product quality and enhance shelf life and storage stability of products. Economical methods are also needed 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 should also work toward the development of 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 (HUFAs), antioxidants, and vitamins.

Anticipated Products:

- Improved techniques to measure product quality attributes.
- Improved methods to collect, preserve, and store co-products of the Alaskan fishery processing industry.
- Methods, processes, and procedures to increase the utilization and value of fish processing byproducts.
- Cost effective strategies for optimizing the nutritional quality of edible aquaculture products.

Potential Benefits:

Improving product quality by improving the traits of importance to U.S. consumers may increase the demand for domestic products. Furthermore, efforts to improve the utilization of coproducts and reduce the total loss of important marine protein and oil supplies will improve production efficiency and promote environmental responsibility.

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Component 5 Resources

The following ARS locations have research projects addressing the problem statements identified under Component 5:

- **Fairbanks, AK**
- **Ft Pierce, FL**
- **Hagerman, ID**
- **Leetown, WV**
- **New Orleans, LA**
- **Newport, OR**
- **Oxford, MS**
- **Stoneville, MS**
- **Stuttgart, AR**