National Program in Aquaculture (106)

Retrospective Accomplishment Report

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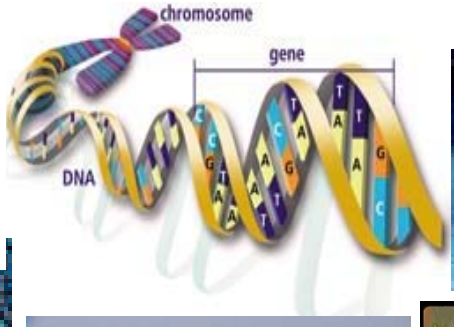

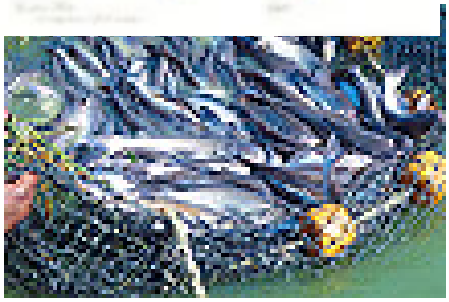

Table of Contents

BACKGROUND AND GENERAL INFORMATION

Aquaculture will be the most likely source of food fish in the 21st century. With increasing seafood demand and declining capture fisheries, global aquaculture production will have to increase by 500 percent by the year 2025 to meet the projected needs of a world populated by 8.5 billion people. The United States has the natural resources and broad interest from private individuals and industry to be in a strong position to help meet this need. The application of new tools in molecular biology and re-circulation systems engineering in support of traditional husbandry, bio-control, and environmental sciences have the potential of improving the economic competitiveness and sustainability of U.S. aquaculture. A missed opportunity in U.S. agriculture is the integration of water use between agricultural irrigation and fish culture, i.e. over 150 million acre-feet of water are currently used in irrigated agriculture, careful reuse of this water could produce 20 million pounds/year of fish valued in excess of $20 B.

The U.S. Department of Agriculture Agricultural Research Service (USDA/ARS) has the national capability and demonstrated performance to lead U.S. aquaculture research and technology development to dynamically enhance the U.S. aquaculture industry. USDA/ARS and partners conduct an aquaculture research program that has contributed to national growth and global competitiveness of U.S. producers. A strong USDA commitment to aquaculture research, technology development, and technology transfer through ARS and the Cooperative State Research, Education, and Extension Service (CSREES), in cooperation with university and private research programs, and linked to State and regional agricultural extension programs, is critical to energize industry development, improve production efficiency, and assure quality and wholesomeness of farmed aquatic animals.

The USDA/ARS is the intramural research agency for the U.S. Department of Agriculture, and is one of four agencies that make up the Research, Education, and Economics mission area of the Department. The USDA/ARS budget in 2007 of $1.13B is allocated to research conducted in 21 national program areas. Research is conducted in 108 laboratories spread throughout the United States and overseas by over 2,100 full-time scientists within a total workforce of 8,010 USDA/ARS employees. The USDA/ARS national program addressing aquaculture (NP 106) involves research conducted at 17 U.S. locations by 63 full-time scientists, organized in 25 appropriated research projects with a total annual appropriated budget of approximately $35 M.

The vision for this national program is *"to furnish scientific information about biotechnologies and management practices that ensure an abundant supply of competitively priced aquaculture products"*. Consequently, the overall mission is to conduct high quality, relevant, basic and applied aquaculture research and technology transfer to create jobs and economic activity that will improve the international competitiveness and sustainability of U.S. aquaculture, and reduce dependence on imported seafood and threatened ocean fisheries. This National Program mission follows from the USDA/ARS Strategic Plan (www.ars.usda.gov/SP2UserFiles/Place/00000000/ARSStrategicPlan2006-2011.pdf) which, in turn, is directed towards achieving goals mandated by the USDA Research, Education, and Extension Mission Area Strategic Plan and the USDA Strategic Plan for 2005-2011.

The products of research conducted in this national program contribute toward broader goals (termed "Actionable Strategies") associated with the following specific Performance Measure from the ARS Strategic Plan for 2005-2011.

Strategic Goal 2: Enhance the Competitiveness and Sustainability of Rural and Farm Economies

Performance Measure 2.2.1: Develop new technologies, tools and information contributing to improved precision animal production systems to meet current and future animal production needs of diversified consumers, while simultaneously minimizing the environmental footprint of production systems and enhancing animal well-being. Baseline: Ten new technologies developed and used by ARS customers to 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. Target: Cumulatively, 35 new technologies developed and used by ARS customers.

PLANNING AND COORDINATION FOR NP 106

USDA/ARS established national programs for organizing and communicating its research programs with customers, stakeholders and partners in 1996. A national program on aquaculture was established at that time to provide leadership in aquaculture research for the U.S. aquaculture industry. The ARS Aquaculture National Program Team held a National Program Planning Workshop on June 5-6, 1997 in New Orleans, Louisiana to establish program direction and implementation of the plan. An Action Plan was written which provided the basis for individual prospectus and project plan development, peer panel review and the authority for the research conducted in the national program from 1997-2002.

The second *Joint ARS-CSREES Aquaculture National Program Stakeholder Planning Workshop* was held on November 20-22, 2002 in St. Louis, Missouri (www.ars.usda.gov/research/programs/programs.htm?np_code=106&docid=1263). The objectives of the workshop were to: 1) validate and update the ARS Aquaculture National Program Action Plan; 2) learn about customer, stakeholder, and partner needs; and 3) communicate ARS capabilities and accomplishments. Review of output from the workshop indicated that the USDA/ARS Aquaculture National Program was generally on target. There was a sense of urgency detected in customer presentations for information to solve problems with over-arching themes or contexts such as reducing costs of inputs, increasing production efficiency, improving fish and shellfish health, developing and protecting domestic genetic resources, increasing environmental compatibility and sustainability, and improving product quality. It was recommended that the national program Action Plan incorporate aquaculture research holistically involving the production system, and, integrate biological, engineering, and social sciences to focus on environmental stewardship, animal well-being, biosecurity, and other contemporary societal issues in food production.

The NP 106 Action Plan was subsequently drafted by writing teams composed of ARS scientists and members of the USDA/ARS National Program Staff (NPS). The writing teams combined input from the workshop, their own knowledge of the subject matter area, and input from other ARS scientists and their cooperators to identify the key priority needs that could be addressed by ARS research in the coming five year period. These needs were aggregated into Problem Areas for each National Program Research Component. After a public comment period, the draft Action Plan was revised, edited, and completed in 2003 (see http://www.ars.usda.gov/research/programs/programs.htm?np_code=106&docid=276 .

Once the Action Plan was completed, ARS project teams each developed a project prospectus containing objectives of specific research from the framework of the Action Plan. Prospectus writing was delayed until 2004 for NP 106 to balance the work load for managing the agency's peer-review process. Project Plans were then written by project teams of scientists after NPS approval of their project prospectus. Project Plans included statements of the anticipated products or information to be generated by the project, how they contributed to solving the larger National Program Problem Areas, and time lines and milestones for measuring progress toward achieving the project goals. All project plans were then evaluated for scientific quality by external peer review panels. The project peer reviews were administered by the ARS Office of Scientific Quality Review (OSQR). Project Plans were revised in response to review panel recommendations, and were then approved for implementation. Project plans were approved for the period of December 2004 to December 2009.

Five years since the NP 106 Customer- Stakeholder Workshop in 2002, the progress achieved in attaining the Action Plan goals is now being assessed by an external retrospective assessment panel. This program assessment is in preparation for the beginning of the next five-year national program cycle (December 2009-July 2014). **The next cycle, which will begin with a national program stakeholder workshop in early 2008, will be driven by a new action plan that will incorporate information from this assessment process**

Day-to-day coordination of the ARS national program in aquaculture is the task of the National Program Leaders who comprise the NP106 Leadership Team. The national program is also coordinated with other ARS National Programs and with activities of other agencies. For example, discussions and analyses of the National Science and Technology Council's Joint Subcommittee on Aquaculture, and Interagency Working Group on Domestic Animal Genomics; as well as the USDA Task Group on Animal Welfare, coordinate and align NP 106 research with efforts in other agencies across the Federal government. These interagency working groups include representatives from the USDA (ARS and CSREES), National Science Foundation, National Institutes of Health, Department of Commerce, Department of Energy, Department of the Interior, Food and Drug Administration, U.S. Agency for International Development, Department of Homeland Security, Office of Science and Technology Policy, National Oceanic and Atmospheric Administration, and the Office of Management and Budget.

In addition to ongoing planning and coordination during the NP 106 Program Cycle, USDA/ARS National Program Staff and Area Offices organized and conducted several workshops focused on specific research issues relevant to the needs of U.S. aquaculture. Most of these workshops also involved coordinating and integrating ARS NP 106 efforts with those of cooperating agencies, and with university and industry partners. A partial list of workshops appears in **Appendix 1.**

HOW THIS REPORT WAS CONSTRUCTED AND WHAT IT REFLECTS

In this Report, information about NP 106 achievements and their impact is organized according to National Program Research Components and their constituent Problem Areas, described in the current National Program Action Plan. The report first outlines the seven NP 106 Research Components. This is followed by a section for each of the components. The actual language from the current Action Plan is given to outline the **committed goals, planned approaches, expected outcomes, and engaged USDA/ARS locations** for each of the Problem Areas within each Component. These are followed by **selected accomplishments** achieved under

the current Action Plan and include the **impact and/or potential anticipated benefits** of those achievements on solving the problems and meeting the high priority needs identified by customer/stakeholders in the NP 106 action plan.

For the most part, the content of this report is derived from responses to a recent survey of the NP 106 scientists who were asked to summarize their project's major accomplishments in terms of impact, and key references documenting those accomplishments. Consequently, this report does **not** include **all** accomplishments achieved in the national program but, rather, only those **selected** by the ARS scientists polled and the National Program Leader who authored this report. As a result, the scope of this report encompasses a subset of the total spectrum of NP 106 accomplishments, chosen to illustrate and exemplify the total progress and achievements ***at the national program level***.

Finally, a word about how NP 106 achievements and accomplishments were documented. Just as only selected accomplishments are reported, the details of those accomplishments are documented selectively so as to illustrate the overall variety of products and knowledge generated by this National Program. In the report text, selected accomplishments found in the narrative are cross-referenced, by numerical citation [e.g., "(1)"], to supporting information presented in **Appendix 2.** Appendix 2 is organized according to the seven NP 106 component areas.

NP 106 encompasses 25 appropriated research projects. The titles of the individual projects, objectives, funding levels, and scientific staffing are listed in **Appendix 3 – Listing of Individual Appropriated CRIS Projects by Geographic Location**, which is organized according to the geographical location of the research unit. Each project is coded to reflect the National Program Action Plan components to which it contributes.

Lastly, **Appendix 4 – Annual Report Information (2001-2006),** provides an aggregated copy of the introduction of the annual reports of the national program for fiscal years 2001-2006. Full copies of the annual reports are available on the ARS website (http://www.ars.usda.gov/research/programs/), but this information has been excerpted as it provides an overview of new scientists, awards and recognitions, new funding, and additional activities associated with the national programs not reflected by any other section of this report.

National Program 106 – Aquaculture – Action Plan (2004-2009)

Significant expansion of U.S. aquaculture is in the national interest. The United States is the world's largest seafood market. However, rising global populations and steady or declining natural fisheries stocks threaten future supplies of seafood. The United Nations projects a global shortfall of 10 to 40 million metric tons of seafood needed for human consumption by 2010. At present the United States is heavily dependent on imported seafood with over 40 percent of its fish and shellfish supplied by other nations. The U.S. seafood trade deficit is the largest for any agricultural commodity, and the second largest, after petroleum, for any natural product. Further development of a strong, sustainable domestic aquaculture industry can offset dependence on imported seafood and help assure safe, affordable, and high quality appealing products for U.S. consumers.

U.S. aquaculture expanded steadily in the 1980's and 1990's, but is now experiencing slower growth. Most of the growth during the previous 20-year period was attributable to catfish farming, which accounts for over two-thirds of U.S. aquaculture production and over half of its value. Despite this growth, the United States ranks only tenth in the world in the value of its aquaculture production, and over $1 billion of imported seafood now comes from farm-raised fish and shellfish grown in other countries. There is major potential and opportunity for a substantially larger U.S. aquaculture industry comprised of multiple fish and shellfish species.

A national investment in research and technology development will be the foundation for the industry's growth. As the Department of Agriculture's in-house research agency, the Agricultural Research Service will be in the forefront of fundamental and applied research to enhance the production efficiency, sustainability, and quality of cultivated aquatic organisms.

Anticpated Products from this Action Plan:

- Improved germplasm, health products, fish feeds, and environmentally friendly production systems technology for use by fish farmers.
- Increased availability of high quality, safe, competitively priced, nutritious and appealing aquaculture products.
- Reduced U.S. trade deficit.
- Decreased pressure on threatened commercial capture fisheries.
- Expansion of domestic and export markets for U.S. aquaculture products and supporting ancillary industries.
- Job creation and contribution to long-term economic growth, particularly in rural and coastal areas.

Program Components and Problem Areas of the Action Plan:

Component I: Genetic Improvement

- Conserve, Characterize, and Utilize Genetic Resources
- Selective Breeding for Economically Important Traits
- Genomic Resources
- Specific Breeding Aids
- Bioinformatics and Statistical Analysis Tools

Component II: Integrated Aquatic Animal Health Management

- Pathogen Identification and Disease Diagnosis
- Vaccines and Medicines
- Immunology and Disease Resistance
- Mechanisms of Disease
- Epidemiology
- Microbial Genomics
- Aquatic Animal Health Management

Component III: Reproduction and Early Development

- Control of Reproduction
- Control of Gender and Fertility
- Gamete and Zygote Quality
- Gamete and Embryo Storage, Cryopreservation, and Use
- Early Life Stage Development and Survival

Component IV: Growth and Development, and Nutrition

- Regulating Feed Intake
- Tissue Growth and Development
- Sustainable Sources of Nutrients
- Nutrient Use and Feed Evaluation
- Interaction of Gene Regulation and Nutrition
- Interactions Affecting Reproduction
- Effective Probiotics
- Immune System Enhancement (Nutrients and Immunostimulants)

Component V: Aquaculture Production Systems

- Biosecurity
- Production Intensity
- Integrated Production Systems
- Predator and Fowl Control
- Live Aquatic Animal Handling, Transport, and Inventory
- Culture of Marine Species in Low-Salinity Water

Component VI: Sustainability and Environmental Compatibility of Aquaculture

- Aquaculture Feeds
- Water Use and Reuse
- Effluent Management Control
- Social Sustainability
- Environmental Sustainability

Component VII: Quality, Safety and Variety of Aquaculture Products for Consumers

- Tissue Quality
- Interaction of Genetics and Nutrition
- Predicting Product Quality or Defects
- Off-Flavor Delayed Harvesting
- Off-Flavor Methodology
- New Uses for Byproducts
- Processing

RESEARCH COMPONENT I: GENETIC IMPROVEMENT

There has been limited genetic improvement of aquaculture stocks so there are major opportunities for improvement through traditional animal breeding, broodstock development, germplasm preservation, molecular genetics, and allied technologies. Research will address improvement of growth rates, feed efficiency, survival, disease resistance, fecundity, yield, and product quality; genetic characterization and gene mapping; and conservation and utilization of important aquatic germplasm.

Vision: Genetically efficient, sustainable, and humane production of food from aquatic organisms will be the goal.

Mission: Accelerate genetic improvement of aquatic species leading to more efficient and profitable production of healthy, nutritious and palatable aquatic products.

Impact: Reduced production costs and losses for farmers, creation and maintenance of globally competitive aquatic products in diverse production environments, increasing consumer choice and reducing consumer costs.

Linkages: 1) USDA/ARS National Programs: 101 Food Animal Production; 103 Animal Health; 107 Human Nutrition; USDA/ARS National Animal Germplasm Repository (NAGP). **2)** Other Agencies and Departments: U.S. Colleges of Agriculture and State Agricultural Experiment Stations, U.S. Fish and Wildlife Service, U.S. Geological Survey. **3)** Private Sector: U.S. Aquaculture and Grower Associations, U.S. Aquatic Animal Farmers

Engaged ARS Locations: *Stuttgart, AR; Aberdeen, ID; Orono, ME; Stoneville, MS; Newport, OR; Leetown, WV.*

Problem Area IA – Conserve, Characterize, and Utlilize Genetic Resources

Problem Statement: U.S. aquaculture production occurs in a wide array of environments and management systems. The efficiency of livestock and poultry production has dramatically improved due to advances in genetic selection programs; however, genetic improvement of cultured aquatic species has lagged behind traditional livestock species. Maintaining genetic diversity will be essential for providing a diverse genetic basis for current and future breeding programs that will develop stocks with efficient performance in different climates and production systems, when exposed to new diseases, and provide quality products that meet the changing demands of consumers. As breeding programs develop and progress, research is needed to determine physical and genetic characteristics of domestic and wild species, develop the means to identify and protect improved stocks from foreign encroachment, and conserve valuable germplasm. DNA of relevant germplasm will be stored and provided to researchers for genomic characterization and analysis. The National Animal Germplasm Program will assist in conservation efforts for future use, and the information will be stored in the animal component of the GRIN (Genetic Resources Information Network) database.

Committed Goals: 1) Isolate and maintain genetic resources (sperm and eggs, DNA, tissue and genomic information) in a repository for potential use by industry and research. **2)** Quantify genetic variation and genetic distance between and within strains of aquaculture species. **3)** Identify phenotypic and molecular differences between stocks and species, which will be used to characterize and develop markers for unique traits or characteristics identified or developed within the United States.

Expected Outcomes: 1) Quantify the status of the nation's available aquatic genetic resources. **2)** Enhance and improve the development of strains and stocks for commercial production. **3)** Protect and conserve developed strains. **4)** Recommend to industry how to utilize diverse genetic resources in different production systems and environments and maintain genetic diversity.

Problem Area IB – Selective Breeding for Economically Important Traits

Problem Statement: Improving biological efficiency and reducing production losses is achieved through selective breeding. There has been limited improvement of aquaculture stocks so major opportunities exist to improve methods of genetic evaluation, genetic characterization, and understanding of genotype by environment interactions of economically important traits. Applied breeding programs for aquatic species are critically needed to accelerate selection response toward efficient and profitable production of healthy, nutritious, and palatable aquatic products and improve the health and well-being through enhanced adaption to different production environments and with greater resistance to disease.

Committed Goals: 1) Clearly define target species and economically important traits for genetic improvement of aquaculture stocks. **2)** Initiate applied breeding programs for genetic improvement and evaluation of aquaculture stocks in multiple environments. **3)** Identify genes controlling relevant traits and the genetic correlations of economically important traits. **4)** Increase biological efficiency through selective breeding.

Expected Outcomes: 1) Relative economic values for traits of importance in aquaculture stocks will be identified. **2)** Improved systems for regional and national evaluations of aquaculture stocks. **3)** Technologies for accelerating genetic improvement in efficiency and profitability will be delivered to aquaculture producers. **4)** Deliver genetically improved lines or broodstocks to producers and consumers.

Problem Area IC – Genomic Resources

Animal genomics will play an increasingly important role in assuring the continued profitability and competitiveness of U.S. agriculture. Advances in the field of genomics are rapidly being implemented in strategies aimed at the genetic improvement of all agricultural species. The United States aquaculture industry is an important segment of U.S. agriculture, yet genomic resources are currently underdeveloped for most aquaculture species. Identifying, mapping, and understanding the function and control of genes will permit the utilization of new genetic technologies and increase our ability to realize the full genetic potential of important aquaculture species. Efficient use of genomic technologies will also require the development of specific molecular genetic resources for each species of interest. Genomic information will be integrated with genetic selection programs to increase genetic gains, develop new products for aquaculture production, and provide new management tools to increase production efficiency.

Committed Goals: 1) Obtain suitable high-density genetic linkage maps for aquaculture species. **2)** Initiate physical maps for aquaculture species; yielding integrated physical/genetic maps that serve as comprehensive maps for comparative mapping with whole genome sequences of model organisms. **3)** Develop genomic resources (molecular markers) for integrating functional genomics into existing aquaculture research programs.

Planned Approaches: 1) Develop and map microsatellite and single nucleotide polymorphisms in well-characterized reference populations. **2)** Develop bacterial artificial chromosome physical (contig) maps for aquaculture species that integrate with the genetic linkage maps through common markers. **3)** Develop tissue specific cDNA libraries and sequences for inclusion in the construction of microarrays and other functional genomic technologies. **4)** Correlate phenotypic information with genetic markers in resource populations to identify quantitative trait loci for use in breeding programs. **5)** Utilize novel molecular genetic techniques (knockouts or insertions) or organisms to investigate the biological activity of specific

Expected Outcomes: 1) Microsatellite/single nucleotide polymorphism genetic linkage maps with sufficient marker coverage to choose subsets of markers for the identification of quantitative traits in resource families. **2)** Physical bacterial artificial chromosome maps anchored to genetic linkage maps, with integrated comprehensive maps serving as comparative maps with whole genome sequences of model species. **3)** Uniquely expressed transcripts and microarrays containing subsets of those transcripts for functional genomic experiments will be identified. **4)** Biochemical pathways and their modulation through controlled manipulation of genes will be defined.

Problem Area ID – Specific Breeding Aids

In addition to traditional selective breeding methodologies, specific breeding aids can be used to further genetic progress following application to breeding programs. Specific breeding aids typically focus on chromosome set manipulations to alter phenotypes (sex), produce inbred lines, and impact reproduction. Control of reproduction is also a critical limitation for most aquaculture species. There are problems related to enabling reproduction in hard to spawn species and throughout the year in seasonal spawners, and conversely to limiting reproduction in animals at risk of escaping to the wild. Cryopreservation of gametes and embryos is also a critical need for both germplasm conservation and applied breeding programs. Cryopreservation may allow year round availability of gametes and permit crosses between animals that do not spawn contemporaneously.

Committed Goals: 1) Induce spawning in hard to reproduce species. **2)** Enable year-round production of gametes. **3)** Limit reproduction and control sex (e.g. chromosome set manipulation, sex reversal). **4)** Suitable cryopreservation of gametes and embryos.

Expected Outcomes: 1) Year round availability of seed for aquaculture animals will be accomplished. **2)** Reduced risk associated with commercial culture near natural populations of aquatic species. **3)** Unrestricted availability of gametes, especially sperm will be realized.

Problem Area IE – Bioinformatics and Statistical Analysis Tools

Genomic and proteomic technologies used in breeding programs create large datasets that quickly outpace simple genetic analysis methods. Bioinformatics tools are needed to assimilate

and process information into functional components in breeding programs. Statistical analysis software needs to be developed to interpret results from genomic/proteomic data and integrate this information into selective breeding programs. Bioinformatics and statistical analysis tools specific for aquacultured species are poorly developed. However, the types and format of molecular data from aquatic species are similar to those of other species. Therefore researchers can integrate and optimize existing bioinformatics tools for aquaculture genomics and proteomics data.

Committed Goals: 1) Relational databases containing genomic and physiological data.
2) Computer software for analysis and application of molecular data.

Expected Outcome: Genomic/proteomic information for aquaculture species and software for data management and application will be publicly available.

Selected Accomplishments for *Genetic Improvement*:

Conserve, Characterize and Utilize Genetic Resources

Aquaculture species added to the National Animal Germplasm Program (1). Since 2002 the National Animal Germplasm Program has acquired 18,605 units of germplasm from 563 individual animals from 17 aquatic species. The majority of the aquaculture collection is comprised of fresh water fin-fish. Major portions of the collection are from agronomically important species: blue and channel catfish, white bass, striped bass, and rainbow trout. The rainbow trout collection consists of five different lines, including base populations that are undergoing selection at the ARS - National Center for Cool and Cold Water Aquaculture (NCCCWA) facility and the Hofer population (which is purported to be resistant to whirling disease). Diploid and tetraploid pacific oysters and a limited number of samples from five species of marine fin-fish have been acquired. This collection has been developed with the NAGP Aquatic Species Committee. In addition to collection activities several experiments have been conducted using cryopreserved milt quantifying fertility levels for rainbow trout

Production traits of different hybrid striped bass crosses (2). In cooperation with hybrid striped bass producers, pond production characteristics (feed consumption, feed conversion rate, growth, and fillet yield) of the two major crosses, sunshine bass (white bass female x striped bass male) and palmetto bass (striped bass female x white bass male), were determined. Fillet yield was greater for sunshine bass whereas, all other production characteristics were not different including IGF-I levels in the blood. Further studies compared the two crosses with regard to stress response by measuring plasma cortisol, glucose and chloride concentrations immediately after seining and low-water confinement. Although there were some seasonal differences, stress responses to harvest were the same for both crosses except in winter when palmetto bass showed some advantage.

Genetic variation analyses of rainbow trout broodstock (3). NCCCWA broodstock populations were initiated and these fish were characterized for molecular genetic variation between and among the strains contributing to broodstock, and subsequently to estimate levels of inbreeding and relatedness between individuals. To increase the efficiency of genotyping broodstock, a single-step method of co-amplifying microsatellite markers for genetic studies

and determining parentage was developed and optimized to reduce the high costs associated with these analyses.

Genotype x environment interactions in rainbow trout (4) Genotype x environment interactions are an important consideration for selective improvement. Looking at diet as an environmental variable, families of rainbow trout have been reared simultaneously on plant meal or fish meal based diets. The initial results show little evidence for genetic x diet interaction; however, these studies have been somewhat small scale (up to 33 full-sib families). Preliminary results from a larger study (~100 full-sib families) suggest that genetic x diet interactions could be significant when comparing diets containing plant protein/oil versus diets with only fish protein/oil.

Obtained and evaluated five North American Atlantic salmon stocks for inclusion in the breeding program and initiated Atlantic salmon selective breeding program (5). U.S. salmon production is constrained by lack of genetic improvement, disease, and low production efficiency. U.S. salmon producers are required to culture salmon of North American origin; however, no genetically improved native stocks are available. The research program at the National Cold Water Marine Aquaculture Center was initiated in the summer of 2003. Four separate year classes (2004-2007) of Atlantic salmon have been obtained representing 5 different North American salmon stocks from state, federal and private industry hatcheries. The Atlantic salmon were from the St. John's River (2 sources), Penobscot River, Gaspe Peninsula, and Maine Landlocked. Early growth evaluation of the different genetic stocks were conducted during the parr stage, and juveniles were pit tagged, vaccinated, and stocked into replicated communal tanks for evaluation of growth to smolts. Smolts from the first two year classes have been transferred into sea cages at aquaculture lease sites operated by industry collaborators. Fish stocked into industry sea cages from the first year class were reared to market size under commercial culture conditions and harvested in February 2007 and data were collected on harvest weight and stage of sexual maturity. The first adult salmon from the breeding program will be spawned in the fall of 2007. Some eggs from selected broodstock will be kept in the breeding program as the nucleus of selected families for the next generation, and some eggs will also be available for transfer to industry.

Genetic characterization of farm-raised catfish (6). A study compared production traits of USDA 103 strain channel catfish, USDA 102 strain channel catfish, USDA 103 x USDA 102 crossbred, and USDA 103 strain channel catfish x blue catfish hybrids. Hybrids had the best fingerling performance of all groups. Of the channel catfish groups, the USDA 103 x USDA 102 crossbred was superior to the USDA 103 line and USDA 102 line catfish for fingerling production traits. The same crosses are being evaluated for foodfish production and processing traits. Initial results indicate crossbreeding and hybridization could enhance catfish production.

Channel catfish x blue catfish hybrids exhibit favorable meat yield and resistance to some diseases compared to purebred channel catfish**,** but reproductive barriers limit large-scale production of hybrids. F_1 channel x blue hybrids were backcrossed to purebred channel catfish and then intercrossed to close this population in order to produce a reproductively viable synthetic, hybrid catfish line that retains the superior growth, disease resistance and meat yield of the hybrid. After two generations of backcrossing the population successfully spawned in ponds and offspring had superior resistance to ESC, a bacterial disease of catfish. Another trial was conducted to compare production traits of blue catfish, channel x blue catfish hybrids, and two channel catfish strains (Norris and NWAC103). Fingerling growth and survival were better for blue catfish and channel x blue catfish hybrids than for the channel catfish strains. Feed

records indicated hybrids consumed the most feed, followed by blue catfish, NWAC103s, and then Norris strain channel catfish during the fingerling to market weight portion of the study.

Meat yield is affected by catfish strain/species and season of the year. Hybrid catfish had higher whole carcass and fillet yield than blue or channel catfish during both the spring and fall. Blue catfish had higher carcass yield than channel catfish during both the spring and fall. Channel catfish had higher fillet yield than blue catfish in the fall, but blue catfish had higher fillet yield than channel catfish in the spring. Blue catfish had higher nugget yield (lower valued rib-meat) than channel or hybrid catfish.

Pacific oyster germplasm resources characterized and augmented (7). Tissue samples suitable for genetic analysis were obtained from all of the known naturalized populations of Pacific and Kumamoto oysters on the west coast of the USA and Canada, from hatchery-propagated breeding populations currently in use, and from wild populations in Japan. In addition, live Pacific and Kumamoto oysters from the Ariake Sea, the southernmost region of Japan were collected and propagated in quarantine systems in the USA. The tissue samples were prepared for AFLP analysis to quantify the genetic variation within and the genetic distances among the current and potential sources of germplasm for the Pacific and Kumamoto oyster culture industry. The first generation of hatchery-spawned oysters from these parents was spawned in 2006 for the Pacific oysters and 2007 for the Kumamoto oysters and are being reared entirely in quarantine systems in accordance with both ICES protocols for importation and state-mandated requirements for disease testing and certification before they can be spawned and their second-generation progeny released to industry.

The Kumamoto oyster is listed as endangered in its native habitat, but genetic analyses indicate that at some sites, it is common. The tissue samples were prepared for detailed AFLP analyses in order to quantify the genetic variation within and the genetic distances among the current and potential sources of germplasm for the Kumamoto oyster culture industry.

Microsatellite markers in the native Olympia oyster (*Ostrea concaphila*) were developed for use in analyzing tissue samples that were collected from a large number of remnant populations in Washington, Oregon, and California to estimate genetic differentiation and gene flow among these populations.

Severity of inbreeding depression demonstrated in Pacific oysters (8). Molecular marker based estimates of the genetic relatedness between parents collected from a naturalized population and paired at random demonstrated that matings among even distantly-related parents results in detectable inbreeding depression for survival in Pacific oysters.

Selective Breeding for Economically Important Traits

Breeding rainbow trout for economically important traits (4). Selective breeding at the NCCCWA has focused on growth rate (in the even year line) and resistance to bacterial coldwater disease (CWD, in the odd year line). Two generations of selection for improved growth rate and one generation of selection for resistance to CWD were completed.

In 2005, 75 full-sib families were challenged with *Flavobacterium psychrophilum*, the causative agent of bacterial CWD. The goal is to determine whether there is genetic variation for resistance to this bacterium. Mortality ranged from 28 to 99% and was similar in replicates. Genetic relationships among multiple families with low mortality indicate that there is a genetic

basis for resistance. Selective breeding should offer a method to improve resistance to CWD. In 2007 trial groups of select and control fish were placed on commercial farms to evaluate performance under industry conditions.

Stress has a negative impact on many traits including growth rate, feed efficiency, disease resistance and reproductive performance. Investigations into the performance benefits of altering stress response via breeding have been inconclusive. Families of rainbow trout from the 2002 broodstock development program that differed by about two-fold in cortisol response to stress were identified, and increased stress response was correlated with increased growth and feed efficiency. Stress response was shown to be moderately heritable (h^2=0.40) in this population. Stress response, measured as plasma cortisol levels, was not associated with resistance to enteric redmouth disease in either stressed or unstressed fish.

Nutrient utilization efficiency was identified as a key concern by stakeholders. This character not only influences the cost of production, but nutrient retention is important for reducing phosphorus and nitrogen excretion to the environment. Significant variation for this trait was identified, both between strains and families within strains. Importantly, a significant correlation between efficiency measured on individually reared fish and efficiency of groups of their siblings was detected.

Genetic improvement of rainbow trout stocks for growth on formulated plant-based diets (9). A rainbow trout broodstock program was developed to select families better suited to utilize a plant-based diet compared to a trout fed a fish-meal based diet. Utilizing variability from eight separate original stocks, germplasm has been developed for spawning on even and odd years. Traits being evaluated are growth rate, weight at 6 months and two years, feed conversion ratio, morphology, and immune status.

Selected trout families are being evaluated for improved growth rates on alternative feeds. Families from this program have been crossed with fish from Clear Springs Foods.

Research was completed that defined the relationship between muscle accretion and growth in rainbow trout by analysis of metabolic and muscle specific genes at different life stages, under different planes of nutrition, and upon feeding varied plant-based diets containing differences in carbohydrates, protein, and lipid levels. This work has further refined the existing model demonstrating which specific genes are involved in muscle growth under varying experimental conditions.

Selective breeding for farm-raised catfish (6).
Selective breeding for increased meat yield will lead to the development of catfish germplasm with higher meat yield and increased economic value. Meat yield was measured in offspring from more than 200 channel catfish families, and the results demonstrated a positive response to selection for increased meat yield.

Genetic basis of heavy metals content in Pacific oysters demonstrated (10). Quantitative genetic analysis revealed that the heavy metal content of Pacific oysters is under substantial genetic control and could be modified through selective breeding. These analyses also revealed that for the metal of highest concern, cadmium, there is a genetic correlation with growth such that selection for reduced cadmium content is expected to produce a reduction in growth and selection for enhanced growth is likely to increase cadmium content.

Quantitative trait loci for survival and growth in Pacific oysters (11). Twelve outcrossed full-sib families were produced and deployed in the field in 2005. This permitted the non-lethal sampling of tissue samples from over 100 individually-identifiable animals per family before significant mortality occurred. This enabled the collection of genotypic information from animals that eventually die from summer mortality and permitted the analysis of associations between marker genotypes and survival.

Genomic Resources

Construction of genetic and physical maps for rainbow trout (12). A genetic map was constructed to facilitate genome analyses aimed at identifying regions of chromosomes affecting aquaculture production traits including stress response, feed efficiency, and disease resistance. The map was constructed by evaluating two generations of five families containing 30 offspring each with ~1,500 genetic markers. A total of 1,124 markers were placed on the sex averaged map, many of which were assigned to orthologs in the zebrafish, tetraodon, pufferfish, and/or medaka genomes. This map enhances the genome research in rainbow trout, especially as it is integrated with the bacterial artificial chromosome (BAC) physical map to facilitate the identification, cloning and characterization of candidate genes for improved production efficiency.

Characterization of the rainbow trout transcriptome (13). A project was conducted to identify as many uniquely expressed genes from the rainbow trout genome as possible. These data, in the form of ~126,000 expressed sequence tags, have been used extensively in candidate gene and functional genomic experiments associating genetic variation and/or function with phenotypes including responses to chemical contaminants and stress.

Characterization of candidate genes affecting aquaculture production traits in rainbow trout (14). Candidate genes including inhibitors of differentiation, toll-like receptors, chemokines, uncoupling proteins, major histocompatibility genes, caplains, tapasins, cyclins, and estrogen receptor were targeted for in-depth characterization based on sequence similarities to genes of known function from better studied species. As candidates affecting growth, stress tolerance, and/or disease resistance, these genes were analyzed by determining 1) mRNA and genomic sequences; 2) map locations in the genome; 3) tissues/stages of gene expression; and 4) changes in expression due to experimental treatments.

Development of genetic markers for striped bass (15). A total of 345 microsatellite genetic markers were developed to enable genome mapping, parentage determination, common garden studies, and estimations of population genetic parameters for striped bass, white bass, and their hybrids.

Development of catfish genomic resources (16). Several thousand DNA sequences (ESTs) of channel catfish were produced and added to public databases. A first-generation microarray of 19,000 catfish sequences was also developed and used to identify genes that were differentially expressed in the catfish spleen after exposure to bacterial cell wall components. This microarray was used as the backbone for a second-generation microarray used to measure gene expression after exposure to pathogenic bacteria. The full DNA sequence of the catfish mitochondrial genome was produced which is useful for identification of broad range population structure and removal of contaminating mitochondrial sequences from nuclear DNA libraries. A 7-fold genomic library (BAC library) consisting of 55,000 bacterial clones that each

contained a large fragment (160,000 base pairs) of catfish DNA was produced. A technique was developed to efficiently identify variable DNA sequence markers that were within or near genes of interest, and the existing first-generation catfish genetic map was enhanced by the addition of many genes that are conserved between species. Not only did this provide more genetic markers for the map, but it also permitted direct comparisons of genome structure and gene order between catfish and species with sequenced genomes such as zebrafish and pufferfish. Use of cross-species comparisons will permit more efficient identification of genes that are controlling important production traits.

A physical map of the catfish genome was developed using a high-throughput DNA analysis system to efficiently perform DNA fingerprinting on the clones of the BAC library. Then specialized software was used to join the overlapping clones based on similar DNA fingerprints. These adaptations were incorporated into a user bulletin by the vendor of the DNA analysis system. Also, more than 30,000 genomic DNA sequences were obtained from these clones to provide information on the repetitive structure of the catfish genome and also provide several thousand potential DNA markers for the genetic map.

The similar physical characteristics of all channel catfish confound methods to identify individuals, families, and strains. Using DNA markers identified in the catfish genome, a catfish strain identification system based on DNA fingerprinting has been developed. This method was used by the U.S. catfish industry, via the Mississippi Seed Improvement Association, to manage populations of the NWAC103 strain and maintain its genetic integrity on commercial farms. The DNA fingerprinting technology can be used by catfish producers to genetically define their catfish populations.

Genes involved in Pacific oyster responses to heat stress and bacterial challenge identified (17). DNA microarrays, cDNA-AFLP, and quantitative PCR techniques were used to identify approximately 50 genes whose expression levels differ between genetic families characterized by high and low survival. Some of these genes were differentially expressed in response to experimental heat shock or exposure to bacterial pathogens. This accomplishment is important because so-called “summer mortality” – mass mortalities of 50-80% due to a combination of heat stress, reproductive stress, and opportunistic bacterial pathogens, is the oyster culture industry’s most important problem. These studies enabled future research to characterize polymorphisms in these genes and in the regulatory elements that control their transcription.

Specific Breeding Aids

Development of tetraploid lines of rainbow trout (18). Fish carrying four sets of chromosomes (tetraploids) offer the opportunity to produce 100% triploid offspring, which are desirable because they are sterile and potentially faster growing than typical diploid production animals. In order to effectively make tetraploid rainbow trout strains, it is necessary to define the timing of the first cell division in fertilized eggs. At a given incubation temperature the time to first cleavage varied by as much as 40 min. among the strains.

First generation tetraploid male and female rainbow trout were reared to maturity and crossed to produce a number of tetraploid and triploid crosses for further evaluation. The offspring from these crosses provide (1) the initial generation of potential tetraploid lines for aquaculture and (2) the initial evaluation of the potential for the lines to produce superior triploid offspring for aquaculture production. Each tetraploid female was crossed with (1) a tetraploid male to

produce a second generation with four sets of chromosomes and (2) a diploid male to produce triploid offspring. The tetraploid males were also crossed with diploid females to produce triploids from the reciprocal cross. All crosses yielded the expected results with respect to ploidy level (100% tetraploids for the first crosses and 100% triploids for the latter two).

Evaluated triploid and all-female Atlantic salmon (19). Triploid salmon, which are expected to be sterile, reduce the potential for interaction with wild salmon stocks if they were to escape from net pens. An additional benefit resulting from the utilization of triploid salmon in commercial culture would be to reduce the potential loss of growth and meat quality associated with the onset of sexual maturity. Diploid and triploid all-female Atlantic salmon from separate families were obtained in 2004 and evaluated in indoor tanks and commercial sea cages. Fish were harvested from the net pen in February 2007 and initial data analysis indicates fish were sterile, but triploid survival was less than normal diploids. The evaluation of triploids is also being repeated on individual families of diploid and triploid salmon obtained in 2007 from two different salmon stocks.

Bioinformatics and Statistical Analysis Tools

Database development. A database recording all individuals contributing to ARS NCCCWA rainbow trout lines, the genetic relationships among fish, and phenotypic information and has been developed.

Technologies for mixed-family breeding of Pacific oysters developed (20). Software to identify the smallest subset of a pool of available genetic markers that can reliably assign progeny to their parents was developed. The software enabled the use of data from pedigreed progeny or simulated progeny incorporating realistic complications such as linkage among loci, null alleles, and genotyping errors. The point during the early larval stages when separately-reared oyster families can be mixed without unacceptably skewing their representation in field evaluations was identified. This finding provides the basis for a transition from very labor-intensive approaches to selective breeding of oysters based on selection among separately-reared genetic families to a more effective and efficient mixed-family approach that can be implemented at the commercial scale with minimal added effort and record-keeping.

RESEARCH COMPONENT II: INTEGRATED AQUATIC ANIMAL HEALTH MANAGEMENT

The U.S. aquaculture industry is dependent, among other factors, on the control of endemic, emerging and catastrophic diseases that result in losses of aquatic animal production. Despite the progress that has been made in aquatic animal health, significant losses to disease still occur. Estimated losses to all aquatic animal producers are about $1 billion annually in the United States alone. The lack of adequate technologies for early and rapid detection, prevention and treatment of diseases has hindered the growth and competitiveness of the U.S. aquaculture industry. Some tools have been generated to detect the major disease agents in aquatic animal production, but few can be used on the farm. Testing of these tools at production systems to show the U.S. farmers the practical value of being able to detect the disease agents in a rapid fashion is needed. Effective vaccines against ESC have been developed by ARS and are being used in health management plans by aquatic animal farmers. Further research is needed to provide new vaccines and methods for mass vaccination of aquatic animals as is utilized in other food animal industries. Only a few drugs are approved for

treating sick fish. The overall strategy is to develop health management technologies and biosecurity plans that are safe for the environment and for the consumer of aquaculture products.

Vision: Establish a globally competitive, sustainable aquaculture industry in the United States through the development of successful aquatic animal health management strategies that will limit losses to disease, permit lower production costs, and improve product quality.

Mission Statement: Conduct high quality, relevant, basic and applied research and technology transfer to solve problems related to aquatic animal health.

Impact: The development of new animal vaccines and medicines as well as the establishment of health management strategies that can be used by aquaculture producers will decrease the impact of infectious and non-infectious diseases in U.S. and world aquaculture. Annual savings of about $700 million by a 70 % reduction in disease losses may be realized.

Linkages: 1) Other USDA-ARS National Programs: 101 Food Animal Production, 103 Animal Health, 108 Food Safety. **2)** Other Agencies and Departments: U.S. Colleges of Agriculture and State Agricultural Experiment Stations, U.S. Colleges and Schools of Veterinary Medicine, U.S. Fish and Wildlife Service, U.S. Environmental Protection Agency, USDA-APHIS-CVB, USDA-APHIS-VS, U.S. Food and Drug Administration. **3)** Private sector: U.S. Aquaculture and Grower Associations, U.S. Aquatic Animal Farmers, U.S. Vaccine, Diagnostics and Medicine Manufacturers.

Engaged ARS Locations: *Auburn, AL; Stuttgart, AR; Orono, ME; Oxford, MS; Stoneville, MS; Newport, OR; Leetown, WV.*

Problem Area IIA – Pathogen Identification and Disease Diagnosis

Methods and reagents to rapidly (within hours) detect pathogens and diagnose diseases in aquatic species are still unavailable or have not been applied at the farm. Methods and reagents to identify strains of aquatic animal pathogens also need to be developed. Automated detection systems are currently not available or in use. Further, non-lethal tests are needed in detection and diagnostic methods. Accurate determination of preclinical infections (i.e., prior to disease) will enhance the opportunities to determine the potential risk of disease occurring and allow for earlier intervention with preventative measures to reduce or eliminate the impact of emerging or catastrophic diseases in the United States. Domestic and international trade of aquatic animals needs rapid, automated and accurate tests to demonstrate that aquatic animals, seed stocks and products are free of harmful pathogens to prevent the introduction and spread of harmful diseases.

Committed Goals: 1) Develop rapid and automated methods to detect infectious and non-infectious pathogens and toxins in aquatic animals. **2)** Apply the rapid and automated detection methods to protect U.S. trade, food and aquaculture industries. **3)** Develop methods that allow the identification of strains and sources of the pathogen prior to overt disease. **4)** Develop non-lethal detection and diagnostic methods for aquatic animals.

Expected Outcomes: 1) Rapid, early and automated detection of pathogens, diseases and carrier states will be realized using immunoassays and PCR in non-lethal formats. **2)** Rapid,

early and automated detection methods will be applied on farms and by USDA- APHIS and State Animal Health facilities. **3)** Techniques for identification and serotyping pathogens prior to disease will be realized.

Problem Area IIB – Vaccines and Medicines

Aquatic animal farmers have a lack of available vaccines and medicines to either prevent or treat infectious and non-infectious disease agents. Development of new vaccines will likely rely on similar techniques that are currently employed 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. Vaccination strategies for mass vaccination will also need to be addressed. Presently, some vaccines are available but are only effective when administered by injection or with adjuvant. These treatments are impractical and not usually economically feasible. The ultimate goal of vaccine research is to develop a product that is safe, easy to administer and effective on the farm. The approach to new aquatic animal medicines will require novel strategies for identification of effective medicines that are safe to the environment, to the target animal, and to the consumer. A better understanding of the pharmacokinetics (i.e., dose, uptake, residue accumulation, reactions, safety) of currently available and new medicines is needed. Methods for delivery of new or approved medicines also need to be studied. Strategies such as oral application, improving palatability and/or the use of water treatment must be determined. Cooperation in the approval process and application of medicines is needed for establishment of safe medicines legal for use in aquatic animals.

Committed Goals: 1) Develop safe and effective vaccines and medicines for prevention and control of economically important pathogens of aquatic animals. **2)** Identify effective mass delivery strategies for aquatic animal vaccines and medicines. **3)** Conduct research and development to support approval and licensing of new aquatic animal vaccines and medicines.

Expected Outcomes: 1) New killed, modified live, DNA and recombinant vaccines and methods for mass vaccination will be developed. **2)** Environmentally friendly, effective and food safe medicines to treat aquatic animal diseases will become available.

Problem Area IIC – Immunology and Disease Resistance

Limited information is available on immune system function of economically important species of aquatic animals. The lack of information is partly due to the lack of methods and reagents available to study the immune response of fish. Historically, most research has been invested in human and other production animal immunology. Part of the reason for the lack of information is that reagents developed in other animal species to identify immune cells and humoral components do not always react with fish cells and components. New reagents need to be developed that correctly identify the immune cells and humoral components of the fish immune system. Little information is available on the immune response of the cells in the fish skin, gut, mucus tissue, and nare. This information is important to understand the first line of defense in fish due to the intimate contact with the surrounding water. More information is needed on factors such as genetics, the environment, husbandry, stress, nutrition, species of fish and the pathogens that influence disease resistance and immunity in fish. Future studies will likely concentrate on the immune response of the fish following vaccination.

Committed Goals: 1) Develop methods to characterize cells and regulatory substances (cytokines) important in natural resistance and acquired immunity in aquatic animals. **2)** Assess the importance of the immune response (i.e., antibody or cell mediated) following vaccination that results in the protective immune response. **3)** Answer basic questions about the immune responses of the skin, gut and nare to economically important pathogens. **4)** Answer questions about the influence of various factors (i.e., environment, genetics, nutrition, husbandry) that affect innate and acquired immunity.

Expected Outcomes: 1) The cells and cytokines important in natural resistance and acquired immunity in aquatic animals will be characterized. **2)** The immune response following vaccination that results in protective immunity against the economically important pathogens will be determined. **3)** The immune responses of the skin, gut and nare will be characterized. **4)** The importance and interactions of environment, stress, genetics, nutrition, husbandry, aquatic animal and pathogen that affect innate and acquired immunity will be determined.

Problem Area IID – Mechanism of Disease

The understanding of the pathogenesis of aquatic animal pathogens at the cellular and organismal level is inadequate. Basic information is also needed on the sources of infection (i.e., water, carrier fish, birds), modes of transmission (i.e., vertical from mother to offspring or horizontal from fish to fish), routes of entry (e.g., nares or gills), mechanisms of pathogen virulence and host response. The lack of *in vitro* and *in vivo* models to investigate mechanisms of pathogenesis and virulence is hindering a better understanding of disease mechanisms.

Committed Goals: 1) Develop challenge models in the laboratory that reflect on farm conditions to assess pathogenesis of disease. **2)** Develop in vitro methods to determine mechanisms of pathogenesis. **3)** Develop basic information on the sources of infection, modes of transmission, routes of entry, virulence mechanisms and host response to economically important infectious and non-infectious diseases. **4)** Discover novel natural product-based compounds for use in managing diseases.

Expected Outcomes: 1) In vitro and in vivo models for parasites, viruses, and bacteria investigations will be developed. **2)** The sources of infection, modes of transmission, routes of entry, virulence mechanisms and host response to infectious and noninfectious aquatic animal pathogens will be identified. **3)** Basic information on the mechanism of pathogenesis will be discovered.

Problem Area IIE – Epidemiology

Epidemiology by definition is the science that deals with the incidence, distribution and control of disease in a population. Fish farmers have suggested that epidemiology is needed to solve aquatic animal health problems on the farm. Many times aquatic animal farmers do not or are not able to identify where all the fish or animals stocked “go”. For example catfish farmers cannot account for approximately 70 % of their fish at time of harvest. Studies need to address and/or identify the risk factors controlling the presence or absence of disease. Use of epidemiology will allow for the identification of the problem areas that can be addressed by health management plans and/or cost effective control strategies (i.e., vaccines and medicines).

Committed Goals: 1) Develop methods to assess risk factors associated with the economically important pathogens of aquatic animals. **2)** Carry out basic epidemiology studies to identify disease prevalence, incidence, sources and origin of economically important aquatic animal pathogens. **3)** Establish estimates of the economic impact of various pathogens on aquatic animal production.

Expected Outcomes: 1) Identification of major risk factors found in aquaculture production systems. **2)** The prevalence, incidence, source and origin of economically important pathogens will be determined. **3)** The estimated costs of diseases in aquatic animal production in different types of aquaculture systems will be determined.

Problem Area IIF – Microbial Genomics

There is a critical need to obtain a better understanding of the molecular basis by which microbial pathogens cause disease in and interact with their hosts. Whole-genome sequencing is a powerful method for rapidly identifying all of the genes of a microbe and serves as the basis for future functional analysis of the newly discovered genes. Large-scale analysis of the microbial pathogen's genome will identify novel antigens, biochemical pathways, and virulence mechanisms that are important for pathogen survival, pathogenesis and immunity. In the future, genomic research will provide the basis for designing new and effective vaccines, medicines and diagnostic reagents to help prevent and control infectious diseases. Presently, no whole-genome of an aquatic animal pathogen has been sequenced.

Committed Goals: 1) Increase the amount of available genetic information of aquatic animal pathogens (virus, bacteria and protozoa). **2)** Develop and/or adapt bioinformatic tools to properly process the information generated. **3)** Apply this new genetic information to functional genomic approaches in order to correlate sequence with gene function.

Expected Outcomes: 1) Genomic sequence information on genes important in pathogenesis and virulence will be generated. **2)** Knowledge of functional genomics of pathogenesis and virulence will be realized. **3)** Genes and gene products useful in the development of diagnostic tests, vaccines and medicines that can reduce or eliminate the impact of these pathogens in aquaculture will be identified.

Problem Area IIG – Aquatic Animal Health Management

More information is needed on fish responses to different stressors. Husbandry techniques need to be developed that minimize the stress of the aquatic animals in production systems (i.e., information on handling and transport, stocking densities, water qualities). Environmental factors including toxicogenic algae that effect animal well-being also need to be addressed. This is especially important because water quality is important for aquatic animal well-being. More information is needed on the sources of current and potential pollutants that may enter aquaculture production systems and negatively impact overall health of the aquatic animals. Basic information is needed for development of biosecurity plans to prevent the spread of disease in aquaculture production systems, between wild and cultured fish, and between geographically isolated locations.

Committed Goals: 1) Improve health management practices currently used in aquaculture and at hatcheries. **2)** Identify factors of intensive aquaculture operations that can enhance animal well-being and decrease stress. **3)** Develop biosecurity plans to curtail the spread of diseases between geographical locations and between cultured and wild fish populations.

Expected Outcomes: 1) Cost effective health management plans will be developed and employed by aquaculturists. **2)** Factors that affect animal well-being will be identified. **3)** Basic health management practices useful for the development of biosecurity plans for aquaculture production systems will be realized.

Selected Accomplishments for *Integrated Aquatic Animal Health Management*:

Pathogen Identification and Disease Diagnosis

Real-time PCR assays development for ESC disease in catfish (1). *Edwardsiella ictaluri*, the cause of enteric septicemia of catfish (ESC), and *Flavobacterium columnare*, the cause of columnaris disease are the two most serious pathogens affecting the culture of catfish fingerlings. A patent was awarded in 2005 for a real-time PCR assay for the identification and detection of *Edwardsiella ictaluri*. The assay is designed to detect genetic material of bacterial cells in small amounts of blood or other tissue samples. This assay is capable of detecting the equivalent of as few as 2.5 cells of *E. ictaluri* and is species specific.

Monitoring technologies developed for ESC and columnaris disease pathogens (2). Control of bacterial infections in catfish culture is dependent on early detection and prompt treatment. A method of predicting potential eipzootics would aid in the control of disease. Several approaches are being developed to identify sources of pathogens prior to overt disease. An “immuno-capture” system to extract *E. ictaluri* or *F. columnare* cells from pond water was developed, and is being optimized. In this assay, antibody-coated magnetic beads are used to extract the target organism from the water. The organisms can then be quantified by plating on microbiological media or by using molecular techniques (real-time PCR). To date the ESC and columnaris assays have been successfully used to measure the number of bacteria in filtered pond water to which known numbers of bacteria have been added. These procedures will be combined with real-time PCR assays and used to quantify number of bacterial pathogens in the water. The goal is to determine the threshold number of bacterial cells necessary to initiate mortalities.

Real-time PCR assay developed for causative agent of proliferative gill disease in catfish (3). Proliferative gill disease (PGD) caused by the myxozoan parasite *Henneguya ictaluri* is one of the most devastating parasitic infections in channel catfish (*Ictalurus punctatus)* aquaculture. Currently, there is no effective treatment for *H. ictaluri* and outbreaks can result in >50% mortality in commercial channel catfish ponds. A real-time PCR assay was developed to identify and quantify *Henneguya ictaluri*. The real-time PGD PCR is now being utilized to characterize the disease progression through the catfish tissues and to quantify levels of the PGD agent in pond water and correlate those levels to disease outbreaks. The goal is to determine the threshold number of spores necessary to initiate mortalities from PGD.

Multiple pathogen detection multiplex-PCR assay developed (4). *Edwardsiella ictaluri, Flavobacterium columnare and Aeromonas hydrophila* are major bacterial pathogens of fish that cause disease with significant economic impact on the U.S. aquaculture industry. Traditional methods of diagnosing infection using culture techniques are laborious and time-consuming, thereby increasing the potential of spreading the disease and delaying implementation of disease control strategies. Development of a rapid, sensitive and cost-effective immunofluorescent test for simultaneous diagnosis of *E. ictaluri* and *F. columnare* infections in fish was accomplished. This test was validated using 303 samples (derived from kidney, brain and nares) from both experimentally and naturally infected fish and was found to be sensitive and specific for simultaneous detection of both pathogens. Further, an innovative multiplex PCR assay using three sets of primers directed at unique target gene-segments of *E. ictaluri*, *F. columnare* and *A. hydrophila* for rapid, simultaneous detection of the three organisms in a single PCR-reaction mixture was developed. A real-time PCR assay for rapid, sensitive and specific detection of *F. columnare* using the TaqMan chemistry was developed. In this test, specific probe and primers were developed targeting a 113 bp DNA fragment of chondroitin AC lyase gene of *F. columnare*.

Identification of disease agents from wild and cultured fish studied (5). Streptococcal species are suggested as emerging species in the United States and other regions of the world where fish culture has intensified. Methods were developed for use of frozen fish in assessment of pathogen prevalence. These methods will aid in furthering the understanding of pathogens from areas where sophisticated laboratories are not available (i.e., shipment of frozen fish for assessment).

Biotype 2 *Yersinia ruckeri* was documented in the United States. Prior studies suggested only Biotype 1 *Yersinia ruckeri* present in the United States with Biotype 2 being reported from trout in England and Spain. Research indicated the presence of this biotype (Biotype 2) in trout hatcheries in South Carolina.

Vaccines and Medicines

Bacterial disease control investigated (6). A method was developed to determine the Minimum Inhibitory Concentrations (MIBs) for several antibiotics to *Flavobacterium columnare*. In another study, the efficacy of amoxicillin for controlling *Streptococcus iniae* infection in tilapia was demonstrated using a laboratory disease model. Experiments have shown that amoxicillin is effective in decreasing the mortalities in infected fish from 96% to 6%. This accomplishment has led the FDA to allow the continued temporary use of this compound for disease control. Similarly, the effectiveness of florfenicol for controlling *Streptococcus iniae* infection in hybrid striped bass was demonstrated. Experiments have shown that florfenicol is effective in decreasing the mortalities in infected fish from 94% to 4%.

In water column treatments, efficacy of potassium permanganate against an acute and a chronic *F. columnare* infection in channel catfish when used as a prophylactic (before clinical signs of columnaris are apparent) was demonstrated. Additiinally, ARS scientists demonstrated that 1 dose of diquat increased the survival of infected fish by approximately 40%.

Aquaflor® use validated for treatment of important warmwater fish diseases (7). Enteric septicemia of catfish (ESC) and columnaris disease are the most serious diseases affecting the culture of channel catfish fingerlings. An effective and palatable antibiotic incorporated in floating feed is needed by the catfish industry to combat these diseases when preventative

measures fail. Laboratory and field efficacy trials were conducted in support of FDA approval of Aquaflor®, a new antibiotic for fish. Results showed that Aquaflor® is palatable, effective, and safe for use in catfish. Efficacy and residue-depletion studies were used to establish a dose rate of 10 mg active ingredient/kg of body weight that was approved by FDA for control of mortality associated with *E. ictaluri.*

Efficacy trials have demonstrated Aquaflor® is effective against columnaris infections and the data was used to obtain a provisional license for control of columnaris in catfish. As opposed to Romet and Terramycin, Aquaflor® is effective against both ESC and columnaris.

Development and technology transfer of modified live and killed vaccines against *Edwardsiella ictaluri and Flavobacterium columnare*, *Edwardsiella tarda, Streptococcus iniae, S. agalactiae and Ichthyopthirius multifiliis* (8). The ESC vaccine was first introduced in 2002. According to the vaccine manufacturer, total benefit to producers from use of this vaccine alone is almost $2,000 per acre because the faster growing vaccinated catfish are ready for market sooner than unvaccinated catfish. Since the ESC vaccine release almost 1 billion fry have been vaccinated. The columnaris vaccine, launched in 2005, is the first efficacious vaccine against columnaris disease, worldwide. Launched in 2005, the 2005/2006 production was sold out. The columnaris vaccine provides at least 72% improved survivability of catfish fry. These two vaccines, in combination, provide fish farmers a cost effective means for preventing the two most economically serious diseases affecting commercial pond-raised catfish. Use of these vaccines significantly reduces the need for antibiotics, thus decreasing environmental contamination and providing a safe fish product for consumers. Currently, fry production is about 1 billion/year and some 25% are vaccinated with one or both of the vaccines. With some 180,000 acres of ponds in catfish production, the potential economic benefit of these vaccines approaches $50 million annually.

Vaccines to control the bacterial pathogens, *S. agalactiae* and *S. iniae*, were also developed and patented. *S. agalactiae* (No. 7,204,993) and *S. iniae* (No. 6,379,677 BI) have been associated with significant mortalities among a variety of freshwater, estuarine, and marine fish species. The streptococcal vaccines protect fish against multiple pathogen strains of these species. The *S. agalactiae* vaccine provides a therapeutic effect in subclinically infected hybrid striped bass by protecting them from further morbidity and mortality. The vaccines do not leave a residue, so are safe for the fish and the consumer. The vaccines may be administered to tilapia weighing between 0.01 to 25 grams or more by hyperosmotic immersion. The benefits of the immersion vaccination technique outweigh the costs of vaccine and immunization. The vaccines are being licensed by the ARS Technology Transfer Office to a major vaccine manufacturer for use as a bivalent vaccine in the USA and worldwide. The expected economic impact of the vaccines is $100 million dollars annually.

A modified live vaccine against *Edwardsiella tarda* was developed and patented (No. 7,067,122) and is being licensed by a major vaccine manufacturer for use domestically and internationally. Edwardsiellosis is a disease problem in a number of species of cultured fish and eels, with an estimated cost of more than $40 million yearly, worldwide. This vaccine can be used in mass immunization programs.

Ichthyopthirius multifilis (Ich) is a major parasitic pathogen of many different fish species, including catfish. Catfish were protected against ichthyopthiriosis following bath immersion immunization with live theronts or by injection. Catfish immunized with sonicated trophonts

were 90% protected against Ich infestation. Thus, cutaneous antibody plays an important role in protecting against Ich including those produced in the skin.

Developed an *in ovo*, hyperosmotic immersion, oral route of vaccine administration and cohabitation immersion vaccine methods (9). The modified live *Edwardsiella ictulari* vaccine against enteric septicemia of catfish and a combination vaccine of Aquavac-ESC and Aquavac-COL to prevent ESC and columnaris diseases were tested *in ovo* in channel catfish eyed eggs. A significant proportion of fingerlings hatched from eyed catfish eggs immunized by bath immersion with either the *E. ictaluri* vaccine or the combination vaccine were protected against ESC and columnaris. This is among the first examples of successful immunization of fish eggs and another means of mass immunization of fish that is not stressful and is cost effective.

The patented *S*. *iniae* vaccine was formulated in the OralJect feed vaccine and tested for efficacy in tilapia. Tilapia fed the OralJect *S. iniae* vaccine were protected against *S. iniae*.

A vaccination and challenge cohabitation model was established and evaluated using Nile tilapia, the fluorescent chromophore calcein, and a *Streptococcus iniae* vaccine and *Ichthyophthirius multifilis* immunization. Tilapia fish were non-invasively calcein marked, sham-vaccinated and cohabited with non-marked *S. iniae* or Ich vaccinates as a single unit. The results showed that the cumulative mortality of the calcein-marked sham vaccinated fish was significantly greater than those of non-marked vaccinated fish. The calcein did not have an effect on the vaccination or the *S. iniae* challenge. Calcein marks were visible in the calcified skeletal structures of the head and fins using a portable UV lamp, and persisted for at least 45 days. Calcein appears to be a valuable tool for non-invasive, non-lethal, non-stressful, mass marking of fish (animal welfare) and allows differentiation between controls and vaccinates in a cohabitation model.

Copper sulfate for control of Ichthyophthiriasus and egg fungus in channel catfish (10). There are no legal water column therapeutants available that can be used in a 15 to 20 acre catfish pond. ARS copper sulfate research focuses on gaining an FDA-label for use to control Ichthyophthiriasis (Ich) in catfish in ponds. Studies determined the margin of safety of this compound to channel catfish; there was no mortality and no histological changes at five-times the therapeutic dose. An environmental assessment (EA) on the effect of use in the catfish industry was submitted to FDA in December 2006. . A supplemental FDA-label for fungus control using copper sulfate on catfish eggs has been initiated. An effectiveness range-finding study determined that treating catfish eggs daily with 10 ppm copper sulfate until eyes develop will prevent fungus from growing and destroying the eggs in high alkalinity/moderate hardness water at 74º F.

Compounds to treat water for snail and tapeworm control (11). Rams-horn snails are commonly found in production ponds and are a vector for the catfish trematode that can cause significant losses to the catfish industry. Experiments determined the best treatment for killing rams-horn snails along the shorelines of ponds with slurried hydrated lime and copper sulfate. For another snail, the invasive red-rim melania snail that vectors trematodes to wild and cultured fish species, Roccal D-Plus® was determined to be an effective disinfectant treatment for dip-nets. These snails can live on dried surfaces for several days and there was no previous treatment rate to kill them. These findings provide an effective disinfectant with an established rate for use on small fisheries equipment to control the spread of this snail.

Anesthetics for effective and non-injurious handling of fish (12). In cooperation with, and under the umbrella of the U.S. Fish and Wildlife Service's investigational new animal drug permit, the effective dose of two anesthetics (tricaine methanesulfonate and Aqui-S) for the handling and harvesting of Florida pompano were determined. Juvenile pompano held at two different salinities were exposed to various concentrations of two commercially available aquatic anesthetics and the effective dose for each chemical at both salinities which would allow handling and subsequent recovery was determined. This accomplishment establishes practical working rates for each anesthetic over a range of salinity levels which can be used by pompano producers to allow effective and non-detrimental handling of fish. In addition, the U.S. Fish and Wildlife Service is incorporating the data set with their own to seek approval by the U.S. Food and Drug Administration for extension to currently non-approved species to use Aqui-S as a zero withdrawal time anesthetic in aquaculture and fisheries.

Immunology and Disease Resistance

Genetic basis for immune responses to catfish diseases investigated (13). Enteric septicemia of catfish (ESC) is the most prevalent and costly bacterial disease affecting commercial channel catfish farms. Using the ARS-developed DNA-based detection method for the *Edwardsiella ictaluri* bacteria that causes ESC permitted rapid, specific, and sensitive pathogen detection in catfish blood and solid tissues. The test revealed that catfish surviving an experimental challenge with *E. ictaluri* contained lower levels of the bacteria than diseased catfish from the challenge.

Other research involving experimental challenge with *E. ictaluri* showed evidence that plasma cortisol levels during an *E. ictaluri* challenge correlated to the genetic predisposition of a family for *E. ictaluri* resistance or susceptibility.

Catfish administered recombinant bovine growth hormone grew faster and cleared *E. ictaluri* to a greater extent than controls.

Toll-like receptor 5, involved in cell signaling in the immune system, was identified as a potential biomarker for ESC exposure because it showed high levels of gene activity in challenged catfish. When exposed fish were compared to controls, expression of the TLR5 gene in stomach and liver was elevated 200 fold in fish with low susceptibility to ESC compared to only a 2-fold increase in fish with high susceptibility.

The patterns of gene expression after bacterial exposure can differ, however, between channel catfish, blue catfish, and their hybrid.

Channel catfish virus (CCV) outbreaks can produce large losses of fingerlings on individual farms. An extensive series of viral challenges was performed to determine the relative susceptibility of various catfish lines to CCV. Among the catfish strains tested, there were no significant differences in susceptibility to CCV.

A quantitative DNA test was optimized for detection of CCV in fish showing no symptoms of infection, and 10-50% of broodfish and fingerlings from commercial farms and research populations were determined to be carriers of CCV.

The Major Histocompatibility Complex (MHC) contains genes whose products regulate vertebrate immune systems, and the MHC class I and II molecules were identified in channel catfish. Unlike mammals, the catfish class I and II gene regions were found on different chromosomes. Preliminary functional studies showed that spontaneous non-specific cell killing responses between cells of different fish were mediated by the MHC class I region molecules. The CD45 gene, whose product plays in important role in communication within immune cells, was also identified.

Antibody titer and disease resistance in catfish (2). Preliminary trials focusing on ESC were conducted on a commercial fingerling farm in 2005, demonstrating a strong correlation between sero-positive populations of fish and increased disease resistance.

Options for vaccine development enhanced by further defining the roles of antibody and macrophages in protective immunity and mechanisms of disease (14). Serum and mucus antibody responses against *Streptococcus iniae*, *S. agalactiae* and *Ichthyophthirius multifillis* were assessed. Passive transfer with serum antibody from *S. iniae* and *S. agalactiae* immune tilapia protected susceptible tilapia from infection with *S. iniae* and *S*. *agalactiae*, respectively. Cutaneous antibody from the skin of channel catfish immune to *Ichthyophthirius* immobilized theronts of Ich and prevented further development in the skin of susceptible catfish. Furthermore, cutaneous antibody from the skin of channel catfish immune to *Ichthyophthirius* protected susceptible co-habited catfish against Ich. These findings demonstrate that serum and cutaneous antibody play a role in protective immunity against these pathogens.

Macrophage stimulating activity of *S. iniae* and *S. agalactiae* extracellular products vaccines was characterized to help better elucidate immune mechanisms. These extracellular products possess both macrophage chemotactic and chemokinetic *in vitro* activity. Semipurified extracellular products from *S. iniae* and *S. agalactiae* were similar in chemotactic activity, but had some differences in molecular weights of their chemotactic components. This research demonstrated that extracellular products are important pro-inflammatory molecules that help recruit macrophages and initiate the cellular immune response to streptococcal infections and vaccination. This information was used to help formulate the *E. ictaluri* and *F. columnare* vaccines which are dependent on cell-mediated immunity rather than antibody-mediated immunity.

Gene expression research identifies important immune response genes in catfish (15). The gene expression profile in channel catfish consequent to infection with *Edwardsiella ictaluri* was evaluated. Using subtractive-suppression hybridization methods a number of novel and important gene transcripts associated with innate immune response of channel catfish (up-regulated during infection with *E. ictaluri*) were discovered. These include the genes encoding for Immunophilins, nitric oxide synthase, the ubiquitin-proteasome system, homologs of zebrafish cytoplasmic domains (CD147, CD81, the complement-cascade attack system inhibitor CD59, and CD63 involved in the immune response), and extracellular matrix proteins (galactin-4, laminin receptor-1 and fibronectin-1a). In addition, a number of transcripts homologous to metabolic pathway-regulatory genes were demonstrated to be up-regulated during infection. These new gene sequences have been deposited in the National Centers for Biotechnology Information (NCBI) database.

Complete gene sequences of the channel catfish hemoglobin-β gene, peroxiredoxin 6 gene, cyclophilin A & B and the complement-cascade regulatory pathway CD59, have been determined and annotation provided.

Rainbow trout immune gene identification and selection of bacterial coldwater disease resistant trout (16). A number of novel rainbow trout immune genes were discovered and described that belong to the chemokine and tumor necrosis factor families of immune genes, including two new chemokine genes: CXCd1 and CXCd2. These genes were expressed in most tissues of healthy rainbow trout. Levels of gene expression increased in the major immune organs (spleen and anterior kidney) following either vaccination with dead bacteria or infection with the live *Yersinia ruckeri* bacteria. Infection of trout with another pathogen, the infectious hematopoietic necrosis virus, did not increase levels of gene expression indicating that the CXCd genes are not up-regulated in response to all pathogens. These findings suggest that measurement of CXCd1 and CXCd2 gene expression may be of future interest as biomarkers for an ongoing immune response to *Y. ruckeri*.

A comprehensive analysis of the TNF superfamily in teleost fish was completed. Members are involved in inflammation, cellular activation, and immune system development. Forty-two novel genes were identified from database searches and sequencing efforts. Results included: 1) teleosts possess orthologues of BAFF, APRIL, EDA, TWEAK, 4-1BBL, FasL, LIGHT, CD40-L, RANKL, and possibly TL1A; 2) the BAFF-APRIL subfamily is enriched by a third member, BALM, unique to fish; 3) lymphotoxin alpha and beta are absent in teleosts, but are replaced by a related ligand, TNF-New; 4) as many as four TRAIL-like genes are present in teleosts, as compared to only one in mammals; and 5) orthologues of T cell activation ligands OX40L, CD27L, CD30L, and GITRL were not identified in any fish species examined. Finally, mRNA expression of TNFSF members CD40L, LIGHT, BALM, APRIL, BAFF, FasL, RANKL, TRAIL-like, and TNF-New in rainbow trout immune and non-immune tissues was characterized. This work furthers the understanding of the fish inflammatory response and how vaccines elicit protective immunity.

Catfish stress and disease responses studied (17). The bacterial pathogen *Edwardsiella ictaluri* is a prevalent source of substantial economic loss to catfish farmers, and stress has been shown to increase *E. ictaluri* susceptibility. Trials were conducted to determine the effect of cortisol in the presence and absence of stress on mortality during an *E. ictaluri* challenge. Cortisol is the primary stress hormone in fish, and may adversely affect feeding, growth, disease resistance, reproduction, and overall fish health. Increased plasma cortisol levels due to stress correlated to increased mortality; however, stress-free cortisol administration had no effect on mortality. These results suggest that although cortisol is considered the primary stress hormone in fish, it in itself is not immunosuppressive. Understanding the relationship between stress and disease resistance is crucial to the development of biomarkers and management strategies for improving disease resistance.

Mechanisms of Disease

Model system developed for ESC (18). Laboratory protocols that better reflect the growth characteristics of pond-raised fish and pathogenesis of natural *E. ictaluri* infection were developed. Channel catfish fry were fed commercial diets supplemented with natural pond biota and vaccinated 10-12 days of age post-hatch. Following vaccination, resistance of fish to *E. ictaluri* infection was evaluated using standard single- and continuous-dose challenge models. Mortality differences were not detected between vaccinated and non-vaccinated fish or among dietary treatments using a standard single-dose challenge model. In contrast, vaccination and diet significantly affected mortality when disease resistance was evaluated using the continuous-dose model. Using this method mortality was lowest in vaccinated fry fed supplemented diets. These results suggest that diet is critical in the growth and development of

fry and that the methods used to induce disease in the laboratory influence the outcome of disease challenge trials.

Disease modeling and host identification (19). Two model systems were developed to test aquatic therapeutants. A model system was designed to simulate the pond environment to study the effect of therapeutants at controlling mortality associated with Ichthyophthiriasis in catfish. Infected fish exhibited 100% mortality. Studies with copper sulfate in local pond water were conclusive of efficacy and indicate that half of the recommended dose of copper sulfate is needed to effectively control an occurrence of Ichthyophthiriasis. In the second model, columnaris was induced through waterborne exposure of the bacteria to channel catfish after abrading the skin. Infected fish exhibited 95-100% mortalities and clinical signs or lesions that closely resembled a natural infection.

Disease mechanisms explored (20). Reproducible challenge methods were developed for *F. columnare* and *E. tarda*.

Source of infection, modes of transmission, routes of entry, virulence mechanisms, and host response to economically important infectious and non-infectious catfish diseases elucidated (21).

A previously unknown streptococcal bacterial disease was diagnosed in brood-sized channel catfish on three Mississippi delta farms. The disease is characterized by severe weight loss, arching of the back due to progressive destruction of the vertebral column, and draining sores located primarily at the angle of the jaw and fin bases. Challenge trials were conducted to fulfill Koch's postulates demonstrating that the organism in question is a true pathogen. Complete characterization of the bacterium is being conducted in collaboration with the Centers for Disease Control. At present the significance of this emerging disease is unknown, but monitoring efforts are ongoing to determine its prevalence in the industry.

Infections caused by the trematode, Bolbophorus damnificus, were first reported in Louisiana in 1994 and later in Mississippi in 1999. The disease is associated with poor production efficiency and high mortality, there are no scientific studies addressing these relationships. Studies were conducted to determine the production potential and disease resistance of fish infected with the trematode *Bolbophorus damnificus*. Light trematode infections reduced feed consumption and overall production by approximately 30%. These experimental results are comparable to decreases in production observed on commercial farms. Aquaria studies demonstrated that once fish are removed from the source of infection, the growth potential of trematode infected fish is equal to that of non-infected fish. Data indicate that breaking the life cycle of the trematode is an effective cure for this disease and that the long term implications of the disease are negligible once the source of infection has been removed. Experimental investigation concerning the interaction of *E. ictaluri* and *Bolbophorus* sp. infection demonstrated that concurrent exposure to both pathogens significantly increased mortality associated with ESC. However, no significant increase in mortality was observed when exposure to *Bolbophorus* sp. cercarieae occurred 28 days prior to *E. ictaluri* exposure.

Development of a disease challenge model for *Y. ruckeri* in salmonids (22). An engineered strain of the salmonid pathogen, *Yersinia ruckeri,* was developed which constitutively expresses the green fluorescent protein (GFP). GFP expression was exceptionally stable and the strain retained its ability to colonize and cause disease in immersion-challenged rainbow trout. This strain has been used for detecting and imaging the

interactions between this pathogen and its natural salmonid host. This strain is also easily identified in complex environmental samples making it useful for characterizing factors that allow its persistence and spread outside its host. A standardized disease challenge model for *Y. ruckeri* was established with this strain.

Epidemiology

Epidemiology of proliferative gill disease in catfish (23). Studies were conducted to gain a better understanding of the prevalence, seasonal occurrence and severity of proliferative gill disease (PGD) in catfish production ponds and determine environmental factors that contribute to severe epizootics. Monitoring efforts indicated that fish in essentially all intensively managed production systems develop some degree of PGD and that the most severe manifestations of the disease occur during the spring when fish are initially stocked into the ponds. Laboratory and field studies indicate that severe manifestations of the disease are dependent on the rate spores are shed into the water and the susceptibility of the fish to infection. The protocol used in these studies appears to be an accurate and quantitative method of monitoring infection rates. A molecular-based "PCR" assay capable of quantifying numbers of infective PGD spores in pond water and fish tissue has been validated for specificity. These procedures are being used to develop rapid methods of assessing the risk for the occurrence of PGD related losses. Initial experimental trials have demonstrated that the disease causing agents are evenly dispersed in the pond environment and validate the use of single point monitoring procedures in a risk assessment program. It has been demonstrated on commercial operations that the use of these procedures can effectively eliminate PGD related losses in fish that are stocked in production ponds during the spring.

Economic impact of trematode infection assessed (24). The trematode identified as *Bolbophorus damnificus* has been associated with high mortality rates, decreased feed consumption and poor production efficiency in commercially raised catfish. To assess the economic impact of this disease, sampling protocols were established to determine presence and severity of trematode infections. To date, approximately 2,000 ponds in 8 counties in Mississippi have been sampled. It is estimated that 20-30% of the ponds used in the commercial production of channel catfish contain infested populations of fish. The severity of infection ranged from mild to severe with most of the farms sampled testing positive for this disease. Farms that were unable to implement an effective bird management program and were within close proximity to pelican loafing sites were much more likely to suffer from severe infections. Compared to trematode negative ponds, ponds in light, moderate and severe categories produced 13.8%, 36.0%, and 40.5 % less pounds of fish per acre, respectively. Decreases in production were associated with significant decreases in net returns. Net returns for ponds in the light category were reduced by 80.8% ($87 net return/acre) and production from ponds in the moderate and severe categories were not shown to cover variable costs of production. Ponds in the moderate category produced a net loss of $506 per acre and severely infected ponds produced a net loss of $631 per acre. The overall economic impact of this disease to the catfish industry in the southeastern United States is estimated to be $45.4 million annually.

DNA sequence method for aquatic epidemiology (25). Columnaris is a serious infection to the channel catfish industry; however, there is no reliable diagnostic technique to definitively identify the causative agent (*Flavobacterium columnare*) of the disease. Restriction fragment polymorphism (RFLP) and sequencing methods of the 16S rRNA gene and the 16-23S rDNA spacer were developed as an epidemiological tool for bacterial columnaris outbreaks in aquatic

species. These methods have the advantage of being universal and capable of comparing the genotypes from different geographic locations. The methods were used to show the correspondence between the genotypes of isolates in North America with those in Asia and Europe and for the first time demonstrate the presence of genotype III in the United States.

Microbial Genomics

Differential expression of virulence genes in *Flavobacterium columnare* (26). A shot-gun genomic library of the *Flavobacterium columnare* ALG-530 virulent strain was previously constructed by ARS and more than 3,000 clones have been sequenced to date (800 contigs). Based on sequence identity with putative known virulence genes of related species, four genes were selected for differential expression analysis. A panel of 31 *F. columnare* isolates was tested for the presence of these four genes. However, not all of the isolates contained all the genes. Isolates belonging to the same genomovar presented an almost identical gene profile. Ten isolates of *F. columnare* representing both genomovars I & II were chosen for gene expression studies. The expression profile of the genes tested varied when all strains were grown *in vitro* under identical conditions. Strain ALG-530 was selected for gene expression comparison when cultured in normal broth, iron-limited broth and in perfused catfish skin. Expression of the thioredoxin gene (*trx*), and glycosyltransferase gene (*gft*) were not detected under any condition. The genes ferrochelatase gene (*hem*H) and nitric-oxide reductase gene (*norB*) were strongly expressed within 2 hours of incubation in normal Shieh broth, but the expression under iron-limiting conditions varied. Gene expression level in the presence of catfish skin was lower than under other conditions. This is the first study demonstrating differential gene expression in *F. columnare*.

***Streptococcal* antigenic variation explored (27).** In recent years, streptococcal infections have emerged as a significant cause of septicemic disease affecting both captive and free-living (recreational) fish in freshwater, estuarine, and saltwater environments worldwide. Examination of the correlation between genetic variation and pathogenicity at the molecular-level is focused on unraveling the mechanisms that interplay in the phenomenon of antigenic variation in these two species of streptococci. Research results demonstrated that *S. agalactiae* isolates from cattle were phenotypically and genotypically distinct from those isolated from fish (lacking receptor specificity) thereby revealing the inability of cattle isolates of *S. agalactiae* to infect Nile tilapia or channel catfish. Further examination showed that *S. agalactiae* isolates from fish display considerable genetic variation and can be divided into distinct clones. This research further advances the concepts of genetic (pathogenicity islands) and consequent antigenic variability among fish-pathogenic *Streptococcus* species.

Drug resistant plasmid identified and sequenced (22). A multiple drug resistance (MDR) plasmid was identified in *Y. ruckeri* and completely sequenced. This plasmid was shown to be highly related to MDR plasmids present in clinical isolates of *Yersinia pestis* and *Salmonella enterica*. The high degree of sequence identity and gene synteny among these plasmids suggests recent dissemination from a common ancestor. Subsequent screening has shown that this plasmid type is currently broadly disseminated among several human and animal pathogens isolated from retail meat samples. These plasmids represent a reservoir of mobile resistance determinants and have the potential to disseminate to *Y. pestis* and other human and zoonotic bacterial pathogens of public health importance. Additionally, rapid detection and typing methods were developed that could be used to monitor this antimicrobial resistance plasmid in animal production systems, foods, and may be useful for detecting these plasmids in biothreat organisms.

Genome sequencing of the trout pathogen *Flavobacterium psychrophilum* (28). The genome sequence of *Flavobacterium psychrophilum* strain CSF-295-93 was determined. A single circular chromosome of 2,900,735 bp containing 2,654 open-reading frames has been identified and annotated. This information has been used to select vaccine candidates and identify putative virulence factors.

Genomic technology informs virulence diversity of catfish bacterial agents (29). *Flavobacterium columnare* is a major disease agent of pond-raised catfish in the southeastern United States. Pulsed-field gel electrophoresis (PFGE), in which the entire genome can be represented as a distinct pattern of DNA restriction fragments, is used for molecular typing of microorganisms. Methods were developed for conducting PFGE on *F. columnare*, and its efficacy determined for characterizing *F. columnare* strains isolated from different locations in the Southeastern United States. On the basis of PFGE-derived profiles, similarity dendrograms were constructed for more than 30 *F. columnare* isolates, resulting in two major genetic groups with more than 60% similarity. Virulence diversity was observed in two different immersion challenge experiments conducted with 16 different isolates in channel catfish fingerlings. A direct correlation was found between the PFGE clustered groups and virulence. Challenged fingerlings with PFGE - Group A isolates resulted in mortalities greater than 60%, whereas PFGE-Group B isolates resulted in mortalities of less than 9%. These results suggest that two genetic divisions of *F. columnare* channel catfish isolates exist, one that contains strains that are “primary” pathogens of channel catfish (Group A), and another that are “secondary” or opportunistic pathogens of catfish (Group B).

An *E. ictaluri* genome sequencing project has discovered the presence of numerous mobile elements. A total of 88 isolates of *E. ictaluri*, isolated between 1978 through 2006 from Mississippi, Louisiana, Alabama, Arkansas, Georgia, and South Carolina, were analyzed by repetitive sequence PCR using BOX and GTG-5 primers. Twenty of these strains were subsequently analyzed by restriction enzyme digest with *Pme*I and *Spe*I followed by separation by pulsed field gel electrophoresis. Three or more molecularly distinctive groups have been observed.

Aquatic Animal Health Management

Population health management and diagnostic techniques (12). Eggs and larval Florida pompano of different ages (1, 8, and 16 days post-hatch) were subjected to short-term exposure (24 h) to varying concentrations of either ammonia or nitrite, and the tolerance of each life stage was determined in order to establish the relative sensitivity of early developmental stages of pompano to these waste products.

Similarly, acute tolerance of juvenile Florida pompano and black sea bass to ammonia and nitrite in low salinity environments was determined to assist producers in maintaining the health of marine fish stocks reared in low salinity. Juvenile fish were evaluated for 24, 48, and 96 hr exposures to increasing ammonia and nitrite concentrations at decreasing salinities to determine toxicity in low-salinity environments and establish tolerance limits, as tolerance is compromised at reduced salinities. This information provides criteria for maintaining health and production of these species in low-salinity culture.

Hematological profiles, stress, and health status were determined for a variety of species transferred to or reared in low-salinity environments to assist producers in establishing the health and well-being of pompano, black sea bass, cobia, and hybrid striped bass in low-salinity

environments. Following long or short term exposure to various salinities, blood samples were collected and evaluated for blood chemistry, osmolality, electrolytes, and total protein and fish were examined for signs of stress and disease.

Determination of the impact feeding practices on disease resistance (30). Manipulation of feeding strategies was shown to be very effective in controlling ESC-associated losses in channel catfish fingerlings. Laboratory studies demonstrated withholding feed from fish during infection reduced losses by 50-80%. Field trials support these observations where the implementation of restricted feeding regimes reduced ESC mortality by approximately 75%. Implementation of restricted feeding practices on commercial farms has dramatically reduced losses associated with ESC. This information is also being used to develop strategies for controlling disease related losses in food fish production ponds.

Nevertheless, withholding feed for four weeks resulted in weight loss, suppressed hematopoiesis, decreased non-specific immune response and increased susceptibility of catfish to *Edwardsiella ictaluri* and *Flavobacterium columnare* infection as indicated by high mortality. Long term withdrawal of feed during enteric septicemia of catfish (ESC) epizootics also increased mortality.

RESEARCH COMPONENT III: REPRODUCTION AND EARLY DEVELOPMENT

Successful and efficient reproduction is essential for sustainable aquaculture. There are many opportunities for improving reproduction and early development of cultivated aquatic organisms. Development of domestic strains with increased fertility will allow more efficient production of aquatic species with desirable culture characteristics and consumer appeal. Inefficiencies in maintaining viable seedstock, sexing of individuals, storing and utilizing gametes, and maintaining embryos and early life stages limits hatchery production, and utilization and conservation of valuable germplasm. Suboptimal fertilization rates and poor development during early life stages reduces production efficiency and profitability. Understanding requirements for proper gamete production and development of early life stages will lead to improved reproduction techniques. Research efforts will improve the understanding of environmental, neuroendocrine and genetic factors controlling reproduction in aquaculturally important species, and development of methods to enhance reproductive output, embryo development, and rearing of cultivated aquatic organisms during early life stages.

Vision: Produce reproductively efficient aquatic strains that require fewer resources, are environmentally friendly, and supply aquaculture products that more fully meet consumer expectations.

Mission: Research will seek to improve gamete and seedstock quality with year-round availability; improve development and survival during early life stages; and ultimately achieve optimum reproductive efficiency.

Impact: Decrease overhead and unit cost of production of all aquatic species, resulting in greater profitability for U.S. aquaculture producers and in lower food costs for consumers.

Linkages: 1) Other USDA-ARS National Programs: 101 Food Animal Production. **2)** Other Agencies and Departments: U. S. Colleges of Agriculture and State Agricultural Experiment Stations, U.S. Fish and Wildlife Service, and U.S. Food and Drug Administration. **3)** Private Sector: U. S. Aquaculture and Grower Associations.

Engaged ARS Locations: *Stuttgart, AR; Fort Collins, CO; Orono, ME; Stoneville, MS; Leetown, WV.*

Problem Area IIIA – Control of Reproduction

Problem Statement: Control of reproduction is essential for genetic selection, cost efficient and reliable production of seedstock and product quality. Currently there are few species where specific seedstock can be induced to produce gametes year-round and the timing of gamete availability is optimal for grow-out strategies or genetic selection efforts. Furthermore, the percentage of animals successfully producing high quality gametes is low, increasing hatchery costs; while on the other hand, high energy demands of gonadal development in pre-market size animals reduces product quality and production efficiency. Reproduction is regulated by environmental factors detected by higher brain centers, which in turn activate the neuroendocrine system controlling gonadal development and spawning. There is genetic variation in how the individual animal responds to these signals. Controlling reproduction requires characterizing then managing the environment for optimum reproductive efficiency, which in turn requires understanding basic neuroendocrine regulatory mechanisms and gonadal development and function. Since optimal or even minimal requirements may not be met in captivity, hormonal intervention and other strategies may also be required.

Committed Goals: 1) Elucidate pathways for neuro-endocrine regulation of gonadal development and function, and identify candidate genes for enhancing reproductive efficiency. **2)** Elucidate environmental influences and the impact of stress on critical control points limiting reproductive efficiency. **3)** Develop methods to identify reproductive stage. **4)** Develop methods to alter timing of reproductive development.

Expected Outcomes: 1) Management strategies and techniques for controlling reproduction and enhancing efficiency, improved reproductive efficiency and on-demand production of gametes. **2)** Ability to delay, shift, or prevent the onset of gonadal development.

Problem Area IIIB – Control of Gender and Fertility

Among many aquatic species, one gender has characteristics that make it more desirable for food than the other. The desirable gender for aquaculture is determined by several characteristics and different species may have a different preferred gender. This difference in sex-associated characteristics can be taken advantage of by developing methods to produce only the desirable sex. Monosex populations can be produced directly by exogenous hormone treatment if the fish are treated during early development, or by gynogenesis and androgenesis. Indirect production of monosex populations can be produced by hormonal sex reversal combined with genetic backcrosses, gynogenesis, or androgenesis. The indirect technique is often more desirable because parental strains are produced, and their progeny are then sold for aquaculture production. Under some conditions, sterile animals, such as those produced by polyploid induction, are useful in aquaculture. These animals cannot reproduce if they escape to the environment and often demonstrate increased growth.

Committed Goals: 1) Identify desirable gender-specific characteristics for aquaculture species. **2)** Determine the conditions by which gender can be controlled. **3)** Develop new technologies and improve current methods to control gender and fertility. **4)** Develop genetic techniques to determine the sex genotype.

Expected Outcomes: 1) Monosex seedstock to maximize desirable characteristics for improved production efficiency and market value. **2)** Seedstock that can be ownership protected since only the progeny are sold for grow-out. **3)** Understanding of gender-related genetic make-up and the plasticity of the sex determining system. **4)** Sterile animals to reduce risk of environmental damage will be produced if feasible.

Problem Area IIIC – Gamete and Zygote Quality

Improvement of gamete quality or zygote (fertilized egg) quality is essential for cost efficient and reliable production of seedstock and genetic selection. Egg quality is unacceptably low for all fish species. The multitude of factors contributing to egg quality makes this a particularly difficult problem to address. Determination of egg quality has genetic and environmental components. Environmental factors affecting egg quality include, but are not limited to, improper environmental cues leading to failed neuroendocrine regulation of gonadal development and function, stress, and diet. Lacking is an understanding of what makes a good egg or high quality gametes. Improving egg quality requires understanding basic processes involved in the assembly of the egg, and needs of the developing embryo; then providing the seedstock with the environmental conditioning and nutritional requirements to produce quality gametes (egg and sperm), followed by appropriate methods for spawning and fertilization.

Committed Goals: 1) Elucidate processes involved in gamete production. **2)** Elucidate environmental and neuroendocrine influences on processes of gamete production. **3)** Develop methods to evaluate quality of gametes and zygotes. **4)** Identify diets appropriate for optimal gamete quality. **5)** Identify superior seedstock.

Expected Outcomes: 1) Management strategies and diets resulting in high quality gametes and zygotes will be developed. **2)** Reduced loss from maintaining non-productive seedstock, and reduced mortality during hatchery operations. **3)** Reliable methods for identifying high quality seedstock standards will be realized.

Problem Area IIID – Gamete and Embryo Storage, Cryopreservation, and Use

Artificial fertilization utilizing stored gametes extends longevity and use of superior germplasm many fold. However, this technology is currently limited for aquatic species to the cryogenic preservation of sperm. New and commercially applicable methods for cryopreservation of gametes and embryos could greatly enhance reproductive efficiency. These new reproductive technologies can further increase the rate of genetic improvement and reduce costs of aquatic animal production. Storage of embryos, eggs, and somatic cells would enable the preservation of genetic information.

Committed Goals: 1) Develop new and improved existing methods for cryogenic preservation of sperm, somatic cells, eggs, and embryos of aquatic species. **2)** Develop improved methods of in vitro oocyte maturation and fertilization. **3)** Improve developmental competence of embryos.

Expected Outcomes: 1) Reliable methods for cryogenic preservation of sperm, eggs and embryos will be developed. **2)** Increased reproductive efficiency that expands use of superior germplasm.

Problem Area IIIE – Early Life Stage Development and Survival

Maximum production efficiency requires all fertilized eggs to result in healthy, normally developed offspring that survive and grow to a juvenile stage. Although 100% survival is unrealistic, survival to a juvenile life stage in many aquatic species is unacceptably low, often less than 10%. Others are born with developmental abnormalities that contribute to losses throughout development or impact processing and consumer acceptance. Factors contributing to losses during early life stages and/or inappropriate development are numerous and interacting.

Committed Goals: 1) Mitigate and prevent abnormal and inefficient growth and development during embryonic and early life stage development of cultured aquatic species. **2)** Improve embryo and early life stage handling, rearing and culture techniques to enhance survival and health.

Expected Outcomes: 1) Enhanced embryonic and early life stage development and survival. **2)** Reduce inputs used to obtain healthy offspring and improve production efficiency and profitability.

Selected Accomplishments for *Reproduction and Early Development*:

Control of Reproduction

Control of reproduction in marine species (1). Broodstock domestication programs for both Florida pompano and black sea bass were initiated because lack of techniques for sustained production of seedstock for grow-out operations is one of the foremost bottlenecks of marine finfish aquaculture. Additionally, broodstock domestication is known to enhance seedstock quality. Adult fish of both species were collected from the wild and transferred to the HBOI fish hatchery, acclimated to culture conditions, and gradually transferred to pelleted feeds. Fish rapidly adapted to culture conditions, a healthy status was achieved and maintained, and the pelleted feeds were readily accepted. In subsequent years experiments were conducted to evaluate hormone-induced spawning of captive broodstock for both species using a synthetic hormone analogous to gonadotropin releasing hormone (GnRH-a) and larval rearing methods were developed. While some differences between the two exist, it was determined successful reproduction of both species can be achieved over a sustained period of time, and larvae can be produced using hatchery procedures similar to those employed in the culture of other larval marine fish. The development of technologies for year-round seedstock production, enhanced biosecurity, domesticated strains of broodstock for genetic selection, and diet development for optimal gamete quality to produce quality larvae enhances U.S. competitiveness in marine fish culture. Year-round production of pompano and black sea bass seedstock for commercial farmers ameliorates one of the principal bottlenecks of marine fish aquaculture.

Gamete and Zygote Quality

Genes regulating oocyte development studied (2). Improving egg quality, reflected by better fertilization and hatching rates is important for the trout industry. ARS researchers are examining the localization and time course of gene expression of a suite of genes thought to regulate oocyte development in the stages just prior to spawning. This research has shown differences in the location of expression of several key genes, Activin A and BAMBI in the follicle cells, and BMP4, BMP7 and GDF9 predominantly expressed in the oocyte. Additionally, this work has revealed the time course of events leading up to spawning. These data suggest that these peptides may be critical to the process of egg development and therefore may be important for egg quality.

Early Life Stage Development and Survival

Management practices identified to improve catfish fry survival (3). Survival of catfish fry after stocking into ponds is not well documented, and reported averages among farms range from about 55% to 80%; survival within a farm can range from 0 to100%. This variable, unpredictable return leads to decreased efficiency in the fry to fingerling production stage of culture. Several studies were conducted to identify areas of management that could improve fry survival.

The zooplankton selection patterns by catfish fry were determined. Catfish fry selectively feed on cladocerans from 450 to 1000 micrometers, copepods greater than 650 micrometers, and ostracods greater than 450 micrometers. Rotifers and copepod nauplii were not consumed by the catfish fry. Collectively, cladocerans, copepods, and ostracods met or exceeded all nutritional requirements of catfish. These large zooplankton are high in protein, contain essential amino and fatty acids, and are excellent sources of vitamins and minerals. In laboratory feeding studies, catfish fry grew 50% more over a 20-day feeding study when fed commercial feeds supplemented with zooplankton compared to commercial feeds alone. In preliminary studies, catfish fry performed better in challenges to a bacterial pathogen (*Edwardsiella ictaluri*, the cause of enteric septicemia of catfish) when fed a diet supplemented with zooplankton. Impacts of this research should help fish farmers adopt management practices to maximize profitability of fry culture through more efficient utilization of natural pond productivity. Many catfish farmers have adopted the practice of monitoring zooplankton and basing their stocking decisions on the numbers of large (preferred) zooplankton taxa present. Utilizing more of the ponds' natural productivity is providing both economic and environmental advantages to the farmers.

Commonly used fertilization practices for catfish nursery ponds were developed over 40 years ago in Alabama and have not been studied in Mississippi Delta catfish ponds. Studies showed that natural productivity of catfish fry nursery ponds in Mississippi are not phosphorus-limited as previously thought, but are nitrogen-limited. By fertilizing with inorganic nitrogen fertilizers, desirable phytoplankton blooms develop more quickly and preferred zooplankton numbers were increased. The new fertilization recommendations have been adopted by many farmers and are now considered the standard practice for catfish nursery ponds in the Mississippi Delta. Farmers using the new recommendations have reported higher fry survival and less labor costs than when using the previous recommendations; thus improving economic efficiency of fry production.

Variability in fry survival may also be related to handling and stocking methods currently used. When stocking, fry are transferred relatively quickly from hatchery to pond water. Generally, temperature differences are monitored between hatchery and pond water, but little attention is paid to pH differences. Studies conducted to determine the tolerance of catfish fry to pH changes showed that catfish fry tolerate decreasing pH values relatively well, but have low tolerance for increasing pH values. It is estimated that a rapid increase in pH of 0.7 units will cause mortality in catfish fry of 10%, and an increase of 1.4 pH units will cause 50% mortality. Recommendations were developed for farmers to monitor pH before stocking fry and stock ponds that tend to increase in pH throughout the day. By monitoring pond pH at stocking, the major problem of poor, variable fry survival should be mitigated.

Certain pathogens may also play a role in early fry mortality. Chemical control of host organisms (e.g., snails and *Dero* worms) may reduce the spread of these pathogens and increase survival. Uniform pond application of copper sulfate at 5.0 ppm is an effective treatment against snails. Rotenone, Bayluscide, chloramine-T, formalin, and potassium permanganate may be useful as pond sterilization strategies for control of *Dero* worms before stocking fry. Application of these results have provided farmers experiencing chronic disease problems an additional management tool.

Management practices to increase catfish hatching rates (4). Catfish eggs often become infected with aquatic fungi or bacteria that lower hatching success. The optimal concentration of hydrogen peroxide was determined for use as a daily therapeutant in static and continuous-flow situations. Treatment with hydrogen peroxide significantly improved hatching success by ~30% compared to iodine and formalin treated eggs. Hydrogen peroxide treatment methods were optimized to account for differences between channel and hybrid catfish under different culture temperatures, and dependent upon developmental stage at the time of treatment. By following management recommendations, commercial catfish producers could realize up to 30-40% increase in annual fry production.

Fungal and bacterial egg infections can be a significant problem in commercial catfish hatcheries. Studies were conducted to optimize chemotherapeutic treatments for maximal hatching success of hybrid catfish. Hybrid catfish eggs were treated with increasing doses of hydrogen peroxide, formalin, copper sulfate and povidone iodine, and the optimal frequency of treatment application was determined. Hatching success was greatest among eggs treated with 100-ppm formalin, 100-ppm iodine, or 2.5-ppm copper sulfate. The optimal frequency of treatments was determined to be thrice daily, and a treatment-sensitive, developmental stage was identified. These data were used to formulate an optimal chemotherapeutic treatment regime for managing maximal hatching success of hybrid catfish and increasing production efficiency.

Channel catfish x blue catfish hybrids are superior to channel catfish for several production traits, but poor fertility and hatch are factors which impede large-scale production of hybrids. Catfish eggs are adhesive and are typically hatched as a large egg mass, but poor fertility typical of hybrid egg masses leads to increased bacterial and fungal infection and reduced hatch. ARS scientists compared jar-hatching of hybrid eggs after chemical elimination of the adhesiveness to traditional hatching of egg masses and found use of jars increased hatch to 6.8 hybrid fry per gram of eggs compared to 4.8 fry per gram with traditional hatching methods. Increased hatching rate can help offset inherent inefficiencies in production of catfish hybrids.

Catfish hatchery oxygen requirements and management elucidated (5). Approximately 2 billion catfish eggs are incubated each year by the channel catfish industry. The process is simple, and the methods have changed little since the inception of the industry nearly 75 years ago. With no empirical data available, recommendations on egg and fry oxygen requirements vary widely, and accordingly the Dissolved oxygen (DO) concentrations observed in commercial hatcheries range from 40-100% saturation. A study was conducted to determine for the first time both metabolic rates and critical oxygen concentrations for catfish eggs, sac fry, and swim-up fry. It was determined that eggs require over 90% oxygen saturation during the last day of incubation. At lower concentrations, the eggs hatch prematurely, and suffer a higher mortality during the sac fry stage. It was recommended that hatcheries run supply water through a packed column to bring the DO concentration up to air saturation. LOX should then be used to maintain DO at or above air saturation through incubation. In addition to eliminating premature hatch and higher mortality, the LOX also provides a margin of safety during electrical outages. ARS worked with five hatcheries to improve oxygen management during the 2006 hatching season. These hatcheries represent approximately 15% (350 million eggs incubated yearly) of the total industry capacity. These recommendations are being rapidly accepted by the industry due to the large improvement in hatchery production, low cost, and ease of implementation, resulting in a 15-20% increase in industry fry production.

Optimizing production of hybrid striped bass fingerlings (6). Production of many species of fish requires feeding tiny live food organisms (plankton) during the early life stages (fry-fingerlings). One approach to providing these organisms is to manipulate conditions in rearing ponds so that appropriate plankton are present at the time of fry stocking. Pond rearing of larval hybrid striped bass is not yet optimized. Patterns of live food succession in fingerling ponds were determined in a series of studies to predict the most advantageous time for fry entry. The influence of several water quality characteristics, fertilization methods, aeration methods, and chemical treatments on live food production in ponds was statistically modeled to improve fingerling production. Automated harvest of freshwater zooplankton from outdoor ponds for tank culture of fingerlings was demonstrated to further efforts toward off-season fry production. These results represent major milestones in bringing indoor, year-round production of hybrid striped bass fry to fruition.

Another approach to providing live food organisms (plankton) to early life stages of fish is to control all fry-rearing operations indoors; however, indoor methods of hybrid striped bass fry production have several bottlenecks that need to be addressed. A relationship between stocking density and growth of fry raised in tanks with unlimited feed was discovered that optimizes commercial hatchery production. The nutrient profile of live foods is often enhanced with algae or lipid mixtures to subsequently improve survival, growth, health, and fatty acid profile of larval marine fish. Live food enhancement requires significant investments in space, equipment, materials, and labor. The value of several different combinations of commercially available algal pastes and fatty acid enhancements to hybrid striped bass fry were determined in a series of studies. An indoor automated feeding system was developed to deliver highly condensed algae paste to feed rotifers for fingerling fish production, as well. Rotifer densities far in excess of what were expected during traditional culture were obtained with a system that was inexpensive, easy to construct, occupied comparatively little space, and was modular and expandable in design. These results represent major milestones in bringing indoor, year-round production of hybrid striped bass fry to fruition and these findings are eagerly sought and currently used by producers of marine fish larvae.

Marine species studied to develop practices to increase early survival (1). Captive and wild southern flounder brood stock were spawned under recirculating, closed-culture conditions to assist hatchery managers in addressing the need for technologies to produce year-round high quality seedstock of flounder. Captive and wild flounder were placed in broodstock conditioning units for hormonal and temperature manipulation, with the resulting larval fish cultured in closed recirculating systems. System and culture management protocols were established. In subsequent years trials were conducted to compare reproductive success of broodstock flounder fed formulated feeds vs. a frozen whole fish diet. Fish fed formulated feed preformed equally well as fish fed the traditional broodstock diet of frozen fish. These accomplishments established that captive southern flounder fed practical formulated diets successfully reproduce, and that resulting larvae can be cultured through the hatchery stage in recirculating culture. The use of captive broodstock, formulated feeds, and recirculating culture provides hatchery managers with enhanced biosecurity provided the impetus for establishing a broodstock domestication program to produce year-round high quality seedstock of flounder.

In addition, a model was developed to predict appropriate sized feeds for first feeding larval southern flounder through metamorphosis to assist hatchery managers in providing optimal nutrition and increase survival of larvae at first feeding. With imaging analysis technology larval southern flounder hatched were tracked for growth and development from the time of hatch to metamorphosis. As a result scientists are able to evaluate alternative feeds of better nutritional quality and southern flounder producers can select appropriate sized feeds for larval southern flounder from the onset of feeding to metamorphosis. Increasing survival and efficiency through the hatchery phase will remove one of the major bottlenecks to further development and enhancement of the U.S. mariculture industry.

An ammonia neutralizer for the reduction of ammonia concentrations in a small-scale rotifer culture system was tested for its ability to increase rotifer production and survival to assist hatchery managers produce live feeds for larval fish with greater efficiency. Rotifers were cultured either with or without the addition of an ammonia neutralizer to the culture unit with the units receiving the treatment exhibiting lower total ammonia levels, higher reproductive capacity and higher population numbers. Utilization of this technology allows hatchery managers to culture live feeds at higher densities reducing requirements for space, labor, and decreasing costs per unit production.

Early life stages of Florida pompano and black sea bass were also characterized via determination of growth and development to assist hatchery managers in implementing appropriate management strategies for critical life stages for both species. Larval Florida pompano and black sea bass were collected daily from culture tanks (from one day after hatching through complete metamorphosis) and microscopic images were captured and analyzed using digital imaging analysis to determine rate of increases in length, yolk absorption rate, time of complete yolk absorption and first feeding, mouth size and development, and time of complete metamorphosis. This established the timeframe of various physical and developmental changes that marine finfish larvae undergo. Characterization of these early life stages provides information that can be utilized to develop management protocols such as day of first feeding, appropriate size of live feed organisms to be offered, and time of complete metamorphosis. This information will be used by fish hatchery managers to establish developmentally appropriate management methods for larval rearing of both species.

Additionally, the effect of environmental salinity on early life stages of Florida pompano was determined to assist hatchery managers target the appropriate salinity range for optimizing

reproductive success and survival from egg stage through metamorphosis of Florida pompano. Eggs and larval Florida pompano of different ages (1, 8, and 16 days post-hatch), as well as post-metamorphic juveniles, were subjected to short-term exposure (24 h) to varying salinity levels and the effects of salinity on each life stage was determined. This accomplishment established the range of salinities supporting acceptable egg buoyancy and hatching, as well as larval and juvenile survival and will increase the success rate and production efficiency of hatchery operators. Increasing survival and efficiency through the hatchery phase will remove one of the major bottlenecks to further development and enhancement of the U.S. mariculture industry.

RESEARCH COMPONENT IV: GROWTH AND DEVELOPMENT AND NUTRITION

Growth and development, and nutrition are imperative for all animal production. These components form the basis for economic production of high quality and healthy aquaculture products for the American public and for export. Efficient growth and development is dependent upon an adequate supply of essential nutrients. Altering dietary nutrients and energy, and feeding management can have a direct impact on the growth and development at different life stages, disease resistance, production efficiency, quality and quantity of final product as well as waste production. Nitrogen, phosphorus and organic substances from unconsumed and undigested feed as well as metabolic wastes are the major sources of environmental pollution. These compounds, at high concentrations, may lead to toxicity, health risk and growth depression. Moreover, alternative sources of feed ingredients must be found to reduce the current heavy dependence of the aquaculture feed industry on fish meals and oils. Thus, appropriate nutrition to lower feed costs, improve production efficiency, reduce disease-related production losses, improve the quality of final product and reduce environmental impacts is fundamental for sustaining the growth and competitiveness of the U.S. aquaculture industry.

Vision: Develop efficient diets and feeding practices that optimize growth and development, enhance production efficiency, product quality, disease resistance, and minimize environmental impacts.

Mission: To develop cost-effective feeds and feeding strategies for commercially important aquaculture species that maximize nutrient utilization, reduce disease-related production losses and reduce the impact on the environment arising from aquaculture.

Impact: Increasing production efficiency and providing healthy and nutritious food products from aquaculture species raised in culture systems that are environmentally sustainable.

Linkages: 1) Other USDA-ARS National Programs: 101 Food Animal Production; 103 Animal Health; 107 Human Nutrition; and 206 Manure and Byproduct Utilization. **2)** Other Agencies and Departments: U.S. Colleges of Agriculture and State Agricultural Experiment Stations. **3)** Private Sector: American Feed Industry Association; American Fish Farmers Associations

Engaged ARS Locations: *Auburn, AL; Stuttgart, AR; Hilo, HI; Aberdeen, ID; New Orleans, LA; Orono, ME; Oxford, MS; Stoneville, MS; Leetown, WV.*

Problem Area IVA – Regulating Feed Intake

A major controlling factor of growth across species is feed intake. Feed costs represent the primary economic input into aquaculture production systems. Metabolic and sensory factors affect short-term feeding behavior. Long-term feeding behavior is controlled by the animal with its attempt to adjust to a defined equilibrium within its environment. Understanding the mechanisms involved in regulating feeding behavior and appetite will lead to more efficient production of aquaculture species.

Committed Goals: 1) To understand the mechanisms regulating feed intake in order to optimize the utilization of feed resources. **2)** Improve energy balance of commercially important aquaculture species at different phases of production.

Expected Outcomes: 1) Improved efficiency of feed use by commercially important aquaculture species at various phases of production. **2)** Increased knowledge and understanding of mechanisms involved in regulating feeding behavior and appetite will be developed.

Problem Area IVB – Tissue Growth and Development

Improved growth and development are vital for the sustainability and profitability of the aquaculture industry. Optimum growth performance and efficiency have limited value if product quality is not acceptable to consumers. The impact of tissue development and growth, and nutrition on flesh quality and composition is not completely understood. Knowledge of genetic, environmental, and nutritional factors that control development and growth of muscle, fat, and reproductive tissue is needed to develop practical methods for improving flesh quality and composition, and reproductive performance.

Committed Goals: 1) Understand the physiological and environmental conditions that enhance expression of growth and reproductive potential of aquaculture species. **2)** Alter characteristics of flesh quality of commercially important aquaculture species to meet consumer preferences and improve nutritional value. **3)** Determine the genetic and physiological basis for development and differentiation of adipocyte precursor, skeletal muscle cells, gonadal and epithelial cells. **4)** Identify tissue specific bioregulatory mechanisms for adipose, bone, muscle, and reproductive tissue growth, and function.

Expected Outcomes: 1) Increased growth rate and decreased culture period of various stages of production cycles. **2)** Produced aquaculture products with improved sensory attributes and nutrient composition for consumers. **3)** Increased reproductive performance, and gonad quality of aquaculture species.

Problem Area IVC – Sustainable Sources of Nutrients

Aquaculture feeds, especially those for carnivorous species, are heavily dependent on fish meal and fish oils to meet their critical protein and lipid requirements. The global supply of fish meal will likely remain static or decline because captured fisheries have reached maximum sustainable yields. There is also increasing competition among consumer segments for these

products. Thus, for the U.S. aquaculture industry to expand and remain competitive, cost-effective, sustainable sources of protein and oil must be identified or developed.

Committed Goals: 1) Identify and develop alternative sources of ingredients of economical importance to the United States to replace or reduce fish meal and fish oils in aquaculture diets. **2)** Develop nutritionally efficient diets using alternative sources of ingredients.

Expected Outcomes: 1) New sustainable sources of ingredients as replacements for fish meal and oils in aquatic animal diets. **2)** Consistent supply of low-cost, nutritionally efficient diets for various life stages of aquaculture species will be developed.

Problem Area IVD – Nutrient Use and Feed Evaluation

Changes in dietary nutrients and energy can have a profound impact on growth and development, reproductive success, composition of growth and carcass quality, disease resistance, and waste accumulation. Individual feed ingredients, whether traditional, novel and/or genetically-modified, greatly impact these characteristics though few ingredients have been evaluated for their potential nutrient bioavailability. Knowledge of feeding strategies to increase nutrient utilization and assimilation and decrease waste output are important for sustainable and profitable aquaculture production. Furthermore, the impact of diet alteration on product quality and how this relates to consumer preferences must be evaluated.

Committed Goals: 1) Develop diets based on ingredient nutrient bioavailability to conform to the nutrient and energy requirements of commercially important aquaculture species at different life stages reared in different culture systems. **2)** Develop feeding practices and strategies to improve nutrient retention, increase feed conversion efficiency, improve product quality, and reduce wastes. **3)** Establish feed manufacturing processes to improve feed palatability, pellet quality and nutrient availability, inactivate anti-nutritional factors, and reduce environmental impacts.

Expected Outcomes: 1) Aquaculture diets that optimize growth and development, provide desirable flesh quality, and increase production efficiency with minimal environmental impacts. **2)** Feeding management strategies for various production systems that enhance or optimize production efficiency with minimal environmental impact will be developed. **3)** Optimum feed and ingredient processing methods and conditions that enhance pellet physical characteristics, palatability, and nutrient bioavailability. **4)** Knowledge and strategies of nutritional modulation of metabolic processes in aquaculture species will be realized.

Problem Area IVE – Interaction of Gene Regulation and Nutrition

Current state of knowledge on gene regulation for growth and metabolism in aquaculture species is limited. Nevertheless, several research institutions have devoted considerable resources and efforts to genetically manipulate stocks of aquaculture species to improve growth and development. Genes responsible for regulating these physical traits must be better understood. Nutrient requirements must be determined and met to realize improved genetic potential. Feeding practices and strategies to deliver nutrients to optimize production while minimizing nutrient losses to the environment must be developed. A comprehensive understanding is required of the metabolic or physiological functions that limit production potential.

Committed Goals: 1) Improve understanding of functional genomics associated with growth and development and nutrition. **2)** Alleviate metabolic or physiological limitations restricting performance. **3)** Optimize animal nutrient efficiency to maximize conversion of nutrients to food products and balance environmental impacts with costs of production.

Expected Outcomes: 1) Improved nutrient management systems that enhance performance of existing genetic potential of aquaculture species. **2)** Improved genetic selection targeting specific metabolic or physiological limitations to production.

Problem Area IVF – Interactions Affecting Reproduction

Reproductive processes are affected by numerous nutritional factors such as diet composition, regulatory metabolic hormones, and body contents of certain nutrients. Changes in diet affect pituitary hormone secretions and reproductive performance. Understanding of basic regulatory cell interactions affecting gonadal development, maturation and spawning, and gonadal and larval quality, as influenced by changes in dietary nutrient and energy profiles, and feeding strategies, is needed to develop effective maturation diets and refine feeding systems and aid in cost effective management of broodstock and seed production.

Committed Goals: 1) Improve diet formulations and feeding management strategies to enhance reproductive performance, and improve gonadal and larval quality of commercially important aquaculture species. **2)** Improve understanding of nutritional effects on regulatory cells affecting gonadal development, maturation and ovulation.

Expected Outcomes: 1) Nutritional management systems and culture practices that enhance or maximize reproductive efficiency will be developed. **2)** Increased knowledge of nutritional modulation of cellular functions affecting reproductive physiological pathways in commercially important aquaculture species.

Problem Area IVG – Effective Probiotics

As with terrestrial monogastric animals, aquaculture species also host microbial populations in their gut that can influence the efficiency of nutrient utilization. Regional differences in types and availability of feeds and the introduction of new, alternative feed ingredients with improved nutritional properties have increased options for formulating rations to improve production. There is a paucity of information on the interactions of these feeds and microflora in the gastrointestinal (GI) tract of aquaculture species. Understanding how these interactions affect digestibility, digestion kinetics, and nutrient absorption is important for optimizing feed efficiency.

Committed Goals: 1) Develop strategies to optimize populations of specific, beneficial microbial species. **2)** Elucidate the biological and environmental factors affecting composition of microbial species of the GI tract and growth efficiency of GI tract microorganisms. **3)** Determine efficiency of microbial species to improve water quality and digestibility of various feed components under different production systems and environmental conditions.

Expected Outcomes: 1) Nutritional systems to enhance and maximize efficiency of nutrient use through optimization of relationships between endogenous gut microflora and diets. **2)** Production systems to improve water quality, growth and product quality will be developed.

Problem Area IVH – Immune System Enhancement (Nutrients and Immunostimulants)

Diseases cause considerable losses to aquaculture production. Currently only a limited number of therapeutics/chemicals are approved for disease treatments. These treatments are often ineffective, costly, and result in product and environmental contamination. A better alternative is to improve the disease resistance by enhancing the immune response. It is commonly known that nutrition is a key factor affecting aquatic animal health. A deficiency or excess of most, if not all, dietary nutrients has profound effects on infection and survival, largely through effects on host immune function. Other factors such as nutrient bioavailability and interactions, the presence of immunostimulants and/or toxins, and feeding management also influence on the immune system function and disease resistance of aquaculture species.

Committed Goals: 1) Elucidate the effects and modes of actions of dietary nutrients and immunostimulants, and identify natural compounds in feed ingredients capable of enhancing immune system function and disease resistance in aquaculture species. **2)** Delivery methods and optimum concentrations of nutrients/immunostimulants to enhance innate immunity and disease resistance will be identified. **3)** Feeding regimens and feeding strategies will be evaluated in relation to environmental parameters and life stages on immune response and disease resistance.

Expected Outcomes: 1) Diet formulations effective for improving fish health as well as optimizing production efficiency. **2)** Feeding regimens effective for stimulating immune responses and disease resistance, providing optimum growth and minimizing environmental impacts. **3)** Reduce production losses due to diseases and improve product quality and reduce environmental contamination.

Selected Accomplishments for *Growth and Development and Nutrition*:

Regulating Feed Intake

Seeking catfish with enhanced efficiency of nutrient utilization (1). Since feed costs account for about 50 % of the operating costs of commercial catfish production, improvement of growth rate and or feed efficiency would decrease the costs of catfish production. USDA103 line channel catfish grow 10-20% faster than average industry catfish, and had superior feed efficiency compared to Norris catfish, with observed differences between families. Comparison of USDA 103 strain catfish with the average industry fish showed no differences with regard to nitrogen and energy utilization.

Experiments were conducted to study the growth promoting effects of recombinant bovine growth hormone (rbGH) on growth performance, body composition, and endocrine responses in two strains of channel catfish. The rbGH increased final weight and improved feed conversion ratio in the USDA103 catfish line without a change in body composition due to treatment.

A study was conducted to determine the effects of feed restriction and the age at which the restriction was imposed on subsequent growth of channel catfish. A 1 week feed restriction was imposed at 7 or 28 days post-hatch, followed by return to satiation feeding. Compared to unrestricted controls, weight at 1 year post-restriction was lower in both feed restricted treatments and lower for fish for which feed restriction was imposed at 7 days than 28 days.

Study of the endocrine growth axis reveals fundamental information on energy balance and nutrient partitioning (2). Research revealed that novel GH-secretogogues (GHS) stimulate GH release (via the GHS-receptor) in tilapia, channel catfish and rainbow trout, thus providing the first evidence for the presence of this regulatory axis in teleosts. Ghrelin has been found to be the natural ligand for the GHS-R. This work was extended to rainbow trout and has revealed that ghrelin, and other GH secretogogues, do stimulate the growth axis (GH, IGF-I and IGF-binding proteins) and feed intake in this species.

The POMC transcript is a pre-pro-hormone which codes for multiple peptide hormones. Some the resulting hormones, α-melanocyte stimulating hormone and β-endorphin, are involved in feed intake through neuropeptide signaling in the hypothalamus. The tissue distribution of multiple genes and splice variants encoding POMC was determined.

Tissue Growth and Development

Gene expression of the growth hormone axis elucidated in catfish (3). The growth hormone-insulin like growth factor hormonal pathway is central to tissue growth. The genes and protein sequences for several members of this pathway, including growth hormone, growth hormone receptor, insulin-like growth factors I and II (IGF-I, IGF-II) and their receptors, and an insulin-like growth factor binding protein were determined. An IGF-I radioimmunoassay was developed and validated.

Injection of growth hormone in catfish stimulated both IGF-I and IGF-II gene expression. In fact, IGF-II gene expression was induced three-fold greater than IGF-I. Levels of IGF-II mRNA were higher in faster growing catfish families compared to slower growing families while IGF-I mRNA levels were unchanged. However, IGF-I protein levels in plasma were higher in faster growing families. Faster growing catfish strains also showed higher levels of plasma IGF-I protein.

Two genes known to regulate growth and feeding in other animals are growth hormone receptor and ghrelin. Research was conducted to identify these genes in catfish, determine their roles in catfish growth, and identify differences in sequence and gene expression patterns. Both genes were identified in catfish, found to be potent regulators of growth hormone, and to play central roles in the regulation of catfish growth.

Effect of gene regulation on tissue growth explored using the myostatin gene (4). Molecular genetic analyses have determined channel and blue catfish DNA sequences for myostatin, myogenin, myoD, and follistatin, whose products influence muscle growth and development in other species. DNA sequence variants were examined in these genes in order to determine if these polymorphisms are associated with meat yield and growth. The effects of passive immunization against myostatin, a negative regulator of muscle growth, on muscle growth in developing catfish embryos were determined. There was no increase in weight gain due to passive immunization against myostatin.

Catfish were exposed to glucocorticoids or a bacterial lipopolysaccharide (LPS). Expression of the myoD gene was upregulated in response to LPS, while expression of the myostatin gene was downregulated in response to glucocorticoids and LPS. These results were opposite to the responses seen in mammalian models of muscle growth and metabolism. This research

demonstrates the need to further characterize the mechanisms involved in muscle growth and breakdown in catfish, rather than just extrapolating from mammalian models.

Sustainable Sources of Nutrients

Evaluating alternative feed ingredient sources for catfish diets (5). Phytase, an enzyme produced by fungi, has been shown to be effective in improving phosphorus bioavailability in monogastric animals. Results demonstrated that a level of 250 units of phytase per kg of diet could be used to completely replace inorganic phosphorus supplements in channel catfish diets without affecting fish growth and bone phosphorus deposition. Using phytase to replace inorganic phosphorus supplements reduced fecal phosphorus concentrations and thus reduced phosphorus input to the pond. A level of 500 units of phytase per kg of feed and a total phosphorus level of 0.6% and above can be used to ensure adequacy. This research has led to commercialization of the use of supplemental phytase in catfish feeds.

New protein sources for catfish diets (5). Two products that are potential substitutes for soybean meal in catfish diets are cottonseed meal and distillers dried grains with solubles (DDGS). Cottonseed meal is a local product that is generally priced competitively (on a protein basis) with soybean meal. Distillers dried grains with solubles, may become abundant as ethanol plants come on line. Two pond studies were conducted to evaluate the use of DDGS, cottonseed meal, and supplemental lysine to partially or totally replace soybean meal in channel catfish diets. Results show that about 50% of soybean meal can be replaced with cottonseed meal + supplemental lysine in channel catfish diets without negatively affecting fish performance. Further, DDGS can be used in channel catfish diets up to at least 30% of the diet when the diet is supplemented with lysine. Use of DDGS in the diet appears to improve feed efficiency.

Genetic tools for improving barley and oats for aquafeeds (6). Significant progress in molecular genetics and molecular biology of the low phytic acid trait in barley has been made. Low phytic acid content is a very important nutritional trait in crop seeds because of decreased environmental pollution and increased availability of minerals for animals and humans. Increase in inorganic phosphorus and decrease of ground water pollution make low phytic acid barley a good candidate component for aquafeed utilization. Fifteen of the 17 mutants are now mapped to possibly six loci. In addition, a good candidate gene of *lpa1* using genomic approaches has been identified.

Tropical agricultural by-products and ingredients characterization (7). Disposal of numerous by-products that have potential use in the aquatic feed industry results in economic loss and potential environmental impacts. This work addressed two problems: developing cost-effective shrimp feeds that use locally available ingredients, and finding new uses for by-products that would otherwise require costly disposal. A shrimp growth trial was conducted using experimental diets containing meat & bone meal at four inclusion levels; spent fruit-fly media; and macadamia nut oil press cake at two inclusion levels; a fish meal-based diet, which contained none of these by-products, was used as a control. The trial was conducted in an outdoor, zero-water exchange system that permitted the development of high levels of floc, which has been shown to provide a portion of the nutrient needs of shrimp. After eight weeks of feeding, there were no significant differences in final weight, growth, or survival between shrimp fed the experimental diets and those fed the control. A second trial was conducted with whole

papaya, papaya flesh, papaya seeds, okara (soybean pulp), and wheat mill run. Papaya seed could replace 11.6% of fish meal, okara (soybean pulp) could replace up to 21.1% of fish meal, and wheat mill run could replace 100% of the whole wheat.

Nutrient Use and Feed Evaluation

Evaluating poultry by product meal for hybrid striped bass production (8). The relative value of pet-food grade poultry by-product meal (PBM) as a substitute for fishmeal was determined in a series of trials. In the laboratory, fish fed poultry by-product meal as the only protein source performed as well as those fed fishmeal, when PBM was supplemented with the two most limiting amino acids. In production trials in tanks and ponds, a substitution rate of 50% (PBM for fishmeal) was achieved in commercial test diets containing a typical mix of practical ingredients without compromising production goals. Digestibility trials with the commercial test diets underscored the need for more amino acid availability data in hybrid striped bass to avoid essential nutrient imbalances. Ammonia production trials confirmed that supplementing diets with free-amino acids did not result in more nitrogen loss to the system when compared to unsupplemented diets. Additionally, the modest decreases in production observed at higher levels of PBM substitution may be offset by lower costs of PBM-based diets.

Methods to increase nutritional value of plant products for rainbow trout (9). The distribution of chemical components within a cereal kernel is heterogeneous. Many nutrients are concentrated in the outer layers of cereal grains. For value-added utilization of cereal grains, it is important to efficiently and economically separate each of these layers. A simple, efficient and reliable lab processing method was developed to abrade grains into different layers or fractions. The method offers a valuable tool for conducting cereal chemistry research. It was used to determine effects of low phytic acid (LPA) mutation on contents and distribution patterns of different types of phosphorous and minerals within a barley seed. Seeds of one conventional barley cultivar and its 4 *low phytic acid* isolines were processed into different fractions. Concentrations of different types of phosphorus and other mineral elements in the fractions were measured and compared. It was found that except for phosphorus there was no significant difference in mineral concentrations and distribution patterns between conventional barley variety and LPA mutants. This finding is significant since it implies that localization of phytic acid synthesis has little effect in mineral distribution during seed development and that breeding *low phytic acid* crops does not lead to reduction or shifting within seed tissues in minerals, which, unlike phytate P, are valuable micronutrients for humans and animals.

Dry fractionation has been a primary means to enrich protein in cereal grains since it is least expensive as compared with some other protein enrichment approaches (such as wet extraction). A study was conducted to compare different dry milling methods followed by sieving for enriching barley protein contents and producing high protein fractions for fish feed. It was found that milling followed by sieving separated fractions with varying protein contents. Yet, the method of milling significantly affected the efficiency of protein enrichment and the protein content in individual sized fractions. Abrasive milling produced sized fractions with much higher variation in protein content than the impact milling. With a combination of abrasive milling and sieving methods, a larger fraction with almost doubled protein content could be produced. Using a modified wet extraction method, a barley protein concentrate with protein content in the range of 65% (dry matter basis) was produced. This sample is to be used for studying nutritional implication of 100% fish meal replacement for trout feed.

Co-production of protein concentrates and carbohydrates from grains used to produce ethanol is an approach that would benefit both products. By separating protein from the carbohydrates before fermentation, protein quality remains high making it more valuable and the ethanol less expensive. Barley protein was concentrated from 14% to over 50% on a dry matter basis. Barley has a balanced amino acid pattern and is devoid of known anti-nutritional factors.

The probiotic effect of non-nutritive components of plant products can also be of value to trout. Barley varieties with high levels of beta-glucans have been evaluated in rainbow trout feeds. Fish were directly challenged with infectious hematopoietic necrosis virus (IHNV) and trout fed the barley beta glucans had reduced mortality compared to trout not fed the barley beta-glucans.

Solid substrate fermentation (9). Solid Substrate Fermentation using food grade microorganisms is a method of altering substrate under low moisture levels thus reducing drying costs. Initial trials utilizing a blend of barley and soybeans have resulted in high protein products (> 60%), free of phytic acid, stachyose and raffinose. Nutrient digestibilities were very high, but palatability of the products was reduced.

Feeding practices and strategies developed to improve nutrient retention in catfish production (10). It is not clear how much dietary protein is optimal for fish fed every other day (EOD) to satiation, a feeding strategy that may be used when harvest is delayed because of off-flavor or other issues. A study was conducted to evaluate dietary protein requirement for fish fed once daily or EOD. Results demonstrated that feeding EOD to satiation improved feed efficiency and used less aeration compared with fish fed daily. However, feeding EOD resulted in lower growth, net production, and processed yield. For fish fed daily, a diet containing 24% protein provided the same growth and production as a 36% protein diet. However, for fish fed EOD to satiation, net production and weight gain per fish generally increased as dietary protein levels increased, suggesting that channel catfish may require higher dietary protein when fed EOD.

A pond study was conducted to evaluate effects of dietary protein concentration and carnitine supplementation on growth and body composition of channel x blue catfish hybrids. Carnitine is a product that is naturally occurring in the animal body and is approved for adding to animal feeds to reduce fattiness. Results demonstrated that growth, feed conversion, and visceral fat were not affected by dietary protein levels ranging from 28% to 36% or by addition of carnitine. As dietary protein increased fillet fat generally decreased. Use of high-protein diets appears to reduce body fatness.

Channel catfish were fed to satiation with a commercial feed once daily in the morning, once daily in the afternoon, or twice daily in both morning and afternoon for two growing seasons. There were no improvements in weight gain and net production by feeding catfish twice daily from advanced fingerlings to market size. Feed efficiency appears to be reduced for fish fed twice daily. Fish fed twice daily appear to have a better processing yield. Pond-raised catfish can be fed either in the morning or in the afternoon during the growing season. Feeding once a day improved feed efficiency, and reduced labor cost compared with feeding twice a day.

Research showed that feeding six days a week reduced net production by 3.3% and feeding five days a week reduced net production by 6.9%, compared with fish fed seven days per week for one growing season. Feed conversion ratio (feed/gain) was reduced by 4.8% and 7.9%,

respectively for fish fed six days and five days a week, compared with fish fed seven days per week. Considering the slight decrease in net production, slight improvement in feed efficiency, and possible reduction in other costs associated with feeding, feeding six days per week may reduce production cost for food-sized catfish.

A pond study was conducted to evaluate feed conversion ratios of channel catfish varying in initial sizes from 0.23 to 1.4 kg. Results showed that as fish sizes increased feed conversion ratio increased. For example, fish raised from 0.23 to 0.45 kg had a feed conversion ratio of 1.79, whereas fish raised from 1.36 to 1.82 kg had a feed conversion ratio of 3.74.

Results from aquarium and pond studies have shown that it is feasible to substantially increase omega-3 HUFA levels in catfish fillets by including 2-4% refined menhaden oil in the diets while maintaining the natural, mild flavor of farm-raised catfish. Feeding pond-raised catfish a diet containing 2.5% refined menhaden oil for a growing season resulted in a 3-fold increase in fillet omega-3 HUFA contents compared to catfish fed 2.5% catfish offal oil.

A study was conducted to evaluate effect of organic diets on growth and body composition of food-sized channel catfish. There were no differences in production characteristics and carcass yield between conventionally and organically grown fish. However, organic fish had lower fillet yield and higher body fat than control fish. The slightly higher body fat observed in organic fish resulted from the slightly higher fat content in the organic diet. This was because of the higher fat content of mechanically extracted organic soybean meal compared with solvent extracted conventional soybean meal that is typically used. However, the amount of deposited fat in organically grown fish was within the range typically found in channel catfish.

Nutrient requirements and use in hybrid striped bass production (11). A series of studies was conducted to define nutrient requirements in hybrid striped bass. The dietary requirement for the essential amino acid tryptophan was determined. The requirement for *n*-3 highly unsaturated fatty acids was determined at different dietary lipid levels. Carbohydrate utilization was investigated both *in vitro* and *in vivo* in advanced juvenile fish. Tracer studies confirmed that carbohydrate is not a major oxidative substrate for this fish. Plasma concentrations of IGF-I in sunshine bass were found to be highly correlated with culture temperature, photoperiod, feed intake, dietary carbohydrate and tryptophan levels. IGF-I levels can be used to identify diets and culture conditions which optimize growth.

Nutritional value of alternate feed ingredients assessed (12). A series of studies is ongoing to determine the effects of extrusion cooking, the primary method of aquafeed production, on the nutritional value of fish meal- and soybean meal-based diets. Extruder temperature, and the amount of time feed was exposed to that temperature, affected both fish growth and nutrient digestibility. Time in the extruder barrel significantly affected feed intake and weight gain; longer extrusion time significantly decreased feed intake and weight gain. Higher temperature in the extruder barrel significantly improved feed utilization (feed conversion ratio). Pre-cooking a soybean meal before inclusion in the diet significantly improved the apparent digestibility coefficient for organic matter, energy, and carbohydrates. These results demonstrate the importance of extruder processing conditions on fish performance, and indicate high temperature (127^{o}C) and short time in the extruder barrel results in the greatest weight gain of rainbow trout.

Digestible protein, energy, and available amino acids from ingredients in extruded complex diets have been quantified for more than 30 ingredients. Some of these ingredients are currently

included in rainbow trout diets and other ingredients that are being developed or have potential as replacements for fish meal have also been evaluated.

Development of fish meal free, all-plant protein, diets for rainbow trout (13). To develop base-line data, a series of diets were fed to juvenile rainbow trout to determine the effect of protein source and energy density on fish performance. A 4 by 2 factorial treatment design with four protein sources (fishmeal/barley, plant concentrates, plant meals, animal/plant-no fish) and two nutrient densities (43% protein/13% fat, and 48% protein/18% fat) were used. A reference diet (commercial fish-meal based trout diet) and a control diet (fish meal/wheat flour based) were also fed. Protein source and nutrient density affected feed intake, weight gain, and feed conversion ratio. Weight gain for fish fed diets with plant concentrates or meals was equivalent to fish fed the fish-meal based diets. Protein retention was affected by protein source, but not nutrient density, and was highest for the fish fed diets containing fish meal and lowest for the fish fed diets containing plant meals. Differences in apparent digestibility coefficients and apparent amino acid availabilities of the diets were reflected by differences in weight gain. This study provides evidence that growth rates of trout fed fish-meal free diets, using conventional and concentrated plant protein ingredients, can support growth up to ~80% of that observed when feeding fish meal-based feeds.

The amino acid taurine is conditionally indispensable, dependent upon life stage, in the diets of juvenile marine carnivorous fish and is a naturally occurring component of fish meal. Taurine was identified to be conditionally indispensable for rainbow trout fed diets that contain only plant proteins. Additionally, the mechanism by which methionine supplementation may spare taurine and the changes in expression of key enzymes in the bioconversion of methionine to taurine were quantified.

Feeding practices and strategies developed to improve shrimp production (14). An open shrimp diet formulation with defined ingredients and a defined nutrient profile, including for amino acids, fatty acids, minerals, and vitamins, was developed. This practical shrimp feed formulation was profiled and manufactured under the following National Institutes of Health Standards (NIH) specified herein: NIH STD 1 – Animal Feed Processing and Milling Sanitation Standard and NIH STD 5 – For Nutrient and Chemical Contaminant Analysis of Laboratory Animal Diets by a commercial feed company Zeigler Brothers Co. (Garnder, PA). This defined research shrimp feed is currently being used to defined processing parameters of particle size reduction to optimize shrimp performance.

Various floc extract fractions were incorporated into complete formulated diets for shrimp. Seven diets were processed with inclusions of whole floc (intact floc and ground floc) and floc fractions: polar (Fraction A), medium-polar (Fraction B), non-polar (Fraction C), and the residue (Fraction D), individually and recombined (A+B+C+D). An indoor growth trial demonstrated that inclusion of floc materials in the shrimp feeds had significant growth-enhancing effects without influencing survival rate. The feed containing floc Fraction B (medium-polar extract) achieved higher weekly growth than the control feeds, and much higher growth than the commercial feeds.

Feed formulation and nutrient requirements defined for Florida pompano and black sea bass (15). Appropriate protein and lipid concentrations for maximum growth and efficiency of black sea bass reared in saltwater were determined. Two protein sources differing in quality and four lipid levels resulting in different digestible protein and energy levels were evaluated for growth and production efficiency in black sea bass.

Hybrid striped bass were reared throughout a growout cycle in low-salinity recirculating systems with feeds containing partial replacement of fish meal with poultry by-product meal to evaluate its suitability as a partial protein substitute. Two different levels of poultry by-product substitution (35% and 70%) of the dietary protein were tested relative to a commercial hybrid striped bass diet and the fish fed the feed with 35% poultry by-product substitution grew as efficiently as fish fed the commercial diet.

Optimal dietary protein and energy levels for rearing juvenile Florida pompano were determined. Florida pompano were fed diets containing a wide range of protein and energy levels, with fish receiving moderate levels of protein and high levels of energy performing as well as or better than fish receiving higher levels of protein and less energy.

Protein, energy, and other nutrient digestibility (availability) values were determined for six protein sources to assist feed manufacturers in identifying alternatives to fish meal for use in Florida pompano diets. Three plant-based (soybean meal, soy protein isolate, and corn gluten meal) and three waste by-product-based (poultry by-product meal, meat and bone meal, and distillers dried grains) feed ingredients were fed to Florida pompano, and availability of protein, energy, and other nutrients were determined to initiate a database of feed ingredients.

Immune System Enhancements

Gossypol improves growth and disease resistance in tilapia but not in catfish (16). It has been reported that gossypol has antioxidant, anti-microbial, and anti-parasitic activities. Based on this information, studies evaluated if dietary gossypol from gossypol-acetic acid (GAA) and natural free- gossypol in glanded-CSM at various concentrations, in purified and/or practical diets, were responsible for improving immune response and disease resistance of Nile tilapia and catfish. The toxicological and pharmaceutical effects of GAA and the two gossypol enantiomers [(+)-gossypol and (-)-gossypol] to *E. ictaluri* were also evaluated. Results demonstrated that the concentrations of gossypol adversely affecting growth, feed efficiency, body composition and hematological parameters in catfish were lower than those reported earlier. The toxic concentrations of gossypol to catfish were higher in practical than in purified diets. Dietary gossypol levels necessary to enhance the immune response and resistance of catfish to *E. ictaluri* challenge were also established. However, gossypol levels found to improve immune response and resistance to *E. ictaluri* infection were higher than those found toxic to fish, gossypol from GAA supplemented to purified diets is of little benefit in improving disease resistance of catfish. Compounds in CSM other than gossypol may enhance the resistance of catfish against ESC.

Nile tilapia can tolerate relatively high level of dietary gossypol (1600 mg/kg diet). A dietary gossypol level of 100 mg/kg was sufficient to improve growth, feed efficiency, lysozyme activity and the resistance of tilapia to *Streptococcus iniae* infection. It was also discovered that natural gossypol was more effective in stimulating serum lysozyme activity and superoxide anion production than GAA.

Anti-nutritional factors or other compounds present in soybean meal (SBM) and distillers dried grains with solubles (DDGS) may be beneficial or detrimental to fish health (17). SBM and DDGS are readily available at much lower costs than fish meal. Incorporation of high levels of SBM usually results in depressed growth, and pathological and immunological changes due to the presence of anti-nutritional factors, particularly trypsin inhibitors (TI). Trypsin

inhibitors are heat-labile. Treatment of raw SBM at 130 ^{o}C for 40 minutes, was sufficient to inactivate TI and improve growth performance and feed efficiency. Heating duration had no effect on hematopoiesis or histological changes associated with anti-nutritional factors as has been reported in salmonids. Results also showed that catfish fed diets containing raw and insufficiently heated raw SBM had increased specific and non-specific immune responses, and improved resistance to *E. ictaluri* infection. Raw SBM contains heat-sensitive compounds or factors that enhanced the resistance of juvenile catfish to *E. ictaluri* infection. DDGS can be incorporated in Nile tilapia diets at a level of 20% as a replacement of soybean meal without negatively affecting growth, hematological values, immune response, or resistance to *S. iniae*. For channel catfish, growth performance and immune responses were not affected by inclusion of up to 40% DDGS (supplemented with lysine) as partial replacement of SBM, but a level of 10% DDGS appeared to improve the resistance of catfish to *E. ictaluri* infection.

Interactions between dietary nutrients, immunity and disease resistance in fish (18). Magnesium (Mg) content of bone and whole body were found as sensitive indicators in evaluating the adequacy of dietary Mg. The level of dietary Mg required for good growth, feed efficiency and survival, prevention of various deficiency signs, and enhancement of macrophage chemotaxis was identified. Although catfish have a requirement for Mg for normal growth and physiological functions, dietary level of this mineral had no effect on the resistance of channel catfish to *E. ictaluri* infection.

Effect of feed additives and anesthetics on fish stress resistance, immune response and resistance to infectious diseases explored (19). Research was conducted to determine if immunostimulants (bovine lactoferrin and yeast or yeast subcomponents (YYS) [β-glucan or mannan oligosaccharide]) had positive effects on fish health. Thus far, five and seven different bacteria in Nile tilapia and channel catfish, respectively, have been tested for use as potential probiotics, but none affected growth, immune function, or disease resistance in either species. It was discovered that dietary supplementation of bovine lactoferrin is effective in reducing susceptibility of Nile tilapia to *Streptococcus iniae* and channel catfish to *Edwardsiella ictaluri* infections. In Nile tilapia, 800 mg/kg of lactoferrin in the diet appears to be optimal; while channel catfish fed the highest level of lactoferrin tested (1600 mg/kg) had the greatest resistance to *E. ictaluri*.

NaCl in diets of channel catfish is effective in reducing mortality from nitrite toxicity. Resistance to nitrite toxicity increased with increasing dietary NaCl to 4% in diet. Sodium chloride at levels greater than 4% in diet caused a significant reduction in growth. Feeding Nile tilapia a 6% sodium chloride diet for 6 weeks not only improved fish growth and feed efficiency but also reduced fish stress after direct transfer from freshwater to 20 ppt salinity water.

An MS-222 concentration of 90 mg/L was optimal in reducing the stress response to handling.

RESEARCH COMPONENT V: AQUACULTURE PRODUCTION SYSTEMS

Aquatic animals are grown in a wide variety of environments using many different management systems to provide consumers with consistently uniform, safe, and nutritious products. Aquatic animal producers continually are challenged to produce fish, shellfish, and crustaceans efficiently and economically. 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 be improved through the development and application of innovative biological and engineering approaches. Producers must be provided with the necessary information and technology to meet consumers' needs with desired fish and shellfish products.

Vision: Improved production efficiency, economic competitiveness and environmental compatibility of aquatic animal production systems.

Mission: Optimize aquatic animal production systems in terms of productivity, economic performance and environmental compatibility through application of biological and engineering approaches.

Impact: Reduced cost of production for all aquaculture species resulting in greater profitability for U.S. aquaculture producers, globally competitive products, and lower food costs for consumers.

Linkages: 1) USDA-ARS National Programs: 101 Food Animal Production; 108 Food Safety; 201 Water Quality and Management. **2)** Other Agencies and Departments: U.S. Agricultural Colleges and Agricultural Experiment Stations, Other Universities, USDA-CSREES, U.S. Fish and Wildlife Service, USDA-APHIS, USDC-NMFS. **3)** Private sector: Harbor Branch Oceanographic Institution, Mote Marine Laboratory, Freshwater Institute, U. S. Aquaculture Associations.

Engaged ARS Locations: *Auburn, AL; Pine Bluff, AR; Stuttgart, AR; Hilo, HI; Aberdeen, ID; Orono, ME; Oxford, MS; Stoneville, MS; Leetown, WV.*

Problem Area VA – Biosecurity

Intensification of aquatic animal production systems results in concentration of pathogenic microorganisms associated with losses from infectious disease outbreaks or toxin occurrence. Inter- and intra-facility transmission of pathogenic microorganisms can occur by many routes. Breeding programs result in locally developed and enhanced stocks of aquatic animals. Unauthorized export of improved stocks detracts from the U.S. industry's competitiveness, while escape of improved stocks may have a negative environmental impact. Defining risk, development of quarantine systems, pathogen detection and monitoring, increased understanding of the relationship between pathogenic and non-pathogenic microorganisms, and control mechanisms of improved stock are critical areas of investigation.

Committed Goals: 1) Define engineering and biological components of bio-secure production systems. **2)** Develop systems to prevent loss of improved stocks of aquatic animals.

Expected Outcomes: 1) Effective quarantine systems and facilities will be developed. **2)** Methods and strategies for pathogen detection and monitoring will be produced. **3)** Mechanisms to prevent loss of improved stocks of aquatic animals will be identified.

Problem Area VB – Production Intensity

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

Committed Goals: 1) Optimize production, increase economic competitiveness, and reduce environmental impact of aquatic animal production systems. **2)** To increase reliability, efficiency, and cost-effectiveness of production through the use of new and improved technologies. **3)** To develop models of fish farming systems that assist producers in decision-making.

Expected Outcomes: 1) Economically viable, globally competitive, and environmentally responsible aquaculture production systems will be developed. **2)** Increased application of technology to aquatic animal production. **3)** New tools to analyze aquatic animal production systems.

Problem Area VC – Integrated Production Systems

Aquacultural production systems can incorporate synergistic, multi-enterprise production systems to recover valuable inputs (e.g., nutrients) that would otherwise accumulate within the production system or be discharged into the environment. Integration of components of fish culture, shellfish culture, hydroponics, or micro/macro algae culture can enhance input utilization efficiency and farm profit potential. However, matching the requirements of the different component species is difficult, and interactions among components are not understood fully.

Committed Goal: 1) Develop new and improve existing integrated production systems for aquaculture.

Expected Outcome: 1) Polyculture systems that maximize nutrient utilization.

Problem Area VD – Predator and Fowl Control

Predators (notably mammals, turtles, and snakes) and fowl (fish-eating birds, such as cormorants, pelicans, egrets, herons, anhingas, storks, and diving ducks) can cause significant direct and indirect losses to the aquaculture industry. The economic impact of predators and fowl on aquaculture facilities in terms of revenue loss and expenditures on control efforts sums to tens of millions of dollars annually. Damage by predators and fowl is caused not only by the consumption and wounding of aquatic species, but also when the aquatic animals are forced to seek shelter and stop feeding, they become more susceptible to disease because of stress, crowding, or decreased environmental quality. Also, birds carry and transmit disease agents to aquatic animals and humans. An increased understanding of predator and fowl behavior will foster development of improved control strategies at aquaculture facilities.

Committed Goals: 1) Develop new, improve existing, or adapt alternative techniques to minimize depredation at aquaculture facilities. **2)** Assess regional populations of fish-eating birds and evaluate other potential predators and fowl.

Expected Outcomes: 1) Reduced depredation by predators and fowl at aquaculture facilities.
2) Surveys of regional fish-eating bird populations will be completed.

Problem Area VE – Live Aquatic Animal Handling, Transport, and Inventory

Handling and transport of live aquatic animals between the hatchery and the farm, within production units on the farm, or between the farm and the processing plant or live market is inefficient, and survival, condition, and performance of the aquatic animals following transport are variable. Inventory management of aquaculture stocks is essential for optimal production management. Current methods and technology for handling and transporting live aquatic animals are labor intensive, constrained by deterioration of water quality, or utilize intrusive handling methods. Suitable methods do not exist currently for monitoring the population number or average size of most aquacultured species. Moreover, biological and engineering technologies are needed to decrease size variation of animals within culture units to increase efficiency and provide consistent quality products to enhance U.S. competitiveness and profitability. Increased understanding of aquatic animal behavior and physiological responses, and application of innovative engineering design approaches are needed to optimize handling, transport and inventory management of live aquatic animals.

Committed Goals: 1) Develop new and improve existing methods or technologies of handling and transporting live aquatic animals. **2)** Develop methods or technologies to track population number and average size of cultured aquatic animals within the culture unit. **3)** Develop methods or technologies to reduce size variation of cultured species within culture units.

Expected Outcomes: 1) Live aquatic animal handling and transport equipment and procedures that are efficient and minimize stress. **2)** Enhanced technologies to measure aquatic animal size and numbers in the culture unit. **3)** Technologies to provide more consistent quality products to improve competitiveness and profitability of U.S. aquaculture products will be developed.

Problem Area VF – Culture of Marine Species in Low-Salinity Water

A number marine species are able to adapt and thrive in low-salinity water normally encountered in estuarine environments. Availability of low-salinity water from inland aquifers expands the range of environments where marine species can be cultured, providing opportunities to diversify farm production strategies and to develop new markets. However, because the ionic composition of low-salinity ground water may differ substantially from dilute seawater, survival and growth of marine species may be sub-optimal. Understanding physiological and biological responses to low-salinity water and developing strategies to enhance ionic composition of ground water may lead to increased culture of marine species in low-salinity water. Moreover, little is known about the waste production, and its handling, and elimination from production units, used for rearing marine species in low-salinity environments.

Committed Goals: 1) Increased culture of marine species in low-salinity water at inland sites.
2) Systems of production for disease-free stock.

Expected Outcome: 1) Increased production of marine species at inland locations in the United States.

Selected Accomplishments for *Aquaculture Production Systems*:

Biosecurity

Development of water recirculation systems and novel phage biotherapeutics (1). A 34-tank recirculation system was completed and used to rear rainbow trout from the ARS's selective breeding program. The recirculating aquaculture system uses biological and mechanical filtration to remove ammonia and suspended solids. Additionally, carbon dioxide is stripped and dissolved oxygen levels increased prior to returning the water to the culture tanks. This system has enabled ARS to maintain its rainbow trout genetic stock, while conserving water resources. Growth of brood fish was compared in recirculated and flow through systems to examine the impact of culture environment on production characteristics. Fish reared in tanks receiving recirculated culture water gained more weight than fish reared in the flow-through tanks, even though water quality in the recirculating aquaculture system was poorer, with respect to ammonia and organic concentrations. Additionally, the results indicated that the strains maintained the same rank order in terms of growth while being reared in either environment. The implications of this work suggest that a single selective breeding program may be sufficient to improve growth characteristics of rainbow trout when reared in a recirculating or flow-through environment.

Production Intensity

Improved efficiencies of scale and reduced water quality constraints for sustainable production in controlled intensive aquaculture systems (2). Research was conducted to develop and evaluate functional, operational, and economic efficiencies through close integration of engineering, biological criteria, and product flow within large-scale coldwater recirculating systems. Improvements in culture tank and biofilter design, and in carbon dioxide and dissolved oxygen control, were developed to help fish farmers improve culture tank water quality, increase production capacity, and reduce fish farm production costs. Crowding, lifting, sorting and slaughter methods during fish harvest were developed or improved. Reduced labor requirements, improved worker safety, improved food quality, and increased animal well-being resulted from this research. Engineering and management practices were also developed to improve biosecurity in intensive aquaculture systems to protect the cultured stock from infection with specific pathogens. The new or improved aquaculture production practices, technologies, and engineering design criteria produced through this research are described below.

The large circular culture tanks commonly used by fish farmers for high density production of finfish create large, dilute effluents that make water quality restoration difficult and expensive. Water inlet and outlet structures were developed to better control rotational velocities, mixing, and solids removal within large dual-drain culture tanks. These findings have helped fish farmers improve culture tank water quality, increase the rate of particle removal from large dual-drain circular culture tanks, and reduce fish farm capital costs by combining the function of high density rearing containment with the ability to separate settleable biosolids into a relatively small bottom center drain flow. Dozens of private and public fish culture systems have now incorporated the dual-drain circular fish culture tank and have benefitted from this research.

Research on two settling basin designs that remove solids from the flow exiting the bottom center drain of the dual-drain tanks were compared; the radial-flow settling basin design was found to approximately double the efficiency of suspended solids removal as compared to the swirl separator. Equipment suppliers are now marketing the improved settling basin design and commercial fish farmers have retrofitted to the improved settling basin design.

Dynamic systems for dissolved carbon dioxide and oxygen control were developed and assessed within intensive recirculating aquaculture systems. Research was completed to determine how to better design forced-ventilated cascade aeration columns to improve dissolved carbon dioxide removal in both freshwater and full-strength seawater applications. The gas transfer and process control systems developed are now being used to improve water quality and increase carrying capacity within high-intensity fish culture systems.

This research contributed to increased application of technology to aquatic animal production and the development of economically viable, globally competitive, and environmentally responsible aquaculture production systems. This research has been used to improve the production efficiency of warm (e.g., tilapia), cool (e.g., walleye, yellow perch, hybrid striped bass, barramundi, and sturgeon), and cold water (e.g., rainbow trout, Arctic char, and Pacific and Atlantic salmon) aquatic species that are cultured within large-scale recirculating aquaculture systems.

Technology development and evaluation for catfish production systems (3). Two commercial products (LASE and PhytoMax) are formulations of organic acids, enzymes, vitamins and micronutrients designed to stimulate microbial activity leading to higher levels of dissolved oxygen, healthy algae blooms, reduced aeration costs, and overall improvement in water quality. Research showed no benefits of adding either product to production ponds.

Most U.S. aquaculture production comes from ponds. Traditional ponds need continuous management of oxygen concentrations and are susceptible to algae-related fish off-flavors, losses to avian predators, difficulties in disease control, inefficient fish harvesting, and the finite limit on fish production. Engineers at Clemson University developed a culture system that allows routine fish production at the limits of the potential for culture systems with no water exchange. The partitioned aquaculture system (PAS) as currently configured at Clemson consists of an extensive, shallow "waste-treatment" basin representing about 95% of the total system area and an intensive fish-confinement area in which fish are crowded at about 20 to 40 times the density of traditional ponds. A low-speed, energy-efficient paddlewheel circulates water between the fish-holding section and the waste-treatment section. A modified PAS system was constructed that confines fish at a much lower density than the Clemson system. The overall concept is to take advantage of the fish confinement benefits of the PAS (facilitation of inventory, harvest, health management, and protection against predation) while avoiding the need for intensive system management. In two years of optimization studies, the simple version of the PAS was found capable of sustained fish feeding rates of 150 to 200 kg/ha per day without deterioration of water quality. Net fish production (harvest weight minus stocking weight) has been 12,000 to 16,000 kg/ha at feed conversion of less than 1.9. This system shows promise as an alternative to traditional pond aquaculture.

Modular system shown to be effective in catfish systems (4). A fingerling to stocker phase (2nd phase) evaluated as part of a three phase (modular) channel catfish production system was experimentally shown to be feasible and economically practical. The per kilogram total cost of production for two higher stocking rates was respectively eight and fifteen cents less than corresponding production costs for the multiple-batch production system. In addition, to being a cost effective, alternative management strategy for the farming of channel catfish, the modular system offers several advantages based upon better inventory control and minimizing loss due to bird depredation. The fingerling to stocker phase also affords flexibility in the choice of management options to meet an array of different goals of individual operations.

Oxygen management practices developed for ponds (5). Catfish farmers have long known that aeration allows them to feed and grow more fish. Over the last 30 years, farmers have progressed from having only tractor-powered aerators that could be moved to a pond with low D.O. concentration, to having over 2-hp/acre of electric paddlewheels permanently installed. Production has increased concurrently from 1,000 lbs/acre to 5,000-10,000 lbs/acre. However, prior to ARS research, most aeration recommendations were based on minimizing visible oxygen stress in fish, not on maximizing fish performance or profit. Use of computerized technology enabled researchers to both control the D.O. concentration and record aerator usage in a multi-pond research facility. In several production studies, it was determined that allowing the D.O. concentration to fall to 1.5 mg/L (as compared to 4.5 mg/L) reduced food consumption, growth, and net production by 45%, 31% and 54%, respectively. At higher dissolved oxygen concentrations, a net production of 23,547 kg/ha was achieved, a record for pond production systems. For the first time, D.O. management can be based on empirical data. In addition to increased profits resulting from increased food input, this research demonstrated that catfish can be grown to market size in two years instead of three with increased feed consumption made possible by increased aeration. These data have been used by the Catfish Farmers of Mississippi in an on-going process to negotiate "off-peak" electric rates from the Mississippi Electric Power Association. Following a meeting of the Association with representatives of the catfish industry, the Association provided a $3M rebate to Mississippi fish farmers while they continue to develop an off-peak rate structure.

Aerator placement in large ponds (6). ARS has devoted several years of research to determine D.O. requirements of pond-raised catfish. The focus of this research has now shifted to finding economical means of meeting those requirements. A non-funded cooperative agreement with Dillard & Company, Inc. was initiated to evaluate a new aerator placement strategy on a commercial catfish farm. Ten 17-

acre ponds were selected and brought into the study in pairs, with one pond using the conventional aerator placement strategy and the other pond using the new strategy. The ponds were stocked similarly and managed according to normal commercial practices. This was the first study to determine how aerator *placement* in commercial ponds can affect oxygen concentrations. This new aerator placement strategy was a radical departure from conventional practices and shows great potential for increasing production while reducing energy costs. Commercial ponds using the new strategy had higher morning D.O. concentrations, higher net production, with fish consuming more feed and converting that feed more efficiently. Ponds using the new aerator placement strategy used 18% less electricity for routine aeration, and used tractors 22% less frequently for emergency aeration. Overall savings on this farm from use of this new strategy is estimated to be $100,000.

Comparison of a diffused oxygen aeration system with a tractor-powered paddlewheel (7). During hot summer weather, channel catfish producers historically have used tractor-powered paddlewheels to aerate and move water through grading nets loaded with food-size fish. New technology using diffused oxygen to provide supplemental oxygen to catfish held in grading nets was compared to the aeration effectiveness of a tractor-powered paddlewheel. An informal economic analysis showed that a diffused oxygen system could be operated for about the same cost per hour as a tractor-powered paddlewheel. Results of the study indicate that the diffused oxygen system is a viable alternative to the tractor-powered paddlewheel for aeration of channel catfish in grading nets.

Tank-hatched Golden Shiners reared to market size in one season (8). Final stocking densities of 47,000-57,000 fish/ha of tank-hatched golden shiner fry yielded 560-670 kg/ha of jumbo (> 12 g) fish, whereas stocking 741,000 fish/ha, where survival averaged 44%, yielded 2,200 kg/ha of medium (~7 g fish) in one growing season. Normally, two growing seasons are needed to produce jumbo-sized fish, and baitfish yields in general are low, which results in low profits to the farmer because of high fixed costs. Researchers stocked ponds with 124,000-741,000 tank-hatched golden shiner fry per hectare to determine the effect of stocking rate on production of jumbo-sized golden shiners in one growing season. These results demonstrated that high yields of jumbo-sized golden are possible in one growing season, which should improve profitability.

Potential of blue catfish explored (9). Blue catfish have potential as a commercial culture fish but it was believed that their oxygen requirements were higher than channel catfish. A study was conducted to determine the relative impact of low oxygen on food consumption and production of channel and blue catfish. It was determined that while channel catfish food consumption decreased 9.5% and net fish production decreased 851 lbs/acre when the dissolved oxygen concentration dropped below 3.0 mg/L, blue catfish actually consumed slightly more feed (+0.9%) and had slightly higher net production (+156 lbs/acre) than blue catfish maintained at a dissolved concentrations above 4.0 mg/L. Blue catfish may have more potential as a commercial culture species than previously thought, and certainly merit further examination.

Aquascanner catfish SONAR (10). Previous research indicated the utility of using acoustics (SONAR) in commercial catfish ponds to observe and inventory the fish population. The ability to accurately inventory the standing crop of mixed-size catfish in commercial ponds would facilitate most production and water quality management decisions. Such information would also aid banking and insurance decisions regarding farm operations. The initial research utilized modified commercial ocean fisheries SONAR systems that cost in excess of $100,000 and were large and difficult to deploy. Work was initiated to develop a smaller, cheaper and more portable system. The Jamie Whitten National Center for Physical Acoustics at the University of Mississippi, in conjunction with subcontractors and researchers at MSU-MAFES and MS Valley State University, developed the AquaScanner side scan sonar system to fit the desired design goals. In order to work in a shallow pond, a high frequency is desired to produce a narrow acoustic beam, and to limit interactions with the top and bottom of the water column, but a low frequency is desired to reduce absorption and thus increase range. To balance these competing needs,

the unit operates at a frequency of 460 kHz which is significantly above the audio range (20 kHz) but below the medical ultrasonic range (typically a few MHz). As such, the unit fits into an operating niche which is not supported commercially and which required the development of customized electronic components. The unit, by definition, operates in a shallow environment and as such receives significant acoustic returns from the gas filled sediment bottom which can often mask or obscure echoes from fish. This bottom contribution compromises the use of standard biomass estimator models often used in off-shore sea pens. To address this concern a unique empirical model was developed which correlates the individual acoustic echoes observed to biomass in ponds with known densities under a wide range of stocking densities (~1000 lbs/acre to ~10,000 lbs/acre). Several prototype units have been assembled, tested and used at commercial and research ponds in the Mississippi delta. The licensing process is underway with a patent filing in March 2007 and several potential manufacturers and vendors have expressed an interest in the technology.

Ancillary acoustic measurements (11). As part of the development of the Aquascanner™ SONAR system, additional technologies were developed and measurements were made which may form the basis of future research on fish behavior with an eye toward increased production or improved harvests. As part of the investigation of meteorological effects on SONAR operations and fish population predictions, an inexpensive (~$100) temperature probe, capable of measuring and logging the vertical temperature profile in a catfish pond, was developed. Temperature variations of as much as 18˚ F between the top and bottom of the pond were encountered during the late afternoon while more isothermal conditions were prevalent late in the evening. These gradients cause refraction which reduces the operating range of the SONAR. Colleagues in the biological sciences have expressed an interest in the technology for potential research on water quality and fish behavior issues. Field usage of the Aquascanner™ indicated that the optimal operation occurs at night which conflicts with the need to operate aerators to raise dissolved oxygen levels. While the aerator operation corrupts the acoustic biomass measurements, the acoustic data can image the extant and duration of the aerator bubble cloud. A commercial catfish farm has expressed interest in using the technique to help place aerators for optimal pond coverage as well as to determine recirculation patterns and mixing. Work has been performed to develop a user-friendly graphical interface to determine the surface area covered by the bubble cloud. While there has been an expressed interest in using the technique to map bubble clouds, measurement plans are still under development and represent a potential future research avenue. In parallel to the initial field testing of the active pulse echo SONAR work, passive measurements on fish sounds during spawning and ambient sounds in the pond from normal farm operations were made over a number of seasons. This work was geared to identify signals of interest to either improve spawning or at the least to discourage unwanted sounds during spawning. It is envisioned that this work could form the basis of future research on fish behavior such as the use of acoustic queues to aid seining.

Integrated Production Systems

Optimizing pond fertilization to improve catfish survival (12). Commonly used nursery pond fertilization practices were developed over 40 years ago and had not been studied in Mississippi ponds. MSU and USDA researchers evaluated responses to several combinations of organic and inorganic fertilizer. Ponds responded more to nitrogen additions than phosphorus additions, and organic fertilizer did not produce a response. Increasing nitrogen application stimulated the algal bloom more quickly and significantly increased preferred zooplankton densities. Several different nitrogen application levels are now being evaluated. Changing the previous fertilization methods can increase densities of zooplankton and provide a healthy algal bloom which should lead to improved fry growth, survival and health. Large zooplankton are readily consumed by catfish fry, but the nutritional contribution of these zooplankton was not known. The nutritional value of zooplankton from catfish nursery ponds was determined. The zooplankton consumed meet or exceed all nutritional requirements of channel catfish fry, are high in protein (65%), contain essential amino and fatty acids, and are excellent sources of vitamins and

minerals. Because of the high nutritional value of zooplankton present in catfish nursery ponds, it is critically important to maintain high densities of this nutrient source.

Remote sensing to detect harmful algal blooms (13). High suspended solids and chlorophyll concentrations are typical in aquaculture grow-out ponds. High fish stocking density requires both rapid mineralization of nitrogenous waste and production of oxygen by algae. To rapidly and accurately monitor algae, remote sensing methods offer utility. ARS researchers have used both airborne and handheld remote sensing spectral radiometer/cameras to detect algal biomass in ponds. Digital imagery is less useful than high resolution radiometers for detecting specific wavelengths critical for accurate biomass assessment. Utilization of handheld units allowed modeling measured field concentrations with 80% accuracy using pond-specific models. Ground-truthing methods have also been modified to more accurately extract cyanobacterial pigments.

Predator and Fowl Control

Assessment of integrated management techniques to minimize the impact of bird predation on aquaculture (14). The collaborative efforts by ARS scientists and cooperators determined the effectiveness, benefits, and logistical factors (i.e., labor, duration of effect, and inconvenience to fish production) for simple physical barriers, blue pond dye, robotic boats, or doing nothing during over-wintering months (December-April) of birds at commercial catfish farms in Arkansas. Cormorant and other fish-eating bird numbers were quantified using three different techniques and compared to control (i.e., no treatment) ponds at several commercial catfish farms. Utilizing simple physical barriers was the most effective and cheapest, and provided the best outcome when compared over time.

Using six privately owned catfish production facilities across a large area within southeastern Arkansas, scientists further fine-tuned and developed a low-cost, physical barrier system for deterring fish eating birds under commercial settings. The technique limited cormorant access to aquaculture ponds by 4 to10 fold, prevented other fish-eating birds from landing at similar rates to complete exclusion at some farms, and limited the duration cormorants stayed on ponds. This relatively economical, nonlethal method has gained industry support with at least 10 major catfish farms now employing this approach to greatly reduce losses of cultured fish by double-crested cormorants and other fish-eating birds.

Identified spatial and temporal differences between fish-eating bird use patterns (15). Double-crested cormorant and other bird predation on cultured fish is often cited as one of the major economic losses for the aquaculture and sport fish industry. Proper management of fish-eating birds requires accurate knowledge of bird numbers at catfish production facilities. Aerial surveys in a fixed wing aircraft were conducted for the fourth and final consecutive year to document double-crested cormorant, American white pelican, and other fish-eating bird numbers within the catfish production regions of southeastern Arkansas. Major accomplishments of these research projects included ameliorating depredation issues by diving ducks, herons, and egrets at baitfish ponds and spatially assessing cormorant numbers in relationship to roost sites. Double-crested cormorant numbers have significantly increased throughout most of their range, and present day numbers are at historic highs, with a large percentage of the population wintering within the aquaculture production regions of the southeastern United States. Winter double-crested cormorant populations in the Delta Region of Mississippi have increased nearly 400% since 1994. Currently, channel catfish production in Arkansas exceeds 34,000 acres of water and 155 operators. Losses to the catfish industry in 1996 due to double-crested cormorant depredations were estimated at over $13 million. Specific findings provided improved management strategies for limiting fish-eating birds at baitfish facilities and increased our knowledge of bird numbers at catfish production facilities.

Live Aquatic Animal Handling, Transport, and Inventory

Development of new harvesting technologies for the catfish industry (16). Inefficient harvesting, improper sizing, and stresses related to handling and hauling of fish are seriously affecting profitability of the catfish aquaculture industry in the southeastern United States. It has been estimated that more than $100M of revenue is lost annually due to problems associated with inefficient harvest. These problems include poor harvest efficiency of market-sized fish, premature harvest of undersized fish, and death and morbidity of fish stressed during harvest, grading, or transport. Studies were conducted to develop new harvest technologies to improve efficiency of harvesting, grading, and transport of catfish.

a. Development of a more efficient commercial seine. This project focused on the development and evaluation of a new commercial seine and live car system with improved fish handling and grading characteristics designed to greatly reduce the stresses imposed on fish during harvest as well as reducing the overall harvest time. The new seine was constructed by modifying a conventional seine based on recommendations of the National Marine Fisheries Service's Harvest System Team. Specific modifications are as follows: 1) use of braided, polyethylene mesh hung in a square pattern rather than twisted polyethylene mesh hung in a diamond pattern; 2) significant enlargement (width and height) of the funnel section of the seine; 3) use of long marine zippers instead of standard small metal frames to attach the live cars to the seine; 4) use of a "traveling" mud line with mud rollers instead of the conventional mud line; 5) use of braided, polyethylene mesh hung on a square pattern to build the live cars; 6) use of larger, more closely spaced floats with grommets on the seine. Several versions of the prototype seine with some or all of the modifications were built and tested in commercial and research catfish ponds in Mississippi, Arkansas, and North Carolina. Catfish producers participating in the project liked the marine zippers for attaching the live car and seine funnel and the larger, more closely spaced floats with grommets but preferred a conventional rolled, mesh mud line instead of the newer "traveling" mud-roller mud line. Although farmers and seining crews disliked the "traveling" mud-roller mud line system, they agreed that the system had merit in ponds with very muddy bottoms and they indicated that the primary advantages of the experimental seine and live car were; 1) ponds could be seined more quickly with less pushing needed to dump mud; 2) fish transitioned into the live car more easily and appeared to be in better condition; 3) fish graded out of the live car faster; 4) little if any aeration was needed when loading the fish into the live car; and 5) the braided, polyethylene webbing appeared to be much more durable. This project has stimulated a major change in the harvest technologies used by the catfish industry. Since the conclusion of this project in 2004, one large net manufacturer now puts enlarged funnels and marine zippers in 80 to 90 percent of the new seines and live cars built; the manufacturer has also installed 400 to 500 of marine zippers and funnels in existing seines and live cars. These changes have resulted in reduced harvest costs (shorter harvest times) and improved conditions of harvested fish. Farmers indicate using the larger throat and marine zipper system maintains the fish in much better condition (dark gray instead of light in color with improved vigor) because they load much quicker with less struggling and disorientation; in addition, the seine, seine funnel, and live car do not accumulate as much mud as the old system thus improving the water quality in the harvest area.

b. Design and test an electrically enhanced seine for use in harvesting channel catfish from ponds. Inefficient harvesting is seriously affecting the profitability of the catfish industry because market-sized fish that escape harvest continue to grow and create additional inefficiencies resulting from higher feed conversion ratios and carry-over of large fish that are unacceptable to processing plants. At least $50M of revenue is lost annually due to inefficient harvesting. The best solution to these problems appears to be the development of new or improved harvest gear and methods. One possibility to improve the efficiency of harvesting catfish from ponds is to use electricity as a means of repelling fish out of the holes and away from the seine as it is pulled through the pond. Initial studies used a programmable commercial backpack electrofisher to evaluate over 600 varying

combinations of wave-form types, voltages, pulse frequencies, pulse widths, and pulse sweeps to determine their effectiveness in moving channel catfish away from electrodes used to "seine" catfish held in concrete vats. The studies demonstrated that an electrical system using a low to high frequency waveform with a 6.0-60 Hz sweep, and a 2-millisecond pulse width over a 4 second sweep time could be used to capture 92% of the fish present in a concrete vat in a single pull. These results were used to construct a 70 feet long by 9 feet deep electrically enhanced seine for use in a comparative harvesting study. The comparative studies were conducted in twelve rectangular 0.1-acre ponds which had been stocked with 1,200 or 1,800 fingerlings the previous spring. Six ponds were harvested with a conventional seine and 6 with the electrically enhanced seine. The results demonstrated for the first time that an electrical stimulus can be used to significantly improve the catch efficiency of a conventional seine when used to harvest catfish from ponds. The electrically enhanced seine captured an average of 22% more fish than the conventional seine. New studies are underway to design, build, and test commercial electrically enhanced seine based on the results obtained from previous studies.

The Sock-Saver (17). Catfish are typically held in a net "sock" overnight to grade out small fish prior to delivery to the processor in the morning. Over 70,000 pounds of catfish may be held at densities exceeding 20 lbs/ft^3. This is risky, especially when water temperatures are high and D.O. (dissolved oxygen) concentration is low. Most farmers have had at least one disaster in which an entire sock of fish was lost; more typical situations may result in several hundred pounds of "weighbacks", dead fish arriving at the plant for which the farmer is not paid. ARS scientists believed that LOX (liquid oxygen) could remedy this situation and sought out and collaborated with a commercial catfish farmer to build the "Sock-Saver", a portable trailer capable of holding and delivering 150 gallons of LOX to remote farm areas where fish are being held in a sock overnight. This equipment was thoroughly tested by a commercial harvest crew and proved useful in reducing fish mortality. The concept and the initial plans were developed in collaboration with farmers and an area aquaculture extension agent was added to the team during the testing phase to help with subsequent technology transfer. This new technology has been widely publicized through numerous newsletters, abstracts, posters, and one journal article, and over 15 units in the industry have adopted the technology.

Culture of Marine Species in Low-Salinity Water

Information developed for low salinity production systems (18). A rotifer culture system that utilizes an automated feeding system to deliver a highly condensed algal paste to feed rotifers was developed to assist hatchery managers produce live feeds for larval fish with greater efficiency and reduced labor costs. Rotifer densities are achieved far in excess of those obtained using traditional rotifer culture using inexpensive and easily obtainable materials, and it is straightforward to construct, saves space, and is modular and expandable. Utilization of this system greatly increases the hatchery manager or producer's ability to culture sufficient live feed organisms on a year-round basis while decreasing costs and increasing the efficiency of larval production resulting in increased profitability. The design of this rotifer culture system is currently being marketed as a high-density rotifer production system by a commercial aquaculture supply company.

Also, three methods for measuring dissolved carbon dioxide (CO_2) in a marine recirculating aquaculture system were developed to overcome interferences imparted by compounds and salts when determining dissolved carbon dioxide concentrations in marine aquaculture systems. Water at three increasing salinities was infused with three concentrations of carbon dioxide gas and the dissolved carbon dioxide in each combination of solutions was measured by each of the three methods. It was determined removing carbon dioxide gas from water and measuring it directly with a portable hand-held gas analyzer proved to be a more reliable and cost-effective method for fish culturists to measure CO_2 concentrations in saltwater environments than traditional methods. This method will allow marine fish culturists to more

accurately and cost efficiently determine dissolved CO_2 concentrations in recirculating culture systems where build up of CO_2 can adversely affect growth, efficiency, and health during the culture cycle.

In addition, rate of ammonia removal and performance curves for a fixed-film biofilter with floating bead media were determined to provide engineers and fish culturists criteria to facilitate selection among different types of biofilters. Prior to this investigation there was previously no standard method for determining and reporting biofilter performance and efficiencies. Four recirculating aquaculture systems with two fixed-film biofilters, each containing a different type of floating plastic bead media, received one of two different ammonia concentrations to evaluate ammonia removal at salinities ranging from 3 g/L to 15 g/L. The resulting database provides aquaculturists, design engineers, and filter manufacturers the criteria needed to make well informed decisions on biofilter selections over a wide range of salinities. These data will allow further development of low salinity husbandry techniques and serves as baseline design criteria for engineering low-salinity recirculating systems.

Similarly, efficiencies of solid waste and ammonia removal were determined for a variety of recirculating aquaculture system components. These systems, under intensive culture conditions accumulate uneaten feed and waste generated by the fish, which if not removed become toxic and kill the fish. Ammonia removal efficiencies for two recirculating aquaculture system components, and particle size removal efficiencies for three other components, were determined under increasing feeding rates. The data obtained provides practical field performance rates of specific recirculating aquaculture system components for the aquaculture industry. This accomplishment advances design criteria for developing technologies that will improve market competitiveness for domestic producers through maximizing environmental and production efficiencies, minimizing waste production, decreasing energy and regulatory costs, and optimizing product quality and safety. This information will be used by culturists to design cost-effective, energy efficient aquaculture systems and management strategies capable of sustainable, year-round production of pompano and black sea bass in land-based low salinity environments.

A survey of aquaculture industry and other stakeholders was conducted to systematically quantify experiences with different types of biological filtration and requirements for use in recirculating systems. The survey canvassed over 400 people in the aquaculture industry including scientists and technicians at universities; private, public, and government research facilities; State and Federal hatchery managers; and aquaculture farmers for the purpose of quantifying filtration needs. Results of this survey provide biofilter manufacturers the demographics, requirements, and needs specific applications of biofilter users for recirculating aquaculture. These data will allow further development of low salinity husbandry techniques and serves as baseline design criteria for engineering low-salinity recirculating systems.

RESEARCH COMPONENT VI: SUSTAINABILITY AND ENVIRONMENTAL COMPATABILITY OF AQUACULTURE

The domestic aquaculture industry will face strong competition from imports of foreign aquaculture products as well as competition from the other protein food commodities. Enhancing the economic production of aquaculture in the United States by using responsible aquaculture systems is a continual challenge. Inputs used for aquaculture food production must be economical for the producer, non-impacting on the environment and such that would not warrant consumers to discriminate in the market place.

The development of sustainable systems for aquaculture must include considerations for producer profit; effects on environmental quality including soil, water, and air; and be compatible with worker safety and requirements for food safety and quality. These considerations will govern the ongoing development of

new technology to provide and promote economically viable and environmentally sustainable aquaculture systems. A number of approaches are collected under this component even though they appear in other components of this Action Plan. Most notably these issues involve feed, water and water recycling, effluent management, biosecurity and animal health management.

Vision: Improved aquatic animal and integrated production systems that are both economically and environmentally sustainable and acceptable to consumers in the global market place.

Mission: Develop and transfer sound, scientifically-based knowledge that will result in the economical production of aquaculture products that are safe for food and have minimal impact on the environment.

Impact: Economic advantage and improved marketplace competitiveness for domestic producers through new technology and practices that maximize environmental and production efficiencies, minimize wastes, energy input and regulatory costs, optimize product quality and safety, and provide clear value to consumers.

Linkages: 1) Other ARS National Programs: Food Animal Production (101); Quality and Utilization of Agricultural Products (306); Soil Resource Management (202); Water Quality and Management (201); Integrated Agriculture Systems (207). **2)** Other Agencies and Departments: U.S. Colleges of Agriculture and State Agricultural Experiment Stations; National Marine Fisheries, Environmental Protection Agency, Freshwater Institute, Oceanic Institute. **3)** Private Sector: Catfish Farmers of America; U.S. Trout Farmers Association; Striped Bass Growers Association; U.S. Marine Shrimp Farmers; American Society of Agricultural Engineers; American Fisheries Society; Aquaculture Engineering Society; America Tilapia Association.

Engaged ARS Locations: *Fairbanks, AK; Auburn, AL; Pine Bluff, AR; Stuttgart, AR; Hilo, HI; Aberdeen, ID; Orono, ME; Newport, OR; Stoneville, MS; Leetown, WV.*

Problem Area VIA – Aquaculture Feeds

The negative impact of poorly managed aquaculture systems on the environment, and particularly on water quality, is becoming an important issue and constraint to the sustainability and expansion of the aquaculture industry. The main input and greatest cost in most aquaculture production systems is feed, which is not only a source of nutrients for fish growth, but also the major source of pollutants. To reduce costs and harmful pollutants, diets need to be formulated from lower cost highly digestible alternatives. Uneaten and undigested feeds are also main factors affecting environmental pollution. Development of new diets with good quality alternative ingredients and alternative feeding strategies may reduce feed costs and minimize the accumulation of organic substances and metabolic wastes that may lead to toxicity, health risks, and growth suppression in fish.

Committed Goals: 1) Develop alternative feed ingredients from plant protein sources and reduce the amount of minerals and other compounds released into the environment after consumption. **2)** Develop feed formulation and manufacturing, and feeding practices for optimal nutrition, increased production, and improved water quality. **3)** Improve understanding of the nutrient retention and energy requirements of cultivated aquatic species to reduce undigested or uneaten feeds.

Expected Outcomes: 1) Reduction in the nutrient and mineral content of fish manure released into the environment. **2)** Development of least-cost, environmental friendly diets, and optimum feeding practices for various fish species cultured under different production systems. **3)** Improve culture water and effluent quality of fish production systems.

Problem Area VIB – Water Use and Reuse

Concerns over the environmental impact of aquaculture have grown and with these concerns have also come increased regulations on aquaculture discharges. Traditionally, aquaculture often relies upon relatively large volumes of water, which produces high-volume, low-strength wastewater that can be difficult to treat economically. Re-circulating aquaculture systems (RAS) however, require relatively little make-up water and concentrate their wastes into relatively small discharges. Discharge from a RAS is more amenable to treatment allowing 95% or more of the particulate wastes to be captured. For larger commercial scale systems that recirculate freshwater and saltwater, research and development is required to improve the reliability and reduce the costs of fish production under increasingly intensive conditions. Research is also necessary to develop best waste management practices and technologies for effective removal of solids and improving effluent quality to meet discharge limits and guidelines.

Committed Goals: 1) Improve system designs and treatment technologies to improve the effluent quality of aquaculture systems. **2)** Improve understanding of factors and processes contributing to efficient water utilization and quality conservation in aquaculture systems.

Expected Outcome: 1) Generate wastewater management strategies and technology that producers can select as the most appropriate for their existing aquaculture operation.

Problem Area VIC – Effluent Management Control

Concerns over the environmental impact of aquaculture have grown and have led to increased regulations on aquaculture discharges. Flowing water aquaculture systems often have two separate discharges: (1) the system primary flow which is high-volume, low-strength wastewaters; and (2) the system solids flow which is low-volume, high-strength wastewaters, i.e., backflush from filters, purge materials from swirl separators. The concentration of waste in the primary flow discharge is relatively low; however, the cumulative waste load discharged to the receiving watersheds can be significant when water flows are large and continuous. Aquaculture effluents typically are treated before discharge and the captured solids waste has potential for beneficial reuse.

Committed Goals: 1) Provide appropriate waste management technologies and practices that are integrated with aquaculture system complexity and environmental regulation criteria. **2)** Produce more reliable and accurate monitoring methods for aquaculture effluents. **3)** Identify and develop opportunities for the reduction and reutilization of aquaculture waste products.

Expected Outcomes: 1) Development of criteria that will facilitate the selection of appropriate, integrated wastewater management strategies through decisions regarding system design and operational criteria. **2)** Decrease aquaculture's reliance on water resources and improve the utilization of captured wastes from aquaculture systems.

Problem Area VID – Social Sustainability

Modern day aquaculture enterprises must exist within and depend upon a dynamic ecosystem with finite resources. In order to remain competitive in a global marketplace these enterprises need new knowledge, techniques, and equipment that will improve productivity and protect the environment, protect worker health and safety, and sustain the quality of "common use" natural resources.

Committed Goals: 1) Improvement of aquaculture facilities to minimize conflict of common-use water resources. **2)** Identify and develop opportunities for the improvement of aquaculture worker health and safety.

Expected Outcome: 1) Technology and practices useful in traditional aquaculture production and activities that improve production efficiency and product quality while reducing worker exposure to risk of injury and illness, and foster conservation of resources.

Problem Area VIE – Environmental Sustainability

As the U.S. aquaculture industry expands, it must be sustainable and environmentally compatible. Inevitably the aquaculture industry will be examined in the context of environmental sustainability. Knowledge about aquaculture production systems is needed to minimize the potential for habitat degradation, disease transmission, genetic dilution of wild stocks through interbreeding with cultured strains, introduction of non-indigenous species into natural waters, and discharges of wastes, toxins, and excess nutrients.

Committed Goals: 1) Promote and encourage technological and management solutions for the environmentally sustainable development of the growing aquaculture industry. **2)** Improve our understanding of interactions between aquaculture production systems and the surrounding environment.

Expected Outcomes: 1) Technology applied by the domestic aquaculture industry that is sustainable, profitable, and environmentally safe, and maintain high water quality standards. **2)** Knowledge to assist farmers with solving problems regarding environmentally sustainable practices will be developed. **3)** Knowledge of the interactions between aquaculture waste and the receiving environment, and how to minimize negative effects will be generated.

Selected Accomplishments for *Sustainability and Environmental Compatability of Aquaculture*:

Aquaculture Feeds

Low input management practice evaluated for freshwater prawn production (1). A preliminary economic analysis of a low input management practice using low cost forms of inorganic fertilizers, corn gluten pellets and/or wheat midds pellets (as an alternative to sinking catfish feed) for pond culture of freshwater prawn reduced feed costs per pound of shrimp produced to $0.30 to $0.62/lb, (up to a 56% decrease); thereby reducing production costs to $3.04 to $2.94/lb, respectively. These production costs dramatically increase the prospects for economic success of freshwater prawn farming in the United States because selling prices would be highly competitive with foreign imports. Organic fertilization as part of the low input strategy eliminates the need of comparatively expensive formulated feeds, allows flexibility in the selection of sources of organic material used to fertilize, produces a large proportion of a large, highly value product, and is an example of highly sustainable aquaculture.

Water Use and Reuse

Developing sustainable waste management technologies (2). Recirculating systems conserve water and concentrate wastes into smaller and more treatable discharges. Best waste management practice

guidelines for recirculating systems were developed and published in a report prepared by the Joint Subcommittee on Aquaculture for the United States Environmental Protection Agency under an Interagency Agreement with the USDA CSREES.

When a recirculating aquaculture system is operated at high salinities for culture of marine species, recovering the saltwater contained in the backwash effluent would allow for its reuse and reduce salt discharge to the environment. A pilot-plant membrane biological reactor was evaluated and determined to require little membrane maintenance while removing more than 99% of suspended solids, biochemical oxygen demand, and bacteria found in high solid-and nutrient-laden filter backwash flows. This research has provided design and management recommendations that can be used by fish farmers to reduce waste discharge and increase water reuse, especially in applications where inland marine recirculating systems can save money by reclaiming their saltwater discharge.

An inclined belt filter using coagulation and flocculation aids (i.e., alum and/or polymers) was evaluated for removing and thickening suspended solids and phosphorus from the microscreen backwash discharged from intensive recirculating aquaculture systems. By eliminating the need for settling tanks or ponds, the leaching of nutrients (phosphorus, nitrogen) is minimized and the dewatered sludge is in a form that fish farmers could readily transport, store, or send for disposal. Inclined belt filter design and management recommendations were developed to improve waste capture, dewatering, and disposal, which has now been implemented at both private and public aquaculture facilities.

Optimum conditions required to produce coagulation, flocculation, and settling of suspended solids and phosphorus in microscreen filter backwash flows using alum, various polymers, or a combination of the two were identified so as to improve waste removal from aquaculture biosolids thickening and settling treatment systems. Results are being used to improve waste capture, dewatering, and disposal at both private and public intensive aquaculture facilities.

A high-rate aerobic treatment process was evaluated for removing ammonia, soluble biochemical oxygen demand, and some soluble phosphorus from the overtopping flow discharged from biosolids thickening tanks. Findings will be used to design aerobic treatment basins that can rapidly remove wastes from what is arguably the dirtiest effluent.

A more effective technology was developed to remove dissolved waste products from large, but relatively dilute aquaculture effluents. Fluidized sand biofilters consistently removed 90% of the total ammonia nitrogen and 60-80% of the carbonaceous biochemical oxygen demand in the effluent of an intensive trout production facility. The biofiltration technology requires minimal attention, never requires backwashing, and can be readily scaled to reduce the pollution discharged within large volume aquaculture effluents.

Development of recirculating system technologies for marine fish production in land-based systems (3). U.S. marine aquaculture is constrained by the high cost of and limited availability of coastal lands, high production costs, and restricted growing seasons; therefore, innovative, cost-effective recirculating system technologies to produce marine fish in land-based systems must be developed to overcome these constraints. To test such systems an 11,400 ft^2 Sustainable Tank Aquaculture Recirculating Research facility with two different types of recirculating system technologies, each equipped with remote sensors for constant monitoring, was constructed for the collection of production-scale scientific, engineering, and economic data.

Since energy consumption is one of the major variable costs in aquaculture production facilities, energy use, energy efficiency, and water consumption were determined for production scale recirculating systems to provide economic data and target efficiency opportunities for culturists utilizing recirculating aquaculture systems. Complete energy load and daily water consumption analyses were conducted on

four low-salinity production scale recirculating aquaculture systems consisting of four tanks each throughout a six-month growout production cycle of hybrid striped bass. The information generated provides farmers utilizing recirculating culture systems an analysis based on production-scale technologies advancing design criteria to develop cost-efficient management strategies that maximizes environmental and production efficiencies.

Production characteristics and body composition of Florida pompano reared from juveniles to market size at two different densities in low salinity recirculating systems were determined. Juvenile pompano were stocked into commercial-scale low salinity recirculating aquaculture systems at two initial culture densities and reared for a period of 110 days resulting in the production of fish of suitable size for wholesale and retail markets. This accomplishment establishes the feasibility of rearing market-ready pompano in low salinity recirculating systems while identifying the effect of varying culture densities on fish production characteristics and body composition, thereby allowing established and developing commercial pompano aquaculture operations to implement this production format. Rearing pompano to market-size in low-salinity recirculating culture allows intensive production of fish with minimal effluent and establishes low-salinity culture of marine species as a viable alternative to traditional marine aquaculture methods.

Effluent Management

Effect of dietary phosphorus on waste discharge from catfish ponds evaluated (4). Compared to other agricultural animals, fish require phosphorus at relatively high dietary levels and a significant fraction of the total phosphorus in practical fish diets is not assimilated. Dietary phosphorus in unavailable forms or supplied in excess of fish metabolic requirements will be excreted into the culture water. If the water is discharged from the culture unit, phosphorus may cause eutrophication in effluent-receiving water bodies. One approach to reducing mass discharge of waste is to modify feeding strategies or feed formulations to increase the assimilation and retention of nutrients so that less waste is generated per unit of feed consumed. Although the environmental benefit of optimizing phosphorus retention by fish raised in flow-through and net-pen culture systems is obvious, the value of optimizing dietary phosphorus retention by fish in ponds is less clear because the long hydraulic residence time in ponds provides ample opportunity for various biological, chemical, and physical processes to affect the amount of phosphorus that is eventually discharged. To assess the effect of dietary phosphorus on potential waste discharge from ponds, three studies were conducted. In experiment 1, a basal diet with 0.20% available phosphorus was compared to diets supplemented with 0.5% or 1.0% dicalcium phosphate to provide 0.27% or 0.35% available phosphorus. In experiment 2, fish were fed diets supplemented with either dicalcium phosphate or defluorinated rock phosphate to contain 0.40% available phosphorus. In experiment 3, fish were fed one of three diets containing 250 or 500 FTU (phytase units) phytase/kg (0.27% available phosphorus) or 0.75% dicalcium phosphate (0.39% available phosphorus). Husbandry practices in all three experiments were typical of commercial culture conditions. Quantitative and qualitative modifications of dietary phosphorus did not affect waterborne phosphorus concentrations or phytoplankton abundance and; therefore, will not reduce phosphorus or organic matter mass loading in pond effluents. Lack of effectiveness is the result of high baseline nutrient loading from phosphorus contained in practical feed ingredients combined with high internal phosphorus loading (recycling) within ponds. These factors overwhelm any effect of small changes in external phosphorus loading associated with diet modification. Therefore, the source and level of dietary phosphorus in channel catfish feeds should be based primarily on nutritional and economic considerations rather than potential environmental impact. These results have been incorporated into environmental management plans for pond-raised fish.

Effluent management practices shown to be effective in catfish ponds (5). Effluents from aquaculture facilities have come under scrutiny by non-governmental organizations and regulatory agencies as potential sources of pollution. A wide variety of culture systems are used to grow aquatic

animals, and each type of system has unique waste discharge characteristics. For example, flow-through culture systems (raceways) produce a constant effluent stream that, for individual facilities, varies little in volume and quality over time. Such systems are physically amenable to traditional "end-of-pipe" approaches to waste treatment, in which performance can be easily documented by periodic sampling of the effluent. By contrast, pond culture systems discharge water intermittently (only after heavy rains or when ponds are drained) and effluent quality varies widely over time and among individual ponds. These characteristics make it difficult to identify suitable waste treatment technologies and monitor their performance. The intermittent and unpredictable discharge from ponds impacts the cost and potential effectiveness of nearly all "end-of-pipe" treatment options. Treatment systems for pond effluents would be idle for many more days than they are used and the average annual hydraulic loading to the system will be low. However, when discharge occurs, the volume may be relatively large for a brief period. This is a difficult engineering problem because the system must be designed to rapidly treat a large volume of dilute wastewater. The intermittent nature of pond effluent discharge also makes it impossible to assess the performance of waste treatment technology by simply monitoring waste concentration. Therefore, approaches that reduce effluent volume and improve water quality prior to discharge will more effectively reduce mass discharge from aquaculture ponds.

Adoption of an Environmental Management System (EMS) consisting of a set of management practices to minimize environmental impacts is one such approach. A set of effluent-management practices was therefore evaluated over 3 years in 2.4-ha earthen ponds in northwest Mississippi. Mass discharge—which is the product of concentration and volume—is usually more important than concentration alone in determining the impact of an effluent on the environment. Practices were therefore implemented that addressed both factors affecting mass discharge. One practice, water-level management, was implemented to reduce effluent volume. The other three practices were implemented to reduce the concentration of substances in effluents: 1) limiting daily feed inputs to 110 kg/ha per day, 2) using a low-protein (28%) feed, and 3) maintaining a modest fish density (18,500 fish/ha). Pollutant discharge and fish production from the "managed" ponds were compared to "traditional" ponds managed without these practices. Concentrations of total nitrogen, total phosphorus, 5-day biochemical oxygen demand, and total suspended solids were only slightly lower in effluents from managed ponds relative to traditional ponds. However, mass discharge (kg/ha) of those substances from managed ponds was reduced by more than 60%, primarily because water-level management reduced effluent volume to half of that from traditional ponds. Water-level management was originally developed in catfish farming as a water conservation tool, so it is not surprising that average annual groundwater use was only 18 cm in managed ponds while 45 cm of water was added to traditional ponds. Average annual fish harvest was 6,425 kg/ha in managed ponds and 6,250 kg/ha in the traditional ponds.

These results show that a simple management system can be used to dramatically reduce pollutant discharge and water use in catfish ponds without affecting fish production. The centerpiece of the management system is pond water-level management, which can reduce overflow volume by 50% and groundwater use by more than 60%. Combining water-level management with practices to improve water quality inside the pond reduces pollutant discharge to less than 40% of that from unmanaged ponds. This study demonstrates that improved environmental performance of catfish culture can occur without sacrificing profitability because large-scale and costly changes to the existing production system are not required. This is the first study to quantify the effectiveness of a farm-level environmental management plan to reduce potential pollution from aquaculture ponds. Components of this plan have been implemented into comprehensive sets of environmental best management practices for pond aquaculture.

Further research to address catfish farm effluent quality (6). Considerable research has been conducted to characterize effluent quality and potential environmental impacts, especially for pond facilities. However, no assessment has been made of catfish hatchery effluents despite the fact that hatcheries can discharge significant volumes of water. Assuming a 10-week spawning season and a

water flow of 400 L/min for a hatchery designed to produce 10 million fry/year, about 40 x 10^3 m^3 of effluent will be discharged from the hatchery each year. A study was conducted to describe the solids, phosphorus, nitrogen, and organic matter content of effluents from typical commercial channel catfish hatcheries in northwest Mississippi. Net pollutant loads (effluent concentration minus inflow concentration) were low for all variables. Concentrations of all potential pollutants are lower than corresponding concentrations in effluent-receiving streams in northwest Mississippi and total effluent volume from catfish hatcheries constitutes less than 0.02% of total annual streamflow in the region. It is therefore highly unlikely that catfish hatchery effluents will have a negative effect on receiving stream water quality. These results have been incorporated into effluent-management plans for warmwater aquaculture.

Development of effective waste management plans for pond aquaculture depends on characterization of the water discharged from ponds. A study assessed the quality of pond effluents when ponds are drained and characterized the nature of the material discharged. When ponds are drained, the initial flush of water discharged consists of pond water and a slurry of sediment that has accumulated over the screen inside the pond, but the effluent clears in 5 to 30 minutes and all water subsequently discharged is simply pond water. Since only a small proportion (1 to 4%) of the total solids discharged during pond draining was contained in the initial flush, it will be uneconomical to design elaborate treatment facilities to remove the material in that small volume of water.

Processes that rely on gravity settling are the most economical method of removing solids from the initial flush of water released when ponds are drained. Scientists measured the settling characteristics of solids in catfish pond effluent and used equations to calculate design criteria for settling basins. For average pond discharge rates, removal of 95% of solids requires a basin area ranging from 95 to 125 square meters. The capacity of common drainage ditches to remove solids and nutrients from pond effluents was also assessed. At typical pond effluent discharge rates, more than 95% of the solids in initial pond draining effluent were removed after the effluent traveled 120 to 220 meters downstream in a 1-meter wide ditch. This was consistent with the results of basin-design modeling and shows that it is unnecessary to build elaborate facilities to improve catfish pond effluent quality because considerable improvement in quality occurs as effluent flows down the simple drainage ditches that are common features of most commercial catfish farms.

Environmental Sustainability

Diuron efficacy and evaluation of other herbicides for control of blue-green algae (7). Selective control of problematic algae in aquaculture ponds has been an elusive goal. Diuron is presently available for control of off-flavor producing algae, however little information on the selectivity of this compound is available. Field and laboratory studies were conducted to assess trophic impacts of diuron. Diuron reduced off-flavor occurrence, but was not effective in controlling species of algae capable of forming toxins (i.e. microcystin). Efficacy of various compounds was tested and one compound has been identified that exhibits desirable control at reasonable application rates (0.3 mg/L to kill 50% of algae present). This chemical should provide a cost effective alternative to copper compounds and diuron for control of off-flavor and toxin-producing algae if EPA permitting is obtained.

Impact of water-borne chemicals on hybrid striped bass fry production (8). Much of fish farming in the United States occurs in close proximity to row crop farming. Row crop farming requires the use of aerially applied herbicides that have the potential to damage pond ecosystems and water quality and endanger fish production. Altercations between fish and row crop farmers over perceived losses on either side can also damage the sustainability of aquaculture in the public eye. A series of long-term studies were undertaken to determine the effects of various concentrations of several commonly used cotton, corn, and rice herbicides and defoliants on pond water quality, phytoplankton, zooplankton, and fish health. Commercial formulations evaluated included Rimsulfuron, Nicosulfuron, Atrazine, Basis

Gold™, Diuron™, Propanil, and several others. Among the major findings, Atrazine was found to significantly reduce pond primary productivity and this also can reduce fry production; however, potential damage directly to fish was insignificant. Only Diuron™ used as either an herbicide or as a defoliant caused such sudden collapses in primary productivity that oxygen levels were adverse to fish health. This information is now used by row-crop farmers to make informed decisions about the application of herbicides in fields adjacent to fish farms and dispels much of the apprehension on the part of fish farmers regarding direct toxicity of these aids to row cropping. Other water-borne chemicals are used in aquaculture as water amendments or disease treatments; however, all such chemicals can potentially harm aquatic food chains. Potassium permanganate is a potential therapeutant for killing diseases in fish. Aquashade™ is a commercial dye used to retard the growth of rooted aquatic plants. Several trials were conducted to determine the environmental impacts of these chemicals on hybrid striped bass fry production in tanks and ponds. These data are being used by FDA to set the limits and conditions of future use of potassium permanganate as a disease treatment in hybrid striped bass and by fish farmers to improve pond management practices.

Burrowing shrimp integrated pest management improved (9). An expanded monitoring program that quantified burrowing shrimp populations in Washington and Oregon estuaries was implemented which provided new information needed to limit the damage the shrimp cause to commercial oyster production. Data revealed that larval recruitment of burrowing shrimp from the nearshore coastal ocean is both spatially and temporally variable. Adult ghost shrimp populations outside shellfish beds were relatively stable in most estuaries; recruitment has been relatively low for the past decade in Willapa Bay, Washington and appears to fluctuate in Oregon estuaries. Mud shrimp recruited in high numbers to Oregon estuaries in 2006, but adults were highly infested with a bopyrid isopod that prevents reproduction and may be a limiting factor in recruitment to Washington estuaries where adult populations have declined. These data provided new biological data regarding the shrimp life cycle that will be critical in developing integrated pest management approaches to replace use of the pesticide currently employed for shrimp control. Understanding and predicting how shrimp larval recruitment from the nearshore coastal ocean influences shrimp populations within the estuaries and consequently, the abundance of these pests on shellfish beds provided the conceptual basis for a decision tree tool to decide when shrimp control should be implemented. This accomplishment demonstrated that the post-larval recruitment stage is a critical point in the life cycle of these shrimp where they are vulnerable to more environmentally sustainable control measures since the animals are small and their burrows are shallow.

Aquaculture practices influence habitat and associated species in west coast estuaries (10). The first map of intertidal habitats including eelgrass, burrowing shrimp and aquaculture in Willapa Bay, Washington (which produces over 9% of US oysters) has been completed. This is important because aquaculture practices at a landscape scale appear to be important determinants of the health of estuarine ecology. This map enabled the use of spatial analyses to evaluate the impact of shrimp, oyster culture, and harvesting operations on eelgrass populations; eelgrass is considered important habitat for juvenile stages of salmonid fishes, some of which are listed as threatened or endangered. Although eelgrass is viewed as a critical habitat for juvenile salmon and Dungeness crabs, commercial oyster beds also provide habitat for juvenile crab and a diverse infaunal and epifaunal community which serve as prey for salmon. The map will be utilized by the National Marine Fisheries Service who seek to maintain critical habitat for other species of concern and the U.S. Army Corps of Engineers who issue new nationwide permit for aquaculture.

RESEARCH COMPONENT VII: QUALITY, SAFETY, AND VARIETY OF AQUACULTURE PRODUCTS FOR CONSUMERS

Aquaculture products provide consumers with consistent high quality, nutritious foods. Maintaining superior product quality and developing novel aquaculture products are imperative to increasing consumer demand, developing new markets for aquaculture products, and meeting consumer expectations for safety, variety and nutritional value. Proactive safety/quality assurance programs, improved management of off-flavor in aquaculture production systems, development of new product forms, and new and improved technologies for processing, packaging, and preserving aquaculture products must continue to meet producer, processor, and consumer needs. Improvements of processes for traditional uses of byproducts are needed, as are discoveries for new high-value uses of byproducts.

Vision: Provide consumers with a variety of high quality, safe, nutritious and innovative aquaculture products.

Mission: Ensure and optimize the safety, freshness, flavor, texture, taste, nutritional characteristics, and shelf life of cultured fish and shellfish products, and to develop new value-added products and processes through research, development and technology transfer.

Impact: Increased consumer satisfaction and demand for aquaculture products.

Linkages: 1) USDA-ARS National Programs: 101 Food Animal Production; 108 Food Safety and 306 Quality and Utilization of Agricultural Products. **2)** Other Agencies and Departments: Auburn University, Mississippi State University, University of Alaska, University of Idaho, University of Mississippi, National Marine Fisheries Service, The Oceanic Institute, U.S. Environmental Protection Agency. **3)** Private sector: Catfish Farmers of America, The Catfish Institute, other processing groups.

Engaged ARS Locations: *Fairbanks, AK; Auburn, AL; Pine Bluff, AR; Stuttgart, AR; Hilo, HI; New Orleans, LA; Oxford, MS; Stoneville, MS; Wyndmoor, PA.*

Problem Area VIIA – Tissue Quality

Research is needed to identify strategies and develop technologies to ensure delivery of consistent, high quality, and innovative aquaculture products.

Committed Goal: 1) Identify techniques and technologies for post-harvest handling and processing of aquaculture products to enhance product value, quality, functionality, and sensory characteristics.

Expected Outcome: 1) The development of processing technologies/strategies that increase consumer demand for aquaculture products derived from sustainable, profitable production systems.

Problem Area VIIB – Interaction of Genetics and Nutrition

Research on the interaction between genetics and nutrition in aquaculture is essential to improve the development and production of high quality aquaculture products. The development and use of improved germplasm and diets have played important roles in the profitable production of high quality products in many livestock species and the same principles apply to aquaculture species.

Committed Goal: 1) Identify key needs for germplasm improvement, diet development, and potential interactions between genetic improvement and nutrition that will allow development and production of high quality aquaculture products from profitable production systems.

Expected Outcome: 1) Identification of critical research areas in genetics and nutrition of aquaculture species that lead to the improvement in production of high quality aquaculture products from profitable production systems.

Problem Area VIIC – Predicting Product Quality or Defects

Mislabeled imports and large variations in production practices lead to considerable variation in quality of aquaculture products. Lack of consistent product quality can lead to consumer dissatisfaction and subsequent loss of future purchases of aquaculture products. Producers and processors need methods to identify defects and ensure product quality stocks.

Committed Goals: 1) Methods that distinguish between domestic and foreign aquaculture products. **2)** Pre-harvest and post-harvest techniques and systems to ensure product quality.

Expected Outcomes: 1) Consumers will be provided with more information and confidence about the quality of aquaculture products. **2)** Enforcement of labeling regulations will be easier.

Problem Area VIID – Off-Flavor Delayed Harvesting

Off-flavors cause delays in the harvest of aquaculture products and result in severe economic losses to producers. In addition, off-flavors can adversely affect consumer demand and aquaculture industry development due to inconsistent product quality. Producers need better management practices and methods to prevent and control environmentally derived off-flavors.

Committed Goal: 1) Management practices that result in consistent, high quality and excellent tasting aquaculture products will be realized.

Expected Outcome: 1) Improved, off-flavor management practices that reduce producer costs.

Problem Area VIIE – Off-Flavor Methodology

Producers lack methods to easily detect the occurrence of environmentally-derived off-flavors. Factors promoting the occurrence of such off-flavor episodes are not completely understood. The biochemical pathways involved in the production of the most common environmentally-derived off-flavors are unknown, and the regulation and control of these pathways have not been determined.

Committed Goals: 1) Methods to easily detect common off-flavor compounds. **2)** Methods to control biological processes that will help maintain consistently high quality products.

Expected Outcome: 1) Consistent and excellent tasting aquaculture products will be provided to consumers.

Problem Area VIIF – New Uses for Byproducts

Most byproducts produced from the processing of aquatic animals are either not utilized or are underutilized. Processors often incur additional expenses due to the disposal of these unused

byproducts. The identification of high-value uses and development of methods for the utilization of byproducts will help reduce the economic burden on processors and create sustainable agricultural systems. Aquatic animal processing byproducts are being used to make food products for humans, as animal feed ingredients, industrial products, fertilizers, and composts. New high-value uses need to be discovered to improve the utilization of processing byproducts and knowledge about the properties of their biochemical constituents will benefit fish processors and consumers and reduce environmental impact.

Committed Goal: 1) Discover high-value uses for fish processing byproducts for commercialization.

Expected Outcomes: 1) New high-value products. **2)** Enhanced use of fishery processing byproducts that results in additional sources of revenue for processors and reduced byproducts wasted.

Problem Area VIIG – Processing

The economics of aquatic animal processing are greatly influenced by product yield and further processing to create value-added products. Improved methods for removing more flesh from the animal frame would increase the recovery yield for processors and provide edible tissue for existing and new high-value product forms. The development of new and improved products that utilize trimmings, tissue minces, mechanically de-boned tissue, broken and mis-cut tissue, and other edible tissue would help processors.

Committed Goals: 1) More efficient processing methods to enhance recovery of the edible portion of the aquatic animal and development of new product forms that use these smaller, edible portions in novel value-added products.

Expected Outcomes: 1) Increased processing efficiency and recovery. **2)** Increased variety of aquaculture products to consumers.

Selected Accomplishments for *Quality, Safety, and Variety of Aquaculture Products for Consumers*:

Tissue Quality

Harvesting and transport environmental effects on meat quality elucidated in catfish (1). Exercise and stress during transport negatively impact meat quality in most livestock species, including channel catfish. Water temperature had little effect on meat quality, but increasing dissolved oxygen levels during transport resulted in improved fillet color and reduced drip-loss.

Research was conducted with isoeugenol (a product currently undergoing FDA approval for use in foodfish with zero withdrawal time) to determine effects of rested-harvesting on physiological responses and fillet quality of channel catfish. Trials demonstrated that rested-harvesting reduced serum cortisol, blood lactate, blood glucose and increased blood and muscle pH. Thus the detrimental physiological responses associated with harvesting were reduced. The color and texture of catfish fillets were also improved compared to current harvest methods.

Product quality evaluated for Pacific aquatic food species reared on developed feeds (2). The effect of lipid content in a finishing diet on fillet yield and sensory quality of Pacific threadfin was determined. Juvenile threadfin were grown close to market size, and then fed with a finishing diet (containing 43% protein, and either 13% or 24% lipid) for 14 weeks before harvest. Higher lipid finishing diet did not lower fillet yield and the sensory results from a trained taste panel for sashimi and baked samples did not show consistent differences in fillet color, texture and flavor between low and high lipid finishing diets.

Shrimp are very nutritious low fat food but they are very high in cholesterol. Consuming one portion of shrimp meat (114 g) provides more than half (170 mg) of the Daily Reference Value (300 mg/day) for cholesterol. An experiment was conducted with 9 g shrimp (initial weight), fed with experimental and control diets and grown in outdoor culture tanks (1100 liters functional volume, 75 animals/tank, 2 L/min flow rate) to the market sizes of 20 g and 27 g, followed by evaluation of the effects of diet on cholesterol and DHA content of shrimp tail muscle tissue. The shrimp fed a diet containing a very low level of cholesterol and a high level of docosahexaenoic acid (DHA) resulted in a lower level of cholesterol and a higher level of DHA in the tail muscle tissue of market-size shrimp, compared to shrimp fed a commercial control diet.

Effects of dietary protein levels and sources on catfish meat yield (3). Meat yield was generally not affected by diets with protein levels ranging from 28 to 32 % or diets with protein sources that were all plant in origin versus a combination of plant and animal proteins. This research indicates that it may be possible to lower diet costs without negatively impacting meat yield of channel catfish.

Predicting Product Quality or Defects

Rapid assay to correctly identify channel catfish fillets developed (4). Marketing of imported basa, *Pangasius boucourti*, fillets as catfish has resulted in serious economic losses to the farmed catfish industry in the United States. A monoclonal antibody specific for a channel catfish fillet protein was produced and characterized. The monoclonal antibody was used to develop an indirect enzyme linked immunosorbent assay (ELISA) specific for fish of the species *Ictalurus* (i.e., channel catfish, blue catfish and hybrid channel X blue catfish). The assay correctly identified raw or cooked channel catfish fillets in less than 3 hours from other species (basa, striped bass, sea bass, red snapper, tilapia, and flounder) tested in a single blind study. Use of this technology would allow the correct labeling of channel catfish and thus allow compliance with truth in labeling laws.

Off Flavor Methodology

Research on factors leading to off-flavor (5). A number of research studies and approaches have been utilized to develop basic information to better understand the underlying causes and contributors to off-flavors in catfish. These include:

Identifying and characterizing genes and enzymes involved in the biosynthesis of off-flavor metabolites. A 1.8 kb PCR product was isolated that contains a sesquiterpene cyclase gene from O. *splendia*. This enzyme is homologous to cyclases from *S. coelicolor*, *S. avermitilis* and *M. xanthus*. A knockout mutation of the *S. coelicolor* gene eliminated geosmin biosynthesis. The cyclase gene was cloned into an *E. coli* expression plasmid and high level expression was observed. The enzyme has been purified to near homogeneity.

Bacteria to remove off-flavor compounds from catfish ponds. Bacteria that degrade 2-methylisoborneol (MIB) and geosmin are being isolated and characterized for use in eliminating MIB and geosmin in catfish ponds as they are formed. Although it has been difficult to isolate bacteria capable of growing with MIB and geosmin, bacteria that grow on various analogous terpenes including camphor,

limonene, and alpha-pinene have been isolated. Many of these bacteria are able to act on MIB, converting it (1) by hydroxylation to form 2,3-dihydroxy-2-methylbornane, 2,6-dihydroxy-2-methylbornane, or more oxidized products or (2) by dehydration to form 2-methylenebornane. The latter compound lacks the earthy taste and odor of MIB and the demonstration of this metabolic conversion represents a significant step toward controlling MIB in aquaculture.

Off-flavor methodologies evaluated for catfish production (6). A study was conducted to determine whether multi-year exposure of channel catfish to diuron resulted in tissue residues exceeding the EPA tolerance level of 2.0 ppm. The study showed that diuron residue levels in fillets remained well below 1 ppm after treatment and there was no carryover of residues in fish from one year to the next. Results show that this algicide can be safely used to manage this important problem in catfish farming. Data from this study were critical in the recent favorable EPA ruling to allow a full registration for this chemical in the catfish industry.

A study was conducted where low densities (0, 25, or 100 fish/acre) of silver carp were co-cultured with catfish in ponds. Silver carp did not eliminate odor-producing blue-green algae from pond phytoplankton communities and did not reduce the incidence or intensity of off-flavors in catfish.

Similarly threadfin shad, a planktivorous fish, was co-stocked at three densities in multi-batch channel catfish production ponds to determine its impact on plankton biomass and catfish production characteristics. At the densities used in this study, threadfin shad appeared ineffective in impacting phytoplankton biomass or flavor score of marketable catfish at harvest.

Evaluation of sensory versus analytical detection of off-flavors (7). Fast through-put analytical methods were developed for routine use in off-flavor assessment. Human flavor checkers in processing plants are used to assess fillet quality. Four processing plants provided fillets that were processed for analysis by human flavor checkers; samples for gas chromatography/mass spectrometry were also obtained from each fish. The sensory detection limit for human flavor checkers was determined to be 0.1–0.2 ppb for MIB and 0.25–0.5 ppb for geosmin. Results from both analyses were highly correlated; suggesting human flavor checkers provided reliable evaluations.

Trophic relationships in catfish production ponds (8). The relationships of pond age with nutrients, zooplankton and phytoplankton populations, and the incidence of off-flavor occurrence, as well as synoptically assessed off-flavor and algal toxin prevalence in Mississippi, Alabama, Arkansas, and Louisiana were analyzed. Nutrients accumulated in ponds during the first 3 years of production, but leveled off after this time. After year 4, the algal composition became dominated by filamentous cyanobacteria, and zooplankton composition was dominated by larger copepods and cladocerans. Younger ponds have lower incidence and intensity of off-flavor relative to older ponds. Around 60% of ponds in the 485-pond survey had detectable levels of the toxin microcystin, whereas less than 30% had detectable levels of off-flavor compounds present.

Dogs were trained to detect off-flavor compounds in water and fish fillets (9). Off-flavor (an earthy or musty taste) in catfish production costs the industry as much as $50 million annually. Dogs selected from a local animal shelter were trained to sniff water samples for the off-flavor compounds. The dogs were able to smell with 90% accuracy if the fillet was off or on-flavor. The dogs were successfully evaluated at the Alabama Fish Farming Center in Greensboro, AL.

New Uses for Byproducts

Alaska fish protein meal quality assessed (10). The chemical and nutritive quality of commercial fish protein meals produced from the byproducts of the Alaska fish processing industry were assessed. The

chemical and nutritive quality of fish protein meals was assessed relative to a commercial formulation of known high quality in diets for Pacific white shrimp and Pacific threadfin and to anchovy-based protein meal for rainbow trout. The nutritive quality of all byproduct fish protein meals was found to be indistinguishable from commercial fish protein meal for shrimp and, with the exception of one Alaskan salmon protein meal, in formulations for Pacific threadfin and rainbow trout. This work demonstrates that Alaskan fish protein meals are equivalent to high quality commercial fish protein meals. Selected fish protein meals made from byproducts of the Alaska seafood processing industry were screened for pesticides and PCBs, and they did not contain detectable levels.

The results of an 8-week feeding trial indicated that Alaska pollock and salmon oils were able to replace menhaden oil in diets for shrimp. Shrimp that were fed diets containing pollock and salmon oils had excellent growth, feed efficiency and survival rates.

Soy-based dietary formulations have shown reduced palatability that limits effectiveness. Fish hydrolysates derived from Alaskan processing byproducts were shown to enhance the palatability of rainbow trout dietary formulations based on soybean protein meal.

Byproducts from different fish species evaluated (11). Research was conducted to evaluate the properties of the oils and protein for black cod (*Anoplopoma fimbria*) heads and frames and Pacific Ocean perch (*Sebastes alutus*) byproducts. Protein from the byproducts of these two species was of high quality, and the oil extracted from black cod byproducts contained lower levels of polyunsaturated fatty acids compared to most other cold water fish.

Energy and fertilizers from fish byproducts (12). In Alaska there is potential for increasing the recovery of fish oil from underutilized salmon by-products. Studies have been conducted that indicate Alaska salmon oil can be converted to biodiesel. In these studies biodiesel from salmon oil was characterized and found to have comparable properties to biodiesel derived from vegetable oils, such as soybean and corn.

Fish meal, fish bone meal, and hydrolysates made from the byproducts harvested from sustainable Alaska fisheries can be used as fertilizers. These fish by-products are rich in nitrogen and can be processed and used as a nutrient source for crop production. In the laboratory the amount of mineral N released in 56-day incubation was similar among the three fish byproducts and there was very little P release over the course of incubation. A single exponential model was suitable for simulating N release for fish meals, and that was validated by the field incubation results.

Feed ingredients for pets, pigs and reindeer (13). Hydrolysates made from fish by-products have been evaluated as replacers for plasma protein for early-weaned piglets. Results indicated that fish hydrolysate from Alaskan seafood by-products is a suitable replacement for a portion of the plasma protein in piglet diets and may lead to the development of a higher value product being produced from Alaskan seafood byproducts. The use of Alaska fish meals and hydrolysates have also been evaluated as ingredients in the diets of companion animals.

A study was conducted to compare soybean meal and fishmeal as the protein source on reindeer growth performance, feed efficiency and ultimate meat quality. No significant differences were observed in overall weight gain nor in most meat quality attributes; however, the feed efficiency was significantly improved. No “fish-related” flavor was reported by the sensory panel during consumption of the reindeer meat, supporting the use of fish meal as a dietary ingredient.

Gelatins from fish skins (14). Currently in Alaska, fish skins are either used to make fish meal or else discarded. Studies designed to increase the value of fish skins, including; dehydration as a stabilization method for fish skins; improved extraction of gelatin from the stabilized collagen material; and modification of the gelatin's functional properties through a cross-linking process using glutaraldehyde and genipin have been conducted. Glutaraldehyde proved to be a more effective cross-linker for pollock gelatin than genipin. Films made from fish-skin gelatins demonstrated reduced oxygen permeability while providing increased water vapor barrier properties. These studies have identified unique properties of cold-water fish-skin gelatin films with potential application to food products. Additionally, developments have been made to increase the yield of gelatin from dried fish skins.

Antimicrobial enhancements can be used with fish skin gelatin to produce safer food-grade gelatin films and gels. Lysozyme, a food-safe antimicrobial protein, was incorporated into fish-skin gelatin films and gels, and their antimicrobial properties and other useful characteristics were evaluated. Results indicated both films and gels retained their lysozyme activity, Fish-skin gelatin, when formulated with lysozyme, may provide a unique, functional barrier to increase the shelf life of food products.

Hydrolysates from byproduct (15). Studies have been conducted using different enzymes and reaction times to evaluate the protein quality and oil recovery during hydrolysis of salmon heads. The degree of hydrolysis (DH) is an important characteristic when making fish hydrolysates because it influences peptide length, nutritional properties and other protein characteristics. Studies have found differences in the functional properties of salmon head hydrolysates produced using different types of proteases and digestion times. These studies demonstrated that different enzymes as well as the length of incubation influenced both the DH values and oil yield from salmon heads.

Salmon hydrolysate production was improved by changing the acidification agent, and by using drum drying as a method for stabilizing hydrolysates and retaining the valuable properties of the dried salmon hydrolysates.

Oils from fish byproducts (16). Cold water marine fish tend to have higher omega-3 content than many warm water marine fish, which tend to have higher omega-3 content than freshwater fish. Oils from Alaska pollock and salmon rank near the top with omega-3 fatty acids accounting for 20% or more of total triglyceride fatty acid levels. Livers from seven fish species harvested in Alaska were collected and the composition of their oils determined. Liver lipid contents ranged from 3.3 to 50.3% and the percentage of long chain 3-omega fatty acid content also varied between livers form the different species.

Protein powders from fish byproducts (17). Alaska fish processing byproduct is made into fish protein meal or discarded; however, there is the potential to extract specific protein mixtures from byproducts that would have superior physical properties and a greater value in the market place. Two methods have been used to extract protein from byproducts from a variety of fish including salmon, pollock, arrowtooth flounder and herring. An extraction method was developed to extract soluble and insoluble protein fractions from byproducts including heads, frames and viscera. Significant differences in yield, chemical composition, and functional properties including fat absorption, water absorption, protein solubility and emulsifying stability were detected. Feed ingredients with different chemical and functional properties can be made from the insoluble and soluble protein fractions of different pollock byproducts.

Extraction of protein from Alaska fish byproducts at high pH followed by isoelectric point precipitation using pollock heads, whole fish, viscera, and frames and pink salmon heads and viscera collected from commercial processing lines was examined. Protein powders with good functional and nutritional characteristics were made from pollock heads and frame and salmon heads.

Protein meals from livers, roe and testes (18). Salmon livers have both relatively high lipid and cholesterol levels and in females, relatively high concentrations of vitellogenin. In Alaska, salmon livers and other viscera are not utilized for human food and are usually discarded. An industrial scale method was developed for processing these livers into protein meals, which were then characterized. These meals had a high cholesterol content that will be useful in formulating diets for aquaculture shrimp and possibly as a feed augmentation ingredient for younger fish.

Protein meals made from milt have been referred to in the past as spawn powder and large amounts of fresh raw milt are available from the processing of pollock and salmon in Alaska. Ordinarily, testes are either directed towards the production of generic fish protein meal or are discarded. Development of industrial scale methods for the production of high quality milt meal from both pollock and salmon has been completed and the products have been characterized. There are a number of potential uses for this meal based on the relatively high concentrations of nucleic acids and other components.

Solubles from fish meal processing (19). The nutritional value of adding stickwater (aqueous solubles derived from making fish protein meal) to solids during fish protein meal manufacture was examined. The optimum level of stickwater added, on an equal protein content basis, was 15% for trout, 20% for threadfin, and up to 40% for shrimp.

APPENDIX 1 – PLANNING AND COORDINATION FOR NP 106 – NATIONAL WORKSHOPS

- NP 106 National Program Planning Workshop, 1997
- Joint USDA ARS-CSREES Aquaculture National Program Stakeholder Workshop, 2002
- National Animal Germplasm Program Policy and Coordinating, Annual, 1999-2007
- National Animal Germplasm Program Aquaculture Species Committee, Annual, 1999-2007
- Future Trends in Animal Agriculture Annual Symposia 2002-2005
- USDA Animal Genomics Workshop, 2004
- DISCOVER Conference on Animal Germplasm, 2004

APPENDIX 2 - SELECTED SUPPORTING INFORMATION AND DOCUMENTATION FOR ACCOMPLISHMENTS AND IMPACT OF NP 106 RESEARCH

Component I: Genetic Improvement

1. Aquaculture species added to the National Animal Germplasm Program.

See National Animal Germplasm Program at: http://www.ars-grin.gov/animal .

2. Production traits of different hybrid striped bass crosses.

Small, B. and K.B. Davis. 2002. Validation of a time-resolved fluoroimmunoassay for measuring plasma cortisol in channel catfish *Ictalurus punctatus*. Journal of the World Aquaculture Society 33:184-187.

Davis, K.B. and B.R. Griffin. 2004. Physiological responses of hybrid striped bass under sedation by several anesthetics. Aquaculture 233: 531-548.

Davis, K.B. 2006. Management of physiological stress in finfish aquaculture. North American Journal of Aquaculture 68: 116-121.

Davis, K.B. and B.C. Small. 2006. Rates of cortisol increase and decrease in channel catfish and sunshine bass exposed to acute confinement stressor. Comparative Biochemistry and Physiology. Toxicology & Pharmacology 143: 134-139.

Davis, K.B. 2004. Consequences of physiological stress in aquaculture. American Fisheries Society, 2004 Annual Meeting, Madison, WI.

Davis, K.B. 2004. The effect of temperature on the stress response in sunshine bass. U.S. Aquaculture Society, 2004 Annual Meeting, Honolulu, HI.

Davis, K.B. and B. Small. 2004. Use of metomidate to determine the secretion and clearance rates of cortisol in sunshine bass. U.S. Aquaculture Society, 2004 Annual Meeting, Honolulu, HI.

Rawles, S.D. 2006. Hybrid Striped Bass Research Program at USDA/ARS - HKDSNARC. Invited Presentation, Fourth Annual Workshop on Genetic Improvement and Selective Breeding for the Hybrid Striped Bass Industry, Las Vegas, NV.

Davis, K.B. and M. McEntire. 2006. Comparison of the cortisol and glucose stress response to acute confinement and resting insulin-like growth factor-I concentrations among white bass, striped bass and sunshine bass. U.S. Aquaculture Society, 2006 Annual Meeting, Las Vegas, NV.

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Silverstein, J.T., T. King, and C. E. Rexroad, III. 2004. Genetic variation measured by microsatellites among three strains of domesticated rainbow trout. Aquaculture Research 35: 40-48.

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Johnson, N.A., R.L. Vallejo, C.E. Rexroad, III, E.M. Hallerman, and Y. Palti. 2007. Development and evaluation of a new microsatellite multiplex system for parental allocation and management of rainbow trout broodstocks. World Aquaculture Society, 2007. San Antonio, TX.

Silverstein, J., J. Parsons, C. E. Rexroad, III, and Y. Palti. 2005. Heterosis and genetic distance in strain crosses of rainbow trout. Aquaculture America 2005, New Orleans, LA.

Palti, Y. “Genomics implementation into the NCCCWA rainbow trout breeding program”. March 15, 2006 presentation at Virginia Tech University.

4. Established rainbow trout lines, genotype x environment interactions and breeding for economically important traits.

Silverstein, J.T., C. E. Rexroad, III, and T. King. 2004. Genetic variation measured by microsatellites among three strains of domesticated rainbow trout. Aquaculture Research 35*:* 40-48.

Silverstein, J.T. M. Hostuttler, and K. P. Blemings. 2005. Strain differences in feed efficiency measured as residual feed intake in individually reared rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquaculture Research 36*:* 704-711.
Chiou, P., Peter, Bols Niels, Douglas, Sue and Chen, Thomas T. 2005. Regulation of Immune-Relevant Genes in the Trout Macrophage Cell Line RTS-11 by Antimicrobial Peptides. Developmental and Comparative Immunology 30: 797-806.

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Wolters, W. R. 2006. USDA ARS National Cold Water Marine Aquaculture Center Atlantic Salmon Containment Management Plan. Submitted to Maine Department of Environmental Protection, 33 p. (Required prior to construction and operation of Center's research program; 2006 document supersedes document developed in 2005 covering research in temporary facilities)

Wolters, W. R., and S. Summerfelt. 2007. USDA marine aquaculture center breeds Atlantic salmon. Global Aquaculture Advocate 10(2): 56-58.

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Bosworth, B.G., W.R. Wolters, D.J. Wise, and P.H. Klesius. 2004. Genetic effects for response to live *Edwardsiella ictaluri*, killed *E. ictaluri*, and stress in juveniles from all crosses among USDA 103, USDA 102, and Norris channel catfish *(Ictalurus punctatus*) strains. Journal of the World Aquaculture Society 35: 78-86.

Bosworth, B.G., W.R. Wolters, D.J. Wise, and P.H. Klesius. 2004. Genetic effects for growth and resistance to *Edwardsiella ictaluri* estimated from a diallel cross among USDA 102 strain channel catfish *Ictalurus punctatus*, blue catfish *Ictalurus furcatus*, and their F1 hybrid. Journal of the World Aquaculture Society 35: 418-424.

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Component II: Integrated Aquatic Animal Health Management

1. Real-time PCR assays development for ESC and columnaris disease in catfish.

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Component V: Aquaculture Production Systems

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APPENDIX 3
Listing of Individual Appropriated CRIS Projects by Geographic Location

NATIONAL PROGRAM 106 – AQUACULTURE

Auburn, Alabama
Aquatic Animal Health Research Laboratory
(4 projects)

CRIS Project Title: *Vaccinology and Immunity of Aquatic Animals*
(NP 106 -- Components II, IV)

SY (2.75): P. Klesius (Co-Lead -- 75%); J. Evans (Co-Lead -- 50%); D. Xu (25%); C. Shoemaker (50%); V. Panangala (25%); Shelby, R. (25%); J. Bebak (25%)

Appropriated Annual Funding: $ 1,253,067 (NTL)

Related Sibling CRIS Projects:

a) Efficacy of Streptococcal Vaccines and Characertization of Mechanisms of Immunity in Tilapia

b) Efficacy of Bacterial Pathogen Vaccines in Tipalia Under Field Conditions and Isolation and Identification of Fish Pathogens

c) Catfish Pathogen Genomics, Epidemiology and Vaccines

d) Development of Monovalent and Multivalent Fish Vaccines

e) Edwardsiella Ictaluri Vaccine: Efficacy and Dose Titration Study in Channel Catfish

Species Impacted: Channel Catfish, Tilapia, Hybrid Striped Bass

Listing of Project Objectives:

1) Characterization of the mechanisms of immune responses by *in vitro* and *in vivo* experiments to identify antibody and/or cells responsible for protection.

2) Development and experimental testing of non-living and modified monovalent and multivalent vaccines and antibody for passive immunization against economically important pathogens.

3) Development of *in ovo*, bath hyperosmotic immersion and oral routes of vaccine administration as a cost-effective means of mass immunization of fish with cohabitation and immersion as a means to more closely mimic field conditions.

CRIS Project Title: *Nutrition, Immune System Enhancement and Physiology of Aquatic Animals*
(NP 106 -- Components IV, VI)

SY (2.25): Chhorn Lim (Lead -- 100%); Thomas Welker (75%); Vacant (25%)

Appropriated Annual Funding: $ 533,904 (NTL).

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Species Impacted: Channel Catfish, Tilapia

Listing of Project Objectives:

1) Examine the effect of alternative protein sources and dietary nutrients on stress response, immune response, and disease resistance of fish.

2) Evaluate the effect of immunostimulants and probiotics on fish stress resistance, immune response and disease resistance.

3) a) Determine the optimum dietary level of marine fish oil and feeding duration necessary to optimize n-3 highly unsaturated fatty acids (n-3 HUFA) content in fillets of channel catfish

b) Determine the relationship between n-3 HUFA content in diets and the catfish vitamin E requirement with regard to stress, the immune response, and disease resistance.

CRIS Project Title: *Molecular Analysis of Virulence Determinants of Select Bacteria in Fish Diseases*
(NP 106 – Components II, IId, Iif)

SY (2.0): V. Panangala (Lead -- 75%); H-Y. Yeh (50%); Vacant (75%).

Appropriate Annual Funding: $ 1,066,018 **(**NTL)

Species Impacted: Channel Catfish, Tilapia

Listing of Project Objectives:

A muliplex PCR for simultaneous detection of three fish-pathogenic bacteria, Edwardsiella ictaluri, Flavobacaterium columnare and Aeromonas hydrophila. The current project will be expanded to include development of molecular-based vaccines for use in aquaculture to prevent fish disease.

CRIS Project Title: *Aquatic Animal Diagnostics, Pathogenesis and Applied Epidemiology*
(NP 106 -- Components II, V, VII)

SY (4.0): C. Shoemaker (Lead -- 50%); R. Shelby (75%); J. Evans (50%); D. Xu (75%); J. Bebak (75%); H-Y. Yeh (50%); P. Klesius (25%)

Appropriated Annual Funding: $ 1,143,796 (NTL)

Related Sibling CRIS Projects:

a) Field Trial to Test the Efficacy of Aquavac-Col Vaccine for Protection Against Columnaris Disease Outbreaks in Trout

Species Impacted: Channel Catfish, Tilapia, Hybrid Striped Bass

Listing of Project Objectives:

1) Develop and apply rapid non-lethal methods to detect, diagnose and evaluate effects of pathogens, toxins and off-flavor compounds in aquatic animals.

2) Develop in vivo and in vitro models for addressing the mechanism of disease for bacteria, parasites and toxins in aquatic animals

3) Determine risk factors and disease prevalence, incidence, sources and origin of economically important aquatic animal pathogens.

Fairbanks, Alaska
Subarctic Agricultural Research Unit

CRIS Project Title: *Converting Alaska Fish Byproducts into Value Added Ingredients and Products*
(NP 106 -- Component VII)

SY (2.10):

USDA/ARS Scientists

Peter Bechtel, Food Technologist, Lead Scientist	100%
Cynthia Bower, Food Technologist	100%
Alberto Pantoja,	10%

Appropriated Annual Funding: $2,150,778 (NTL)

Related Sibling CRIS Projects:

a) Converting Alaska Fish by-products into Value Added Ingredients and Products.
b) Nutritional Value of Fish Oil on Reproductive Performance in Gilts and Sows and Fish Protein Hydrolysates on Immune Indices in Geriatric Dogs
c) Gasification of Salmon Processing Waste in Alaskan Communities.
d) Evaluating Nutritional Values of Alaska Whitefish by-products for Organic Food Production.

Species Impacted: Fish, Livestock, Reindeer, Companion Animals, Humans

Listing of Project Objectives:

The over-arching goal of this project is to develop new knowledge to increase the value of underutilized seafood processing by-products for aquaculture and agriculture in a sustainable manner.

1) Elucidate the chemical, biological, and physical properties of underutilized Alaska fish by-products and their biochemical constituents to identify properties/compounds that can be used to make new and improved aquaculture and agriculture feed ingredients, and other high value products.

2) Improve processes and methods for analysis, collection, and storage of raw materials, to retain the chemical, biological, and physical qualities of Alaska fish processing raw materials for developing new and improved ingredients/biochemicals.

3) Make and evaluate the value of new and improved aquaculture and agriculture ingredients and feeds from underutilized Alaska seafood by-products and their constituents.

Pine Bluff, Arkansas
Aquaculture Systems Research Unit

CRIS Project Title: *Improved Warm Water Aquaculture Products and Technologies*
(NP 106 Components V, VI, VII)

SY (2): B. Green (Lead – 100%); P. Pearson (100%)

Appropriated Annual Funding: $943,967 (NTL)

Related Sibling CRIS Projects:

a) **Improved Efficient Foodfish and Baitfish Aquaculture.**

b) **Development of Aquaculture Production Systems and Value Added Aquatic Food Products at Pine Bluff, Arkansas.**

c) **Development of a Modified Grading System for Golden Shiner Minnows**

d) **Innovative Technologies and Methodologies for Commercial-Scale Pond Aquaculture**

e) **Development of Channel Catfish Value-Added Products**

f) **Expanding Aquaculture Graduate Program Offering: A need for Aquaculture/Fisheries Research and Educational Library**

g) **Continued Development of a Channel Catfish Value-Added Product: Catfish Patty**

h) **Integrated Teaching and Experience Learning (Intel) Laboratory: Creating a High-Tech Learning Environment for Students**

Species Impacted: Hybrid Striped Bass, Catfish, Baitfish.

Listing of Project Objectives:

1) Develop improved mechanical methods to manage excessive phytoplankton biomass in warm water ponds to increase predictability of food fish production.

2) Develop microbial-based management system to increase yield per unit volume of water in warm water aquaculture systems.

3) Develop or adapt technologies to increase efficiency and reduce stress of fish production and handling.

4) Increase quality aquaculture products by meeting changing consumer preferences and enhancing recovery and further processing of edible tissue.

5) Improve performance efficiency of warm water aquaculture through identifying optimal management practices related to stocking, nutrition, and disease diagnosis and prevention.

Stuttgart, Arkansas
Harry K. Dupree Stuttgart National Aquaculture Center
(4 projects)

CRIS Project Title: *Improving the Production Efficiency and Sustainability of Morone species Culture*
(NP 106 Components -- I, III, IV, V, VI)

SY (6.5): S. Rawles (Lead -- 100%); G. Ludwig (100%); A. Fuller (100%); D. Freeman (50%); C. Weirich (100%); M. Riche (100%); T. Pfeiffer (100%).

Appropriated Annual Funding (NTL): $1,419,925 (NTL)

Related Sibling CRIS Projects:

a) Building Capacity in Food Science Studies at PVAMU

Species Impacted: *Morone* sp., Striped Bass, White Bass and their Hybrids

Listing of Project Objectives:

1) Develop and refine year-round production of hybrid striped bass fingerlings through photothermal, dietary, and culture manipulation.

2) Increase hybrid striped bass production efficiency through physiological control of stress, gender, and hormonal growth factors.

3) Increase hybrid striped bass production efficiency by refining nutrient requirements, manipulating feeding strategy and diet nutrient density, and reducing fish meal and oil content.

4) Develop genetically superior *Morone* sp.

CRIS Project Title: *Development of Strategies to Minimize the Impact of Bird Predation on Aquaculture*
(NP 106 -- Component VII)

SY (1.2): A. Radomski (Lead -- 100%), D. Freeman (20%).

Appropriated Annual Funding (NTL): $323,061 (NTL)

Related Sibling CRIS Projects:

a) Effectiveness of Solar Electric Boats for Deterring Fish-Eating Birds at Commercial-Scale Aquaculture Production Ponds
b) Double-Crested Cormorant Research Study

Species Impacted: Catfish, Hybrid Striped Bass, Baitfish, General Aquaculture species.

Listing of the Project Objectives:

1) Apply innovative engineering design approaches to develop and evaluate new, improve existing, or adapt alternative techniques to minimize and control depredation at aquaculture facilities. Concurrently, assess and evaluate regional populations of fish-eating birds and other potential vertebrate predators that threaten or increase the risk of predation. at aquaculture facilities.

2) Identify spatial and temporal differences between fish-eating bird use patterns within and surrounding aquaculture areas, compare these patterns under regulatory management of lethal and nonlethal roost dispersal sites, and nonmigratory and migratory cormorant populations to enhance management efforts within aquaculture areas.

CRIS Project Title: *Evaluation of Compounds and Strategies for Controlling Aquatic Animal Disease*
(NP 106 Aquaculture – Component II)

SY (5.3): D. Straus (Lead -- 100%); A. Darwish (100%); A. Mitchell (100%); D. Freeman (30%).

Appropriated Annual Funding (NTL): $969,207 (NTL)

Related Sibling CRIS Projects:

a) Potentially Harmful Trematodes to Aquaculture Transmitted by Aquatic Birds

Species Impacted: Channel Catfish, Hybrid Striped Bass, Baitfish (i.e., grass carp, golden shiners, goldfish)

Listing of Project Objectives:

1) Develop data needed to demonstrate safety (animal, human, and environmental) and efficacy of compounds that are relevant to the needs of aquaculture.

2) Determine the efficacy of compounds, biological control strategies, clearance rate and pathogenesis (understanding of the disease process) of parasites and fungi applicable to catfish, baitfish, and *Morone* sp. culture.

3) Improve performance efficiency of important warm water aquaculture species by identifying optimal management practices related by mitigation of disease outbreaks, evaluation of blue/channel hybrid catfish production characteristics and disease resistance, evaluation of oxygen transfer technology related to cost, and harvesting and transport.

CRIS Project Title: *Engineering and Production Strategies for Sustainable Marine Aquaculture*
(NP 106 Components -- II, III, IV, V, VI)

SY (3.4): M. Riche (Lead -- 100%); T. Pfeiffer (100%); C. Weirich (100%); D. Freeman (40%).

Appropriated Annual Funding (NTL): $1,546,781 (NTL)

Related Sibling CRIS Projects:

a) Development of Culture Technology for Production of Marine Fish Species in Low Salinity Closed Systems

b) Improving Stress Resistance of Cultured Hard Clams: Triploid Production

c) Demonstrating Technological and Economic Feasibility of Cobia (Rachycentron Canadum) Aquaculture from Hatchery to Market.

d) National Initiative for Aquaculture Development and Fishery Enhancement of Cobia, Rachycentron Canadum

Species Impacted: Pompano, Black Sea Bass, Cobia, Flounder

Listing of Project Objectives:

1) Determine nutrient requirements and develop feeding strategies and diets for optimal growth, efficiency, and reproductive success of marine fish reared in low-salinity recirculating systems.

2) Develop year-round spawning strategies for captive broodstock and larviculture and early juvenile rearing methods for sustainable seed production of selected marine finfish species.

3) Develop engineering processes and sustainable effluent technologies to enhance water and energy utilization and reduce environmental impacts in low-salinity environments.

Hilo, Hawaii
U.S. Pacific Basin Agricultural Research Center – Tropical Aquaculture Research Unit

CRIS Project Title: *Tropical Aquaculture: Develop Feeds for Optimum Nutrition of Cultured Shrimp and Finfish*
(NP 106 Components -- IV, VII)

SY (0.00): ARS, D. Gonsalves (0%);

Appropriated Annual Funding: $1,387,405 (NTL)

Related Sibling CRIS Projects:

a) **Specific Minimum Nutritional Requirements for Reproductive Performance in Pacific White Shrimp, Litopenaeus Vannamei**
b) **Tropical Aquaculture-Grant: Develop Feeds for Optimum Nutrition of Cultured Shrimp and Finfish**

Species Impacted: Pacific white shrimp (*Litopenaeus vannamei*), Pacific threadfin (*Polydactylus sexfilis*), and Longfin amberjack (*Seriola rivoliana*)

Listing of Project Objectives:

1) To vertically integrate (from raw materials to feeds to food products) the science and technolology of producing Pacific white shrimp, and the model tropical fish Pacific threadfin and longin amberjack.

2) To optimize existing technologies for shrimp growout, using agricultural by-products and reduced fish meal in feeds for these species.

3) To develop new technologies for threadfin (including larvae) and amberjack growout, using agricultural by-products and reduced fish mean in feeds for these species.

Hagerman (Aberdeen), Idaho
Small Grains and Potato Germplasm Research Unit

CRIS Project Title: *Integration of Nutritional, Genetic and Physiological Approaches to Improve Production Efficiency of Rainbow Trout*
(NP 106 Components – I, II, IV)

SY (5.05): F.T. Barrows (100%); J.M. Bonman (5%); T.G. Gaylord (100%); G. Hu (100%); K. Liu (100%); K. Overturf (100%)

Appropriated Annual Funding: $1,916,509 (NTL)

Related Sibling CRIS Projects:

a) Development of Plant-Based Trout Feeds Using a Nutrabiotic Approach to Supplement Ingredients Derived From Non-Traditional Crops (01S)
b) Development of Plant-Based Feeds For Trout (02S)
c) Development of Biologically Enhanced Plant Proteins to Replace Fish Meal in Trout Feed (04T)

Species Impacted: Rainbow Trout

Listing of Project Objectives:

1) Develop a series of trout feeds that replace fish meal and fish oil with traditional and novel plant-derived ingredients.

2) Determine optimal supplementation levels of specific vitamins, and limiting amino acids for plant-based feeds for trout.

3) Identify and evaluate genetic, biochemical and physiological factors affecting nutrient utilization and growth of trout fed plant-based feeds.

4) Identify rainbow trout families with improved phenotypes for growth and utilization of a plant-based fish feed through a genetic selection program.

New Orleans, Louisiana
Southern Regional Research Center
Food Processing and Sensory Quality Research Unit

CRIS Project Title: *Mitigation of Off-flavors in Catfish Aquaculture*
(NP 106 Components – V, VII)

SY (2.1): B. Hurlburt (Lead Scientist -- 100%); R. Eaton (100%); C. Grimm (10%).

Appropriated Annual Funding: $663,203 (NTL)

Species Impacted: Catfish

Listing of Project Objectives:

1) Identify and characterize viruses that kill specific off-flavor-producing cyanobacteria in aquaculture systems.

2) Identify, characterize and develop novel bacterial strains that metabolize (biodegrade) geosmin and 2-methylisoborneol and develop them for use in eliminating those chemicals from aquaculture systems.

3) Identify and characterize genes and enzymes involved in the biosynthesis of off-flavor metabolites in aquaculture systems. Build on an understanding of the mechanisms used in regulating gene expression and in catalysis of individual reactions and develop methods to control the production of geosmin and MIB.

Orono / Franklin, Maine
National Cold Water Marine Aquaculture Center

CRIS Project Title: *Cold Water Marine Finfish Genetic Improvement and Production*
(NP 106 Component I)

SY (1): W. R. Wolters (Lead – 100%)

Appropriated Annual Funding: $656,838 (NTL)

Related Sibling CRIS Projects:

a) Efficacy of Streptococcal Vacinnes and Characterization of Mechanisms of Immunity in Tilapia

b) Development of Cold Water Marine Aquaculture Research

Species Impacted: Atlantic Salmon

Listing of Project Objectives:

1) Obtain and compare performance of selected North American Atlantic salmon stocks for utilization in an applied selective breeding program.

2) Estimate genotypic and phenotypic parameters for commercially important traits and develop a selection index.

3) Evaluate specific breeding aids such as polyploidy (triploidy) and sex reversal (all female stocks) for benefits to Atlantic salmon aquaculture.

4) Conduct molecular genetic analyses to establish that the identity of foundation salmon stocks are North American origin.

5) Conduct preliminary evaluations for producing mono-sex and polyploidy Atlantic salmon in cooperation with the USDA, ARS National Center for Cool and Cold Water Aquaculture in Leetown, West Virginia.

Corvallis (Newport), Oregon
Forage, Seed, and Cereal Research Unit

CRIS Project Title: *Genetic Characterization and Improvement of Shellfish Breeding Stocks for the Pacific Northwest*
(NP 106 -- Component I)

SY (1.0): Mark D. Camara (100%)

Appropriated Annual Funding: $ 346,967 (NTL)

Related Sibling CRIS Projects:

a) Development of Genetic Marker Systems for Use in Research on the Quantitative and Molecular Genetics of Pacific Oysters

Species Impacted: Pacific Oyster (*Crassostrea gigas*), Kumamoto Oyster (*C. sikamea*) Olympia Oyster (*Ostrea concaphila*)

Listing of Project Objectives:

1) Conduct research on the quantitative and molecular genetics of oysters leading to improvement in broodstock management & selective breeding for enhanced production efficiency and product quality.
2) Develop reference families, molecular genetic markers and elucidate key genes and sequence information for traits of economic importance.
3) Assess genetic diversity of potential germplasm resources.

CRIS Project Title: *Integrated Pest Management Practices for Pacific Shellfish Production*
(NP 106 - Component VI)

SY (1.07): B. Dumbauld, G. Banowetz (.07)

Appropriated Annual Funding: $350,503 (NTL)

Species Impacted: Oysters, Clams

Listing of Project Objectives:

1) Identify critical stages in the life cycle of burrowing shrimp in West Coast estuaries that are vulnerable to control measures for aquaculture operations.

2) Map aquaculture operations, eelgrass beds, and burrowing shrimp populations at an estuarine landscape scale and conduct a spatial analysis in order to quantify the interaction between oyster aquaculture practices, fish utilization of these estuarine habitats as predators and parasite hosts, and burrowing shrimp recruitment and movement.

University, Mississippi
Natural Products Utilization Research Unit

CRIS Project Title: *Control of Undesirable Microbes and Off-flavors in Aquaculture with Natural Products*
(NP 106- Components V, VI, VII)

SY (1.0): K. Schrader (Lead Scientist)

Appropriated Annual Funding: $337,453

Related Sibling CRIS Projects: None

Species Impacted: Channel Catfish

Listing of Project Objectives:

1) Discover and develop novel natural product-based compounds for controlling undesirable microbes and off-flavors in aquaculture.

2) Determine the optimal management approaches of new and current algicides and bactericides for controlling undesirable microbes and off-flavors in aquaculture.

Stoneville, Mississippi
Catfish Genetics Research Unit
(6 projects)

CRIS Project Title: *Improving Production Efficiency of Pond-Raised Channel Catfish*
(NP 106 Components – III, IV, V, VI, VII)

SY (0): K. Davis (0.00)

Appropriated Annual Funding: $3,038,673 (NTL)

Related Sibling CRIS Projects: Catfish Health Initiative (NP 106 Component II

Species Impacted: Channel Catfish

Listing of Major Objectives:

1) Develop feeds and feeding practices for optimal nutrition, increased production, and improved water quality of pond-raised catfish.

2) Increase the reliability, efficiency, and cost-effectiveness of catfish fry production through the use of new and improved technologies.

3) Apply engineering approaches to design new or modified live aquatic animal harvesting equipment.

4) Increase the reliability, efficiency, and cost-effectiveness of catfish production through the development of improved aeration technologies and strategies to enhance catfish production in commercial ponds.

Related Sibling CRIS Project Title: Catfish Health Initiative (NP 106 Component II)

SY (0.0):

Appropriated Annual Funding: $828,496 (NTL)

Species Impacted: Channel Catfish

List of Major Objectives:

1) Develop diagnostic tools to detect and monitor diseases in commercially raised channel catfish and determine virulence factors associated with those diseases.

2) Develop fish health management procedures to control economically important diseases of channel catfish.

3) Determine factors associated with emerging diseases in pond-raised channel catfish.

4) Use epidemiological methods to investigate new and emerging diseases, and to identify environmental and management factors that influence the onset and severity of disease outbreaks.

CRIS Project Title: *Catfish Genetics, Breeding, and Physiology*
(NP 106 Components – I, II, III, IV)

SY (9.0): G. Waldbieser (Lead -- 100%); B. Bosworth (100%); L. Clay (100%); K. Davis (100%); S. Quinou (100%); B. Peterson (100%); B. Small (100%); Vacant Microbiologist/Animal Scientist/Fish Biologist (100%); P. Silverstein (100%); G. Waldbieser (100%) E. Torrans (00%)..

Appropriated Annual Funding: $3,548,653 (NTL)

Related Sibling CRIS Projects:

a) Hill Area Aquaculture
b) Sequence Characterization of the Channel Catfish Immunoglobin Heavy Chain Locus
c) Pilot Testing of Experimental Catfish Lines on Commercial Catfish Farms (09N)
d) Pilot Testing on Experimental Catfish Lines on Commercial Catfish Farms (10N)

Species Impacted: Catfish

Listing of Project Objectives:

1) Measure genetic variation in production traits in the USDA103 catfish line and characterize correlations between traits.

2) Develop genomic resources for integrating functional genomics into the catfish applied breeding program.

3) Increase biological efficiency through selective breeding of catfish and transfer improved catfish germplasm to the U.S. catfish industry.

Sibling Project Title: Hill Area Aquaculture (NP 106- Components V, VI)

SY (0.0): 0.0 L. D'Abramo; 0.0 T. Tietjen; .00 B. Small

Appropriated Annual Funding (**NTL):** $256,626 (NTL)

Species Impacted: Freshwater Prawn (*Macrobrachium rosenbergii*);
Channel Catfish (*Ictalurus punctatus*)

Listing of Project Objectives:

1) Develop more efficient and sustainable production systems for the freshwater prawn, *Macrobrachium rosenbergii* by focusing on production based upon different organic fertilization schemes combined with economic analysis.

2) Develop a more efficient and sustainable production system for the channel catfish, *Ictalurus punctatus* through field testing of a three phase production system versus the traditional multiple batch system combined with economic analysis.

3) Evaluate the ability of managers to reduce sampling and monitoring efforts/labor while maintaining high quality waters in aquaculture by developing and assessing surrogate measures of water quality and establishing predictive water quality relationships in aquaculture.

CRIS Project Title: *Optimizing Catfish/Water Quality Interactions to Increase Catfish Production*
(NP 106 Components – II, III, IV, V, VI, VII)

SY (2.0): E. Torrans (Lead – 100%); P. Zimba (100%);

Appropriated Annual Funding: ARS -- $584,740 (NTL);

Related Sibling CRIS Projects:

a) Acoustic Technologies for Evaluating Catfish Production (SCA 58-6402-4-094 with University of Mississippi)
b) Test of novel aerator placement strategy for managing dissolved oxygen on commercial channel catfish farms (NFCA).
c) Development of a powered u-tube aerator ("Power Tube") for use in commercial fish ponds (NFCA).
d) Development and validation of remote sensing algorithms to detect cyanobacteria in catfish ponds (SCA 58-6402-4-075 with Univ of Nebraska).
e) Algal Toxins in Cultures from the Salt River System

Species Impacted: Channel Catfish, Blue Catfish, Channel X Blue Catfish

Listing of Project Objectives

1) Discover, develop, and apply methods to predict off-flavor episodes and manage off-flavor compounds.

2) Identify optimal water column conditions for balanced growth of bacteria, phytoplankton, and zooplankton resulting in reduced secondary metabolite formation, and enhanced fish survival and production.

3) Determine influence of chemical and biological factors on channel catfish respiration, growth and production, and develop and test management methods to minimize limits on production.

4) Develop new equipment and technologies to improve profitability of channel catfish farming.

CRIS Project Title: *Mississippi State University, Mississippi Center for Food Safety and Postharvest Technology, Starkville, Mississippi*
(NP 106 Component VII)

SY (0.55): W. Mikel (25%); J. Silva (10%); M Lawrence (10%); D. Marshall (10%).

Appropriated Annual Funding: $903,104 (NTL)

Related Sibling CRIS Projects: None.

Species Impacted: Catfish and other aquaculture species

Listing of Project Objectives:

1) Optimize the safety of aquaculture products through innovative processes throughout the production chain.

2) Evaluate various mechanisms for reducing microbiological, physical and chemical hazards in aquaculture products.

3) Determine the mechanisms responsible for microbial survival of selected pathogens in aquaculture products.

4) Optimize the market value of aquaculture products through enhanced food safety and quality.

Leetown, West Virginia
National Center for Cool and Cold Water Aquaculture
(4 projects)

CRIS Project Title: *Identification and Characterization of Genes Affecting Cool and Cold Water Aquaculture Production*
(NP 106 Component I)

SY (3.70): C. Rexroad III (Lead -- 100%), Y. Palti (100%); R. Vallejo (70%); S. Gahr (100%);

Appropriated Annual Funding: $1,634,071 (NTL)

Related Sibling CRIS Projects:

a) Development of Genetic Markers for Rainbow Trout (Specific Cooperative Agreement)
b) Functional Genomics Research for Rainbow Trout Aquaculture Production (Specific Cooperative Agreement)
c) Sequencing and characterization of the Rainbow Trout MHC Class I & II (Reimbursable Agreement)
d) Molecular Markers for Genome Mapping and Selective Breeding of Striped Bass (Reimbursable Agreement)
e) Transcriptome Analyses In Salmonids (Non-funded Cooperative Agreement)
f) Production of an Integrated Physical and Genetic Map for Rainbow Trout (Reimbursable Agreement)
g) Production of A Physical Map for the Rainbow Trout Genome Using High Throughput DNA Fingerprinting (Specific Cooperative Agreement)
h) Genetic and Diet Effects On Growth Rate and Reproduction in the Rainbow Trout Strains of Troutlodge, Inc. (Reimbursable Agreement)

Species Impacted: Rainbow Trout, Striped Bass

Listing of Project Objectives:

1) Continued development of a rainbow trout genetic map to reveal qualitative and quantitative trait loci affecting traits associated with aquaculture production.

2) Identification and characterization of genes affecting important aquaculture production traits through functional genomic technologies.

3) Development of strategies to implement functional genomic and genetic mapping information into the NCCCWA rainbow trout selective breeding program using bioinformatics.

4) Develop databases and programs to assimilate and coordinate genomic data on the rainbow trout broodstock developed at the NCCCWA.

5) Develop methodology to utilize rainbow trout genomic information in comparative analyses to identify potential critical functional genes and genetic pathways.

CRIS Project Title: *Utilizing Genetics for Enhancing Cool and Cold Water Aquaculture Production*
(NP 106 Components -- I, III, IV)

SY (4.30): J Silverstein (Lead – 100%), W. Hershberger (100%), G. Weber (100%), R. Vallejo (30%), Vacant (100%)

Appropriated Annual Funding: $2,274,202 (NTL)

Related Sibling CRIS Projects:

a) Genetic and Diet Effects on Growth Rate and Reproduction in the Rainbow Trout Strains of Troutlodge, Inc. (Reimbursable)

b) Evaluation of Genotype by Environment Interactions in Rainbow Trout (Specific Cooperative Agreement)

c) Evaulation of Selected Rainbow Trout Lines Fed Grain-Based Diets under Farm Scale Conditions (Specific Cooperative Agreement)

d) Production for Superior Rainbow Trout Broodstocks by Genetic Manipulation (Specific Cooperative Agreement)

Species Impacted: Rainbow Trout

Listing of Project Objectives:

1) Produce strains of rainbow trout with superior growth and disease resistance performance.

2) Define the architecture of energetic partitioning and the regulatory genes and proteins affecting partitioning of energy into growth, stress, and reproductive traits.

3) Establish a tetraploid line of rainbow trout for the production of triploid, reproductively sterile offspring by crossing with diploids.

CRIS Project Title: *Host, Pathogen and Environmental Interactions in Cool and Cold Water Aquaculture*
(NP 106 Components – II, V, and VI)

SY (3.0): G.Wiens (Lead – 100%), T. Welch (100%), B. Brazil (100%).

Appropriated Annual Funding: $1,098,319 (NTL)

Related Sibling CRIS Projects:

a) Identification of Critical Leukocyte binding Determinants on *R. salmoninarum.*
b) Genome Sequencing of the Vertically-Transmitted Fish Pathogen *Renibacterium salmoninarum.*
c) Genome Sequencing and Identification of Virulence Factors in *Flavobacterium psychrophilum*.

Species Impacted: Rainbow Trout

Listing of Project Objectives:

1) Characterize virulence determinants produced by the rainbow trout pathogens *Yersinia ruckeri* and *Flavobacterium psychrophilum*.

2) Identify rainbow trout genes and factors that are critical to expression of innate and acquired immunity against *Y. ruckeri* and *F. psychrophilum*.

3) Identify water quality factors that influence host susceptibility and pathogen refuge within a recirculating system, and evaluate the use of bacteriophage as a targeted antibacterial strategy in recirculating and serial reuse aquaculture systems.

CRIS Project Title: *Development of Sustainable Land-Based Aquaculture Production Systems*
(NP 106 Components – II, V, VI)

SY (0.0): (0%) W. Herschberger

Appropriated Annual Funding: $1,671,919 (NTL)

Related Sibling CRIS Projects:

a) Development of Sustainable Land-Based Aquaculture Production Systems
(Specific Cooperative Agreement)

Species Impacted: Rainbow Trout, Arctic Char, Atlantic and Pacific Salmon, Tilapia, Sturgeon, Hybrid Striped Bass, Barramundi, Walleye, Yellow Perch, Sea Bream, Halibut, and Ornamental Fish

Listing of Project Objectives:

1) Develop and evaluate solutions that improve efficiencies of scale and reduce water quality constraints for sustainable production in controlled intensive aquaculture systems.

2) Develop and evaluate sustainable waste management technologies that result in environmentally compatible controlled intensive aquaculture systems.

3) Field test selected rainbow trout germplasm resources for performance in intensive recirculating aquaculture systems.

Madison (Milwaukee), Wisconsin
U.S. Dairy Forage Research Center

CRIS Project Title: *Improving Great Lakes Aquaculture Production*
(NP 106 Components - I, II, III, IV)

SY (1.00): Brian S. Shepherd (Lead/ARS -- 100%); M. Neal (.00)

Appropriated Annual Funding: $508,478 (NTL)

Related Sibling CRIS Projects:

a) Improvement in Great Lakes Aquaculture Production
b) Novel Approaches for Improving Perch Growth in RAS Systems

Species Impacted: Great Lakes Finfish - Yellow Perch & Rainbow Trout

Listing of Project Objectives:

This is a new project initiated in FY'04 and with ARS funding beginning in FY'05. The initial project had fairly broad objectives to address aquaculture needs for Great Lakes finfish species and work was conducted by scientists at the Great Lakes WATER Institute, University of Wisconsin-Milwaukee. As a consequence, these participants have made significant scientific progress on rainbow trout and yellow perch using non-ARS funds. Other funding sources include Wisconsin Sea Grant, North Central Regional Aquaculture Center and the USDA/NRICGP. In 2006, the ARS & GLWI hired a lead SY who prepared an approved Project Plan with the objectives stated below.

1) Initiate a genomics program that will enable the future development of a genetically defined improved broodstock(s) of yellow perch. Specifically, genomic DNA from geographically-distinct populations of yellow perch will be collected and genetic diversity will be determined using microsatellite analyses.

2) Develop molecular and proteomic tools needed to advance the genetic improvement of yellow perch.

3) Characterize physiological mechanisms that influence growth in yellow perch.

4) Improve culture technologies for year-round fingerling production and 1-year grow-out for yellow perch.

APPENDIX 4 – ANNUAL REPORT INFORMATION (2001-2006)

National Program 106: Aquaculture

National Program Annual Report Introductions
Fiscal Years 2001 – 2006

2001: The Agricultural Research Service (ARS) Aquaculture National Program expanded in four of the six research program component areas: Genetic Improvement; Integrated Aquatic Animal Health Management; Aquaculture Production Systems; and Sustainability and Environmental Compatibility of Aquaculture.

Dr. Henry (Hank) Parker returned to the National Program Staff in April 2001, after detail assignments as Acting Director, U.S. Horticultural Research Laboratory, Fort Pierce, Florida, and Acting Associate Area Director, ARS North Atlantic Area. Dr. Lewis (Lew) Smith served as Acting National Program Leader for Aquaculture while Dr. Parker was on detail assignment.

Several significant activities occurred during this reporting period. On August 31, 2001, Senator Robert C. Byrd dedicated the new National Center for Cool and Cold Water Aquaculture (NCCCWA) in Leetown, West Virginia, and was the keynote speaker for the dedication ceremony. The Center also held a program planning workshop with customers, stakeholders, and research partners on October 18-19, 2001.

On March 5-7, 2001, ARS and the Oceanic Institute cohosted a workshop, "BiotechnologyAquaculture Interface: The Site of Maximum Impact" in Shepherdstown, West Virginia. The workshop report and related information are available at http://nps.ars.usda.gov/static/arsoibiotecws2001 .

With new Congressional funding in fiscal year (FY) 2001, ARS initiated program planning and design phases for a new ARS National Cold Water Marine Aquaculture Center to be constructed in Orono and Franklin, Maine, in cooperation with the University of Maine. On April 26-27, 2001, ARS held a program planning workshop in Orono with customers, stakeholders, and aquaculture scientists to obtain input into the research programs, target species, and identify facility requirements of the new Center.

With new Congressional funding in FY 2001, ARS also initiated aquaculture research programs with the Canaan Valley Institute, Davis, West Virginia; the Harbor Branch Oceanographic Institution, Ft. Pierce, Florida; and the University of Connecticut.

The public investments in ARS aquaculture research are reaping important dividends.

2002: The Aquaculture National Program expanded in five of the seven research program component areas: Genetic Improvement; Integrated Aquatic Animal Health Management; Aquaculture Production Systems; Sustainability and Environmental Compatibility of Aquaculture: and Quality, Safety, and Variety of Products for Consumers.

In January 2002, Dr. Henry (Hank) Parker, former National Program Leader for Aquaculture, became the Associate Area Director for the ARS North Atlantic Area Office. Dr. Lewis (Lew) Smith became the current National Program Leader.

ARS and the State Cooperative, Research, Education and Extension (CSREES) planned a joint National Program Planning Workshop in St. Louis, MO, November 20-21, 2002.

In cooperation with the University of Maine and the Maine Aquaculture Industry, ARS continued the required program planning and design phases for a new ARS National Cold Water Marine Aquaculture Center to be constructed in Orono and Franklin, Maine,

The Harry K. Dupree Stuttgart National Aquaculture Research Center, Stuttgart, AR, hosted a workshop on Genetic Improvement and Selective Breeding for the Hybrid Striped Bass Industry on October 22-23, 2002.

ARS provided partial funding support for the 4th International Symposium on Aquatic Animal Health in New Orleans, LA on September 1-5, 2002 and the 2nd International Seafood Byproduct Conference in September, 2002

A patent for "Streptococcus iniae Vaccine" was issued April 30, 2002. Two other patents are being filed for the detection and quantification of pathogenic microorganisms in salmonids.

In February 2002, USDA and Mississippi State University completed a joint release of an improved catfish, under the commercial name NWAC103.

2003: The Aquaculture National Program NP 106 had another great year with expanded program, scientific productivity, and recognition of scientific quality. The FY 2003 appropriations expanded aquaculture research in five of the seven research program components areas. ARS implemented a new program on genetic improvement of North American Salmon at the National Cold Water Marine Aquaculture Center, Orono/Franklin, Maine.

A new Action Plan was posted on the Aquaculture National Program (106) home page. This new Action Plan is the product of the Joint ARS/CSREES Program Planning Work Shop held in St. Louis, Missouri, November 20-22, 2002. The writing teams are complimented on the comprehensiveness and quality of the document. The Aquaculture National Program is in the first phase of scientific peer review with prospectuses in the process of being written.

The Catfish Genetics Research Team received an USDA, ARS Technology Transfer Award for creative, sustained, and effective research leading to the development, evaluation, and transfer of NWAC103 catfish line to the commercial catfish industry.

Welcome to 4 new permanent full-time scientists (Ric Barrows, Gibson Gaylord, Richard Eaton, and Charles Wierich) who joined ARS in 2003 to work on aquaculture. Four proposals for post-docs (Class of FY 2004) were awarded to scientists in the Aquaculture National Program.

ARS scientists delivered 15 invitational scientific presentations at national and international conferences. The Agency helped support five symposia, workshops, field days or annual association meetings through partial funding, participation, sponsorship or hosting on use of fishery byproducts, sources of protein for fish feed, genetic improvement of striped bass, re-circulation aquaculture forum and trout production.

Three new patents were filed with the ARS Patent Committee.

2004: The Aquaculture National Program enjoyed an exciting year with expanded program, new hires, scientific productivity, and recognition of scientific quality. The FY 2004 appropriations increased aquaculture research in four of the seven research program component areas. ARS implemented new program on Great Lakes Aquaculture at Milwaukee, WI.

The Aquaculture National Program was peer reviewed during 2004. The program was organized into 26 projects that were submitted to two review panels. I am proud to report that 70 percent of the projects were rated needing only minor revision or needing no revision.

Congratulations to Brian Bosworth, Mid-South Area and Ken Overturf, Pacific West Area for being named Early Career Scientists of the Year.

Welcome to 7 new permanent full-time scientists:
Shawn McNulty, microbiologist-Aquatic Animal Health, Auburn, AL
Sylie Quiniou, Molecular Immunologist, Catfish Genetics, Stoneville, MS
Peter Silverstein, Virologist, Catfish Genetics, Stoneville, MS
LaTonya Clay. Molecular Biologist, Catfish Genetics, Stoneville, MS
Cynthia Bower, Food Science/Microbiologist, Subarctic Agricultural Research. Fairbanks, AK
Philip Pearson, Agricultural Engineer, Aquaculture Production Systems, Pine Bluff, AR
Brett Dumbauld, Ecologist, Newport, OR

Two proposals for post-docs (Class of FY2005) were awarded to scientists (Camara and Bilodeau) in the Aquaculture National Program.

ARS scientists delivered six invitational scientific presentations at national and international conferences. The Agency helped support symposia, workshops, field days or annual association meetings through partial funding, participation, sponsorship or hosting on various aquaculture research related topics.

Seven patents were filled or awarded in FY2004. Les Torrans received Honorable Mention for Excellence in Technology Transfer from the Federal Laboratory Consortium, Southeast Region for the "Sock Saver" method to deliver oxygen to catfish. He also received a Mid-South Area Technology Transfer Award.

2005: The Aquaculture National Program continues to receive strong support from stakeholders. The FY 2006 appropriations increased aquaculture research in four of the seven program components at five locations. Construction for the National Cold Water Marine Aquaculture Center, Franklin, Maine began in fall 2005.

Two research scientists changed positions within ARS and two vacancies were filled. Ken Davis was selected for the Research Leader of the Catfish Genetics Research Unit, Stoneville, MS, and Brian Shepherd was selected to start the new research project on Great Lakes Aquaculture at Milwaukee, WI. Welcome to Keshun Liu, Research Chemist (cereal) and Gongshe Hu, Research Geneticist who joined the ARS aquaculture feeds research group at Aberdeen, Idaho.

Congratulations to Timothy Welsh, National Center for Cool and Coldwater Aquaculture, Leetown, WV for being awarded an Administrator's Post-Doctural, Class of FY 2006 for his proposal entitled "Utilizing phage as targeted antibiotics in aquaculture".

Craig Shoemaker, Joyce Evans, and Phil Klesius received the 2005 ARS Technology Transfer Award for Outstanding Effort and the 2005 FLC Southeast Region Excellence in Technology Transfer for catfish vaccines for prevention of the two major diseases of catfish. The Rapid Identification Assay for Channel Catfish Fillets was a product selected for supporting the ARS Budget presentation at the Congressional Hearing.

One U. S. patent was granted on modified live Flavobacterium columnare vaccine.

Scientists were active in holding customer workshops and field days, organizing and chairing professional scientific meeting sessions, and delivering local, national, and international invitational scientific talks.

Four scientists (Barrows, Hershberger, Silverstein, Rexroad, and Wolters) participated in a trilateral meeting between Norway, Canada, and the United States to determine shared goals and research priorities for genomics and breeding in aquaculture species. Follow-up conference calls will take place in January 2006 and the next meeting is planned to take place in Canada in 2006.

The Freshwater Institute, Shepherdstown, WV (an ARS partner) solved the problem of "what to do with market size research animals when the research is completed". They made an arrangement to donate fish to the Virginia Federation of Food Banks and the West Virginia Mountaineer Food Bank as well as several local shelters. Composting is often the only way to dispose of animals once the research is completed, but it is a more expensive and wasteful solution.

2006: The Aquaculture National Program continues to receive strong support from stakeholders. The FY 2006 appropriations bill for ARS contained increases for aquaculture research in three of the seven program components at five locations. Construction for the National Cold Water Marine Aquaculture Center, Franklin, Maine began in the fall, 2005 and is nearing completion. Construction funds were in the appropriations for the Hagerman Fish Culture Experiment Station, Hagerman, Idaho and the National Cold Water Marine Aquaculture Research Center, Orono, Maine. These items are pending Congressional action.

The National Cold Water Marine Aquaculture Center in Franklin, ME completed 2 years of biannual fish health inspections in 2006 on all year classes of Atlantic salmon. The biannual inspections evaluated fish for ten different pathogens and none were detected. The research facility's salmon stocks in the breeding program now have disease-free status and permits can be obtained from state, federal, and international sources for transfer of fish or eggs from the breeding program to other facilties.

Our scientists in Fairbanks Alaska participated in "ComFish" the largest show and exhibition for the commercial fishing industry in Alaska and "Changing Tides: Wild Alaska Salmon" showing potential new products from Alaska salmon byproducts

Scientists at the NCCCWA were awarded $700,000 from the CSREES-NRI competitive research grants program for Animal Genomics. The award was given to Y. Palti (PI) and co-PIs C. Rexroad III, R. Vallejo and M-C Luo (UC Davis) to complete a scaffold of bacterial artificial chromosome (BAC) physical map for the rainbow trout genome and to integrate the physical map with the trout genetic linkage map.

Scientists at the Catfish Genetics Research Unit published the physical map for catfish, Ictalurus Punctatus at http://www.ars.usda.gov/Main/docs.htm?docid=14234 .

Scientific Recognition

Brian Small, Catfish Genetics Research Unit, received the Area Early Research Scientist Award "For the impact of collaborative research and technology transfer related to the improvement of catfish germplasm and production practices".

Brian Small was appointed to the 2007 editorial board for the journal Comparative Biochemistry and Physiology.

Les Torrans was awarded runner-up for best paper of 2005 by North American Journal of Aquaculture for the article entitled “Effect of oxygen management on culture performance of channel catfish in earth ponds, NAJA 67:275-288.

Scientists delivered numerous invited presentations at national and international workshops and conferences.

Phil Klesius agreed to co-chair the Biologics Committee of the Joint Subcommittee on Aquaculture.
Dr. Chhorn Lim with collaborators edited yet another book: “Tilapia: Biology, culture and nutrition”.

The Fish Disease/Therapeutic Drug Approval research group at the Harry K. Dupree SNARC received the Food and Drug Administration’s Leveraging Collaboration Award as a member of the Aquaculture Working Group/Drug Approval Coordination Workshop.

Patents
A world-wide animal health company is developing and licensing a Streptococcus agalactiae vaccine invented by scientists at the Aquatic Animal Health Research Unit. The vaccine will be protected by 25 foreign patents. Several vaccines are being commercialized that are health management tools for use in biosecurity and preventing the introductions of domestic and foreign disease agents into U.S. fish farms.

Welcome New Scientists

Dr. Adam Fuller, Research Geneticist was selected by the Harry K. Dupree, Stuttgart National Aquaculture Center to lead research on genetic improvement of hybrid striped bass.

Dr. Julie Bebak , DVM was selected by the Aquatic Animal Health Research Unit to lead the epidemiology research on warm water fish pathogens.

Dr. Ted Wu an environmental chemist was hired as a postdoctoral fellow to work with Peter Bechtel on fishery byproducts.