

Aquaculture (NP 106) Annual Report for 2007

Introduction

The mission of the Aquaculture National Program is to conduct high quality, relevant, basic and applied aquaculture research, to improve the genetic foundation of domesticated aquaculture species, and to conduct technology transfer in order to enhance the productivity and efficiency of US producers, and the quality of seafood and other aquatic products. Jeff Silverstein, Ph.D, National Program Leader (NPL), Aquaculture, began managing the program in October 2008.

Dr. Brian Small with the Catfish Genetics Research Unit in Stoneville, MS was honored as the recipient of the “Distinguished Early Career Award in U.S. Aquaculture” presented by the United States Aquaculture Society, a chapter of the World Aquaculture Society.

Veterinarian and experienced epidemiologist, Dr. Julie Bebak joined the Aquatic Animal Health Research Unit in Auburn, AL. Her background and experience brings new strengths to this unit. Dr. William Hershberger, the director of the National Center for Cool and Cold Water Aquaculture, one of the larger aquaculture research centers for ARS, retired in September, 2007 after eight busy and productive years. His replacement is currently being sought.

The Aquaculture National Program completed the fourth year of the five year National program cycle in Fiscal Year (FY) 2007. The Aquaculture National Program currently includes 27 core research projects supported by 60 scientists located at 15 research sites throughout the country. The ARS research budget for the Aquaculture Program FY 2007 was \$ 32 million (NTL).

Construction began on the broodstock facility for the National Center for Cool and Cold Water Aquaculture. This facility is expected to be completed in early 2008 and will hold the rainbow trout brood fish for the Center’s selective breeding program.

Fish health inspections have been conducted on breeding populations of Atlantic salmon at the Franklin, Maine location and rainbow trout at the Leetown, WV location making them eligible for certification status. All populations have been free of reportable pathogens thus far.

Scientists in the Aquaculture National Program were well recognized nationally and internationally with over 40 invited presentations. ARS Aquaculture scientists were successful in applying for extramural grants with 9 awards being made totaling over \$450,000.00.

Scientists within the National Aquaculture Program were very active in their fields during FY 2007, with more than 90 articles in peer-reviewed scientific journals. Many of the discoveries and findings were published in the popular press to reach customers and stakeholders, including 99 articles in trade journals and book chapters. Technology

transfer activities for the National Aquaculture Program included 4 invention disclosures, 13 new Cooperative Research and Development Agreements (CRADA) and Material Transfer Agreements (MTA).

The following section of the report summarizes high impact research results addressing objectives in the current national program action plan.

Genes regulating egg development. Improving egg quality, reflected by better fertilization and hatching rates is important for the trout industry. Research conducted at the National Center for Cool and Cold Water Aquaculture examined 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 work has shown differences in the location of expression of several key genes, some growth factors are expressed in the follicle cells surrounding the oocytes whereas other key growth factors are predominantly expressed in the oocyte. Additionally, this work has revealed the time course of events leading up to spawning. These data suggest that these peptide dynamics may be critical to the process of oocyte development and therefore may be important for egg quality. (National Program 106 and Performance Measure III.C.1)

Scientific Publication:

Weber, G.M., Moore, A.B., Sullivan, C.V. 2007. In Vitro Actions of Insulin like Growth Factor-I on Ovarian Follicle Maturation in White Perch (*Morone americana*). *General and Comparative Endocrinology* 151, 180-187.

Identification of new rainbow trout immune genes. Infectious disease is a significant factor hindering aquaculture and a better understanding the fish immune system is required to improve health and disease resistance. Studies at the National Center for Cool and Cold Water Aquaculture identified a family of forty-four new tumor necrosis factor (an important group of immune system genes) gene sequences from rainbow trout and other fish species. Comparison of the protein sequences and chromosomal locations allowed us to identify which genes were related to those found in mammals and which were unique to fish. We developed methods for measuring gene expression in rainbow trout tissues. These studies have led to a better understanding of how the fish immune system functions and how it differs from that of mammals. The newly developed gene detection methods will advance studies on gene expression and functional analyses in fish. (National Program 106 and Performance Measure II.C.1).

Scientific Publication:

Glenney, G.W., Wiens, G.D. 2007. Early Diversification of the Tumor Necrosis Superfamily in Teleosts: Genomic Characterization and Expression Analysis. *Journal of Immunology*, 178:7955-7973.

Disinfection in recirculating fish culture systems. Pathogens and other microbial populations can accumulate and compromise fish health in fish culture systems that recirculate water. Scientists at The Conservation Fund's Freshwater Institute (Shepherdstown, WV) determined the process requirements necessary to achieve full-flow disinfection of recycled water using ozonation followed immediately by ultraviolet irradiation. The entire recirculating flow could be effectively disinfected when the ozone dose was controlled in a feed-back loop using probes that measured either dissolved ozone concentration or oxidation reduction potential. Thus, combining ozone with ultraviolet irradiation in a recirculating system can prevent the accumulation of most fish

pathogens and significantly reduce the risk of spreading fish disease. These findings will be used to produce more biosecure aquatic production systems that sustain healthier and more growth promoting environments. (National Program 106 and Performance Measure VI.B.1).

Scientific Publication:

Sharrer, M.J., Summerfelt, S.T. 2007. Ozonation followed by ultraviolet irradiation provides effective bacteria inactivation in a freshwater recirculating system. *Aquacultural Engineering* 37, 180-191.

Weaning rock sole onto artificial feeds with chemical cues. Getting larval fish to switch to artificial feeds from the live feeds they begin feeding on, is difficult. Scientists at the NOAA Fisheries in Manchester, Washington in collaboration with scientists at the University of Idaho Hagerman Fish Culture Experiment Station, the University of Alaska's Fishery Industrial Technology Center in Kodiak, Alaska and the Subarctic Agricultural Research Unit in Fairbanks evaluated different Alaska fish processing byproducts for their ability to stimulate the weaning of rock sole (*Lepidopsetta* spp.) larvae onto prepared feeds. L-alanine, Artemia culture water and red salmon stickwater (the soluble protein fraction resulting from protein meal processing) were chosen as the chemical cues, and inert metal oxides were incorporated at known proportions into the feeds to measure their consumption. When red salmon stickwater was added to the culture medium before weaning, the fish were more likely to take up the microparticulate food on the first and second days post-weaning than fish that were not given the chemical cue. These results indicate the potential for using stickwater or compounds derived from stickwater in fish larvae production. Use of this material may enhance the larval survival of marine fish larvae. (National Program 106 and Performance Measure VII.F.1).

Scientific Publication:

Sathivel, S., Bechtel, P.J., Prinyawiatkul, W. 2006. Physicochemical and Rheological Properties of Salmon Protein Powders. *International Journal of Food Engineering*. Vol. 2 : Iss. 2, Article 3. Available at: <http://www.bepress.com/ijfe/vol2/iss2/art3>

Need for taurine in plant protein diets for rainbow trout. The use of plant proteins in aquafeeds is growing. Although rainbow trout have some capacity for taurine biosynthesis from sulfur amino acid precursors, taurine has been identified as a potential limiting nutrient in plant-based diets for rainbow trout. Methionine is a precursor sulfur amino acid that can be supplemented to animal feeds more cost effectively than taurine. Research conducted at the Hagerman Fish culture Experiment Station by ARS scientists in collaboration with the University of Idaho tested the efficacy of supplementing methionine and taurine separately and in combination on production performance of rainbow trout. Using metabolite profiling technologies this experiment determined that bioconversion of methionine to taurine was limiting and supplementing taurine was necessary. The impact of this research is to verify the need for taurine supplementation in plant-based diets for rainbow trout and continue to refine plant-based aquafeeds to

reduce dependence on fish meals. (National Program 106 and Performance Measure IV.D.1).

Scientific Publication:

Gaylord, T.G., Teague, A.M., Barrows, F.T. 2006. Taurine supplementation of all-plant protein diets for rainbow trout *oncorhynchus mykiss*. Journal of the World Aquaculture Society. 37:509-517.

Gaylord, T.G., Barrows, F., Teague, A.M., Johansen, K.A., Overturf, K.E., Shepherd, B.S. 2007. Supplementation of taurine and methionine to all-plant protein diets for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 269:514- 525.

Improved treatment for Asian tapeworm in baitfish. Commercially raised fish infected with Asian tapeworms have limited potential for sale and therefore can be a detriment to the producer. The efficacy of bath treatments of praziquantel against these tapeworms was evaluated by scientists at the HKD Stuttgart National Aquaculture Research Center in heavily infected grass carp, and a 24-h bath treatment of 0.75 mg/L eliminated all tapeworms from the fish. The impact will be an economic benefit by allowing producers to ship their fish to states that require tapeworm-free fish. (National Program 106 and Performance Measure II.B.3).

Scientific Publication:

Mitchell, A.J., Hobbs, M.S. 2007. The acute toxicity of praziquantel to grass carp and golden shiners. North American Journal of Aquaculture. 69:203-206.

Performance of low-salinity recirculating aquaculture system. Low-salinity recirculating systems can eliminate the need for marine aquaculture to be located near limited and expensive coastal land thereby expanding opportunities for marine aquaculture; however, little information exists regarding efficient operation of low-salinity recirculating systems. Juvenile Florida pompano were raised to market-size in a low salinity production-scale recirculating system by scientists at the ARS Sustainable Marine Aquaculture Project in Fort Pierce, FL. Pompano were reared for 300 days from 30 g to 620 g, and the efficiency of ammonia removal, feed and waste particle removal, and oxygen, water, and energy use were monitored on the system components as increasing amount of feed inputs were used to maximize fish growth. The accomplishment provides culturists and engineers baseline performance and design criteria over a complete growout cycle for the construction of production-scale recirculating systems to produce market-size marine fish in low salinity. (National Program 106 and Performance Measure V.F.1).

Scientific Publication:

Weirich, C.R., Riche, M.A. 2006. Tolerance of juvenile black sea bass *centropomus striata* to acute ammonia and nitrite exposure at various salinities. *Fisheries Sciences*. 72(5):915-921.

Riche, M.A. 2006. Analysis of refractometry for determining total plasma protein in hybrid striped bass (*Morone chrysops* x *M. saxatilis*) at various salinities. *Aquaculture*. 264:279-284.

Estimation of phenotypic and genetic (co)variances for economically important traits in catfish. Estimation of phenotypic and genetic (co)variances for economically important traits are required for development of a breeding program to produce superior catfish germplasm for release to catfish producers. Data were collected for growth, resistance to enteric septicemia of catfish (ESC, the most devastating disease for catfish production), and processing yield and resulted in heritability estimates of approximately 0.35, 0.10 and 0.25, respectively. The best performing animals from families of the third generation of selection of a channel catfish line were selected as future broodstock based on an index for these traits. Selected broodstock were mated to produce another generation of families which are currently being evaluated for growth, resistance to ESC, and processing yield. This information is being used in a continued selection program to develop superior germplasm for release which will benefit producers, processors, and consumers. (National Program 106 and Performance Measure I.B.3).

Scientific Publication:

Bosworth, B.G., Wolters, W.R., Silverstein, J., Li, M.H., Robinson, E.H. 2006. Family, strain, gender, and dietary protein effects on production and processing traits of norris and NWAC103 strains of channel catfish, *Ictalurus punctatus*. *North American Journal of Aquaculture* 69:106-115.

Peterson, B.C., Small, B.C. 2007. Effects of GH on Immune and Endocrine Responses of Channel Catfish Challenged with *Edwardsiella ictaluri*. *Comparative Biochemistry and Physiology A* 146:47-53.

Reproductive efficiency among catfish strains. The spawning rate of catfish throughout the industry is low (~30%) and requires the stocking of an excess of broodfish to insure adequate numbers of juveniles are produced. Although breeding programs to improve channel catfish traits for commercial aquaculture exist, there is a lack of data on reproductive parameters related to spawning. In order to evaluate potential strain differences for reproductive traits, four selectively bred commercial strains of channel catfish were evaluated in a 12-month study, during which critical reproductive indices were measured, including various sex steroids, proteins, and enzymes. These data are being used to assess what controls reproductive success in channel catfish in order to develop tools to rapidly identify strains or individuals with greater reproductive potential. (National Program 106 and Performance Measure III.A.1).

Scientific Publication:

Barrero, M., Small, B.C., D'Abramo, L., Hanson, L., Kelly, A. 2007. Comparison of Estradiol, Testosterone, Vitellogenin and Cathepsin Profiles Among Twoyear- old Channel Catfish (*Ictalurus punctatus*) Females from Four Selectively Bred Strains. *Aquaculture* 264:390-397.

Assess antibody and cellular immune responses against major fish pathogens. Disease is the major cause of fish loss. Knowledge of antibody and cellular immune responses is essential for the development of vaccines against major fish pathogens. The protective role of antibodies found in serum and mucus against the pathogens *Streptococcus iniae*, *S. agalactiae* and *Ichthyophthirius multifiliis* was assessed. Antibodies from the blood of tilapia immunized against *S. iniae* and *S. agalactiae* protected naïve tilapia from infection with *S. iniae* and *S. agalactiae*, respectively. Mucus antibody from the skin of channel catfish immune to *Ichthyophthirius* prevented *Ich* infections. Research demonstrated that extracellular products of the streptococcal vaccines are important pro-inflammatory molecules that initiate the cellular immune response to streptococcal infections and vaccination. (National Program 106 and Performance Measure II.C.1).

Scientific Publication:

Klesius, P.H., Evans, J.J., Shoemaker, C.A. 2007. The macrophage chemotactic activity of *Streptococcus agalactiae* and *Streptococcus iniae* extracellular products (ECP). *Fish and Shellfish Immunology*. 22(5)443-450.

Pasnik, D.J., Evans, J.J., Klesius, P.H. 2006. Passive immunization of Nile tilapia (*Oreochromis niloticus*) provides significant protection against *Streptococcus agalactiae*. *Fish and Shellfish Immunology*. Vol 21 Issue 4 pages 365-371.

Alternative protein sources and agricultural byproducts. As a result of the recent expansion and increase in ethanol production for fuels due to the shortage and rising cost of petroleum-based fuel, the distiller's dried grains with solubles (DDGS) production in the U.S. has been estimated to greatly increase in the years to come. Thus, new uses for DDGS are needed. A feeding study indicated that, with lysine supplementation, at least 40% DDGS can be included in catfish feeds as replacement of a combination of Soybean Meal (SBM) and corn meal on an equal protein basis without affecting their growth performance. Incorporating 20 to 40% DDGS with lysine also improved some immune parameters and the resistance of catfish to *E. ictaluri*. For tilapia, 30% DDGS can be used as a substitute for soybean and corn meal without requiring the addition of lysine. With lysine supplementation, DDGS can be included at 60% level as a total replacement of soybean meal without affecting fish growth and feed efficiency for tilapia. Dietary levels of DDGS, however, had no effect on immune response and resistance of tilapia to *S. iniae*. (National Program 106 and Performance Measure IV.C.2).

Scientific Publication:

Lim, C.E., Garcia, J.C., Aksoy, M., Klesius, P.H., Shoemaker, C.A., Evans, J.J. 2007. Growth Responses and Resistance to *Streptococcus iniae* of Nile Tilapia, *Oreochromis niloticus* Fed Diets Containing Distiller's Dried Grains with Solubles. *Journal of the World Aquaculture Society*. Vol. 38, No. 2. p. 231-237.

The influence of multiple pathogens on fish was examined. The major cause of loss of production in aquaculture is disease, and simultaneous infection by multiple pathogens, concurrent infections (i.e. parasite and bacteria), are common in cultured fish. *Gyrodactylus* spp. (monogenetic trematodes) are common fish parasites that cause mechanical injuries on fish skin, and gills and lead to fish mortality under crowded conditions. *Streptococcus iniae* is a severe bacterial pathogen and causes heavy economic losses in aquaculture. No information was available on the association of *Gyrodactylus* and *Streptococcus* in tilapia. To better understand the invasion mechanism and control of the parasite, methods for culturing and maintaining *Gyrodactylus* under laboratory conditions and methods for harvesting the parasite were developed. Techniques were perfected to allow for survival of the parasite for about 3-4 day in the laboratory. Previous results suggested survival for only 24 h without a fish host. This technique was used to demonstrate that parasitism in tilapia with *Gyrodactylus* increased infection and mortality following exposure to *S. iniae*. The mechanical injury from the parasite apparently provided a portal of entry for the bacterium. Study results also suggest that the parasite harbored viable bacteria and may be a vector for transmission. This model will enable research and improve outcomes when facing concurrent infections. (National Program 106 and Performance Measure II.D.3).

Scientific Publication:

Xu, D., Shoemaker, C.A., Klesius, P.H. 2007. Evaluation of the link between gyrodactylosis and streptococcosis of Nile tilapia, *Oreochromis niloticus* (L.). *Journal of Fish Diseases*. 30: 233-238.

Evans, J.J., Klesius, P.H., Pasnik, D.J., Shoemaker, C.A. 2007. Influence of natural *trichodina* sp. parasitism on experimental *streptococcus iniae* or *streptococcus agalactiae* infection and survival of young channel catfish *ictalurus punctatus* (rafinesque). *Aquaculture Research*. Volume 38, Issue 6:664-667.