Aquaculture (NP 106) Annual Report for 2008

Introduction

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

Mission: 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 to enhance the productivity and efficiency of U.S. producers and the quality of seafood and other aquatic animal products.

The primary aim of the ARS Aquaculture Program, as described in the National Program 106 Action Plan, is to help develop and ensure an abundant, safe, and affordable supply of seafood products within a healthy, competitive, and sustainable aquaculture sector; this sector is supported by over 4,300 aquaculture farmers that produced in excess of $1 billion dollars worth of goods in 2005 (NASS, 2005 Census of Aquaculture).

During 2008, 61 full time scientists working at 14 locations across the U.S. were actively engaged in 24 research projects in the program. One year of work on current project efforts remains before the next evaluation by the ARS Office of Scientific Quality Review.

This year saw increased emphasis on the development of alternative feeds for aquaculture with the associated Initiative by USDA and the National Oceanic and Atmospheric Association (NOAA). A scientific expert panel met in late February with broad ARS participation (Kurt Rosentrater, distillers dried grains with soluble; Eliot Herman, soybean genetics; Victor Raboy, cereal genetics; Peter Bechtel, fish processing co-products; Rick Barrows, fish nutrition and feed processing; and Jeff Silverstein, Aquaculture National Program Leader). In late April, a second stakeholder meeting was held in Washington, D.C., to gather input from environmental groups, producers/representatives of non-traditional feedstuffs, feed manufacturers, and aquaculture producers.

Retirement: Retiring from the ranks of the scientists in the national program during 2008 was Victor Panangala from the ARS Aquatic Animal Health Research Unit. He retired in September 2008 after 5 productive years.

Recruitments: New permanent scientists welcomed to the program include Jason Evenhuis and Timothy Leeds (Leetown, WV); Benjamin LaFrentz and Julia Pridgeon (Auburn, AL); Natha Booth (Stoneville, MS). Caird Rexroad III became the Research

Leader and Center Director at the National Center for Cool and Cold Water Aquaculture in April 2008, following the FY 2007 retirement of Dr. William Hershberger.

Awards and Recognitions:
Scientists in the Aquaculture National Program were well recognized nationally and internationally over the past year, with over 100 invited presentations among them. The following scientists in the Aquaculture National Program were recognized with prominent awards:

> Marty Riche, Stuttgart National Aquaculture Research Center,
> Harbor Branch Oceanographic Institute, Ft. Pierce, Florida
> (ARS Early Career Scientist Award, Southern Plains Area)
>
> Les Torrans, Catfish Genetics Research Unit, Stoneville, Mississippi
> (2008 ARS Superior Effort in Technology Transfer Award)
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> Les Torrans, Catfish Genetics Research Unit, Stoneville, MS (with James Steeby, Mississippi State University) **"Improved Oxygen Management in Channel Catfish Hatcheries,"** Federal Laboratory Consortium SE Region 2008 Excellence in Technology Transfer Award

Scientists within the National Aquaculture Program were active during FY 2008, publishing more than 110 articles in peer-reviewed scientific journals. Many of the discoveries and findings were published in the popular press to reach customers and stakeholders, including 40 articles in trade journals and book chapters. Technology transfer activities for the National Aquaculture Program included 7 invention disclosures and 10 new Cooperative Research and Development Agreements (CRADA) and Material Transfer Agreements (MTA).

New Facilities:
The ribbon cutting ceremony for the National Cold Water Marine Aquaculture Center in Franklin, Maine, took place in May 2008 with attendance by Maine Governor Baldacci, Congressman Michaud, University of Maine President Kennedy, and Deputy Under Secretary for REE Dr. Merle Pierson, along with ARS National Program and Area representation.

Construction on the broodstock facility for the National Center for Cool and Cold Water Aquaculture was also completed in the summer of 2008. This facility is expected to be fully operational in early 2009 and will hold the rainbow trout brood fish for the Center's selective breeding program.

Funding: During fiscal year 2008, ARS operated under a budget that was unchanged from the previous year, but many projects that had previously operated on "earmarked" funds were redirected to permanent funding. Total funding in the Aquaculture National Program for 2008 was approximately $32M.

Research Results

The following section of the report summarizes the specific high impact research results addressing objectives in the current National Program Action Plan.

Component 1: Genetic Resources

Atlantic salmon broodstock development. U.S. salmon production is constrained by lack of genetic improvement, disease, low production efficiency, and the mandatory stocking of 100 percent native North American salmon. ARS initiated an applied Atlantic salmon breeding program to increase efficiency and sustainability of Atlantic salmon culture in 2003, when pedigreed families were obtained from two St. John's River sources, Penobscot River, Gaspé (Quebec), and landlocked salmon stocks. Smolts (salmon approximately 2 years old) were stocked into sea cages in June 2005, harvested in February 2007, and evaluated for carcass weight, sex, and stage of sexual maturity. Overall, fish from St. John's River had the fastest growth with a carcass weight of more than 4 kg, while growth was slowest among landlocked fish. Data were used to calculate breeding values on sibling adult brood fish, and in the fall of 2007, researchers produced a genetic line selected for carcass weight; in addition, approximately 500,000 eggs from these fish were released to industry for breeding. (National Program 106, Performance Measure 2.2.2)

Discovery to simultaneously improve growth and disease resistance in trout. Researchers evaluated fish selected for resistance to Bacterial Coldwater Disease, the most important bacterial disease affecting trout culture, which is caused by the bacterium *Flavobacterium psychrophilum* (Fp). Estimates indicate that 5-, 7-, 9-, and 12-month body weights are highly determined by genetics, that Fp resistance is moderately linked to genetics, and that significant genetic gains in disease resistance can be achieved within one generation of selection. In addition, estimates of genetic correlations indicate that body weight traits and Fp resistance can be improved simultaneously. This work will offer growers a fish more resistant to disease without sacrificing growth performance. (National Program 106, Performance Measure 2.2.2)

Improved procedures for tetraploid induction developed. The U.S. Rainbow Trout industry would be greatly enhanced with the ability to efficiently produce 100 percent sterile triploids for improved growth performance and germplasm protection. Current procedures for tetraploid induction are unreliable and labor intensive because of variability in the time at which induction treatment must be applied. In investigating the factors contributing to this variability, ARS scientists found that rearing environment had was strong, and that by maintaining broodfish in a common environment, pressured could be applied at a single time point to induce tetraploidy in eggs of fish from diverse genetic backgrounds. Scientists also established new methods for sampling up to 20 fish simultaneously for verification of tetraploid induction. These results support the development of procedures for tetraploidy induction that are amply efficient for commercial implementation. (National Program 106, Performance Measure 2.2.2)

> Hershberger, W.K., Hostuttler, M.A. 2007. Protocols for more effective induction of tetraploid rainbow trout. North American Journal of Aquaculture. 69:367-372.

Construction of a physical map for the rainbow trout genome. The development of tools for genomic research in rainbow trout will facilitate our ability to identify genes affecting phenotypes of economic importance in trout culture. Recently, scientists at the National Center for Cool and Cold Water Aquaculture in Leetown, West Virginia, constructed a physical map of the rainbow trout genome that includes two billion base pairs of DNA and represents 75 to 80 percent of the genome. This resource will facilitate genetic improvement by enabling identification of genes that affect important aquaculture production traits, such as disease resistance. (National Program 106, Performance Measure 2.2.2).

Salem, M., Silverstein, J., Rexroad III, C.E., Jianbo, Y. Effect of starvation on global gene expression and proteolysis in rainbow trout (Oncorhynchus mykiss). Biomed Central (BMC) Genomics. 8:328.

Gahr, S.A., Rise, M., Hunt, P., Koop, B., Rexroad III, C.E. 2007. Characterization of expressed sequence tags from the pituitary of rainbow trout (oncorhynchus mykiss). Animal Biotechnology. 18(3):213-230.

Component 2: Performance and Physiology

Development of catfish pituitary extract for inducing ovulation in fish. Previous research demonstrated that catfish pituitary extract was effective for inducing ovulation in female catfish. Based on this work, an INAD (Investigative New Animal Drug) was developed to support the use of this extract as a spawning aid, which is currently being sold by a commercial vendor (Hybrid Catfish Company). In the spring of 2008, catfish pituitary extract was successfully used to induce ovulation in female catfish at 2 commercial hatcheries. (National Program 106, Performance Measure 2.2.2).

Development of methods to control reproduction timing in yellow perch. Aquaculture production has been limited by the lack of year-round availability of yellow perch fingerlings for stocking. ARS research at the Great Lakes Water Institute has developed procedures to manipulate light cycle and temperature to stimulate reproduction in yellow perch outside of their normal reproduction season in March. Yellow perch lines have been developed that spawn in January, March (natural cycle), July, and October, thus enabling fingerling production throughout the year. The July spawning strain has already been used in industry. (National Program 106, Performance Measure 2.2.2).

Bower, C.K., Malemute, C.L., Oliveira, A.C. 2007. Preservation Methods for Retaining n-3 Polyunsaturated Fatty Acids in Alaska Coho Salmon (Oncorhynchus kisutch) Products. Journal of Aquatic Food Product Technology. 16(4):45-54.

Wu, T.H., Bechtel, P.J. 2008. Ammonia, Dimethylamine, Trimethylamine, and Trimethylamine Oxide from Raw and Processed Fish By-Products. Journal of Aquatic Food Product Technology. 17(1):27-38. doi: 10.1080/10498850801891140.

Component 3: Nutrition

Amino acid profile balanced in rainbow trout diets. Current diets for rainbow trout may be over formulated with protein to meet individual amino acid requirements. Researchers at Hagerman, Idaho, found that when diets are formulated with regard to amino acid availability instead of crude protein, growth rate can be maintained and total dietary protein can be reduced. Individual amino acids were supplemented with synthetic lysine, methionine, and threonine to reduce total dietary protein by 11 percent and provide a better amino acid balance than that currently suggested in the literature, thereby increasing protein retention by 35 percent. This research will be useful for reducing both feed cost and nitrogenous waste released into the environment. (National Program 106, Performance Measure 2.2.2).

Quality and nutrients of distillers dried grains with solubles (DDGS) enriched. Increased production of ethanol from corn has resulted in large quantities of distillers dried grains with solubles (DDGS), the waste product of ethanol production. The DDGS could be a plentiful feedstuff for fishfeeds, but the quality of DDGS is not consistent. Researchers believe the quality of the DDGS is affected primarily by the quality of the raw material. ARS researchers at Aberdeen, Idaho, found that the important characteristics of chemical composition, particle size distribution, and color varied greatly among different DDGS materials. Nevertheless, if the DDGS were separated by size the composition of the particle within a size category was more consistent. Color and nutrient content were mostly linked to particle size. DDGS was further separated to produce materials of different chemical composition. Size separating DDGS not only enhances nutritional value but also could expand their use in animal feeds. (National Program 106, Performance Measure 2.2.2).

> Liu, K.S. 2008. Particle Size Distribution of Distillers Dried Grains with Solubles (DDGS) and Relationships to Compositional and Color Properties. BioResource Technology 99:8421-8428

Branched chain amino acid requirements determined for hybrid striped bass. The ideal balance of branched-chain amino acids (leucine, isoleucine, and valine) in the diets of hybrid striped bass is unknown, but requirement estimates are needed to formulate and improve commercial diets. In a series of experiments at the Stuttgart National Aquaculture Research Center, scientists determined the required levels of leucine, isoleucine, and valine for hybrid striped bass. These requirements determined are now used by industry to formulate more complete, cost-effective feeds. (National Program 106, Performance Measure 2.2.2).

Component 4: Health and Welfare

Copper sulfate shown to prevent columnaris disease in exposed catfish. Columnaris disease, caused by *Flavobacterium columnaris,* is one of the most costly diseases for warm water aquaculture. Copper sulfate and potassium permanganate were evaluated for their ability to both prevent and treat columnaris infection in channel catfish. Although the experiment showed limited therapeutic (treatment)effects for infected fish, the studies clearly demonstrated the prophylatic (preventive) ability of copper sulfate, which was shown to reduce bacterial presence in the water by 90 percent. The research thus indicates that prophylactic treatments with copper sulfate may reduce incidence of columnaris. (National Program 106, Performance Measure 2.2.2).

> Griffin, B.R., Mitchell, A.J. 2007. Susceptibility of channel catfish, Ictalurus punctatus, to Edwardsiella ictaluri challange following copper sulfate exposure. Journal of Fish Diseases. 30:581-585.

> Darwish, A.M., Mitchell, A.J., Hobbs, M.S. 2008. In vitro and in vivo evaluation of potassium permanganate treatment efficacy for the control of acute experimental infection of flavobacterium columnare in channel catfish. North American Journal of Aquaculture. 70:314.322.

Hepcidin shown to be a candidate marker for disease resistance. In order to better understand why some families of catfish are more resistant than others to experimental challenges with *Edwardsiella ictaluri*, the causative agent of enteric septicemia of catfish (ESC), ARS scientists developed a standardized screening protocol to distinguish between families with high or low levels of susceptibility. They found that resistant families repeatedly demonstrated less than 30 percent mortality following experimental challenge, while susceptible families demonstrated more than 70 percent mortality. By analyzing gene expression in tissues from infected and non-infected fish, scientists found that a gene called hepcidin is differentially expressed in the two family types, showing a significantly higher expression in liver tissue at 24 hours post-challenge and at 72 hours post-challenge in spleen tissue. Scientists will continue to monitor the expression of hepcidin in further experiments to determine its correlation with resistance to *E. ictaluri* and other pathogens. (National Program 106, Performance Measure 2.2.2).

Natural compound shown to inhibit a common catfish disease-causing bacteria. Research is ongoing to discover natural compounds that are environmentally safe and effective alternatives to current management approaches (*e.g.*, medicated feed containing antibiotics) for two common bacterial diseases of pond-raised channel catfish, columnaris and enteric septicemia of catfish (ESC). In evaluating compounds using a laboratory bioassay, scientists found a natural compound, tannic acid, capable of inhibiting the growth of both disease-causing bacteria. This natural compound may be useful as a therapeutant to help manage and prevent common bacterial diseases in cultured catfish.

> Schrader, K. 2008. *Compounds with Inhibitory Activity against the Channel Catfish Pathogens* Edwardsiella ictaluri *and* Flavobacterium columnare. North American Journal of Aquaculture. 70:147-153

Effects of aquaculture production noise on growth and disease resistance in fish examined. Intensive aquaculture production systems often use equipment such as pumps, air blowers, and filtration systems that increase noise levels in the fish culture tanks; the impact of these sources of noise on fish is unknown. In a collaborative study by scientists at the Freshwater Institute, University of Maryland, and ARS, rainbow trout were subjected to noise levels commonly seen in recirculating aquaculture systems (RAS) to determine their effects on fish auditory systems, stress levels, and overall performance. The results of the study suggest that the hearing sensitivity, growth, survival, stress, and disease susceptibility of rainbow trout were not impacted by noise levels typical of RAS.

> Wysocki, L.E., Davidson, J., Smith, M.E., Frankel, A., Ellison, W., Mazik, P.M., Popper, A.N., Bebak, J.A. 2007. The effects of aquaculture noise on hearing, growth and disease resistance of rainbow trout Oncorhynchus mykiss. Aquaculture. 272(1-4): 689-697.

Selection marker of rainbow trout disease resistance identified. Improved fish resistance to infectious diseases is needed to prevent this substantial source of loss to U.S. rainbow trout aquaculture. At the National Center for Cool and Cold Water Aquaculture in Maine, rainbow trout were selectively bred for increased resistance to the bacterial cold-water disease agent, *Flavobacterium psychrophilum,* and resistance was shown to persist throughout their life cycle. Scientists also found that resistant fish crosses had, on average, a larger spleen size than susceptible fish crosses and that spleen size alone was a valid predictor of resistance to this disease agent. Since spleen size is easy to measure, it may be a useful selection parameter for evaluation in other fish populations. (National Program 106, Performance Measure 2.2.2).

> Hadidi, S., Glenney, G., Welch, T.J., Silverstein, J., Wiens, G.D. 2008. Spleen Size Predicts Resistance of Rainbow Trout to Flavobacterium psychrophilum Challenge. Journal of Immunology. 180:4156-4165.

Component 5: Production and Products

Florida pompano successfully reared in freshwater. Rearing marine species in low-salinity environments allows farming of marine fish away from expensive coastal land. In an experiment by scientists in Fort Pierce, Florida, pompano were reared for three months at various temperatures and salinities, including freshwater, and evaluated for their ability to adapt, survive, and grow. Results indicate that pompano can be reared in freshwater, although further research is required to improve growth. This accomplishment will increase the range of potential inland areas of the United States where pompano can be reared, significantly increasing opportunities and crop diversity for inland fish farmers. (National Program 106, Performance Measure 2.2.2).

Alternative recirculating system shown to save energy and reduce cost. Inland aquaculture of marine species using underutilized spaces and low-salinity recirculating aquaculture systems

(RAS) offers an alternative approach to marine fish production, which is hindered by cost and availability of coastal land. ARS scientists in Fort Pierce designed, constructed, and evaluated a newly designed low-salinity RAS for energy efficiency and economic savings. Scientists found that the system utilized one-third less energy than systems with conventional pumps and that the cost of removing ammonia with new biofilters was one third that of removing it with conventional filters, resulting in an increase from 13–36 grams ammonia removed per dollar to 120–180 grams ammonia removed per dollar. This new system reduces capital and operating costs for inland aquaculture of marine species and will increase opportunities for inland aquaculture producers. (National Program 106, Performance Measure 2.2.2).

> Pfeiffer, T.J., Osborn, A., Davis, M. 2008. Particle sieve analysis for solids removal efficiency for recirculating aquaculture system components. Aquacultural Engineering. 39:24-29.

Catfish fry shown to have low tolerance for sudden increases in environmental pH. Early-life stage (fry) survival of catfish is variable, and low survival often cannot be attributed to diseases or malnourishment. Hatchery water, where catfish fry are produced, and nursery pond water, where they are transferred after 3 to 10 days of growth, may have very different pH levels. ARS scientists found that catfish fry have a high tolerance for sudden decreases in water pH but low tolerance for increases, with a sudden increase of only 0.7 pH units causing just a 10 percent loss of fry and an increase of 1.4 pH units causing 50 percent mortality. As a result, farmers have been advised to monitor pH before stocking fry in nursery ponds and to stock only when water pH in the nursery pond closely matches water pH in the hatchery, a simple practice that has been widely adopted and is expected to have significant impacts on fry survival in catfish farming. (National Program 106, Performance Measure 2.2.2).

> Mischke, C.C., Wise, D.J. 2008. Tolerance of Channel Catfish Fry to Abrupt pH Changes. North American Journal of Aquaculture. 70:305-307

New catfish incubator design. For the past century, catfish eggs have been incubated in a screen basket, with rotating paddles used to circulate water. An ARS scientist developed a new method in which the eggs are placed in baskets that are periodically lifted out of the water, vastly improving gas exchange and increasing the oxygen available to developing embryos. A commercial-scale unit was fabricated and tested at a commercial hatchery during the 2008 spawning season and was shown capable of incubating up to 72 pounds of eggs on as little as 2 GPM water flow, as compared to standard troughs, which can be loaded with no more than 18 pounds of eggs and require at least 2½ gallons per minute (GPM) of water flow. Development of this incubator is still preliminary; however, it appears that it will save both space and water compared to existing equipment. (National Program 106, Performance Measure 2.2.2).

Technologies for dewatering aquaculture biosolids. Intensive aquaculture systems utilize capture mechanisms such as settling basins and microscreen filters to remove uneaten feed, feces, and biofloc from fish culture water. Although effective in solids removal from fish production systems, backwashing of these mechanisms produces a waste stream that contains too much water to be cost-competitive for most traditional disposal methods. ARS scientists evaluated and compared three technologies: gravity thickening settlers (*i.e.*, settling cones), belt filters, and geotextile bag filters, finding that the belt filter produced the cleanest discharge and highest treatment efficiencies, but had a more complicated and time consuming operation than the other processes. This research identifies better waste management technologies and practices that can be implemented to improve waste capture, dewatering, and disposal of waste at aquaculture facilities. (National Program 106, Performance Measure 2.2.2).

New water treatment protocol established. As a result of bacterial outbreaks and poor water quality conditions in 2005 and 2006, mass mortalities of larval and juvenile oysters occurred in the industry and in the research hatchery at the Hatfield Marine Science Center. ARS scientists developed an improved water treatment system that includes filtration, UV sterilization, foam fractionation, and biological conditioning. Although work remains to be done, the new ARS-developed system helped prevent the closure of a key commercial hatchery when the hatchery operator replicated and scaled up the existing systems, resulting in dramatic improvement that partially restored production. (National Program 106, Performance Measure 2.2.2).

Method developed to stabilize fish byproducts. High-quality by-products from fish processing in Alaska are sometimes discarded unless fishmeal plants are located nearby, because the material cannot be transported cheaply enough in the short time before the materials spoil. ARS scientists in Alaska evaluated a preservation method known as acidification for processing salmon by-products (heads, viscera, and a mixture). Using this method, by-product components were stabilized through fermentation by lactic acid bacteria and through direct acidification with formic acid; all treatment groups maintained stable pH levels for 120 days, although lipid and protein quality decreased. It was also discovered that viscera and heads preserved separately consistently maintained a more effective pH than when mixed together, regardless of treatment. The research provides fish processors with guidance on collecting and storing by-products to lengthen their stability and maximize their nutritional quality. (National Program 106, Performance Measure 2.2.2).

A geographical information system (GIS) map of Willapa Bay developed. The effects of commercial aquaculture facilities on estuarine ecology and habitat are not known, and detailed maps that display physical relationships between production facilities and specific habitats are not available. A GIS was created by ARS scientists in the Forage Seed and Cereal Research Unit in Corvallis, Oregon (based in Newport, Oregon), using results from a habitat survey of Willapa Bay, Washington, on burrowing shrimp, eelgrass, oyster culture, sediment characteristics and bathymetry. Scientists developed a standard base map, which will be used to design detailed studies of interactions between burrowing shrimp and oysters in Pacific Northwest aquaculture facilities and to enable spatial analyses of habitat interactions; these results will be used to inform the shellfish aquaculture industry and decision-makers about how environmentally-sustainable practices can be conducted on a landscape scale. (National Program 106, Performance Measure 2.2.2).

Wisehart, L.M., Dumbauld, B.R., Ruesink, J.L., Hacker, S.D. 2007. Importance of eelgrass early life history stages in response to oyster aquaculture disturbance. Marine Ecology Progress Series.344:71-80.