2004 Aquaculture
Annual Report for NRSP8

ACCOMPLISHMENTS AND IMPACTS: In this section focus on intended outcomes and potential impacts. This information should be built around the activity's milestones. Also, describe plans for the coming year in no more than one or two short paragraphs. For clarity, it may be helpful to present the accomplishments separately for each objective.

Good progress has been made among aquaculture species in 2004. The current state of aquaculture genomics include availability of relatively high-density genetic linkage maps from Atlantic salmon and rainbow trout (with over 1000 markers), moderate density linkage maps for tilapia and catfish (with several hundreds of markers), and framework linkage maps for oysters and shrimps. BAC-based contigs have been established for Atlantic salmon and tilapia, but are lacking for catfish, rainbow trout, striped bass, shrimps, and oysters. Large numbers of ESTs are available for Atlantic salmon and rainbow trout, and moderate number of ESTs are available for catfish. EST resources are yet low or lacking for tilapia, striped bass, oysters, and shrimps (however, a major grant has been awarded to develop EST resources in shrimps and good progress is expected in 2005). Microarrays have been and are being developed for salmonids, catfish, oysters, and shrimps. The availability of genomic resources and infrastructure allows identification and characterization of various economically important genes. Coupled to QTL analysis, aquaculture genome research is making important steps toward genetic improvement of brood stocks through the use of genome-based technologies.

Progress Toward Objective 1: Enhance and integrate genetic and physical maps of agriculturally important animals for cross species comparisons and sequence annotation.

Good progress has been made toward reaching objective 1 among aquaculture species in 2004. The current state of aquaculture genomics include availability of relatively high-density genetic linkage maps from Atlantic salmon and rainbow trout (with over 1000 markers), moderate density linkage maps for tilapia and catfish (with several hundreds of markers), and framework linkage maps of for oysters and shrimps. BAC-based contigs have been established for Atlantic salmon and tilapia, but are lacking for catfish, rainbow trout, striped bass, shrimps, and oysters. In spite of the lack of one or the other maps, efforts were devoted to enhance and integrate these maps as detailed below:

Salmonids: In 2004 salmonid genome researchers under the leadership of Ruth Phillips integrated rainbow trout cytogenetic and genetic maps by assigning linkage groups to chromosomes. To date over half of the Atlantic salmon linkage groups have also been assigned to chromosomes. Sex chromosomes and linkage groups have also been identified for Coho salmon. Mapping efforts to increase microsatellite marker densities and map QTLs for rainbow trout and Atlantic salmon are ongoing.

Microsatellite markers can be used to compare maps among the salmonids. To this end, Roy Danzmann and collaborators developed comparative linkage maps between rainbow trout, Atlantic salmon, and Artic char.

A project was initiated by NCCCWA and WVU to construct a BAC physical map for rainbow trout using 1X from the NCCCWA 10X BAC library. Also, a larger insert 5X BAC library is under development. A BAC physical map for Atlantic salmon has been constructed by GRASP.

An effort to obtain a whole genome shotgun sequence for rainbow trout was attempted by community submission of a proposal to the JGI. The proposal containing 85 authors from 46 institutions and 12 countries was unsuccessful

Catfish: The USDA, ARS, Catfish Genetics Research Unit (CGRU) developed the STRAP technique to efficiently obtain polymorphic molecular markers from BAC clones containing conserved genes (Waldbieser et al., 2003). This technique was used to place several candidate genes on the channel catfish genetic linkage map. There is no physical map in catfish yet. The lack of a physical map is the greatest bottleneck for catfish genome research. A physical mapping project has been initiated by Auburn University. Researchers at Auburn University and CGRU have fingerprinted approximately 2X genome coverage of BACs. In anticipation for the production of a BAC-based physical map, hybridization using overgo probes was conducted at Auburn University to anchor gene-associated DNA markers to BAC clones. These same set of markers have been genotyped for linkage mapping. As soon as a BAC-based contigs become available, these mutually mapped markers will help in alignment of linkage maps with physical maps.

Tilapia: An updated genetic map for tilapia was completed at the University of New Hampshire (UNH). The map orders more than 550 microsatellite and gene-based markers, and has an average spacing of less than 3cM. The map has been used to map the gene for red color in two strains of tilapia, and researchers at UNH are in the final stages of positional cloning of this gene. Thomas Kocher's group has also identified sex-linked markers on LG 1 in *O. niloticus*, and in *O. aureus* they discovered an unusual epistatic interaction between an XY locus on LG1 and a WZ system on LG3.

Striped Bass: *Morone sp*. were first included as a member of the NRSP8 aquaculture species group in 2004 and thus this is the first official report on genomics efforts in this species. A brief introduction of the industry and the potential economic impacts of genomics driven genetic improvement of these species is provided below followed by a description of the current state of genomics knowledge in these fishes.

The U.S. hybrid striped bass (HSB) aquaculture industry was established in the early eighties and has grown rapidly to become 4th largest finfish species in overall value and 5th in terms of production (~12M lbs annually). The HSB industry was founded on and still continues to largely rely on wild-caught broodstocks for production of fingerlings for later growout in a variety of intensive tank based and extensive pond based production systems throughout the U.S. The principal Morone species used for commercial production are hybrids between a white bass (WB: *Morone chrysops*) female and striped bass (SB: *Morone saxatilis*) male. This hybrid is referred to as the "reciprocal cross" in the HSB industry and is used primarily due to the smaller size and relative ease of obtaining eggs from white bass, as well as, seasonal, low-cost availability of large numbers of naturally ovulated female white bass from many river drainages in the

southeastern states. In addition, the production characteristics of these hybrids are preferred by industry.

Significant progress on the reproductive biology and domestication of *Morone sp*. has resulted from efforts by academic research groups, primarily at North Carolina State University (NCSU), University of Maryland, and Southern Illinois University. The largest and most diverse domesticated *Morone* broodstocks are maintained at the NCSU-Pamlico Aquaculture Field Laboratory, where currently large numbers of fourth generation domesticated SB and seventh generation WB are housed.

The HSB industry is relatively small and none of the commercial producers have been able to fund a comprehensive breeding program for the two species, and thus the industry is still reliant on essentially wild germplasm sources. These issues led to the recognition that for this industry to grow and continue to enhance production efficiencies, genetically improved broodstocks of both SB and WB would be required. This led to a landmark meeting entitled, "Workshop on Genetic Improvement and Selective Breeding for the Hybrid Striped Bass Industry", which was held at the USDA/ARS Harry K. Dupree Stuttgart National Aquaculture Research Center (SNARC) in October 2002. At these meetings, a "National Program for Genetic Improvement and Selective Breeding for the Hybrid Striped Bass Industry" was formed and a preliminary White Paper was drafted describing the industry needs and organization of the program. This White Paper was submitted to the USDA for consideration in early 2003 for the ARS/CREES 5 year plan for aquaculture. The Third Annual "Workshop for the Genetic Improvement and Selective Breeding for the HSB Industry" will be held during the Aquaculture America 2005 meetings in New Orleans on January 19, 2005.

One of the largest efforts currently in the U.S. focused on genomics in striped bass (SB) is a collaboration between researchers at North Carolina State University, Kent SeaTech Corporation, and the USDA National Center for Cool and Coldwater Aquaculture in Kearneysville, WV. This collaboration is currently funded by a grant for the University of North Carolina Office of the President Genomic Initiative to develop 200 polymorphic microsatellite markers for use in large-scale common garden breeding experiments and to develop the first linkage map in this species. To date, 3363 quality sequences have been obtained and 577 unique primers sets have been designed. Of the first 259 designed, 221 have been successfully optimized (85%). An additional 318 primers sets are currently in optimization and should yield ~270 additional primers sets. These new primer sets along with approximately 75 existing primers will allow the first linkage maps to be generated as soon as funding is obtained for this project.

Oysters: Framework linkage maps of more than 100 microsatellite DNA markers have been published for the Pacific oyster (Hubert and Hedgecock 2004). The consensus map has 10 linkage groups, in accord with haploid chromosome number covers 70-80% of the Pacific oyster genome, and has a density of markers such that the expected distance of a new gene to the nearest marker on the map is 4 to 6 map units (cM). This microsatellite DNA scaffold can be fleshed out quickly with several hundred AFLP markers (Li and Guo 2004). For example, Hedgecock et al. (2004) have constructed an AFLP map of 341 markers for the mapping family 35×51 F_2, which has an estimated coverage of up to 94%. Mapping of QTL for growth heterosis is in progress with USDA support (Hedgecock NRICGP #2003-35205-12830). The Reece (VIMS) and Gaffney (Delaware) labs have analyzed inheritance of a set of microsatellite markers in the eastern oyster

(Reece et al. 2004), observing a high incidence of null alleles and distorted segregation ratios. Dr Ximing Guo has led a group at Rutgers University focusing on cytogenetics and physical mapping of both species. His group is continuing to work on the identification of oyster chromosomes with fluorescence *in situ* hybridization (FISH). A preliminary cytogenetic map was producing using P1 clones as FISH probes (Wang et al., in press). Also using FISH, they compared karyotypes and the rDNA-bearing chromosomes from different species of oysters and scallops. Comparisons among oysters indicate that the size and shape of the rDNA-bearing chromosome represent a divide between Asian-Pacific and Atlantic species of *Crassostrea* (Wang et al., 2004). Analysis in scallops suggests that there was at least one round of genome duplication during the evolution of Bivalvia (Guo and Wang, 2004). An AFLP-based linkage map was constructed for the Pacific oyster (Li and Guo, 2004). The Gaffney lab has begun an NOAA-sponsored project to develop a suite of 50-100 type I SNP markers in the eastern oyster, for purposes of mapping and assessment of germplasm diversity. In addition, the Gaffney lab has a USDA NRI project to develop 100 Type I SNP markers in the Pacific oyster (NRICGP #DELR-2003-03620), which will be placed on the existing microsatellite map, and tested for QTL associations in association with Dr. Dennis Hedgecock. Dr Reece and colleagues at VIMS have begun development of genetic markers for the Suminoe oyster *Crassostrea ariakensis*, a candidate for deliberate introduction into mid-Atlantic waters to revitalize the decimated oyster fishery. The project aims to use nuclear, mitochondrial, and microsatellite markers to: 1) assess the amount of intraspecific genetic variation within and among existing native populations of *C. ariakensis*, 2) determine the genetic relationship of current U.S. hatchery stocks of *C. ariakensis* to native populations to aid in determining the strain of *C. ariakensis* best suited for any introductions that may become approved, and 3) provide molecular tools that can be used to identify the source population(s) of any unapproved *C. ariakensis* introductions into Chesapeake Bay. To date, four microsatellite libraries have been developed, and primers for amplification of 30 loci have been designed and are being tested. The current research emphasis is on the northern-type of *C. ariakensis*, as this is the genetic type of the majority of the hatchery samples currently in the US being tested for potential introduction into the Chesapeake Bay region and is the genetic type found at the type location for this species, Ariake Bay, Japan. Results of marker screening to date indicate that a total of 19 loci have amplified well and shown polymorphism in the northern-type *C. ariakensis*; only four such loci were found for the southern-type.

BAC libraries were developed for both the eastern oyster and Pacific oyster, with support from the National Human Genome Research Institute. The libraries were constructed by Clemson University Genomics Institute (Dr. Jeff Tomkins, Director) and are publicly available (https://www.genome.clemson.edu/orders). These are deep coverage libraries (10x and 12x coverage respectively), with average insert sizes of 134kb and 150kb, respectively. The libraries were constructed from sperm cells, which, in the case of *C. gigas*, were taken from a 51×35 F_1 hybrid male.

Shrimps: A USDA Supported workshop: "Advancing Shrimp Genomics Research Workshop" was held as a pre-conference to PAG XIII (January 13-14, 2005). The conference had 19 Attendees. The objective of the Workshop was to conduct a survey on the status of the worldwide genomics toolbox for shrimp, generate a white paper and action plan for "Genomic Enablement" for shrimp, and formally establish an International Shrimp Genomics Consortium. The Workshop attendees were quite international with

participants from Australia, Belguim, China, Japan, Taiwan, Thailand, and the USA, with both Industrial and Scientific representations.

Progress toward Objective 2: Facilitate integration of genomic, transcriptional, proteomic and metabolomic approaches toward better understanding of biological mechanisms underlying economically important traits.

In 2004, great progress has been made in the area of transcriptome analysis using ESTs. A summary of current available ESTs in various aquaculture species is listed below. These ESTs has allowed the development of microarrays in various species as detailed below.

Species	Current ESTs	Approximate unique sequences
Rainbow trout	160,816	50,773
Atlantic salmon	120,000	40,000
Catfish	45,000	30,000
Oyster		
Crassostrea gigas	3,300	
Crassostrea virginica	9,200	5,900
Shrimps	9,400	3,300
Tilapia	1,700	
Striped bass	<500	

The largest progress in this area was made with salmonids. A 16,000 gene array has been developed by GRASP using salmon and trout EST data sets. This array has been tested for use in various salmonid fishes. The array has been used to study developmentally regulated genes and genes induced under various environmental conditions.

Robert Li and Geoff Waldbieser of the USDA, ARS Catfish Genetics Research Unit developed an oligonucleotide microarray containing ~18,000 unique sequences. The microarray was manufactured by Nimblegen (24-mer oligonucleotides). Initial testing of the microarray using experimentally treated catfish demonstrated a good correlation between levels of hybridization to the microarray and real-time PCR expression levels for several candidate genes. A cDNA microarray was constructed in Mississippi State University with 2821 genes from fry EST project and Jx13 mixed leukocyte culture EST project (collaboration between Mississippi State, Catfish Genetics Research Unit and Mississippi Medical Center; array is available from Mississippi State). Efforts are ongoing at Auburn University for the identification of positional candidate genes for disease resistance using linkage mapping, and for the identification of expression candidate genes using microarrays. At University of Mississippi Medical Center, Dr. Melanie Wilson's group have completed the sequencing of the functional CD45 gene locus, the CD45 pseudogene locus and are continuing the sequencing of the immunoglobulin heavy chain locus. They also have continued characterization and functional studies of catfish immune molecules, such as, T Cell Receptors, Novel Immune Type Receptors, Leukocyte Immune Type Receptors, Immunoglobulin D and molecules involved in cytotoxicity. Monoclonal antibodies specific for various immune related genes are being and/or have been produced. At Auburn University, a large set of chemokines (26 CC chemokines and 7 CXC chemokines) has been characterized that changed the previous view of small number of chemokines in fish. A number of catfish antimicrobial peptide genes have also been characterized at Auburn University. Greg Warr's group at Hollings Marine Laboratory, Medical University of South Carolina continued their work on characterization of the structure and function of transcription factors involved in the expression of immune-function genes in catfish (see Hikima et al., 2004).

At the University of New Hampshire, Thomas Kocher's group has mapped the gene for red color in two strains of tilapia, and they are in the final stages of positionally cloning this gene. They have also identified sex-linked markers on LG 1 in *O. niloticus* and *O. aureus* discovered an unusual epistatic interaction between an XY locus on LG1 and a WZ system on LG3.

In shrimps, while establishing reverse-genetic methods for use *in vivo*, Greg Warr's group at Medical University of South Carolina has discovered that long dsRNA evokes a non-specific anti-viral immune response in Pacific White Shrimp, *Litopenaeus vannamei.* (see Robalino et al., 2004).

In oysters, an initial cDNA-based oyster microarray is being developed; groups in Montpellier, France (Escoubas, Bachere), Auburn (Liu), Baltimore (Vasta), Rutgers (Guo, Tanguy) and Charleston (Warr, Chapman, Cunningham, Gross) have pooled their resources. Over 5000 unigenes from *C. gigas* and *C. virginica*, as well as 2,500 unigenes from the oyster parasite *Perkinsus marinus*, have been assembled in Charleston. Amplicons are currently being generated from these clones, and an initial ~7,000 unigene microarray will be printed in spring 2005.

In Dr Dennis Hedgecock's laboratory, a remarkable library of 4.6 million Pacific oyster ESTs is available from a genome-wide survey of gene expression in inbred and hybrid Pacific oysters carried out by Lynx Therapeutics (http://www.lynxgen.com/), using Megaclone™ and massively parallel signature sequencing or MPSS™ technologies (Brenner et al. 2000a, 2000b). Each signature is a 17-bp sequence read from the 3' end of a unique cDNA clone; the collection of 4.6 million sequences comprises 52,828 unique signatures (Hedgecock, D., J.- Zh. Lin, S. DeCola, C. Haudenschild, E. Meyer, D. T. Manahan, & B. Bowen, submitted).

Dr. Ximing Guo's laboratory has focused on genes expressed in the eastern oyster upon infection by *Perkinsus marinus*, a major pathogen. Suppression subtractive hybridization (SSH) libraries were constructed using challenged and unchallenged controls. Clones from SSH libraries were sequenced, and about 170 genes and expressed sequences were identified as up-regulated after challenge with the pathogen (Tanguy et al., 2004). SNPs were developed for 13 of the genes so far. Guo's team has also built SSH libraries using triploid and diploid Pacific oysters, hoping to identify genes related to triploid gigantism and sterility.

The laboratory of Dr. Marta Gómez-Chiarri (University of Rhode Island) is also exploring the molecular mechanisms underlying early responses of the oyster to parasitic infection. They have constructed suppression subtractive hybridization libraries with RNA of hemocytes from oysters experimentally infected with either *P. marinus* or artificial seawater. They screened approximately 400 clones from these libraries and identified 170 genes differentially expressed at early time points (4 – 48 hours) after experimental infection with *P. marinus*. The sequences for these genes have been deposited in the Marine Genomics database at MUSC (www.marinegenomics.org). Sequence analysis indicates an unusually large proportion of unknown genes in the library (82% for the upregulated library) showing no significant matches with the genetic databases. Known sequences included genes involved in metabolism (arginine kinase, LDL receptor; NADH dehydrogenase), gene regulation and immunity (histone H4, QM protein, lipooxigenase), homeostasis and immunity (metallothionein, cavortin), and ribosomal and mitochondrial genes. The patterns of expression of selected genes were further studied in hemocytes and other tissues of oysters experimentally and naturally infected with *P. marinus*. The identification of genes involved in the early response of oysters to parasitic infection provides a starting point for dissecting the mechanisms of immunity in oysters. It will be particularly interesting to undertake comparative analyses of gene expression, as the diseases

that kill the eastern oyster cause only benign infections in *C. gigas.* Greg Warr's group has characterized and integrated the transcriptomic and proteomic analysis of oyster metallothioneins, important components of the responses to stress, immune challenge, and heavy metals (see Jenny et al., 2004).

Progress Toward Objective 3: Facilitate and implement bioinformatic tools to extract, analyze, store and disseminate information. (See Attachment 1 for more details on objectives.)

Overall, bioinformatic tool development is a very weak area in aquaculture genomics.

Thomas Kocher's group continues to build informatic tools to integrate the genetic and physical maps of the tilapia genome with with the genome sequences now available for *Fugu*, *Tetraodon*, medaka and zebrafish. The comparative genome databases and browsers are available at http://hcgs.unh.edu/.

Greg Warr's group continue to maintain the website www.marinegenomics.org for the archiving of EST and microarray data, and as a resource for on-line tools that can be used in the analysis of genomic and transcriptomic data. They have developed tools for the design of microarrays from species with limited genomic information (see Chen et al., 2004).

In collaboration with Dr. Lei Liu at the Keck Bioinformatics Center, an ESTIMA system has been developed that provides searchable databases for the catfish ESTs at Auburn University. As soon as it is tested, the website will be available to the research community.

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