

NRSP-8 Aquaculture Research Progress Report for 2006
Aquaculture Coordinator: John Liu

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

Catfish: Two framework genetic linkage maps were reported. One was constructed using channel catfish intraspecific resource families (Waldbieser et al., 2001), and the other was made with channel catfish x blue catfish interspecific families (Liu et al., 2003). The major objective for intraspecific mapping is to aid genetic improvements using various channel catfish lines, while the major objective for interspecific mapping is to aid genetic improvements through the use of interspecific hybrids and introgression. Several hundred more microsatellites have been added in the past year to the linkage maps, especially the gene-associated type I markers. A gene-based linkage map has been constructed.

The physical maps of the catfish genome have been constructed by fingerprinting CHORI 212 BAC library (5.6X genome coverage) and CCBL1 BAC library (7X genome coverage). A total of 3256 contigs were assembled with the CHORI 212 library, and 1782 contigs were assembled with the CCBL1 BAC library. Estimated map lengths were approximately 930 Mb. A large number of BAC end sequences have been generated and over 20,000 have been deposited to GenBank from Auburn University. A large number of BAC-associated microsatellites have been identified for the integration of linkage and physical maps.

Salmonids: Linkage mapping efforts are ongoing with several salmonids species. In particular, work with the cGRASP Project in Canada has mapped hundreds more microsatellites, especially those from the BAC ends to the linkage map of Atlantic salmon. Over 228,000 BAC clones have been fingerprinted from the Atlantic salmon BAC library, and 4338 contigs have been assembled. For rainbow trout, a NRI grant was just awarded to construct a BAC contig-based physical map of the rainbow trout genome. Efforts to obtain genome sequences for rainbow trout and Atlantic salmon continued. A working group from cGRASP, Consortium for Genome Research on All Salmonids Program, met in Leetown, WV on May 10 - 12 to outline strategies for obtaining whole genome sequences. A subsequent workshop was held at Simon Fraser University, October 10-12, consisting of scientists and representatives from sequencing centers and funding agencies. A meeting report and revised version of a white paper has been generated by the Executive Committee. Initial sequencing has been initiated under the support of Canadian and Norwegian governments.

Tilapia: A BAC-based physical map, and the 2nd generation genetic map was published in the last year by Thomas Kocher's group. His proposal to sequence the ends of 35,000 BAC clones was approved by Genoscope, with sequencing to begin in early 2007. The proposal to sequence the tilapia genome was approved by the NIH, with sequencing to begin in late 2007. NIH-NHGRI has committed to producing a draft assembly of the tilapia genome, together with 2x sequencing from each of three closely related haplochromine cichlid fish.

Oysters: Gaffney's lab at the University of Delaware is completing a project on SNP marker development and mapping in the Pacific oyster. Primer sets have been designed and tested for multiple Type I markers, based on the existing EST database. A total of 53 loci have been amplified and sequenced in multiple individuals representing the geographic range of the species, as well as two parents from a mapping family provided by the Hedgecock lab. Although virtually every locus has been found to be polymorphic, often with multiple SNPs, the inbred mapping family is invariant for some of the loci. For the remaining loci, temperature gradient capillary electrophoresis (TGCE) is being used to score parents and progeny (N = 48-72) to enable these loci to be placed on the framework microsatellite map produced by the Hedgecock lab. As part of the EU-funded project Aquafirst, French researchers have developed approximately 50 SNPs; these will be mapped using an F₂ family segregating for resistance to summer mortality, which has already been mapped for 100 microsatellite loci. A BAC-based physical map of the *C. gigas* genome will be constructed by BAC fingerprinting, in a USDA NRI project scheduled to begin in 2007.

For the eastern oyster, Dr. Ximing Guo's lab at Rutgers developed 53 SSR and 44 SNP markers from putative host-defense gene and ESTs. These are now being scored in two mapping families for placement on the AFLP scaffold linkage map.

The Gaffney lab conducted PCR trials of primers developed for *C. gigas* and *C. virginica* were tested on other Crassostrea species, including members of the Atlantic clade (*C. rhizophorae*, *C. gasar*, *C. corteziensis*) and the Asian clade (*C. ariakensis*, *C. sikamea*, *C. hongkongensis*). Several targets were sequenced for all species, to provide preliminary data for comparative genomics applications.

Shrimps: A genetic linkage map of the Pacific white shrimp was constructed, and more markers were added to the existing genetic linkage map of the tiger shrimp.

Striped Bass: One of the largest efforts currently in the U.S. focused on genomics in striped bass is a research collaboration between researchers at North Carolina State University and the USDA National Center for Cool and Coldwater Aquaculture in Kearneysville, WV. This collaboration was funded by a grant from the University of North Carolina Office of the President Genomic Initiative to Dr. Craig Sullivan (NCSU) to develop polymorphic microsatellite markers for use in large-scale common garden breeding experiments and for future development of the first linkage map in this species. This research project ended in May 2005 and has led to the discovery and characterization of over 500 microsatellite markers in striped bass that have now been deposited in GenBank. Additional work by the same group focused on DNA pooling for estimation of allele frequencies. Based on this progress this same group received funding for development of the first genetic linkage map for striped bass from the Sea Grant National Marine Aquaculture Initiative in 2006. This research should result in development of a genetic linkage map for striped bass in 2008.

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

Catfish: The Joint Genome Institute is sequencing 300,000 EST clones from catfish, of which 200,000 will be sequenced from channel catfish and 100,000 will be sequenced from blue catfish.

Melanie Wilson's group has completed the sequencing of 9 BACs covering part of the catfish immunoglobulin heavy chain locus and are continuing mapping of this important locus. A manuscript concerning the annotation has been published. Characterization and functional studies of catfish immune molecules, such as, T Cell Receptors and their accessory molecules CD4 and CD8, Novel Immune Type Receptors, Leukocyte Immune Type Receptors (LITR), Immunoglobulin D, FcRs and the B cell accessory molecules CD79a and 79b, are ongoing. Monoclonal and polyclonal antibodies specific for various LITRs, IgD, CD79b and IpFcR have been produced and are being characterized. Her group is also producing antibodies to other immune related genes. The University of Mississippi Medical Center is coordinating the channel catfish part of the US Veterinary Immune Reagent Network Grant for production of recombinant proteins and/or peptides for development of reagents for basic research and diagnostic purposes for various animals important in US agriculture.

Work in John Liu's lab was focused on the development of genome resources including previously prepared 23 cDNA libraries from various tissues, and construction of four normalized cDNA libraries to support the JGI approved large-scale catfish EST project. Using existing EST as resources, comparative sets of chromosome-specific ESTs were identified by anchoring catfish ESTs to Tetraodon genome. Much effort was made to characterization of innate immune genes and analysis of their expression in the resistant blue catfish as compared to expression in the susceptible channel catfish after infection with the most serious bacterial disease enteric septicemia of catfish (ESC). A total of 26 CC chemokine genes, 6 CXC genes, 4 antimicrobial peptide genes, interleukin-1 beta gene, 23 selenoprotein genes, 6 toll-like receptors, and a few dozens of other genes were completely sequenced, mapped to BACs, and expression was analyzed. Conserved synteny was analyzed comparatively with zebrafish or Tetraodon genomes. A 28K gene array was constructed with the Nimblegen platform, and used to analyze differentially expressed genes after infection of bacterial disease enteric septicemia of catfish.

Work in Geoff Waldbieser's lab using microarrays for the analysis of catfish response to LPS injection has been published. His lab has also conducted investigations on growth hormone – IGF

pathway and its interaction with the immune system, and prepared several cDNA libraries for the JGI EST project.

Salmonids: The number of ESTs for rainbow trout increased 9% to 262,330 and Atlantic salmon increased 131% to 431,754. This represents the majority of nucleotide data for the salmonids, which has 708,862 nucleotides and 5507 proteins in GenBank. Several microarrays are available for functional genome research. These data have provided an excellent starting point for candidate gene investigation including uncoupling proteins, myostatins, pro-opiomelanocortins, and tapasins. Significant progress continues to be made in identifying genes which affect development rate, oxygen consumption, and sex determination.

Tilapia: USDA-NRICGP has just approved a project to sequence 100,000 ESTs from a variety of tilapia cDNA libraries. These sequences will complement existing EST resources for related cichlid fish, allowing the production of 2nd generation microarrays for tilapia.

Oysters: The Oyster Genome Consortium entered into a user agreement with the US DOE Joint Genome Institute for sequencing of EST and BAC libraries for the Pacific oyster. JGI will do paired-end sequencing of 150,000 cDNAs. JGI will also sequence four BAC contigs containing genes identified by Cunningham et al. (2006), in order to assess levels of DNA polymorphism in coding and non-coding regions, which could ultimately influence whole genome shotgun sequencing strategy. A transcriptomic analysis of inbred and hybrid oysters (Hedgecock et al. 2007) revealed candidate genes for heterosis, several of which have been identified through BLAST searches. Work is in progress to find SNPs in these genes and to place them on the QTL map. For the Pacific oyster, Dr. Hedgecock's lab reported QTL-mapping results on the number, location, and mode of action of genes affecting yield in an F₂ family derived from a naturalized *C. gigas* population in Dabob Bay (WA). Three dominant QTL for yield on three different chromosomes were detected. They also have mapped a recessive QTL for an abnormal "hook-hinge" condition segregating in this same family as well as an additive QTL for shell pigmentation. In France, a study of the relationship between genetic variation in amylase genes and growth in *C. gigas* has been published. Current work on other genes (project "Polygigas") is in progress, supported by the Bureau des Ressources Genetiques. Using a F₂ family segregating for resistance to summer mortality, researchers under the EU project Aquafirst will seek to identify QTL for this trait in 2007.

In the eastern oyster, Dr. Guo's lab identified and mapped 12 putative disease-resistance QTLs. For the flat oyster, French researchers are in the process of mapping QTL of resistance to bonamiosis, a major disease for this species. Gaffney's lab is mapping SNPs using a F₂ family segregating for resistance to summer mortality.

Shrimps: Six high quality tissue specific cDNA libraries have been constructed and analyzed for depth and redundancy, with 1000 EST being collected from each library to date. Redundancy depletion and shipment to JGI is 83% complete (100,000 clones), awaiting JGI to perform full double pass sequencing. Preliminary characterization of the libraries and analysis of existing ESTs was published. Full annotation and metagenomic analysis to follow. - The first generation microarray has been printed and initial validation and QA/QC have been completed. These microarrays contain in excess of 3000 unigenes and are currently in use; the first experimental research work is in press. Studies on the effects of long dsRNA and RNAi in relation to viral challenge and WSSV in the Pacific Whiteleg Shrimp, *Litopenaeus vannamei* are ongoing. Continuing research in the area of antimicrobial peptides and their importance in shrimp immunity has yielded data on the structure of the Penaeidin gene family, its control elements, and specificity for microbial targets.

Striped Bass: Significant progress was made in 2006 involving integration of genomic approaches toward better understanding of biological mechanisms underlying economically important traits in striped bass. Much of this work is described in three dissertations submitted to North Carolina State University (Couch, C.R., 2006; Garber, A.F., 2006) and Texas A&M University (Wang, X., 2006). Each of these dissertations studies used microsatellite markers to assess economically important traits such as growth, disease resistance, and carcass-quality in striped bass. These new studies will be important going forward

as the genetic linkage map is produced for striped bass and will eventually aid in the identification of QTL for economically important traits.

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

Catfish: John Liu's lab continues their efforts on development of comparative genome tools such as chromosome-anchored ESTs of catfish. Large scale informatic mining of microsatellites and SNPs are underway; The CGRU in 2006 will continue development and enhancement of microarray tools, and further use of arrays to identify candidate genes for disease resistance.

Salmonids: The Atlantic salmon and rainbow trout gene indices have relocated to the Dana Farber Cancer Institute and have been updated to versions 3.0 and 6.0, respectively. <http://compbio.dfci.harvard.edu/tgi/tgipage.html>.

Tilapia: Dr. Kocher's group has implemented the Gbrowse software to facilitate viewing of tilapia sequences on the Tetraodon genome assembly (<http://hcgs.unh.edu/gbrowse/>).

Oysters: Within the EU project Aquafirst, QTL data will be hosted by the Roslin Institute on ArkDB. EST data will be held on the INRA platform "Sigenae" in Toulouse.

Shrimps: The marine genomics group at the Hollings Marine Laboratory and MUSC continues to maintain 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, which are being used to archive and analyze shrimp metagenomic and microarray data. In addition, contracting with Clemson University Genome Institute is underway to enhance EST analysis capabilities.

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