# 2011 Aquaculture Genomics Annual Report for NRSP8

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**Species Leaders:** Dr. Sylvie Quiniou – Catfish, USDA/ARS/CGRU
Dr. Patrick Gaffney – Oyster, University of Delaware
Dr. Yniv Palti – Salmonids, USDA/ARS/NCCCWA
Dr. Zhiqiang Du – Shrimp, Iowa State University
Dr. Craig Sullivan – Striped Bass, North Carolina State University

**Species:** Although the aquaculture portion of the NRSP8 project is written in support of catfish, oyster, salmonids, shrimp, tilapia, and striped bass; the community is inclusive of those interested in other species, including those aquatic species that primarily serve as models for aquaculture interests (i.e. Zebrafish). No active members are currently working with tilapia.

**International Collaboration:** Members of NRSP8 continue to collaborate intensively across the international aquaculture genome community, especially towards the development of costly resources such as genome reference sequences and single nucleotide polymorphism discovery and genotyping platforms.

**Funding:** In 2011, Coordinators funds were used for travel expenses and to support students, invited speakers, and logistical expenses for the PAG Aquaculture Genome workshop. Funds for special projects targeted catfish, oyster, salmonids, shrimp and striped bass.

**Communication:** Dr. John Liu distributes via email the quarterly Aquaculture Genome Newsletter, currently at #29. Dr. Liu also maintains a list serve which is used frequently for community announcements.

**PAG XIX Aquaculture Workshop Report, January 14-15, 2012**

The 2012 NRSP8 Aquaculture Genome Workshop was held in conjunction with the International Plant and Animal Genome XX Conference in San Diego, California. Dr. Sylvie Quiniou served as the Program Chair. In attendance were approximately 100 during the course of the workshop, with 33 participants signed in from 13 countries besides the US: Canada, Norway, Japan, The Netherlands, UK, Indonesia, France, Chile, New Zealand, China, Australia, Portugal and Mexico.

The NRSP8 Aquaculture Executive Committee received 25 applications for the $1,000 Travel Awards. Nineteen of the applicants were students or post-docs from US institutions and the rest were international applicants (1 each from, Greece, Mexico, Thailand, Nigeria, Philippines and France). Ten travel awards were granted. The presentations covered a diverse range of fields and species showing the input of Aquaculture Genomics to all physiological fields of research.

Four plenary speakers were invited to the 2012 Aquaculture Workshop: 1) Dr. Robert Chapman (Senior Research Scientist at the SC Dept of Natural Resources Research Institute); 2) Dr. Goro Yoshizaki (Associate Professor in the Department of Marine Biosciences Tokyo University of Marine Science and Technology); 3) Dr. Shane Burgess (Dean of the College of Agriculture and Life Sciences at the University of Arizona); and 4) Dr. John Postlethwait (Professor of Biology in the Institute of Neuroscience at the University of Oregon). On Saturday morning, Dr. Chapman gave a presentation on new methods of analysis of microarray and Next Generation Sequencing data. On Saturday afternoon, Dr. Yoshizaki spoke about the development of xenogeneic transplantation for fish species and how transcriptomics helped the process. On Sunday morning, Dr. Burgess and Dr. Postlethwait discussed genome annotation strategies and pitfalls. Dr. Postlethwait also gave a presentation on evolution and meiotic maps by massive parallel DNA sequencing.

Several of the workshop's contributed talks focused on the development of new genomic resources for Aquaculture species (e.g. genome sequence for Atlantic cod and carp; linkage map and/or SNP array development for the pacific whiteleg shrimp, silver lipped pearl oyster, and sablefish; creation of next generation sequence database for fresh water mussels, diverse other shellfish as well as trout and channel catfish; Development of microsatellites for non-model aquaculture species). In addition, several contributed talks focused on the use of genomic tools (microarray, RNAseq, SNPs) to identify genes and markers associated with traits of interest (e.g. egg quality, body weight, disease resistance, gonadal maturation, migration) for various aquaculture species. In total, besides the plenary lectures, 23 contributed presentations were given during the Saturday and Sunday sessions of the workshop.

Our workshop Chair for next year is Dr. Eric Peatman of Auburn University, our Chair-elect is Dr. Steven Roberts of the University of Washington.

**Objective 1: Create shared genomic tools and reagents and sequence information to enhance the understanding and discovery of genetic mechanisms affecting traits of interest.**

The status of efforts to develop genetic and physical maps and genome reference sequences varies greatly across aquaculture species. In 2011 the first genetic map based on microsatellites for striped bass was published; efforts to increase map densities for all species are focusing on SNPs through SNP arrays or RAD technologies. Efforts to obtain genome reference sequences for are progressing rapidly for most species.

**Rainbow Trout Genetic Maps:** A high density genetic map composed of approximately 5,000 single nucleotide polymorphism markers (SNPs) and derived from a cross between the Swanson and Whale Rock doubled haploid lines was produced.
**Sockeye salmon genetic map:** The lab of Jim Seeb at the University of Washington has generated a map of 1,772 SNPs for Sockeye salmon based on 140 progeny from a

full-sib family. **Rainbow trout physical map:** A significant amount of DNA fingerprinting data was used to update the rainbow trout physical map which is now composed of ~180,000 BAC clones in 2,804 contigs.

**Shrimp:** Efforts to obtain a Genome reference sequence for the Pacific White Shrimp, *Litopenaeus vannamei*, have turned to finding shrimp inbred lines with relatively high homozygosity for sequencing BAC libraries due to the high heterozygosity and complexity of the shrimp genome. Genetic markers for Pacific White Shrimp have been identified. A deep sequencing of restriction-site associated DNA marker (RAD-seq) method was used to find genetic markers involved in disease resistance in Pacific White Shrimp.

**Oyster:** The Hedgecock lab (University of Southern California) is developing SNPs for the Pacific oyster to improve linkage maps and to integrate linkage, physical, and cytogenetic maps in support of the genome sequences to be released imminently by BGI and the Chinese Academy of Sciences Institute of Oceanology. Nearly 900 SNPs have recently been added to the linkage map for the Pacific oyster. The Roberts lab (University of Washington) has generated DNA methylation enriched C. gigas library and published a paper describing library and functional role of DNA methylation in oysters. Gaffney (University of Delaware) and Boudry (IFREMER) were funded by Genoscope (France) for BAC end sequencing of the BAC library constructed with previous USDA NRI support. Sanger sequencing of both ends of 77,000 BAC clones was finally completed in August 2011, and data are currently being analyzed. As part of the current USDA NRI GigaSNP project, the Gaffney lab is screening BAC pools to place SNPS mapped by the Hedgecock lab onto the BAC physical map constructed previously. Gaffney has led the effort to annotate > 2MB of BAC sequence for the analysis of genomic polymorphism in the Pacific oyster (ms. in prep). The Guo lab (Rutgers) is conducting fluorescent in situ hybridization studies to assign BACs and other markers to the cytogenetic map.

**Channel catfish genome sequencing:** Mate pair libraries were produced from the reference catfish to include fragments of approximately 3kb and 8 kb using the Illumina mate pair library kit. A fosmid library containing 34-38 kb inserts was also produced from the reference catfish, and mate-paired sequences were produced by batch shearing of fosmid clones to 8-10 kb, re-ligation of sheared DNA, then amplification of the re-joined inserts using vector based primers. A first round of 100 bp paired read sequencing has produced 5.2M paired reads from 3kb fragments, 29.2 M paired reads from 8 kb fragments, and 20.9 M paired reads from 34-36 kb fragments. These sequences are currently being added to the contigs using ABySS to assess the extent of coverage and level of scaffolding, and the potential need for additional libraries.

**Striped bass:** Scientists from the USDA-ARS National Center for Cool & Cold Water Aquaculture (NCCCWA), VIMS and NCSU published a medium density genetic map (linkage or relational map) for the striped bass based on 289 microsatellite DNA markers. The map will enable us to accelerate gains from selective breeding by allowing direct selection based on (marker) genotypes at positions associated with performance

traits (quantitative trait loci, QTL). The same team evaluated the relationship between the DNA markers and performance traits in the mapping populations of striped bass to discover ~68 QTL, many with very strong potential for predicting performance of growth and body composition. The map also will guide assembly of a draft genome sequence (physical map) for striped bass. The NCSU scientists also obtained ~14.34 Gb of DNA sequence data (short read Illumina sequencing) of genomic DNA pooled from 4 individual striped bass (2 female, 2 male), which will support initial assembly of the draft genome sequence. In addition, 5.4 Gb of sequence data for microRNAs (miRNAs) that regulate gene expression was obtained for these striped bass. Through next generation, high throughput sequencing of mRNA (RNA-seq) of fast- and slow-growing hybrid striped bass, 1076 genes that were differentially expressed (at a 90% false detection rate) in fast- versus slow-growing fish were identified and annotated as potential growth regulators. This analysis also identified 270,000 single nucleotide repeats (SNPs) with large numbers of SNPs being found only in fast growing or slow growing fish and thereby having potential for utilization in DNA marker-assisted selective breeding, especially after they are added to the genome maps.

**Objective 2: Facilitate the development and sharing of animal populations and the collection and analysis of new, unique and interesting phenotypes.**

Members of NRSP8 continue to develop specialized resource populations for genome analyses, including inbred lines and designer crosses which take advantage of hybrid genetics. However, many of these populations are derived from commercial populations, which are the focus of additional studies. Several research institutions also maintain pedigreed well-characterized breeding programs.

**Salmonids:** Four multi-year pedigreed rainbow trout populations phenotyped for plasma cortisol in response to stress, resistance to bacterial cold water disease (BCWD) and spleen size have been characterized and propagated at the USDA National Center for Cool and Cold Water Aquaculture (NCCCWA). Quantitative trait loci (QTL) with major effects were detected for the three traits in single-pair matings and are currently being evaluated and validated for potential use in germplasm improvement. Rainbow trout improved for growth and utilization of a fishmeal-free plant-based feed have been developed and are available for release. Physiological differences related to the phenotype are currently being evaluated and quantified at the USDA-ARS research unit in Hagerman, Idaho. At the University of Guelph, QTL mapping populations were established to study stress tolerance (vis-a-vis) salinity tolerance in salmonids, spawning times in females and maturation timing in males/females and QTL influencing growth and its 'coupling' to determination of maturation timing in salmonids.

**Shrimp:** The majority of resource populations continue to be from commercial populations and focus on disease resistance.

**Oyster** The Hedgecock lab, together with Taylor Shellfish Company, we continue to develop inbred lines, to crossbreed $F_1$ hybrids for use in the oyster farming industry, and $F_2$ families useful for mapping QTL for survival, growth, and sex determination.

**Catfish:** Selection and phenotyping of channel, blue, and hybrid catfish raised in intensive raceway environments.

**Striped bass:** In August 2011, Hurricane Irene destroyed much of the physical infrastructure of one of the two main breeding centers for striped bass and white bass broodstocks for the hybrid striped bass farming industry (NCSU Pamlico Aquaculture Field Laboratory). Through sustained emergency actions, the NCSU scientists were able to rescue large numbers of all of the special lines of striped bass and white bass and restore all critical life support systems by September. The laboratory has been largely rebuilt for full operation during the 2012 breeding season. In addition, scientists at the USDA-ARS Stuttgart National Aquaculture Research Center completed studies of phenotypic variation in growth of white bass necessary for planning of the selective breeding programs for this species.

**Objective 3: Develop, integrate and implement bioinformatics resources to support the discovery of genetic mechanisms that underlie traits of interest.**

Species-specific bioinformatics resources have been developed to support efforts aimed at identifying genes of interest. Most efforts have focused on database development, including development of pipelines for next-generation sequencing data processing, analyses and annotation, and in cooperation with Jim Reecy and Zhiliang Hu at Iowa State University.

**Salmonids Genome Map Data Mining Tool**: The current rainbow trout WebFPC BAC physical map on the Clemson University Genome Institute web site is continually updated with genetic markers and BACs sequence data that are being integrated onto the BAC contigs.
**QTL Database**: A rainbow trout QTL database is now available through the Animal Genome website of the NRSP-8 bioinformatics group (http://www.animalgenome.org/cgi-bin/QTLdb/index) and is being continually updated.

Shrimp: An effort to establish a Shrimp genomics database has been established in cooperation with the NRSP8 Bioinformatics Coordinator.

**Oyster:** The Hedgecock lab developed a pipeline for finding SNPs in a mixture of Sanger and next generation cDNA sequences, for annotating the boundaries of exons in which SNPs candidates occur, for selecting SNPs that are at least 50 base pairs from an exon-intron boundary, and for generating fasta files denoting the focal SNP and replacing additional SNPs in the same exon with their IUPAC codes. The Roberts lab produced two short read RNA-seq libraries and compared different means of bioinformatic analysis. Sequences available in NCBI (SRA043778) and described in

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**Channel catfish Transcriptomics:** Deep sequencing of the doubled-haploid channel catfish transcriptome was performed using Illumina HiSeq 2000 platform, yielding over 300 million high-quality trimmed reads totaling 27 giga base pairs. These short reads were assembled using CLC Genomics workbench, ABySS and Velvet, generating 217,114, 192,558, and 311,734 contigs with a minimum length of 200 bp, respectively. Functional annotation of the assemblies was initially carried out by BLASTX search against NCBI zebrafish Refseq protein and UniProt/Swiss-Prot protein databases. Only one of the contigs from three assemblers with best BLAST match was chosen for further analysis, resulting in 25,144 contigs that have unique protein hits from either one of the protein databases. Of which, around 20,000 contigs matched the 50% length of reference proteins, and over 14,000 transcripts were identified as full-length with complete open reading frames. The catfish RNASeq, ESTs, and related SNP information has been disseminated through our Catfish Genome Database, cBARBEL, http://www.catfishgenome.org/cbarbel/, that has generated tens of thousands of hits from over 30 countries in the world. Continued upkeep and support for the cBARBEL website

**Striped bass:** The collection of over 11,000 high-quality, annotated, contiguous cDNA (transcriptome) sequences that have been deposited in the National Center for Biotechnology Information (NCBI) Short Read Archive (SRA) (GenBank: SRX007394) was maintained for public access on the U.S. National Animal Genome Project website.

## Publications

### Salmonids


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