**2015 NRSP8 Aquaculture Genome Annual Report**

Coordinator: Dr. John Liu

Co-Coordinator: Dr. Caird Rexroad

Administrative Supervisor: Dr. Susan Brown

National Program Leader: Dr. Lakshmi Kumar Matukumalli

Industry Representatives: Mitt Walker, Dr. Scott LaPatra

Species Leaders

Salmonids: Dr. Yniv Palti

Catfish: Dr. Sylvie Quiniou

Oysters: Dr. Dina Proestou

Striped Bass: Dr. Craig Sullivan

The Aquaculture Genome Workshop organized by Chair Mohamed Salem was held Saturday January 9th, 2016 in Dan Diego, CA. There were 84 attendees representing 18 Countries (US, Canada, Mexico, Norway, Australia, China, Netherlands, Thailand, Malaysia, Germany, Chile, UK, France, New Zeeland, Turkey, Columbia, Taiwan, Japan) and 53 institutes. The workshop included four invited presentations, sixteen contributed presentations, and 42 poster presentations.

Invited presentations included:

1. Editing Fish Genome with CRISPR

**Wenbiao Chen**, *Vanderbilt University School of Medicine*

1. The Rainbow Trout Genome Provides Novel Insights into Evolution after Whole-Genome Duplication in Vertebrates

**Yann Guiguen**, *INRA-SCRIBE*

1. Genomics in Fish Breeding Programs for Developing Countries

**John A.H. Benzie**, *WorldFish*

1. Regulatory Approval of Genetically Engineered AquAdvantage Salmon

**John Buchanan**, *Center for Aquaculture Technologies*

Contributed presentations included four travel award recipients **highlighted in blue**:

1. Functional Studies in Atlantic Salmon (*Salmo salar L.*) Reveals Candidates for Sterility Vaccines

**Anna Troedsson-Wargelius**, *Institute of Marine Research*

1. Genomic Selection For Bacterial Cold Water Disease Resistance Reveals Large Within-Family Variation That Cannot Be Exploited In Traditional Family-based Selective Breeding In Rainbow Trout

**Roger L. Vallejo**, *USDA-ARS-NCCCWA*

1. Weighted ssGBLUP Improves Genomic Selection Accuracy for Survival in a Rainbow Trout Population

**Breno O. Fragomeni**, *University of Georgia*

1. Genome Scan for Selection Signatures in Atlantic Salmon Populations Using a High Density SNP Array

**María Eugenia López Dinamarca**, *University of Chile*

1. The vgll3 Locus Controls Age at Maturity in Wild and Domesticated Atlantic Salmon (*Salmo salar L.*) Males

**Fernando Ayllon**, *Institute of Marine Research*

1. Genome-Wide Association Study for Identifying Genome Loci That Affect Fillet Yield in Rainbow Trout (*Oncorhynchus mykiss*)

**Dianelys Gonzalez-Pena**, *USDA-ARS-NCCCWA*

1. A Genome-Wide Association Study for Low Oxygen Tolerance in Catfish using the 250K SNP Array

**Xiaozhu Wang**, *Auburn University*

1. Candidate genes for ESC Disease Resistance of Catfish as Revealed by a Genome Wide Association Study

**Tao Zhou**, *Auburn University*

1. A New and Improved Rainbow Trout (*Oncorhynchus mykiss*) Reference Genome Assembly

**Guangtu Gao**, *USDA-ARS-NCCCWA*

1. Progress of the Shrimp Genomic Sequencing Project

**Jianhai Xiang**, *Institute of Oceanology, Chinese Academy of Sciences*

1. Comparative Transcriptome Analysis of the Swimbladder Reveals Expression Signatures in Response to Hypoxia in Channel Catfish, Ictalurus punctatus

**Qiang Fu**, *Auburn University*

1. The Catfish MicroRNAome: Identification, Annotation and Expression Profiling in Response to Bacterial Infections and Hypoxia Stress

**Shikai Liu**, *Auburn University*

1. Allelic-Imbalance Analysis in Pooled RNA-Seq Samples Identifies Muscle-Associated Genetic Markers in Rainbow Trout: Improved Bioinformatics Practices

**Rafet Al-Tobasei**, *Middle Tennessee State University*

1. Role of Long Non-Coding RNAs in Bacterial Cold Water Disease Pathogenesis in Rainbow Trout

**Bam D Paneru**, *Middle Tennessee State University*

1. Genotyping in Thousands By Sequencing (GT-seq): A Low Cost, High-Throughput, Targeted SNP Genotyping Method

**Nathan Campbell**, *Columbia River Inter-Tribal Fish Commission*

1. Development of the Catfish 690K SNP Arrays for Analysis of Quantitative Traits

**Qifan Zeng, Qiang Fu, Shikai Liu and Yun Li**, *Auburn University*

NRSP8 Coordinator Funds also supported travel for Hsinyuan Tsai from the Roslin Institute who presented “Genomic prediction of host resistance to sea lice (L. salmonis) in Atlantic salmon (S. salar).”

The business meeting was held immediately following the conclusion of the workshop. New officers were nominated and approved through a voice vote as follows:

2017 Workshop Chair – Mr. Nathan Campbell

2017 Workshop Chair Elect – Dr. Geoff Waldbieser

2017 NRSP8 Chair Elect – Dr. Mohamed Salem

Dr. Steven Roberts from the University of Washington was selected to replace outgoing Co-coordinator Dr. Caird Rexroad.

**2015 Progress towards NRSP8 Objectives**

Many species lack sufficient data to inform the development of strategies that will provide reliable sequence assemblies. In addition to supporting continued refinement of whole genome reference sequences and transcriptomes for the traditional US aquaculture species of catfish, trout, and oysters, NRSP8 Coordinators Funds were used to generate preliminary data for sablefish (*Anoplopoma fimbria*), blue tilapia , and Pacific whiteleg shrimp (*Litopenaeus vannamei*). These data provide insights into the complexity of these genomes and transcrptomes, and enable better optimization when developing strategies that target assembly of more comprehensive data sets.

**Objective 1: Advance the status of reference genomes for all species, including basic annotation of worldwide genetic variation, by broad sequencing among different lines and breeds of animals.**

***Catfish***

The channel catfish reference genome sequence was assembled with a N50 contig size of 77.2 Kb and a scaffold N50 of 7.7 Mb. A total of 761 Mb of the genomic sequence has been anchored to the 29 chromosomes through linkage analysis using high density SNP arrays. The reference genome sequence was validated by genetic mapping of over 54,000 SNPs, and annotated with 26,661 predicted protein-coding genes.

***Oyster***

NRSP8 Coordinator funds were used to support resource coordination workshops focused on oysters and other shellfish. Sequencing of the eastern oyster (*Crassostrea virginica*) genome (using a single gynogen oyster) has been initiated and a draft genome of Pearl oyster (*Pinctada fucata*) is in progress (NCBI BioProject ID PRJDB2628). A second generation linkage map, based on >1100 type I SNP markers and 66 microsatellite markers was constructed for *Crassostrea gigas*. This higher density linkage map reveals errors in the original genome assembly.

***Salmonids***

NRSP8 Coordinator funds were used to support the development of a genome reference sequence and the identification of genetic variation associated with economically important traits. A new and improved reference genome assembly was generated for rainbow trout coupled with a 50K SNP linkage map. The total size of the new assembly is nearly 2.2Gb with N50=1.7Mb. Approximately 82% of the new assembly has been anchored and ordered onto the new chromosome linkage maps.

A new de novo transcriptome with gene annotation and tissue gene expression atlas was assembled for rainbow trout (Salem et al., 2015).

***Striped bass***

NRSP8 Coordinator’s funds were used to support genome reference sequencing for striped bass. A 585.1 Mbp striped bass genome sequence assembly containing ~35 K scaffolds was produced from Illumina short-read sequence (66-fold genome coverage) and Pacific Biosciences single molecule, long-read sequence (2.8-fold genome coverage). *Ab-initio* and evidence-based gene predictions performed using the MAKER Annotation Pipeline identified 27,485 protein-coding genes. An jBrowse website providing access to the annotated genome was made available online at N.C. State University at https://appliedecology.cals.ncsu.edu/striped-bass-genome-project/.

**Objective 2: Develop strategies to identify and exploit genes and allelic variation that contribute to economically relevant phenotypes and traits, in part through improving functional annotation of the genomes of our species.**

***Catfish***

Columnaris causes severe mortalities among many different wild and cultured freshwater fish species, but understanding of host resistance is lacking. We have used the interspecific hybrid backcross populations and mapped the resistance genes. To identify genes associated with columnaris resistance, we performed a genome-wide association study (GWAS) using the catfish 250k SNP array with 340 backcross progenies. A genomic region on chromosome 7 was found to be significantly associated with columnaris resistance. Within this region, five have known functions in immunity, including pik3r3b, cyld-like, adcyap1r1, adcyap1r1-like, and mast2. In addition, 3 additional suggestively associated QTL regions were identified on chromosomes 7, 12, and 14. The resistant genotypes on the QTLs of chromosomes 7 and 12 were found to be homozygous with both alleles being derived from channel catfish. The paralogs of the candidate genes in the suggestively associated QTL of chromosome 12 were found on the QTLs of chromosome 7. Many candidate genes on the four associated regions are involved in PI3K pathway that is known to be required by many bacteria for efficient entry into the host. Strikingly, the candidate genes may be arranged as functional hubs; the candidate genes within the associated QTLs on chromosomes 7 and 12 are not only co-localized, but also functionally related, with many of them being involved in the PI3K signal transduction pathway, suggesting its importance for columnaris resistance.

***Oyster***

The fully sequenced *C. gigas* genome has led to the intense study of several expanded gene families associated with innate immunity and stress response. These families include: C1q domain-containing proteins (which act as pathogen recognition molecules), Fibrinogen-related proteins (FREPs; which exhibit versatile immune functions), and the Tumor Necrosis Factor (TNF) superfamily.

RNAseq analysis was performed using an oyster sample with a high viral load to elucidate interactions between host (*C. gigas*) and pathogen (OsHV-1; which causes significant mortality in juvenile oysters worldwide). This research resulted in a high quality OsHV-1 transcriptome and identified several molecular pathways in *C. gigas* that are activated in the presence of OsHV-1.

295 SNPs were identified and validated in 90 genes involved with glycogen content in *C. gigas*. Statistically significant associations between genotype and glycogen content were detected for three SNP markers.

The heritability of DNA methylation variation was investigated in diploid and triploid *C. gigas*. Genome-wide methylation patterns did not differ between diploid and triploid oysters. Transmission of methylation status between parents and offspring was largely stable; however at some loci, methylation was observed more frequently in offspring.

***Salmonids***

NRSP8 Coordinators funds were used to demonstrate usefulness of high-density genotyping arrays for identifying disease resistance genes in rainbow trout.

An evaluation of genome-enabled selection strategies for bacterial disease resistance in a commercial rainbow trout population has demonstrated the advantage of genome selection (GS) over traditional BLUP-based breeding values. The predictive ability of offspring performance using different GS models has more than doubled that of the traditional pedigree-based method, translating to much more rapid gains in disease resistance with the potential of increased profitability for the trout aquaculture industry and large reduction of antibiotic use for trout farming.

The 57K SNP chip was used in genome wide association studies (GWAS) for bacterial cold water disease resistance in an experimental and commercial rainbow trout populations, which identified 14 loci with moderate to strong effects that are shared by the two populations. Similarly, two loci with moderate effect were identified in a GWAS for fillet yield in rainbow trout.

Allelic-imbalance analysis of pooled RNA-Seq samples was used to identify genetic markers that may be associated with muscle development in rainbow trout.

A new study identified differentially expressed long non-coding RNAs in response to F. psychrophilum infection in rainbow trout.

RNA-Seq analysis of miRNAs in myosatellite cells exposed to estrogen at different levels (biological or high) revealed dose dependent miRNA expression profiles.

Bisulfite sequencing of the regulatory region of MyoD gene revealed epigenetic regulation of the gene (methylation of a CpG island) under the influence of estrogen. This epigenetic regulation possibly resulted in a decreased expression of the gene in estrogen treated-muscles.

RNA-Seq analysis of miRNAs from eggs of different qualities (assessed by fertilization rate) identified egg quality-associated miRNAs including 4 known miRNAs (omy-miR-193b-3p, omy-miR-203c-3p, omy-miR-499-5p and omy-miR-7550-3p) and two novel miRNAs (omy-miR-nov-95-5p and omy-miR-nov-112-5p).

***Striped bass***

Artificial neural networks and supervised machine learning were used to further evaluate relationships between ovary gene expression (transcriptome) profiles and egg quality (fertility) in striped bass. Findings compared to those obtained for other farmed fishes using conventional analytical methods were published in an invited review. Advanced proteomics and targeted transcript studies revealed heretofore unknown molecular mechanisms of egg yolk protein and lipid formation in striped bass, white perch, and other teleosts.

**Objective 3: Facilitate analysis, curation, storage, distribution and application of the enormous datasets now being generated by next-generation sequencing and related "omics" technologies with regard to animal species of agricultural interest.**

***Oyster***

Resource coordination workshop focused on oysters and other shellfish held in conjunction with the National Shellfish Association annual meeting in Monterey, CA, March 22-26, 2015. Organizer: Steven Roberts.

*C. gigas* transcriptome information derived from 2.2 billion sequences from 114 RNAseq datasets has been organized and deposited into a publicly available database: GigaTON. The user interface provides powerful and user-friendly tools to search and retrieve annotation, expression, and polymorphism information.

**Publications**

***Catfish***

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***Striped bass***

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