NRSP-8 Aquaculture 2018 Annual Report

Leadership

Coordinator: Benjamin J. Reading, North Carolina State University
Co-coordinators: Steven Roberts, Washington State University
Eric Peatman, Auburn University
Species Leaders:
Catfish: Sylvie Quiniou, ARS Stoneville, Mississippi,
John Liu, Syracuse University, New York
Oyster: Dina Proestou, ARS University of Rhode Island, Rhode Island
Salmonids: Yniv Palti, ARS Leetown, West Virginia
Striped Bass: Benjamin Reading, North Carolina State University, North Carolina

2019 Aquaculture Workshop Report

Workshop Chair 2018-2019: Catherine Purcell (catherine.purcell@noaa.gov)
Chair-elect 2019-2020: Louis Plough (lplough@umces.edu)
Chair-elect 2020-2021: Moh Salem (Mohamed.Salem@mtsu.edu)

Theme

Aquaculture Genomics Workshop 2019

Attendees

Number: 80
Number of institutes: 43

Invited Presentations (4 Plenary Speakers)

1. FAASG – Functional Annotation of All Salmonid Genomes Ben F. Koop, University of Victoria
2. Editing for Animal Welfare and Environmental Sustainability: Are These Traits Important? Tad S. Sonstegard, Acceligen, Inc.
3. Gene Transcription Data for eQTL Analysis, Variance Component Analysis and Gebv Estimation in Atlantic Salmon Anna K. Sonesson, Nofima AS
4. Use of Atlantic Salmon Gene Editing in Research and Development Anna Troedsson-Wargelius, Institute of Marine Research

Contributed Presentations (15)

Poster Session Participants (20)

Business Meeting Minutes

Time: Saturday January 12, 2019, 5:00-5:46 pm
Place: Pacific Salon 3/4, Town and Country Hotel, San Diego, CA
Number of Attendees: 9

1. Call to order. Catherine Purcell, Ph.D. (2018-19 Workshop Chair, NOAA, California) called the business meeting to order at 5:00 pm, following the Aquaculture Workshop.

2. Jim Reecy, Ph.D. (2018-19 Bioinformatics Coordinator, Iowa State University) provided a status update of technological resources and plans for data management: the genome database is still under construction; the NAGRP VCF Data Repository (animalgenome.org) will be funded for only five more years and as such, the impetus to store and maintain web resources for genome data is on the species coordinators and associated researchers; a sustainability plan needs to be put into place and is open to suggestions (a current suggestion is to coordinate with librarians at home institutions). Data curation will be led by now-incumbent Co-Coordinators James Koltes, Ph.D. (Present Bioinformatics Co-Coordinator, Iowa State University) and Fiona McCarthy, Ph.D. (Present Bioinformatics Co-Coordinator, University of Arizona).

3. Benjamin Reading, Ph.D. (Present Aquaculture Coordinator, North Carolina State University) began overview of Species Coordinator reports (not all submitted at the time of meeting due to United States Government shut-down) and status of future meetings and chair positions.

4. Monies for 2020 meeting will be funded through North Carolina State University and are ear-marked to be available in October of 2019. Eric Peatman, Ph.D. (former Acting NRSP-8 Chair 2018-19 with John Liu 2018-2018; Auburn University) is processing the remainder of the funds from the past funding cycle and deposit for 2020 meeting can be supported through these remaining funds if required prior to Oct. 2019.

5. Louis Plough, Ph.D. (University of Maryland, Center for Environmental Science) is the Present and Accepted Workshop Chair-Elect for 2019-20. Mohamed Salem, Ph.D. (Middle Tennessee State University) is the Accepted Workshop Chair-Elect for 2020-21.

6. A note was made for coordinators to mention revenue generated, grants added, and funds leveraged in species updates

7. NRSP-8 is one of the longest-running NRSPs in the history of their offering. This is attributed to a weakness in terms of progress, but a strength in terms of goals left to achieve. Action plan moving forward is to focus on data curation (see above, bioinformatics) and the development of widely accessible resources and applications.

8. Industry support is important for each species to obtain a level of impact via industry deliverables, however garnering industry support is important for each species to obtain a certain level of impact, however, this may fragment the solidarity among species-groups by progress made as a function of industry support
.
9. For the distribution of monies pending cuts to funding, Bioinformatics is considered the most critical. Mohamed suggested reducing student awards, small funding opportunities, and looking towards large center grants or industry-matching funds. Louis suggested having fewer plannary speakers, although some speakers accepted honorariums.

10. Meeting was adjourned.

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 (**Quiniou**, Liu)
Using an innovative approach of a YY channel catfish as the sequencing template and third generation sequencing technologies, we generated, assembled and annotated the YY genome sequence of channel catfish. This represents the very first Y chromosome sequence among teleost fish, and one of the few whole Y chromosome sequences among vertebrate species. The genome sequence assembly had a contig N50 size of 2.7 Mb and a scaffold N50 size of 26.7 Mb.

Oyster (Gómez-Chiarri, Putnam, Guo, Warren, **Proestou**)
Eastern oyster (*Crassostrea virginica*) genome assembly v. 3.0 was completed; 99% of sequences are assembled into the known number of chromosomes (10). Gene annotation was completed using the automated NCBI pipeline. Computational Analysis of gene Family Evolution (CAFE analysis) was performed to compare expansion of

gene families in Eastern oyster with other molluscan genomes. Completed re-sequencing of 92 eastern oyster genomes at 20X coverage. Sequenced specimens derived from 4 geographic regions (Gulf of Mexico, Chesapeake Bay, Delaware Bay, and Maine), 2 salinity regimes within each region (high and low), and wild and selected populations within each region. Sequencing was partially funded through NRSP-8 Aquaculture Program.

Salmonids (Salem, **Palti**)
A chromosome level genome assembly was published for Chinook salmon (Narum et al. 2018) based on the publicly released assembly on NCBI. Atlantic salmon farming in eastern US and Canada is restricted to genetic stocks of North American origin due to ecological and conservation concerns. However, the majority of SNP discovery and SNP chip development efforts in Atlantic salmon have focused on genetic stocks from European origin. High coverage whole genome resequencing within 80 North American Atlantic salmon was conducted to identify 8,395,146 SNPs.

Striped Bass (Fuller, Abernathy, Kovach, Berlinsky, **Reading**)
The striped bass genome assembly v. 2.0 (598 Mb) was completed using a combinatorial approach of Illumina and Pacific Biosciences sequencing and Chicago® and Dovetail™ Hi-C + HiRise™ scaffolding. The number of assembly scaffolds was 629, of which 21 contain most of the genome sequence (L90 = 21 scaffolds), which is consistent with a haploid chromosome number of 24 for striped bass. *Ab-initio* and evidence-based gene predictions performed using the MAKER Annotation Pipeline identified 27,485 coding genes. Genotyping efforts based on ddRAD-Seq to explore population heterozygosity and genetic variation related to growth performance of domestic striped bass and those of wild-origin derived from 7 geographic locations along the Atlantic Ocean are complete. The white bass genome assembly v. 1.0 (645 Mb) was completed using Illumina sequencing combined with Chicago® and Dovetail™ Hi-C + HiRise™ scaffolding. This approach produced a genome assembly (L90 = 23 scaffolds). *Ab-initio* gene prediction using produced 28,356 protein-coding genes while evidence-based prediction from alignments of white bass transcriptome sequences produced 24,176 protein-coding genes. Over 2.8 billion paired-end 150 bp reads were generated for white bass using RNA sequencing (transcriptomics studies). These additional sequences will allow for improvement of our white bass transcriptome, provide for a source of gene-associated variation and serve as a guide for annotation of the white bass genome assembly.

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 (**Quiniou**, Liu)
Genetic linkage and GWAS analyses placed the sex determination locus of channel catfish within a genetic distance less than 0.5 cM and physical distance of 8.9 Mb. However, comparison of the channel catfish X and Y chromosome sequences showed no sex-specific genes. Comparative RNA-Seq analysis between females and males revealed exclusive sex-specific expression of an isoform of BCAR1 gene in the male during early sex differentiation. Coupling of positional and expression candidates suggest the candidacy of BCAR1 as a sex determination gene, and experimental knockout of BCAR1 gene converted genetic males (XY) to phenotypic females. This indicates that alternative splicing may serve as the molecular mechanism for sex determination in catfish. QTL also were sequenced and mapped for growth performance and disease resistance against enteric septicemia of catfish (ESC).

Oyster (Roberts, Putnam, Lotterhoos, Puritz, Johnson, Eirin-Lopez, Allen, Zhang, Plough, **Proestou**)
Functional annotation is underway by Eastern Oyster Genome Consortium (e.g. Blast2GO, Pfam, DNA methylation patterns). Outlier analysis and environmental association analysis for population genomic analysis of re-sequence data is underway. Over 30,000,000 SNPs were detected from oyster resequence data, thinned and pruned to a working set of 200K for population genomic analysis. Microbiome analysis of naïve and Dermo-infected oysters resistant and susceptible to disease was conducted. RNA-seq analyses are ongoing to understand the genetic and genomic basis for Dermo-resistance in Eastern Oyster breeding populations. Investigation of genetic basis for low-salinity tolerance in eastern oyster continues to quantify heritability for salinity tolerance and identifying QTL underlying tolerant phenotypes.

Salmonids (Salem, **Palti**)
A total of 10 moderate effect QTL associated with resistance to Infectious hematopoietic necrosis (IHNV; viral disease of salmonids that can result in mass mortality and significant economic losses), which jointly explained up to 42% of the additive genetic variance were detected in genome-wide association analyses of the commercial rainbow trout breeding population of Clear Springs Foods, Inc. using the 57K SNP chip. Two major QTL associated with Bacterial cold water disease (BCWD), a major disease in rainbow trout aquaculture, resistance on chromosomes Omy8 and Omy25 were reported. Whole genome resequencing of 40 fish from resistant and susceptible trout families was conducted to identify new SNPs and to refine the QTL regions. Over 15 million SNPs were identified from resequencing in this population and the two major QTL were narrowed down to regions much smaller than those reported previously. Genomic SNPs associated with thermal adaptation in redband trout were identified.

Genotype frequencies for GREB1L were estimated in populations in the Pacific Northwest USA along with individual genotype association for migrating individuals. Genomic SNPs associated with (1) premature/mature arrival to spawning grounds in Chinook salmon and estimated genotype frequencies for the candidate gene greb1L in populations across N. America and (2) age-at-maturity and sex in Chinook salmon also were identified. Genome-wide association study using a 50K transcribed gene SNP-chip identified QTL affecting muscle yield in rainbow trout. A study characterized coding and noncoding genes involved in gonadogenesis-associated muscle atrophy in rainbow trout also was published. Muscle atrophy appears to be mediated by many genes encoding ubiquitin-proteasome system, autophagy related proteases, lysosomal proteases and transcription factors. A study characterized correlation between lncRNA and mRNA expression in rainbow trout families showing variation in body weight, muscle yield, fat content, shear force and whiteness also was published. Three differentially expressed (DE) antisense lncRNAs were co-expressed with sense genes known to impact muscle quality traits. Forty-four differentially expressed lncRNAs had potential sponge functions to miRNAs that affect muscle quality traits.

Striped Bass (Berlinsky, Fuller, Abernathy, Woods, McGinty, Borski, **Reading**)
Expressed quantitative trait loci (eQTL) and small molecule profiling (metabolomics) analyses are ongoing to understand gene pathways related to superior growth traits in sunshine hybrid striped bass (white bass female x striped bass male) and domestic striped bass. Adult, male, domestic striped bass (n=60) were disseminated to major aquaculture producers in the U.S. for hybrid striped bass fry and fingerling production (directly contributing to the $50 million farm gate per year industry). Wild white bass gathered from Arkansas, Texas and Alabama along with available domesticated strains are being used to establish a base breeding population for familywise evaluations of growth and nutrient utilization on alternative, sustainable diets. A genotyping-by-sequencing panel has been developed from white bass populations, where single-nucleotide polymorphisms (SNPs) identified can discriminate domestic stocks from wild-sourced individuals. Additional genetic markers are being developed to rapidly identify gender and parentage.

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 (Gómez-Chiarri, Roberts, **Proestou**)
Hands-on comparative genomics workshop was held at the National Shellfisheries Association annual meeting in Seattle, WA, March 18-21, 2018. Eastern Oyster

Genome Consortium planning and writing workshop was conducted in Narragansett, RI, October 3-4, 2018.

Salmonids (Salem, **Palti**)
Contributions to the development of FishGen.net database for storage of large-scale genotypes for genetic tagging and monitoring studies were made.

Striped Bass (**Reading**)
JBrowse integrated web portal of the draft striped bass genome resource is hosted online for use as an unrestricted public resource. Progress is being made to produce a similar resource on NCBI. Database URL: http://appliedecology.cals.ncsu.edu/striped-bass-genome-project/. Ongoing development of different and novel machine learning-based analytical platforms focused on small molecule (metabolomics) and gene expression (RNA-Seq) profiling to better understand hybrid striped bass growth performance (heterosis effects).

Research support mini-grants (coordinator grants)

Three (3) mini-grants (~$10,000/each) supported projects that fall under all three primary NRSP-8 objectives and include a variety of species. Awards listed:

1. Jason Abernathy and Steven J. Micheletti "**Mapping sex-linked genes in temperate basses for improved hybrid striped bass culture**", USDA ARS.
2. Hollie Putnam and Steven Roberts "**Functional Re-annotation of Oyster Genomes with Epigenetic Resources (FROGER)**", University of Rhode Island.
3. Moh Salem "**The landscape of histone modifications in the rainbow trout genome: preliminary data for FAASG**", Middle Tennessee State.

Travel Support & Opportunities for Trainings

The travel of seven students/postdocs was funded to attend the Aquaculture Workshop at PAG 2019. The purpose of the travel award program is to help graduate students and postdoctoral fellows to travel to the annual PAG meetings and present their research. The awardees of PAG 2019 are as follows:

1. Pratima Chapagain, Middle Tennessee State University (TN, USA), "**Gut Microbiome Analysis of Fast- and Slow-growing Rainbow Trout (*Oncorhynchus mykiss*)**".

2. Valentina Cordova, University of Chile (Chile), "**Development of a SNP baseline for genetic stock identification in a commercially important species of the South-east Pacific (*Genypterus chilensis*)**".
3. Konstantin Divilov, Oregon State University (OR, USA), "**Genetics of Pacific Oyster Uniformity in Different Environments**".
4. Garrett McKinney, NOAA National Marine Fisheries Service, Northwest Fisheries Science Center (WA, USA), "**Development of a universal sex assay and identification of y-chromosome haplotypes in Chinook salmon**".
5. Ivan Pocrnic, University of Georgia (GA, USA), "**Exploiting the dimensionality of genomic information in channel catfish**".
6. Noemie Valenza-Troubat, The New Zealand Institute for Plant & Food Research Limited (New Zealand), "**Genomics of New Zealand trevally: exploring the interactions genetic basis of quantitative traits to inform a newly developed breeding programme**".
7. Wenwen Wang (Auburn University (LA, USA), "**Identification of QTL associated with Aeromonas disease resistance in catfish throughout a genome-wide association study**".

Leveraged funds and stakeholders' use of project outputs

Leveraged funds from diverse projects totaling more than one million dollars from federal sources. Selected grants are highlighted below:

Egg Yolk, Egg Buoyancy and Striped Bass Recruitment: A Common Link? (PI Reading) *$205,495*. **North Carolina Coastal Recreational Fishing License Program (CRFL), Division of Marine Fisheries.**

The Hybrid Striped Bass: Understanding Heterosis to Improve Food-Animal Agriculture. (PI Reading) *$300,000* (+*$300,000* industry matching funds). **Foundation for Food and Agriculture Research (FFAR), New Innovator in Food and Agriculture Research Award.**

Major impact products (could be potential impact)

The new genome references should help to identity genes that control economically important aquaculture production traits. Draft genomes were assembled for striped bass v. 2.0, white bass v 1.0, eastern oyster (*Crassostrea virginica*) v. 3.0, and an additional re-sequencing of 92 eastern oyster genomes at 20X coverage also was completed. A chromosome level genome assembly was published for Chinook salmon (Narum et al., 2018) based on the publicly released assembly on NCBI. The shrimp genome sequence also was published in *Nature Communications* (Zhang et al., 2019). An improved reference genome also was reported for YY channel catfish (Bao et al., 2019).

Publications (2018)

1. Bao L, Tian C, Liu S, Zhang Y, Elaswad A, Yuan Z, Khalil K, Sun F, Yang Y, Zhou T, Li Ning, Tan S, Zeng Q, Liu Y, Li Y, Li Y, Gao D, Dunham R, Davis K, Waldbieser G, Liu ZJ. 2019. The Y chromosome sequence of the channel catfish suggests novel sex determination mechanisms in teleost fish. *BMC Biology*, https://doi.org/10.1186/s12915-019-0627-7

2. Tan S, Wang W, Tian C, Niu D, Zhou T, Jin Y, Yang Y, Gao D, Dunham R, Liu ZJ. 2019. Heat stress induced alternative splicing in catfish as determined by transcriptome analysis. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics* 29: 166-172.

3. Xiaojun Zhang, Jianbo Yuan, Yamin Sun, Shihao Li, Yi Gao, Yang Yu, Chengzhang Liu, Quanchao Wang, Xinjia Lv, Xiaoxi Zhang, Ka Yan Ma, Xiaobo Wang, Wenchao Lin, Long Wang, Xueli Zhu, Chengsong Zhang, Jiquan Zhang, Songjun Jin, Kuijie Yu, Jie Kong, Peng Xu, Nansheng Chen, Hong-Bin Zhang, Patrick Sorgeloos, Amir Sagi, Acacia Warren, Zhanjiang Liu, Lei Wang, Jue Ruan, Ka Chu, Bin Liu, Fuhua Li, and Jianhai Xiang. 2019. Penaeid shrimp genome provides insights into benthic adaptation and frequent molting. *Nature Communications*, 10:356. https://doi.org/10.1038/s41467-018-08197-4

4. Li N, Bao L, Zhou T, Yuan Z, Liu S, Dunham R, Li Y, Wang K, Xu X, Jin Y, Zeng Q, Gao S, Fu Q, Liu Y, Yang Y, Li Q, Meyer A, Gao D, Liu ZJ. 2019. Genome sequence of walking catfish (*Clarias batrachus*) provides insight into terrestrial adaptation. *BMC Genomics*, 19: 952. https://doi.org/10.1186/s12864-018-5355-9

5. Zhou T, Yuan Z, Tan Suxu, Jin Yulin, Yang Yujia, Shi H, Wang W, Niu D, Gao L, Jiang W, Gao D, and Liu ZJ. 2018. A review of molecular responses of catfish to bacterial diseases and abiotic stresses. *Frontiers in Physiology* 9:1113. https://doi.org/10.3389/fphys.2018.01113

6. Tan S, Wang W, Zhong X, Tian C, Niu D, Bao L, Zhou T, Jin Y, Yuan Z, Yang Y, Gao D, Dunham R, and Liu ZJ. 2018. Increased alternative splicing as a host response to bacterial infections in catfish. *Marine Biotechnology* 20: 729-738. DOI: 10.1007/s10126-018-9844-2.

7. Yang Y, Wang X, Liu Y, Fu Q, Tian C, Wu C, Liu S, Gao D, Dunham R, Liu ZJ. 2018. Transcriptome analysis reveals enrichment of genes associated with auditory system in swimbladder of channel catfish. *Comparative Biochemistry and Physiology - Part D: Genomics and Proteomics* 27: 30-39. https://doi.org/10.1016/j.cbd.2018.04.004

8. Wang E, Yuan Z, Wang K, Gao D, Liu ZJ, Liles MR. 2019. Consumption of florfenicol-medicated feed alters the composition of the channel catfish intestinal microbiota including enriching the relative abundance of opportunistic pathogens. *Aquaculture* 501: 111-118.

9. Tan S, Zhou T, Wang W, Jin Y, Wang X, Geng X, Gao D, Dunham R, and Liu ZJ. 2018. GWAS analysis using F2 interspecific hybrids reveals superior blue catfish alleles responsible for strong resistance against enteric septicemia of catfish. *Molecular Genetics and Genomics* 293:1107-1120. DOI: 10.1007/s00438-018-1443-4

10. Yuan Z, Zhou T, Tian C, Bao L, Liu S, Shi H, Yang Y, Gao D, Dunham R, Waldbieser G, Liu ZJ. 2018. The annotation of repetitive elements in the genome of channel catfish (*Ictalurus punctatus*). *PLoS One* 13: e0197371. https://doi.org/10.1371/journal.pone.0197371

11. Shi H, Zhou T, Wang X, Yang , Wu C, Liu S, Bao L, Li N, Yuan Z, Jin Y, Tan S, Wang W, Zhong X, Qin G, Gao D, Dunham R, Liu ZJ. 2018. Genome-wide association analysis of intra-specific QTL associated with the resistance for enteric septicemia of catfish. *Molecular Genetics and Genomics* 293: 1365-1378. DOI: 10.1007/s00438-018-1463-0

12. Zhou T, Li N, Jin Y, Zeng Q, Prabowo W, Liu Y, Tian C, Bao L, Liu S, Yuan Z, Fu Q, Gao S, Gao D, Dunham R, Shubin NH, Liu ZJ. 2018. Chemokine C-C motif ligand 33 is a key regulator of teleost fish barbel development. *Proceedings of the National Academy of Sciences* 115 (22): e5018-e5027

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14. Yang Y, Fu Q, Wang X, Liu Y, Zeng Q, Li Y, Gao S, Bao L, Liu S, Gao D, Dunham R, and Liu ZJ. 2018. Comparative transcriptome analysis of the swimbladder reveals expression signatures in response to low oxygen stress in channel catfish, *Ictalurus punctatus. Physiological Genomics* 50:636-647. https://doi.org/10.1152/physiolgenomics.00125.2017

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swimbladder. *Physiological Genomics* 50:67-76. doi:10.1152/physiolgenomics.00089.2017

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19. Thongda, Wilawan, et al. 2018. "Development of SNP panels as a new tool to assess the genetic diversity, population structure, and parentage analysis of the eastern oyster (*Crassostrea virginica*)." *Marine biotechnology* 20.3: 385-395.

20. Li, Li, et al. 2018. "Divergence and plasticity shape adaptive potential of the Pacific oyster." *Nature ecology & evolution*2.11: 1751.

21. Ben-Horin, Tal, et al. 2018. "Genetic variation in anti-parasite behavior in oysters." *Marine Ecology Progress Series* 594: 107-117.

22. De Wit, Pierre, et al. 2018. "Gene expression correlated with delay in shell formation in larval Pacific oysters (*Crassostrea gigas*) exposed to experimental ocean acidification provides insights into shell formation mechanisms." *BMC genomics* 19.1:160.

23. Gagnaire, Pierre-Alexandre, et al. 2018. "Analysis of genome-wide differentiation between native and introduced populations of the cupped oysters *Crassostrea gigas* and *Crassostrea angulata*." *Genome biology and evolution* 10.9:2518-2534.

24. Vezzulli, Luigi, et al. 2018. "Comparative 16SrDNA gene-based microbiota profiles of the Pacific oyster (*Crassostrea gigas*) and the Mediterranean mussel (*Mytilus galloprovincialis*) from a shellfish farm (Ligurian Sea, Italy)." *Microbial ecology* 75.2:495-504.

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