Project Report for 2017 NRSP-8 Aquaculture research support funds:
"Outlining parent-of-origin effects in wild x domestic hybrid striped bass for eQTL potential"
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Status narrative: We again thank the NRSP-8 Aquaculture Grant Panel for funds to improve the status of bass genomics. For this project, our goal was to map loci associated to domestication in hybrid striped bass (HSB) and assess the potential for this hybrid model to be useful in study of parent-of-origin effects. We proposed to do so using a combination of reciprocal cross hybridizations and high-throughput sequencing combined with computational analyses.

Our facility manually spawns white bass (WB) and striped bass (SB) each spring according to the natural photo-thermal period of the fish (April-June). As the award notification was in July 2017, this was already passed our (and collaborators) WB and SB spawning and capture season. Thus, we could only spawn the following spring (2018) to make specific crosses.

In April 2018, to generate wild x domestic crosses of HSB, we collaborated with the Arkansas Game and Fish Commission and Keo Fish Farms to capture wild SB, while WB at our station originated from the NCSU lines and have been domesticated for over nine generations. For SB, fish were captured and transported to the location. The next day, wild captured female SB along with domesticated female WB being reared at the station were anesthetized and injected with a 75 µg slow-release GnRH pellet to induce egg ovulation. Males were assessed at the same time for milt production. After approximately 24 hours, female fish were strip spawned and each clutch fertilized with one male according to our experimental design (sunshine HSB; ♀/domestic WB x ♂/wild SB and palmetto HSB; ♂/domestic WB x ♀/wild SB) to produce wild x domestic HSB. Fertilized eggs were activated with water, treated with tannic acid to reduce adhesion and gently rolled in MacDonald jars until hatching into an individual cross tank. At five days post hatch, fry were placed in ponds that had been fertilized to nourish phytoplankton-zooplankton bloom. The experimental design was such that manufactured diets were never presented. Thus to ensure healthy fish, HSB were collected and flash frozen in liquid nitrogen at a time immediately prior to switching to prepared diets (33 dph; ~ 1 g).

For total RNA extraction, individual whole fish from sunshine (n=24) and palmetto (n=24) HSB were ground in a mortar and pestle under liquid nitrogen. A 50 mg sample of the homogenate was re-suspended in Buffer RLT with 2-mercaptoethanol and processed by the RNeasy Mini Kit. Samples were treated with DNase I to remove any potential contaminating DNA. Samples were then processed by the NEBNext Ultra II Directional RNA Library Prep Kit for Illumina with NEBNext multiplex oligos. Samples are currently being assessed for quality on a BioAnalyzer and quantity by quantitative PCR, with no pitfalls experienced to date.

Use of funds: A commercial vendor is under contract for sequencing service on two lanes of Illumina HiSeq X. The remaining funds were used for RNAseq kits, for reagents to assess library quality (BioAnalyzer) and quantification (qPCR) and associated consumables.

Deliverables: (1) Our experimental design and workflow was published in an Abstract:
(Apr 2018) **J. Abernathy**, **S.A. Fuller**, B. Green, M. Lange, S. Rawles, D. Straus and C. Webster. Coordinated effort to advance genomes-to-phenomes through the integration of bioinformatics with aquaculture research. 4th Annual Meeting of the Arkansas Bioinformatics Consortium (AR-BIC). Little Rock, AR. (2) Excess HSB were gifted to industry; (3) RNAseq database to be prepared; and (4) Manuscript to be prepared.