**Semi-Annual Report**

**Development of Genomic Markers for Environmental Resilience in Mussels**

**Reporting Period: 05/01/2022 – 10/30/2022**

**(A) Project Summary**

Our project seeks to support the sustainable expansion of the shellfish aquaculture industry by investigating the downstream impact of common environmental stressors on the survival and cultivation of marine bivalves.

Our research objective is to describe the response of commercially relevant species of marine mussels to environmental fluctuations commonly experienced within nearshore environments, including ocean acidification (OA) and ocean warming (OW), utilizing cutting-edge molecular technologies to identify genetic markers that confer resilience to environmental change.

The measure of success for this proposal will be the identification of genetic markers that, when used as selection criteria for mussel broodstock, will produce adults with robust attachment to aquaculture lines under near-future OA and OW. By defining these gene-environment interactions, our results stand to support commercial growers in the development of selective breeding programs to ensure the efficient, sustainable, and profitable production of mussels within the United States.

**(B) Summary of Progress and Results**

During the previous reporting periods we completed the first and second phases of the project. The first phase constituted laboratory stressor trials wherein mussels (*Mytilus trossulus*) underwent acute exposure to ocean acidification (OA), ocean warming (OW), hypoxia, and desiccation stress; we also completed mechanical testing of byssal threads produced by mussels within each treatment and measured the oxygen consumption (through closed system respirometry) of mussels before and after exposure to stressors. During the second phase, RNA was extracted from flash frozen gill (ctenidia) and foot tissue samples and submitted to the Genomic Sequencing and Analysis Facility (GSAF) at University of Texas at Austin for 3′-end Tag-Sequencing (Tag-Seq) and library preparation. Sequencing data was received from the GSAF in April 2022, trimmed for adapter sequences, and assessed for quality using MultiQC (<https://multiqc.info/>); 131 samples passed quality assessment. There is currently no available genome assembly for *M. trossulus*. To develop a genomic resource against which we could align our sequencing data, we generated a *de novo* transcriptome assembly with Trinity (<https://github.com/trinityrnaseq/trinityrnaseq/wiki>) using all publicly available sequence read archives (SRA) uploaded to the National Center for Biotechnology Information (NCBI) website. We used the splice aware aligner HISAT2 (<http://daehwankimlab.github.io/hisat2/>) to align tag-seq transcripts against the assembly and achieved an average alignment rate of ~35%.

 **(C) Challenges**

Given the poor alignment rate of our sequencing data against the transcriptome assembly we generated using publicly available data, we have decided to submit additional samples for total RNA sequencing. Pooled gill and foot RNA extracted from all mussels included in our experiments were submitted for the University of Washington PacBio sequencing facility on 10/18/2022. Sequencing and library prep will be performed on the Iso-Seq Express platform and will generate approximately 3 million tissue-specific reads per SMRT cell. The Iso-seq platform should address the issues we encountered using publicly available SRA data by generating full-length transcript sequences of 10kb or longer from animals used in our experiments; this approach will maximize the probability that any transcriptome assembly generated achieves a high alignment rate with our tag-seq data while also capturing rare (low expression level) transcripts that are uniquely expressed (no present in SRA data) following environmental stress exposure.