Progress Report:

PSMFC Subaward 23-084G for the period **February 1 – April 30, 2025**

Summary: Recent heat wave stress in the Gulf of Alaska has resulted in significant declines of Pacific cod, Gadus macrocephalus, in that region. The physiological and transcriptional responses of Pacific cod and whether selective mortality is present under thermal stress are unknown. The proposed project will address these questions critical to their survival under climate change by identifying regions of the genome and epigenome that respond to thermal stress. Juvenile Pacific cod will be reared in several temperatures then an integrated genomic approach will identify genes, gene variants, and epigenetic markers that respond to thermal stress and confer resilience. To complement the genomic approaches and further investigate temperature influences on energy resources, we will perform lipid analyses. This work will inform predictions of genetic selection and molecular response of Pacific cod in the Gulf of Alaska under climate change.

Progress and Results

Brief Summary: During this reporting period (February 1 – April 30), we advanced several core components of the project, including re-sequencing previously ungenotyped individuals critical to the study, refining genotype-phenotype analyses with an emphasis on functionally relevant SNPs, developing a protocol for co-extraction of RNA and DNA, and generating a new temporal dataset to assess population-level genetic responses to historic marine heatwaves. Together, these efforts strengthen our ability to identify genomic regions associated with thermal resilience and to evaluate the potential for selection in a changing climate.

1. DNA Resequencing of Degraded Samples

To enhance the power of our genotype-phenotype analyses, we re-sequenced individuals previously excluded due to poor-quality DNA. These juveniles died during exposure to the highest experimental temperature (16°C), and initial tissue samples were too degraded for sequencing. New tissue was collected, DNA was isolated, libraries were prepared, and samples were re-sequenced. Inclusion of these fish is especially important, as they may harbor genetic variants associated with mortality under heat stress, expanding our ability to detect selection.

2. Refinement of Genetic Analyses with Known-Origin Reference Fish

We refined our genetic analyses by comparing experimental juveniles to an expanded reference panel of adult Pacific cod of known spawning origin. This effort integrates newly generated genetic data with sufficient read depth and places findings within the context of regional genetic structure. In particular, enhanced resolution along the admixed gradient between western and eastern Gulf of Alaska populations is improving our ability to investigate whether genetic variation influencing thermal response is geographically structured.

3. Targeted Genotype-Phenotype Association Approach

To address limitations in sample size and sequencing depth, we adopted a targeted GWAS approach, focusing on SNPs within gene regions identified as temperature-responsive via transcriptomic analysis. This hypothesis-driven strategy reduces the number of multiple comparisons and increases statistical power to detect meaningful associations. By prioritizing functionally relevant loci, we aim to identify genetic variants most likely to affect key traits such as lipid storage, growth, and survival under thermal stress.

4. DNA/RNA Co-Extraction for Epigenetic and Transcriptomic Analyses

We developed and began optimizing a protocol to co-extract high-quality DNA and RNA from the same tissue samples using a Bullet Blender. This dual-extraction approach enables integrated analyses of gene expression, genotype, and DNA methylation within individual fish. Initial tests suggest yields are sufficient for downstream applications, including investigations into immune and metabolic responses (e.g., oxygen transport) under temperature stress. Further validation is underway to ensure compatibility with library preparation and sequencing requirements.

5. New Genomic Dataset to Assess Heatwave Effects Over Time

To assess whether allele frequencies have shifted in wild Pacific cod populations in response to recent marine heatwaves, we generated a new temporal genetic dataset. Archived juvenile tissue samples collected in the Gulf of Alaska before, during, and after the 2014–2016 heatwave were selected. DNA extraction, library preparation, and sequencing were completed. These data will allow us to investigate temporal shifts in population genetic structure, especially at loci previously linked to thermal response or traits such as liver size and lipid storage. Initial quality assessment and data processing are underway.

Next Steps

- We will finalize the DNA methylation and mRNA extractions and assess yields and quality for epigenetic and expression co-analysis.
- A manuscript is currently in preparation that reports the phenotypic, gene expression, and genotypic data and integration.
- Analysis of the new temporal genetic dataset will be a major focus of the next reporting period.

Conclusion: Progress this quarter has focused on expanding and refining key datasets to improve statistical power and resolution in detecting genotype-phenotype associations related to thermal resilience. Sequencing efforts, targeted analytical strategies, and protocol development have laid a strong foundation for integrated genomic analyses. These efforts directly support our overarching goal of understanding the genomic and physiological basis of thermal tolerance in Pacific cod and informing predictions of how this species may respond to ongoing climate change.