Project Report
PSMFC Subaward 23-084G for the period **November 1 – January 31**

Project Title: Gene activity and genetic selection in Pacific cod reared under thermal stress

Objective: Predict organismal and population outcomes of Pacific cod exposed to elevated temperature

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: Using genomic and transcriptomic data, we identified key genetic markers linked to growth, body condition, and fat storage—essential for overwintering. A notable discovery is a serotonin receptor gene variant that may regulate appetite and energy use. We also analyzed historical DNA from cod collected since 2008 to track genetic shifts in response to marine heatwaves. Moving forward, we're refining analyses and collaborating with NOAA to better understand how climate change impacts cod resilience, with the goal of informing future fisheries management. *A detailed report of activities is provided below*

Genotype-dependent performance in warming

Genetic data was previously generated for the majority of experimental fish (157 of 160). Using the genetic data and per-fish measurements, we identified genetic variants that are associated with performance in each temperature. The analysis approach is referred to as a Genome Wide Association Study (GWAS), which scans the genome at sites with single-nucleotide polymorphisms (SNPs) and identifies those where traits vary based on the fish's allele. To facilitate this analysis, we performed genotype imputation of our low-coverage whole genome sequencing (lcWGS, 3x depth), which infers genotypes for missing data based on linkage. For example, we leveraged the variability in the hepatosomatic index, or the liver lipid size relative to body size (Figure 1), to identify putative genetic markers that are associated with juvenile cod's ability to allocate energy into its liver when exposed to heatwave-like temperatures. This may be a particularly important trait for juveniles preparing to overwinter (Abookier et al. 2024).

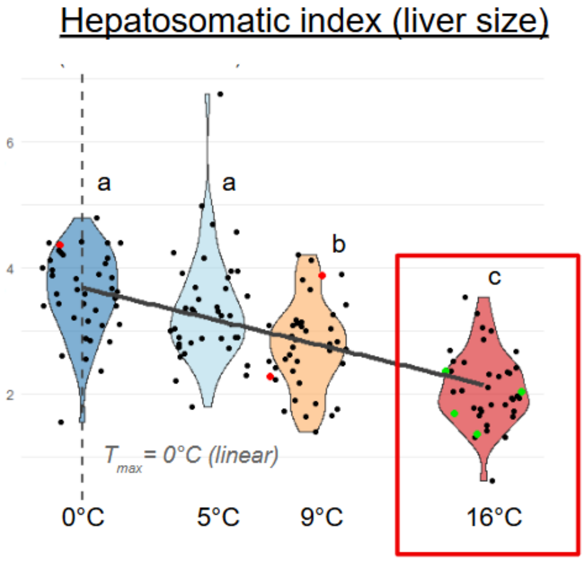

Figure 1: HSI, the liver size relative to body, varied in warm-exposed juveniles. We applied GWAS using whole-genome sequence data to identify genetic variants that are associated with liver size, which was possible due to variation in HSI among juveniles within each temperature (here we highlight HSI variation observed in 16C).

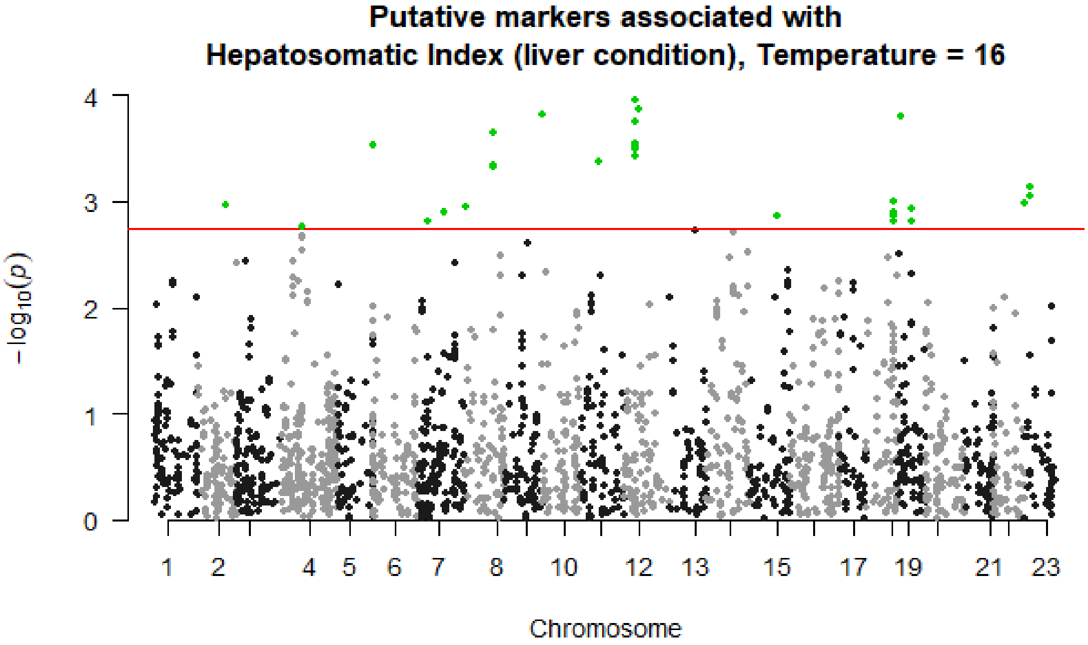

Figure 2: Sites (in green) on the Pacific cod genome that are putatively associated with juvenile cod liver condition index when exposed to warming.

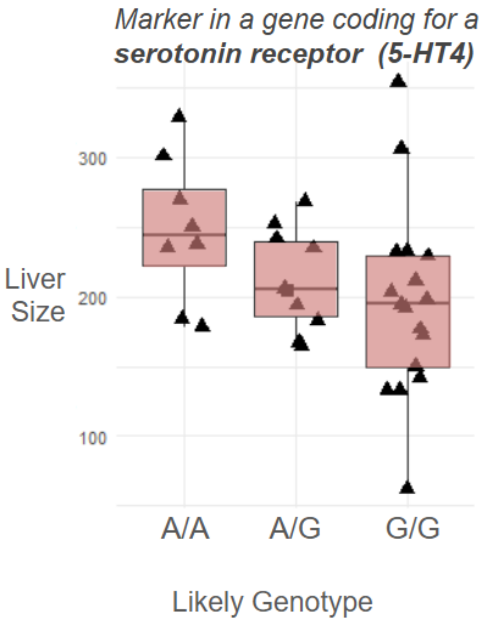

Figure 3. Example data for one of the putative liver-size associated SNPs. The most likely genotypes at this bi-allelic site are A/A, A/G, or G/G, and juveniles with the A/A genotype have the largest liver after the 6-week exposure to warming (16°C), whereas juveniles with the G/G genotype have the smallest livers. This site is located within a gene that likely codes for a serotonin receptor, which may regulate appetite.

Expression profiles associated with performance traits

Using transcriptomic data (RNASeq) and per-fish measurements, we identified genes with activity level that is linearly associated with performance traits in each of the four experimental temperatures, where performance is growth rate, body/liver condition, and lipid allocation. These genes will enable us to develop expression profiles of high-performing fish when exposed to heatwave conditions (16C) and cold conditions (0C) relative to two intermediate conditions (5, 9). For example, Figure 4 shows expression levels for genes that are associated with a composite performance index (CPI) which reflects growth rate, condition index, liver condition, and total lipid concentration in the liver (higher CPI = higher performance).

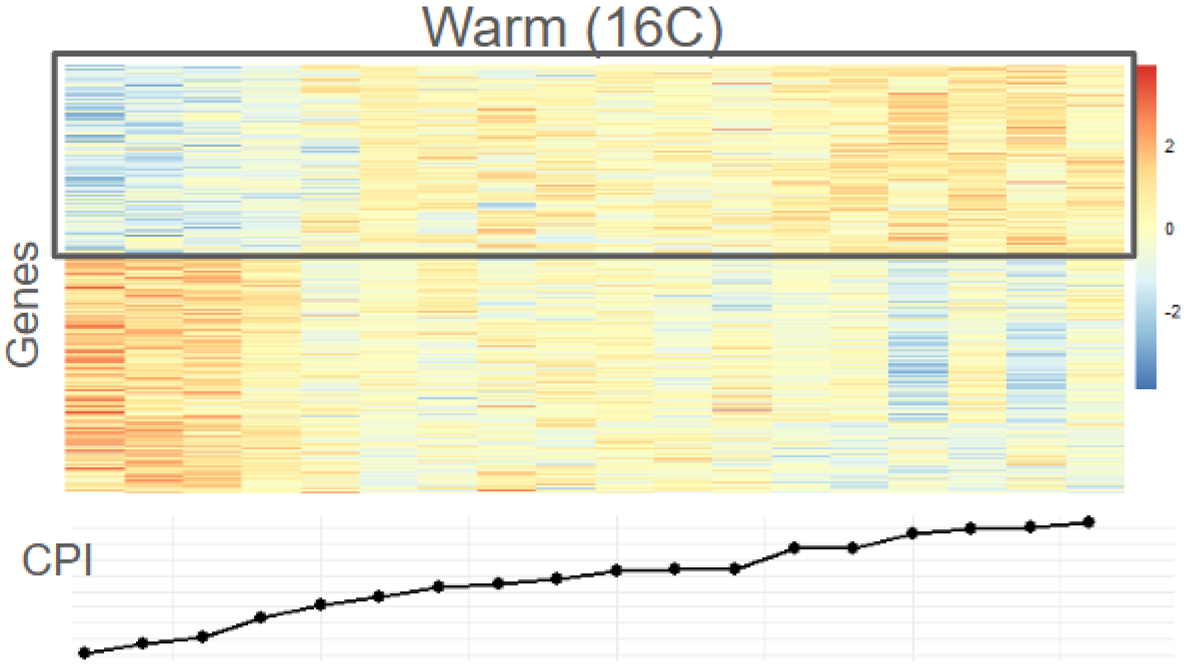

Figure 4. Heatmap showing expression of genes that are linearly associated with performance in warm-exposed juvenile Pacific cod. Rows represent genes, and columns represent individual fish. The line-plot shows the composite performance index (CPI), with higher values indicating higher performance. Color gradient represents expression level (z-score), with genes that have the highest expression (most active) in red, and lowest expression (least active) in blue.

Challenges

DNA methylation extraction process need to be further optimized to improve quantity and quality.