Progress Report:

PSMFC Subaward 23-084G for the period **May 1 – July 31, 2025**

Project Summary: Recent heat-wave stress in the Gulf of Alaska resulted in significant declines of Pacific cod (*Gadus macrocephalus*) in that region. The responses of Pacific cod, and whether selective mortality was present under thermal stress, were unknown. The project addressed these questions by identifying regions of the genome that respond to thermal stress. Juvenile Pacific cod were reared at several temperatures, and an integrated genomic approach identified genes, gene variants, and epigenetic markers associated with thermal stress and resilience. To complement the genomic approaches and further investigate temperature influences on energy resources, we performed lipid analyses. Low-coverage whole-genome resequencing and genome-wide association studies identified single nucleotide polymorphisms (SNPs) associated with growth, liver condition, and lipid content in juvenile Pacific cod, with several loci showing consistent effects across temperature treatments. Integration with transcriptomic data revealed that many of these candidate SNPs are located near temperature- or trait-responsive genes, highlighting genomic regions that may influence survival and adaptive potential in Gulf of Alaska populations.

Progress and Results

Brief Summary: During this reporting period (May 1–July 31), we completed several core project components, including processing and analysis of additional genome re-sequencing data from both experimental and archived individuals, integration with prior analyses, and travel to the AFSC Kodiak research station to discuss findings and retrieve archival samples. These efforts helped in completing two manuscripts: (1) variation in energy allocation across a range of temperatures, and (2) genomic predictors of survival-associated traits, both focused on juvenile Pacific cod from the Gulf of Alaska. The resulting datasets provide an unprecedented level of individual-level information and, beyond supporting these two manuscripts, serve as a valuable resource for the research network investigating the potential for Gulf of Alaska Pacific cod to acclimatize and adapt to warming conditions.

1. Completion of temperature-dependent phenotype models

During this performance period we finalized temperature-dependent models of juvenile Pacific cod growth rate, condition indices, and liver lipid concentrations (e.g. panels a-d in Figure 1).

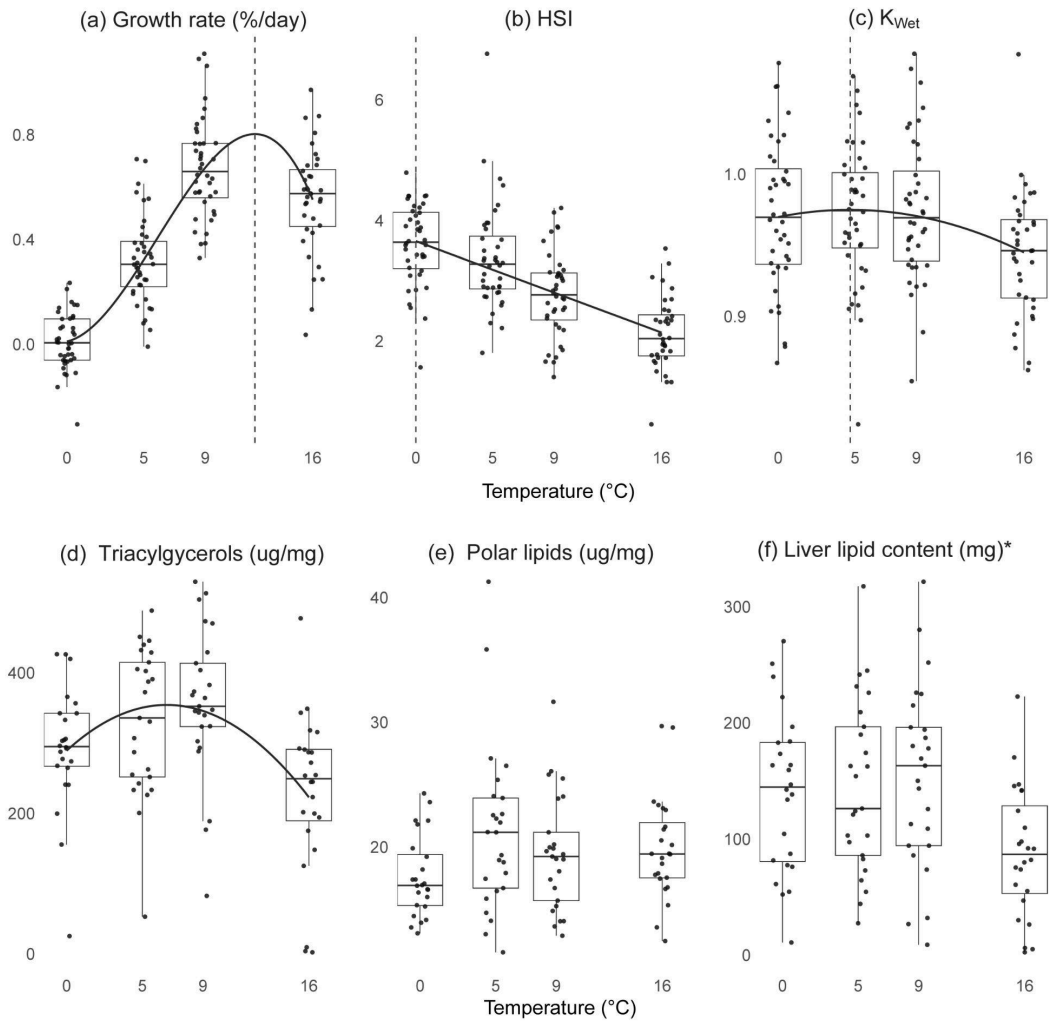

Figure 1. Effects of 1-month temperature exposure on growth (a), condition (b-c), and liver lipid content (d-f) in juvenile Pacific cod. Growth rate, hepatosomatic index (HSI), and condition factor (K_{wet}) are based on wet weight. Predicted temperatures of maximal trait values (T_{max}; dashed lines) are: 12.2°C for growth rate, 0°C for HSI, 4.8°C for K_{wet}, and 6.0°C for triacylgycerols. Temperature did not significantly affect polar lipid concentrations. *Liver lipid content was estimated from the total lipid concentration (triacylgycerols, free fatty acids, sterols, and polar lipids) and liver wet weight, and did not differ among the 0°C, 5°C, and 9°C treatments, but was ~50% lower on average in the 16°C treatment.

2. Integration of DNA Resequencing of Degraded Samples

In the previous performance period, we conducted laboratory work to collect additional tissue, re-isolate DNA, and perform genome re-sequencing for experimental mortalities in the warmest treatment. During this reporting period, these new data were processed bioinformatically and integrated into the existing dataset. The complete dataset was then re-analyzed to evaluate the genetic composition and likely population of origin of individuals most sensitive to warming, and to identify candidate genetic markers potentially associated with mortality under elevated temperatures.

3. Completion of Analysis to Determine Genetic Contribution to Phenotypic Responses

We added a new analysis to our manuscript to assess whether variability in temperature-dependent phenotypic responses was explained by genetic variation. Because all experimental individuals were characterized using low-coverage whole-genome sequencing and had per-individual phenotypes, we were able to apply Redundancy Analysis (RDA). In this approach, principal components from the genetic PCA were analyzed alongside phenotypic measures (growth, condition, and liver lipid concentrations) to identify which traits co-varied and which genetic components predicted individual phenotypes. The figure below from Manuscript #1 shows results for individuals in the coldest (0 °C; panels a–b) and warmest (16 °C; panels c–d) treatments. We found that genetic structure along the axis separating western and eastern Gulf of Alaska populations had no effect on phenotypes in the warmest treatment and only a weak but statistically significant effect in the coldest treatment.

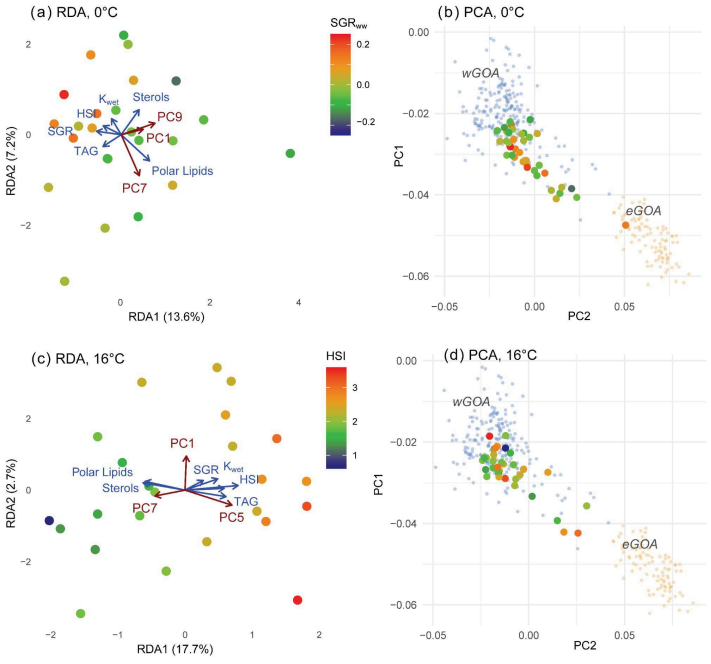

Figure 2: Redundancy analysis (RDA) biplots for juvenile Pacific cod reared at 0°C (a) and 16°C (c), showing relationships between phenotypes and genetic principal components (PCs) that explain significant variation. PC1, representing genetic structure along the wGOA-eGOA axis (see Figure 1 & S2), was tested in both RDA models. At 0°C, PC1 significantly improved model fit, but most phenotypic variation was explained by PC7 and PC9, which are not associated with regional population structure. At 16°C, PC1 did not improve the model and was not associated with any phenotype. Panels b and d show PCA biplot of spawning adults from the wGOA (light blue) and eGOA (light orange), with experimental juveniles projected into this space and color-coded by growth rate (SGR_{WW}, 0°C) or hepatosomatic index (HSI; 16°C). Together, these plots indicate that variation in growth and liver lipid storage at thermal extremes is not determined by broad-scale GOA population structure, although limited eGOA-like juveniles constrained the analysis.

The RDA and additional correlation analyses also revealed how phenotypes covary. For example, individuals with high growth rates also have higher liver lipid concentrations (e.g. TAG) and liver condition index (HSI).

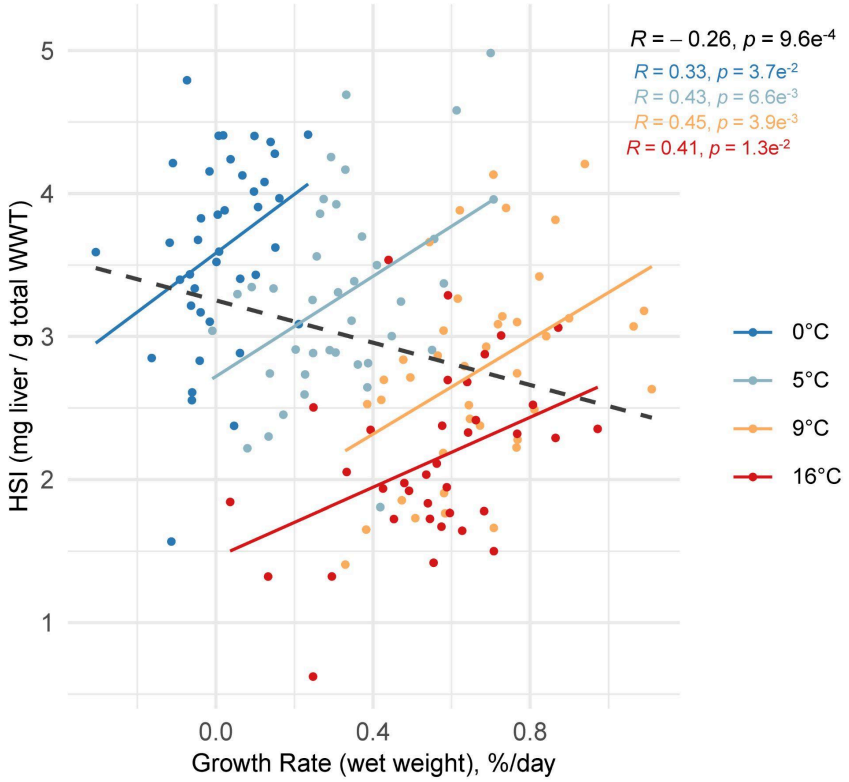

Figure 3: A negative correlation between hepatosomatic condition (HSI, y-axis) and SGR_{ww} (x-axis) was observed across all temperatures (black line), whereas positive correlations were observed within individual temperature treatments.

4. Genotype-Phenotype Association Approach

We completed a series of genome-wide association studies to identify genetic variants (SNPs) linked to fitness traits in juvenile Pacific cod, which are included in Manuscript #2. Specifically, we detected biallelic sites in the Pacific cod genome where allele frequencies were correlated with growth rate, liver condition (Figure 4), or liver lipid concentration. Many of these sites were consistent across temperature treatments, suggesting they contribute to temperature-independent variation in these traits. We also report additional sites that did not meet the statistical significance threshold but may still influence fitness traits, as is common for small-effect quantitative trait loci. These include loci associated with traits in fish exposed to warming conditions.

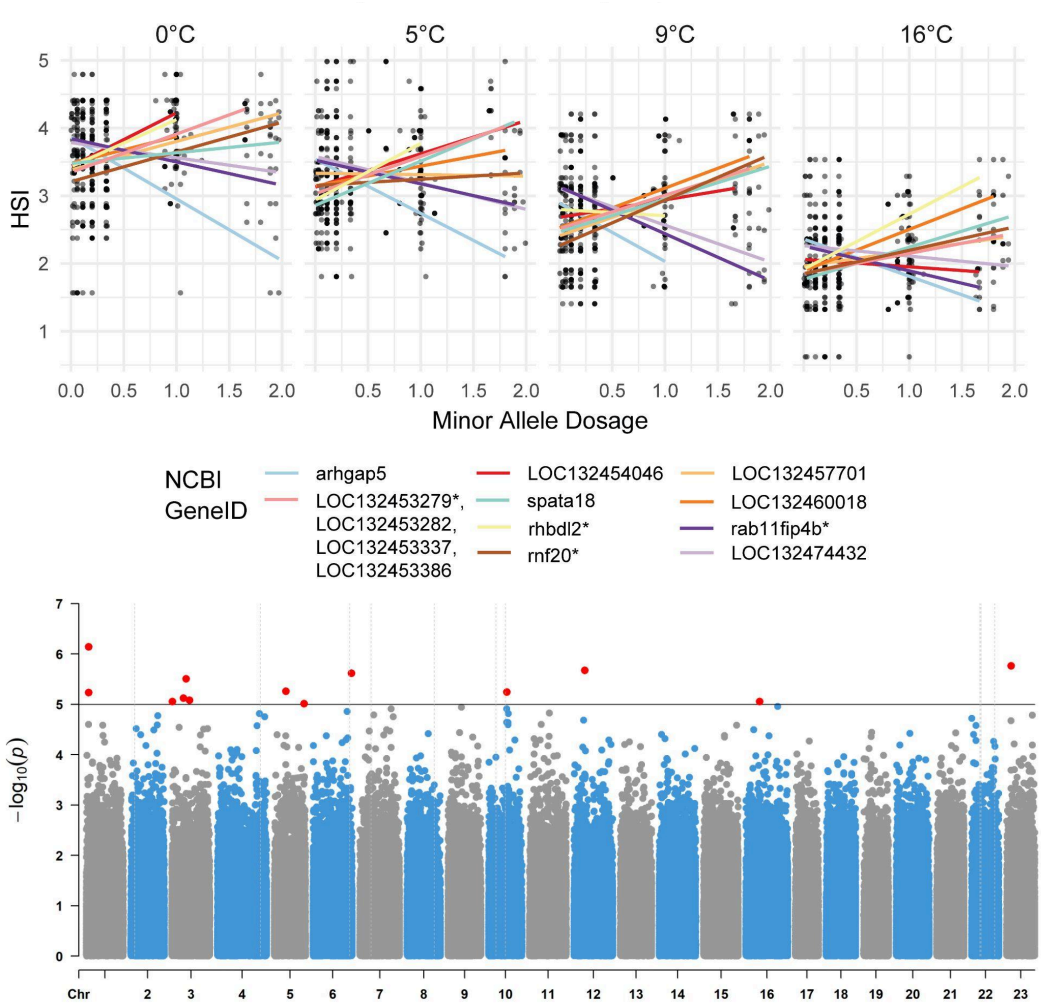

Figure 4. Candidate markers of hepatosomatic index (HSI), which reflects liver size relative to body size, based on GWAS (-log(P)>5). Top: HSI by minor allele dosage, where points represent individual dosages for all markers, and lines are linear model fits by marker. Markers are color coded by their associated NCBI GeneID, and (*) indicates genes with expression profiles that were also temperature- or trait-associated. One marker was located within a region with four overlapping genes (pink, e.g. LOC132453279). Bottom: Manhattan plot showing -log(P) for all sites, with red sites indicating those passing the significance threshold of -log(P)>5.

We also finalized lists of genes from liver transcriptomic data that were either temperature-responsive and/or correlated with an individual's liver lipid content, as reported in Manuscript #2. For example, Figure 5 shows genes (rows) whose expression levels (color scale) were correlated with liver condition in juveniles exposed to warming (16 °C).

Together, the trait-associated genetic variants and gene sets highlight specific regions of the Pacific cod genome that could be targeted to better understand the adaptive potential of Gulf of Alaska Pacific cod.

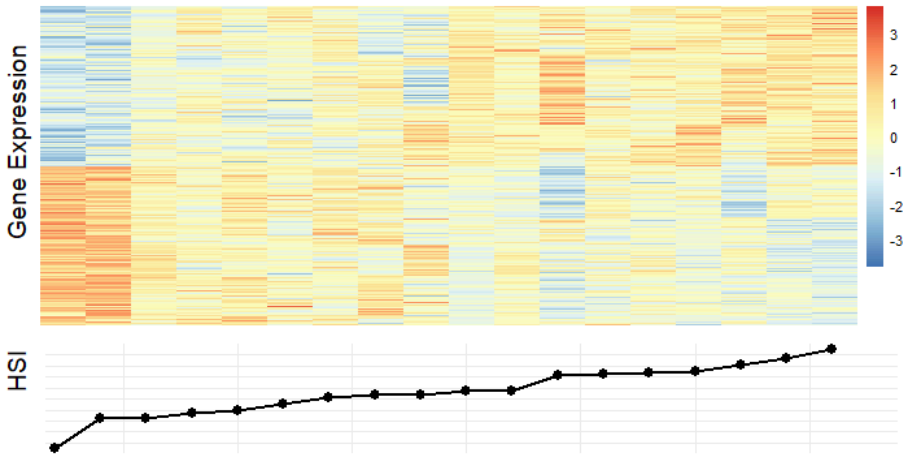

Figure 5. Heatmaps of genes with expression levels that correlated with hepatosomatic index (i.e. liver condition, HSI) in juvenile Pacific cod exposed to the warmest experimental temperature only (16°C, n=18). Columns represent individual fish; points on the overlaid line plot indicate per-fish HSI values. Rows correspond to individual genes (n=1,603 genes), with color indicating normalized expression (Z-score): dark red denotes high expression, and dark blue denotes low expression. Four individuals that died during the experiment are excluded.

5. Blood Epigenetic and Transcriptomic Sequencing and Preliminary Analysis

We completed extractions and generated matched whole-blood methylome and mRNA-seq libraries from experimental juveniles across cold and warm treatments. Sequencing metrics met targets, and datasets are linked to individual phenotypes and genotypes for joint analysis. Analyses show temperature structuring in both data types: unsupervised ordinations separate individuals by exposure, differentially methylated regions are enriched near genes involved in cellular stress responses and energy metabolism, and concordant expression shifts point to coordinated regulation of proteostasis and lipid pathways under warming.

6. Integration of Phenotypes, Genotypes, and Gene Expression Datasets

We used expression data to strengthen inferences about individual genetic variants, showing that allele dosage and transcript abundance often covary with focal traits. This covariation increases confidence in a true causal relationship, even with a limited sample size. For example, Figure 6 highlights a candidate marker on chromosome 10 within the rnf20 gene that is associated with liver condition. Figure 7 illustrates a potential application of these trait-associated SNPs, showing a possible increase in the frequency of favorable alleles from the age-0 stage to adulthood in Gulf of Alaska populations.

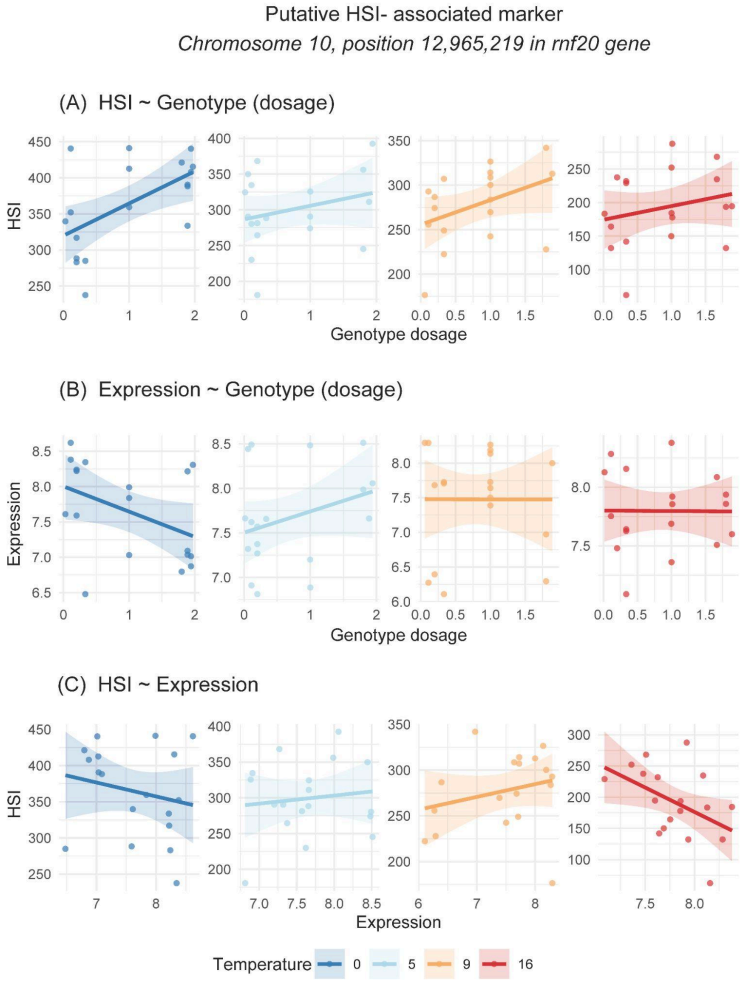

Figure 6: A top HSI-associated candidate marker in transcriptome-identified gene regions which is located in the gene rnf20 on chromosome 10. Panels show (A) HSI by genotype dosage (estimated from genotype likelihoods) (B) gene expression (transformed) by genotype dosage, (C) HSI by expression, and (D) Manhattan plot with -log(p) for all SNPs included in the analysis. rnf20 encodes a ligase that affects histone modification which influences chromatin accessibility. In humans, higher RNF20 levels tend to repress elongation-growth-factor-inducible genes. Higher HSI is associated with higher dosage of the minor allele and lower rnf20 expression, suggesting reduced repression of growth-stimulating pathways.

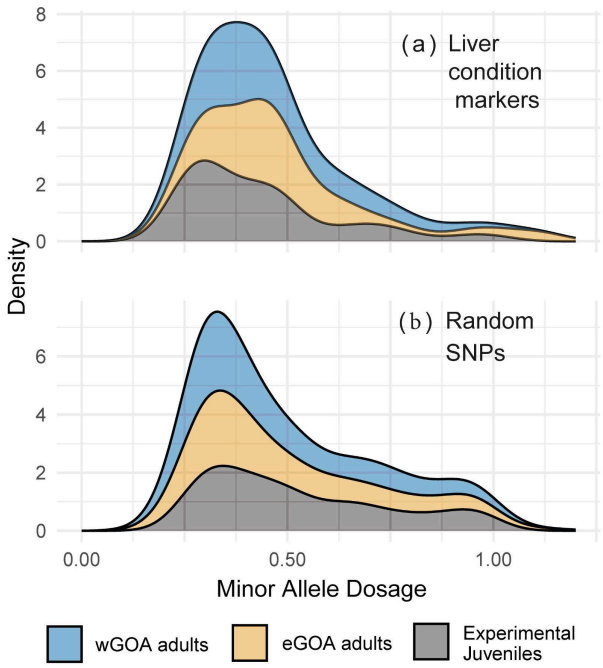

Figure 7: Example application of candidate markers, where favorable minor allele dosages for liver condition (HSI, n=68, panel a) are compared among experimental juveniles and spawning adults from the region, western and eastern Gulf of Alaska (wGOA, eGOA). Adults exhibit higher dosages of favorable alleles linked to liver condition relative to juveniles, whereas no difference was observed in a random SNP set (n=1,000, panel b), suggesting that individuals carrying less favorable genotypes may be lost between the juvenile and adult life stages. Dosages were estimated from genotype likelihoods at sites that were commonly measured in juveniles and reference cod, and which met the suggestive threshold of -log(p)>4. N=160 for juveniles, 208 for wGOA, and 96 for eGOA.

7. Exploration of New Genomic Dataset to Assess Heatwave Effects Over Time

We now have a unique longitudinal genetic dataset for Pacific cod juveniles collected each July over nine years (2008–2023), paired with morphometric data (length, wet weight). This dataset offers substantial potential for future analyses, though full exploration will require additional time or personnel. During this reporting period, we conducted a preliminary analysis to assess whether the broad genetic profiles of age-0 cod shifted in response to the marine heatwave. Results indicate an overall shift in genetic structure among individuals sampled before the heatwaves (2008–2013), during the heatwave years (2014–2019), and after (2021–2023)(Figure 8), however additional analyses are needed to validate these findings. In parallel, our postdoctoral researcher is analyzing allele frequency changes for SNPs previously identified in GWAS of experimental fish to determine whether fitness-related and temperature-sensitive loci were under selection during the heatwave era.

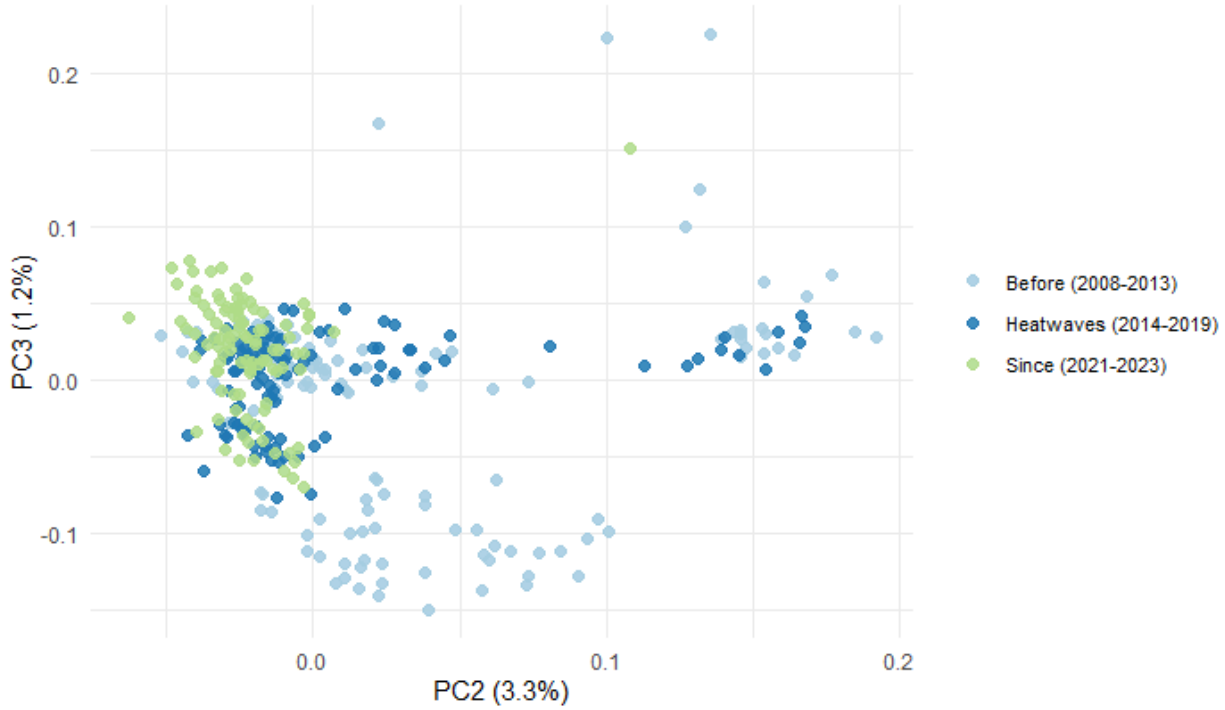

Figure 8: Principal component analysis (PCA) biplot of genetic composition of age-0 Pacific cod (n=344) collected off Kodiak Island, AK before (2008-2013), during (2014-2019), and since (2021-2023) the heatwave era in the Gulf of Alaska. PCA was conducted using genome-wide SNPs (n=3,075,411 sites) with minimum minor allele frequency of 5%, and may indicate changes in genetic composition or diversity in GOA juveniles. Principal components two and three are shown, as PC1 likely captures additional variation due to factors that are not yet characterized.