FINAL REPORT

2023/2024 JOINT INNOVATION PROJECT

Title: Developing improved seed of the Pacific oyster for the Southeast Alaska regions

Lead Entity: Pacific Hybreed, Inc.
Category: Boosting oyster growth at nurseries or farms
Project Location: Baranof Island, Alaska
Project Start Date: August 1, 2023
Project End Date: January 31, 2025
Award Amount: $90,723

Project Team & Partners:

Pacific Hybreed: Francis Pan, Melissa DellaTorre.
NOAA Alaska Fisheries Science Center: Rebecca Cates, Henry Fleener, Heather Fulton-Bennett, Jordan Hollarsmith.

PROJECT OVERVIEW

a. Problem Statement

Genetic improvement of farmed oysters is needed to develop genetic lines suitable for the diverse environmental conditions in the Alaska shellfish industry. Oyster farming in Alaska largely relies on hatchery-produced seeds that have not been optimized for growth and survival in Alaska, presenting a barrier for continued growth of the shellfish aquaculture industry.

b. Background & Context

For generations, oyster farming has provided significant social and economic values in coastal communities. On the U.S. Pacific Coast, the Pacific oyster (*Crassostrea gigas*) is the most farmed mollusk species with reported sales of $150 M (USDA 2023 Census of Aquaculture). As the demand for seafood grows, marine mollusks offer great potential as a source of favorable nutrient profiles and can be produced in sustainable ways that are supportive of the ocean and marine resources. Almost all farmed shellfish, however, are considered undomesticated (domestication here defined as farm stocks having profoundly changed from wild progenitors) and subjected to unpredictable yields and boom-and-bust cycles. Genetic breeding has been shown to improve the yield of farmed shellfish, especially in regions, such as the U.S. Pacific Coast, where farmers depend on seed produced in hatcheries.

In addition to genetics, the environment is a major determinant of animal performances. There is considerable diversity of environmental conditions and growing methods within a shellfish aquaculture industry spreading from Alaska to Baja California Sur. This diversity correlates with different needs among growers that offer an opportunity to develop domesticated breeds for specific environments. This project is aimed to improve yield performance for seeds by using genetic breeding and field testing in Alaska.

c. Proposed Solution

We proposed to develop oysters optimized for colder growing conditions. A set of genetic lines were produced and evaluated for their yield performances in Alaska. Best family lines in Alaska were selected and will be used to produce improved seeds. After field testing, oyster families are maintained in Alaska for further evaluation of yield and to facilitate longer-term development of a local broodstock program. The same families are also maintained in Washington State for more immediate production of improved seed based on data from the ongoing fieldwork.

d. Project Objectives, Tasks & Measures of Success

The primary objectives of this project were to (1) establish a protocol for field testing of family lines with a sufficient level of replication; (2) evaluate field performances for a cohort of hybrid lines of the Pacific oyster; (3) develop broodstock for future breeding and spawning efforts in Alaska. Success metrics include hatchery production of family lines of oysters, deployment of test animals, and field evaluation of oyster families.

e. Project Outcomes

Significant progresses were made toward each objective. A set of test animals were deployed in 2023 to evaluate the feasibility of different deployment methods at NOAA Little Port Walter Biological Research Station (LPW). Field cages were deployed at two different locations in the bay and secured at 2 m below sea surface to minimize freshwater impact on oyster growth and survival. Results of this initial test helped develop a stacking method for outplanting a relatively large number of replications at LPW that allowed sufficient water flow for oysters to grow in the experimental cages.

A set of oyster family lines were produced by crossing broodstock from different pedigreed lines confirmed by single-nucleotide polymorphism genotyping. A total of 132 potential broodstock from 13 genetic lines were genotyped for this project. Spawning, experimental crosses, and larval rearing were completed in the Pacific Hybreed hatchery. In April 2024, 23 different families were outplanted in replicated cages at LPW. A total of 92 cages were deployed at the station for monitoring of seed yield and environmental parameters. We successfully collected data on seed growth and survival at three different time points, in April, July, and September 2024. Across families, yield increased by 4-fold during the summer months relative to the spring. Between families, there was an up to 1.7-fold difference in yield, calculated from the final group weights that were adjusted for confounding factors such as differences in initial weight and count between families.

f. Successes, Challenges, Lessons learned

Oyster families were successfully deployed for 170 days for this project. Seed showed apparent growth and high survival over the course of the project (see DATA for detailed descriptions). Although growth was slower, as expected in colder water, relative to sites in other states, significantly increased yields were evident in top-performing families. The range of yield values between families was smaller than anticipated, likely due to slower growth in the environment. Evaluation of field performances for these families will be continued. We expect greater differences in yield for these families after a second growing season.

g. Continuation & Dissemination of Results

Experimental results of this project will be presented at the Mariculture Conference of Alaska and Aquaculture America. Additionally, PI Pan presented this work to students enrolling in the Applied Fisheries Program at the University of Alaska Southeast. A subsequent phase of the research will be conducted to evaluate the best family lines at commercial farms in Alaska, in addition to continuing the fieldwork at LPW to obtain 2nd-year yield data for the oysters initially deployed in 2024 and to significantly expand the number of family lines outplanted in Alaska.

DATA & PROJECT OUTCOMES

Oyster families were deployed at LPW for a total of 170 days from April to September 2024. Field cages were cleaned periodically to prevent biofouling and ensure proper water flow through cages. Environmental loggers that recorded water temperature and light intensity were attached to the cages. Logger data showed that seawater temperature at an approximately 2-m depth ranged between 7 to 8.5°C between April to June (Figure 1), with an average of 7.9 ± 0.37°C. Started June 15, 2024, temperature increased to above 9°C, with daily averages (filled, black symbols in Fig. 1) being largely above 10°C. The highest temperature was recorded in mid-August at 12.7°C. This warmer temperature then gradually decreased to ~9°C in September. During these summer months, temperature averaged 10.3 ± 0.81°C.

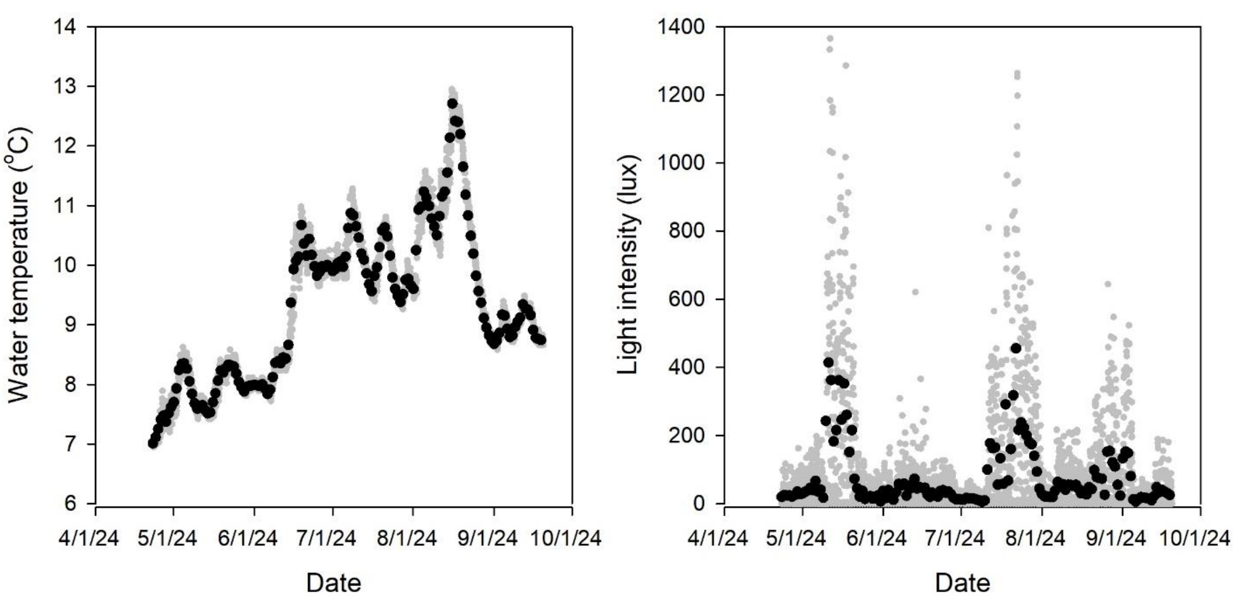


Figure 1. Profiles of seawater temperature (left panel) and light intensity (right panel) at 2 m depth from loggers attached to the field cages. Gray symbols show hourly data, and black symbols show daily averages calculated from hourly data.

The light intensity profile showed two main peaks during the deployment period, in May and July (Figure 1). A smaller peak was evident close to the start of September. Changes in light intensity likely corresponded to local weather events and did not show a clear seasonal pattern. Light availability may also predict the amount of algal food present in the water column, although direct measurements of the abundance of microalgae were not conducted in this project.

Seed performance was evaluated by measuring group weight and count for each of the 92 field cages. Three time-point measurements were conducted that included initial count and weight (in April), mid-point count and weight (in July), and final count and weight (in September). The growth rate for each family was calculated by fitting a regression model to average individual weights per cage. A non-linear, exponential growth curve fit best described the relationship between animal body weight and deployment day (Figure 2). This indicates that the growth rate increased during the summer relatively to the spring months. For this specific family shown in Fig. 2 (left panel), the growth rate between April and July was 4 mg d^{-1} and between July and September 10 mg d^{-1}. Increased growth rates in the summer were consistent across families. On average there was a 3.4-fold increase in growth rate. Different seasonal growth rates were also analyzed for each family (Figure 2, right panel, each data point represents a single family). This analysis showed that growth rates in the spring were a significant predictor for summer growth and explained about 50% of the variation in summer growth rates between families ($R^2 = 0.51$, $P < 0.001$). Seasonal growth differences corresponded to the increase in temperature between July and September (Figure 1), although temperature alone likely did not entirely explain the difference in growth. Laboratory studies generally show a ~2-fold increase in growth rate for every 10°C increase. Given that water temperature only differed by 2°C between spring and summer, other variables such as food abundance likely played a major role in the 4-fold increased growth in the summer months.

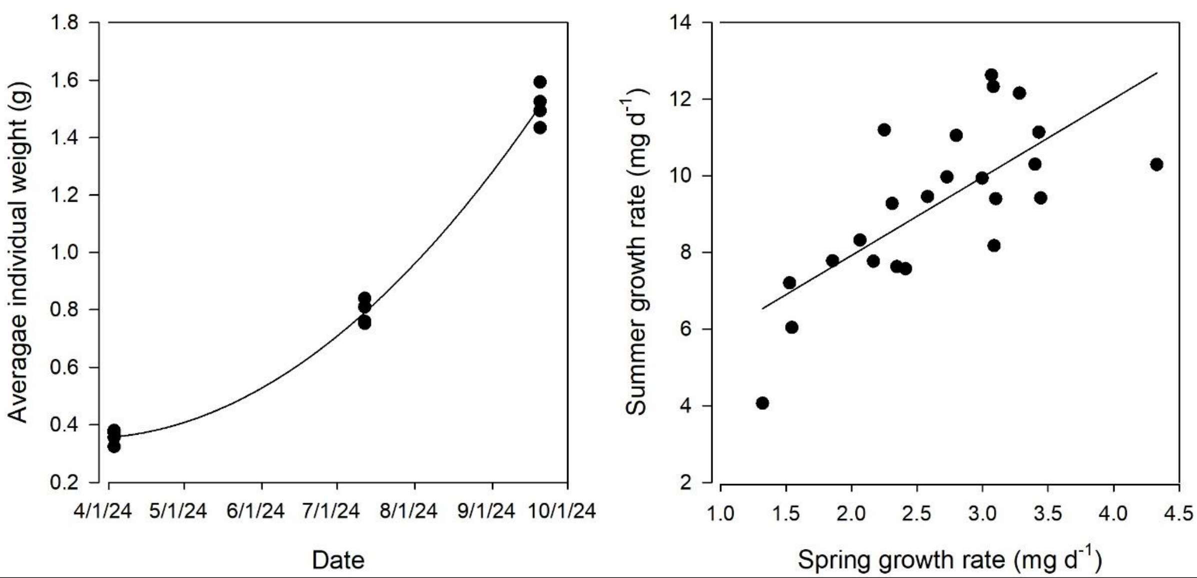


Figure 2. Left panel: Increases in average individual weight, calculated from group weight, over the course of field testing for a single family. Each family was outplanted in four technical replicates, and each replicate was measured three times, producing 12 data points per family. Right panel: Analysis of the relationship between spring and summer growth shows that there is a 3.2-fold increase in growth in the summer across families, and that spring growth rates predict summer growth across the families. Each data point represents growth rates for a family.

Survivorship was calculated by the difference in count for each family. The number of animals that was not recovered at harvest and the number of empty shells were included in the 'mortality' category. Across all families, seed survival was high at 95% ± 8% over the 170-day deployment period with no significant effects of family on survivorship ($P > 0.05$).

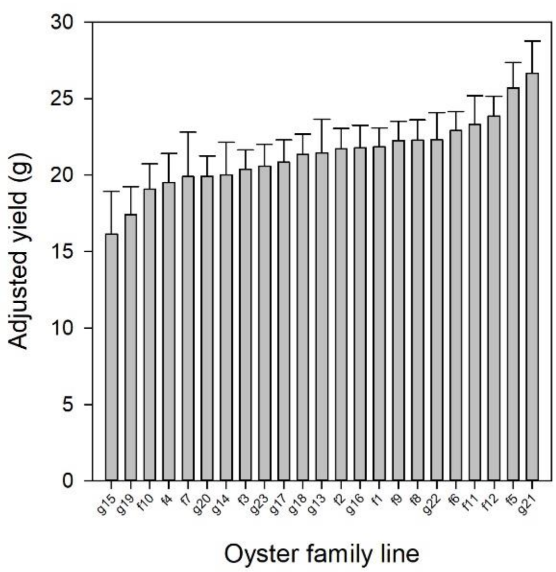
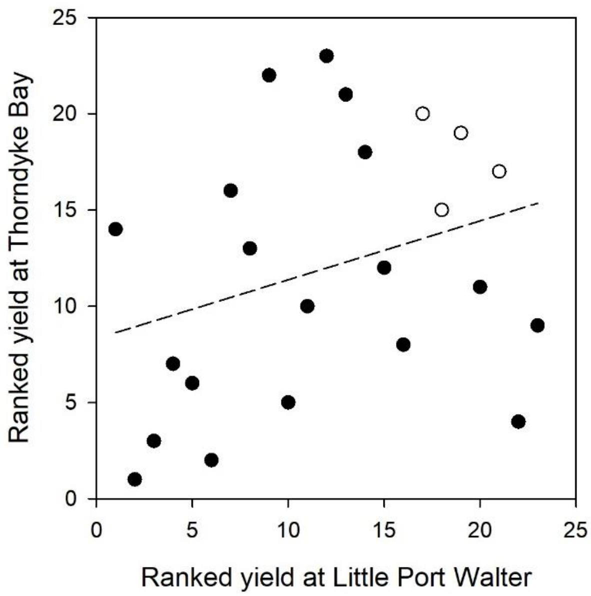


Figure 3. Adjusted yields for 23 different families. Least squares means (± SE) are shown. See text for statistical results.

In addition to the analyses of family growth rate and survivorship, yield was calculated using a general linear model, $\text{Final weight} = \text{initial weight} + \text{final count} + \text{family}$. This model was used to analyze the effect of family line on final weight and to correct for any differences in initial weight and final count between families and experimental cylinders. Differences in animal body weight among families are common at the start of an experiment, as different families grew at different rates in the hatchery. We randomly sampled individuals for the field trial and used statistical models to adjust weight gains for different starting weights. Adjusted yield represents a combined analysis of changes in weight and count (i.e., survival) over time. The model explained 89% of the total variation in the dataset ($R^2 = 0.89$, $P < 0.001$). Family had a significant effect on final group weight ($P = 0.01$). Overall, group weight measurements show that the oysters deployed at LPW grew from 4.2 g to 21.4 g per cage, a 5-fold increase in body weight. The highest-yielding family had a 9-fold gain in group weight, compared with a 3-fold increase in the lower-yielding families. Comparing adjusted yields between families (Figure 3), there was a 1.7-fold, significant increase in yield in the best-yielding family.

Besides the outplant at LPW, these oyster families were also deployed at other farm sites in Washington State, which allowed for a comparison of the yield for the same families across different sites. Although this specific aspect of the research was not included in the original JIP work plan, understanding the effect of genotype-by-environment interaction on yield performances is central to the breeding program. The analysis was conducted using a general linear model that includes site as an independent variable, $\text{Final weight} = \text{initial weight} + \text{final count} + \text{family} + \text{site} + \text{family} \times \text{site}$. Oysters outplanted at Thorndyke Bay, WA had a ~3-fold increase in adjusted yield across all the families compared with siblings deployed at LPW (least squares mean ± S.E.; AK: 21.4 ± 0.66 g; WA: 63.7 ± 0.68 g). More importantly, there was a significant interaction between family and site ($P < 0.001$), indicating that better-yielding families were different at different sites. This was further analyzed using correlation between adjusted yields at the two sites. There was no significant correlation between yields (Pearson Correlation, $r = 0.34$, $P = 0.11$) or ranked yields (Figure 4, Spearman Correlation, $r = 0.31$, $P = 0.15$), indicating the need for breeding efforts focused on different environmental conditions. At the level of individual families, however, there are certain consistencies in yields



between sites. This was illustrated in Figure 4 by the open symbols. Four families ranked in the top 30% at LPW were also among the better-yielding families at Thorndyke Bay. This result further suggests the possibility of identifying generalist families, through field testing and the ability to repeatedly produce seeds of desired genetic lines.

Figure 4. The relationship between ranked yields at two outplant sites in AK and WA. Higher values represent higher yields. Overall there was no correlation between ranked yields, although a number of families (open symbols) showed consistent ranking between sites.

ADDITIONAL INFORMATION

The proposed scope of work was completed in the intended timeframe. The oyster families were produced in Pacific Hybreed hatchery in August - September 2023 and were grown in the hatchery and then nursery until field testing that started in April 2024. A pilot study testing deployment methods was initiated at LPW in September 2023, with experimental oysters outplanted in April 2024. The team made multiple trips to LPW for the duration of the project for deployment, data collection, and maintenance of field cages and experimental animals. In addition to the team members listed above, a number of students and interns affiliated with NOAA Alaska Fisheries and University of Alaska participated in the project.

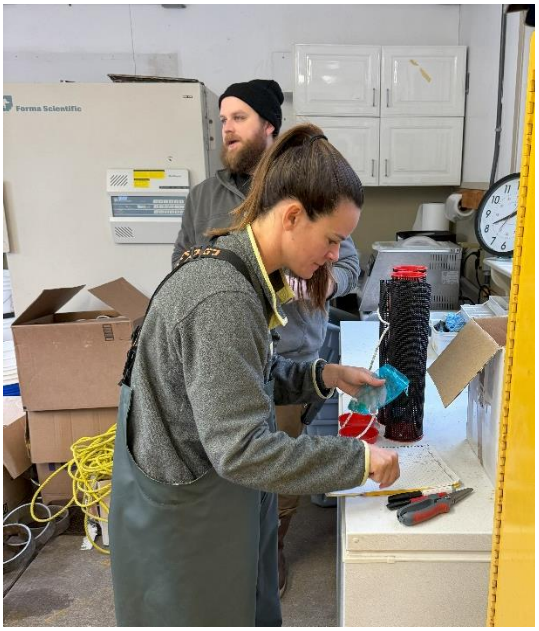
BUDGET

Budget summary

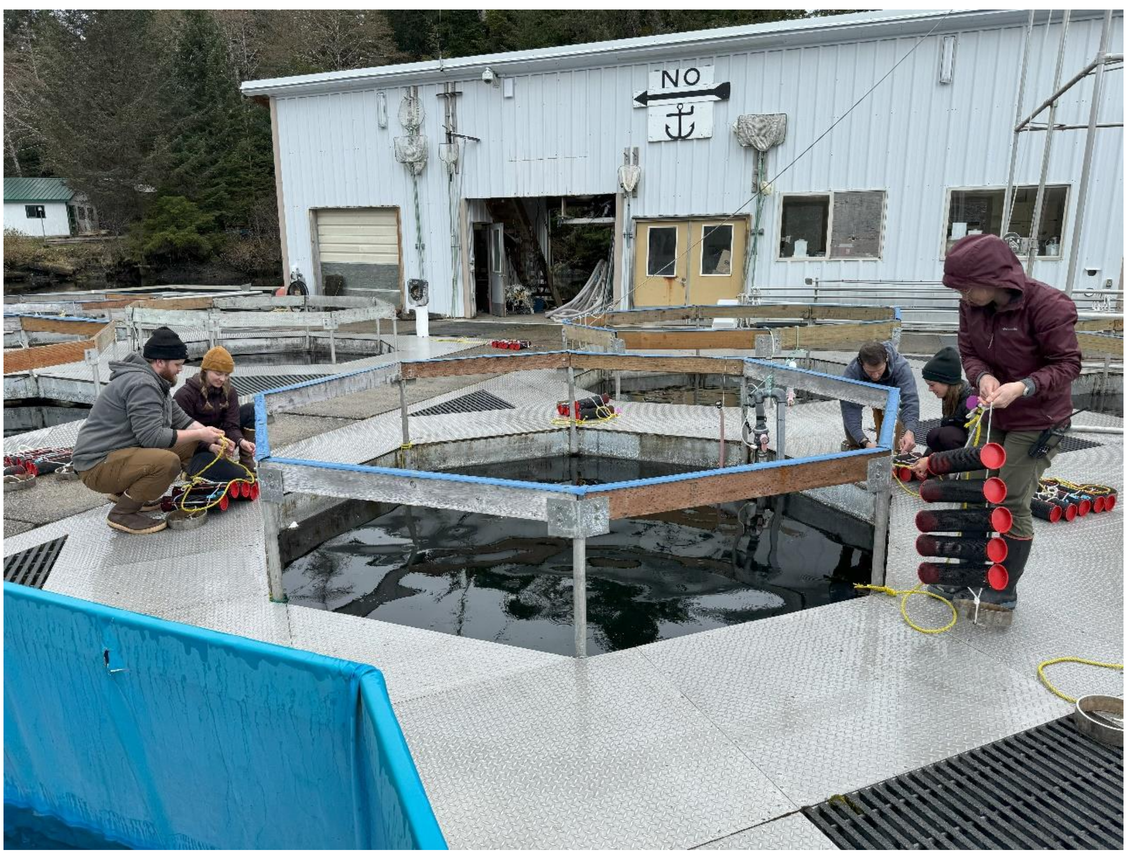
Expense	Amount
Personnel	$ 40,226
Supplies	$ 20,114
Travel	$ 30,343
Total	$ 90,683

The project fell within the originally proposed budget. Table above shows the final budget summary by budget line item. NOAA Alaska Fisheries provided facilities, personnel, and travel coordination and assistance to LPW. Pacific Hybreed provided genetic lines of oysters, facility, and personnel. Leveraging existing resources was a main reason the project fell within the budget.

PHOTOS



Top left: Tagging cages and stocking cages with experimental oysters. Top right: Cages were arranged into stacks with weigh on the bottom. Bottom: Outplanting oysters in 92 field cages at LPW.



Top: Data collection at LPW. Experimental oysters were removed from cages and cleaned thoroughly. Counts of live animals and total weights were measured. Bottom: A representative photo showing oysters from two different families. Labels distinguish the family and cage that the animals were sampled from. Size contrast was evident between the two specific families.

2023/2024 JIP
Developing improved seed of the Pacific oyster for the Southeast Alaska regions

BACKGORUND:

- Farming oysters optimized for growth in Alaska will improve production and resilience of the shellfish aquaculture industry.
- Pacific Hybreed and NOAA Fisheries scientists proposed to conduct field research comparing oysters with different genetic background.

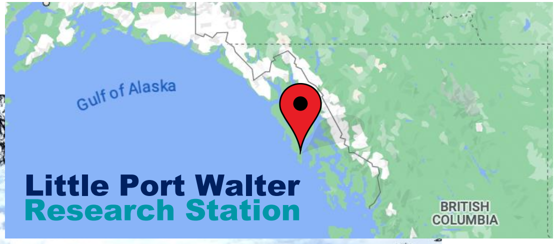
METHODS:

- Oyster families were produced by genetic crosses.
- Seeds were outplanted at the NOAA field station in Southeast Alaska.

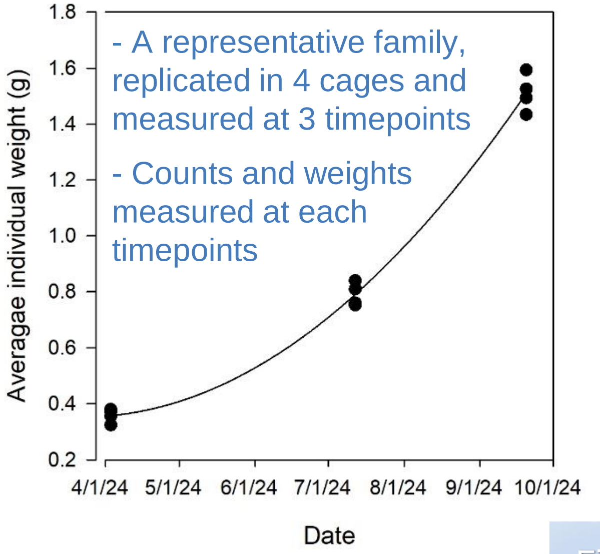
PI: Francis Pan, Ph.D.
Co-PI: Jordan Hollarsmith, Ph.D.
Co-PI: Heather Fulton-Bennett, Ph.D.

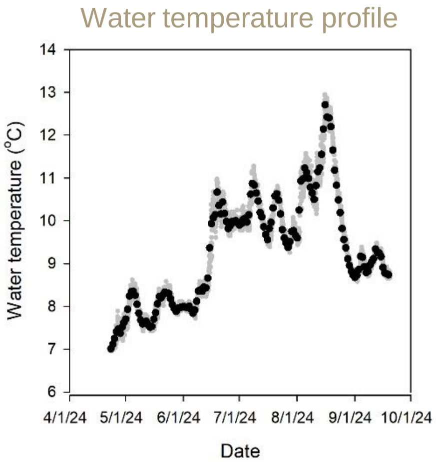
PACIFIC HYBREED

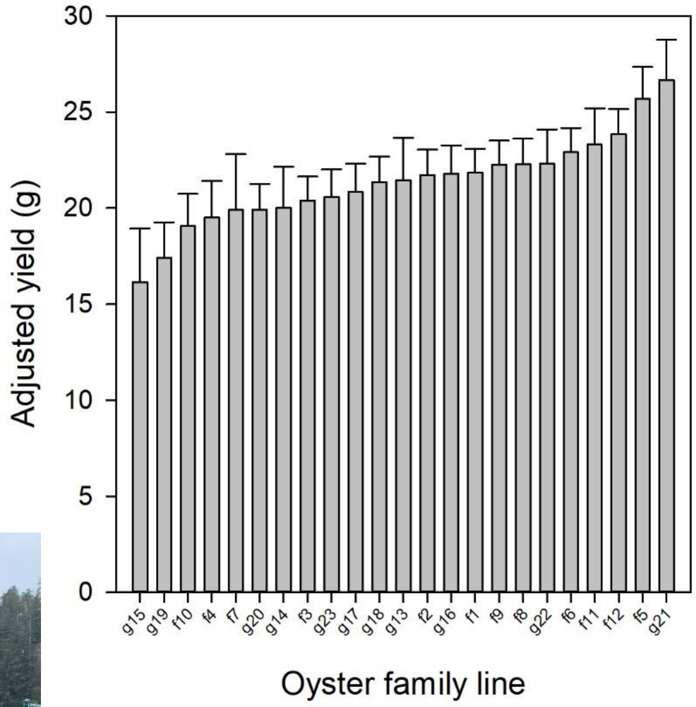
NOAA FISHERIES



92 experimental field cages were outplanted to test seed yields for 23 oyster families.

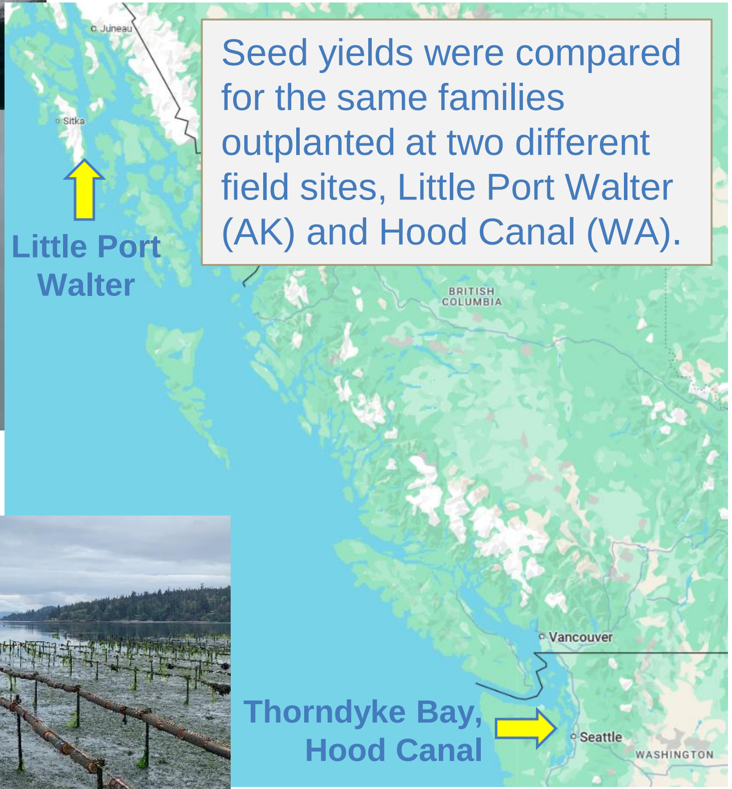




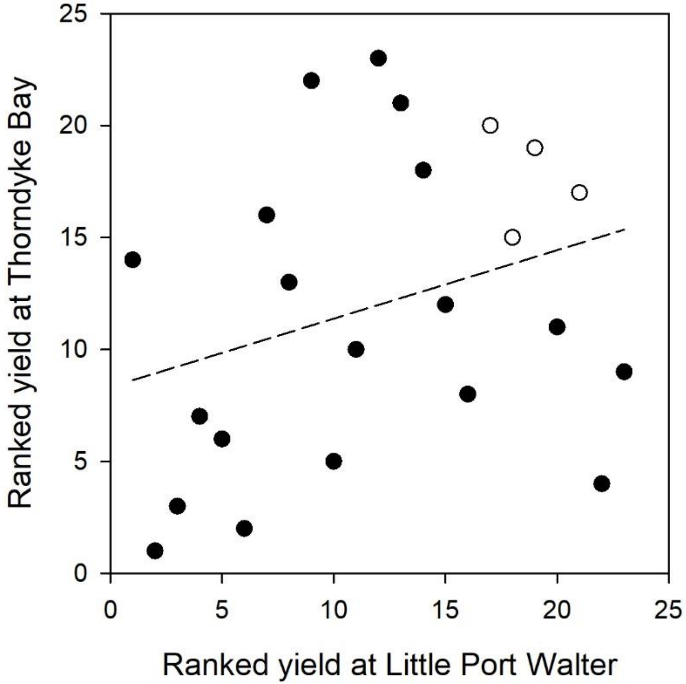


- Adjusted yield: growth corrected for mortality and differences in initial weight

- Best family grew 1.7-times faster.



Seed yields were compared for the same families outplanted at two different field sites, Little Port Walter (AK) and Hood Canal (WA).



- Overall, there was no correlation of yields between the two sites, supporting development of oyster strains specific for Alaska waters.

- Results from this project will inform production of high-yield seeds among this cohort of oyster families.