QUARTERLY PROJECT REPORT

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OVERVIEW

The Alaska oyster industry has solely relied on the Pacific oyster (Magallana gigas) for shellfish farming. The “Other Mariculture Species” project, being conducted by Blue Starr Oyster Co., is working to build resilience and diversity in the sector by assessing the feasibility of growing novel oyster species: Kumamoto oysters (Magallana sikamea), Virginica oysters (Crassostrea virginica), and Olympia oysters (Ostrea lurida). Blue Starr Oyster Co. is leveraging its long-standing shellfish cultivation expertise to evaluate the growth potential of these species in Southeast Alaska and report on the results of each species’ grow-out.

PROJECT UPDATE

This quarter (April - June 2026), the Other Mariculture Species project began its second season, with the main activities being the restart of monitoring of the “Other Species” oysters and the introduction of a new cohort of Kumamoto oysters that are, hopefully, genetically distinct Kumamotos.

In April, the Blue Starr farm reopened for the season, and the 2025 seed was deployed back into the FLUPSY after overwintering in floating gear on the farm. All of the monitored species were sampled as they entered the FLUPSY to understand how they fared over the winter and to begin the season’s monitoring. During this initial sample, we found that the Pacific control group (KC) had strong winter survival as expected, as did the “false Kumamotos” (K1) from 2025. The Virginica (V) oysters had high mortality over the winter; however, some seed showed new growth, which was both surprising and welcome.

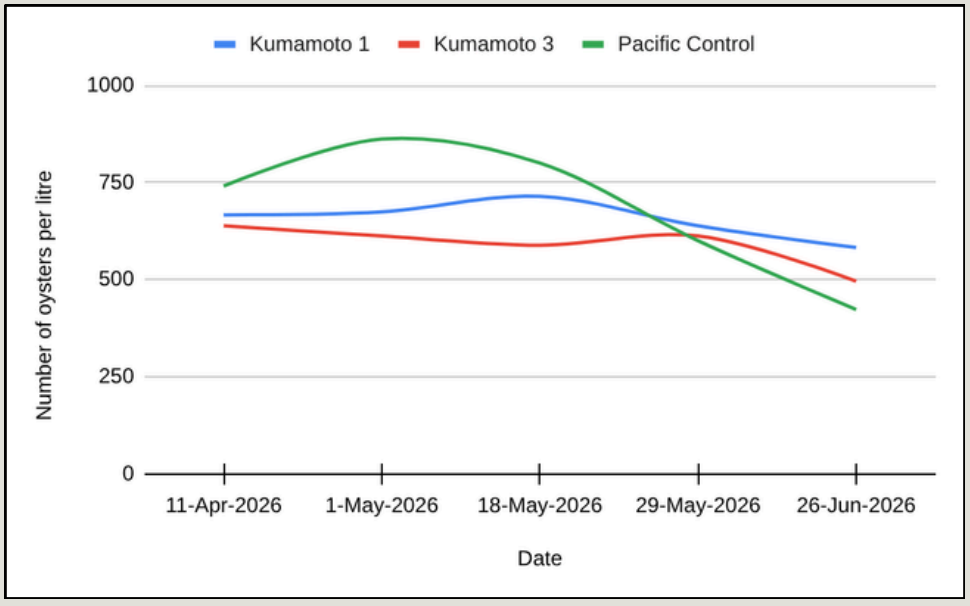
A new cohort of Kumamoto seed (K3) was received in early April from Hog Island Oyster Co., based in California. This seed looked very distinct from the Pacific controls and the “false Kumamoto” seed received in 2025, providing confidence that this seed is true Kumamoto. A sample from this cohort was sent to the lab at UAF for genetic verification using the same methods they employed in 2025.

The lab tests, conducted by PhD student Alorah D. Bliese, found that this new batch of Kumamoto oyster seed was genetically distinct from the original cohort tested. Through specific genetic testing, results showed a sufficient nucleotide difference between the samples of Pacific oysters (*Magallana gigas*) and Kumamoto oysters (*Crassostrea sikamea*) to differentiate them at the species level. This testing has been an unexpected but vital contibution to the project and has helped to solidify confidence in the seed and the ongoing process of growing out the Kumamoto oysters. A full description of the process and report by Alorah can be found attached to this report.

New cohort of Kumamoto seed (K3 - bottom row) compared to the Pacific Control seed (PC - top row).

The new cohort of Kumamotos (K3) was deployed on the farm alongside the 2025 Pacific Control (PC), the “false Kumamotos” (K1), and the Virginicas (V). Over the course of the quarter, all species samples continued to be monitored using the same sampling technique in 2025. The chart below shows the growth trend for the samples for the **2026 season** so far.

Note that the chart shows the total number of oysters in one liter, so a downward trend of lower numbers shows positive oyster growth.



The samples of K1, K3, and PC appear to follow similar growth trends over time this quarter. While there is some variation, their overall growth trends seem similar, which is promising for Kumamoto's feasibility. As the K3 sample is the first true Kumamoto sample to be tested in the FLUPSY so far in this project, it is still early to say how successful it will be, especially since it has not overwintered at the farm yet. To ensure a thorough testing of true Kumamotos, another cohort of smaller seed has been ordered from Hog Island Oyster Hatchery. This cohort will be smaller in size than the K3 cohort, which will allow Blue Starr to test the survivability of smaller, less expensive Kumamoto seed in the farm.

The Virginica oysters are unfortunately not faring well, with high mortality rates and slow, if any, growth. The surviving seed will continue to be monitored through the remainder of the season to observe any positive growth in the warmer months of summer.

As we enter the final quarter of this project, we will continue to collect growth data from all species. Early data is showing that the true Kumamotos may successfully grow on the farm, and we look forward to seeing how they do through this season.

The 2025 K1 (“false Kumamotos”) seen in the left image compared to the new K3 Kumamoto 2026 cohort seen in the right image.

FINANCIALS

The project budget is currently on track for anticipated spending for this quarter. Funds have been requested for personnel time, project supplies, and contractor time as per the budget. Attached to this report are the budget and invoice records for reference.

Total Project Budget Approved	Previously Expended	Amount Currently Requested	Amount Remaining	% Spent
$80,200.00	$34,093.04	$18,988.24	$27,118.72	66%

Cytochrome Oxidase I (COI/COX1) and Internal Transcribed Spacer 2 (ITS2) Analysis in Suspected Kumamoto Oysters (*C. sikamea*)

Lab work by:
Alorah D. Bliese & Ayla Benson

Report by:
Alorah D. Bliese
PhD Student
University of Alaska Fairbanks
Department of Marine Biology
Supervised by Dr. AK Kelley
July 13, 2026

COI Analysis

Protocol Summary:

Sequencing of COI region

DNA that was previously extracted from suspected Kumamoto oysters (n=6) was amplified using the Folmer COI primer set (LCO1490_F GGTCAACAAATCATAAAGATATTGG; HC02198_R TAAACTTCAGGGTGACCAAAAATCA). Approximately 20ng of input DNA was amplified in 25ul reactions with 1x DreamTaq and 0.5uM of each primer under the following conditions: once cycle at 95°C for 5 minutes; 30 cycles of 95°C for 1 minute, 51°C for 1 minute, 72°C for 1 minute; and one cycle at 72°C for 5 minutes. PCR amplicons were visualized on a 1.5% gel to confirm amplification. After verifying all samples successfully amplified, PCR amplicons from 3 randomly selected samples were purified using an Omega Bio-Tek Cyle Pure PCR Cleanup kit. DNA was quantified using the 1x HS dsDNA Qubit kit. Each sample was Sanger sequenced in the forward and reverse directions using the respective Folmer COI primer at Genewiz (South Plainfield, NJ).

Figure 1. Generation

Sanger chromatograms were inspected, trimmed, and aligned using SnapGene and UGENE. Sequences from suspected Kumamoto (*Crassostrea sikamea*) samples were mapped to a *C. sikamea* reference sequence to generate their respective consensus sequences. The consensus sequences from the 3 samples (labeled Test1-3 in the figure) were aligned alongside three COI *C. sikamea* reference sequences obtained from NCBI using the MUSCLE function within UGENE. The multiple sequence alignment was exported as a .png file and inserted into this document. Misalignments between the sequences are noted by dips in the grey bars above the sequence. There are multiple single nucleotide polymorphisms (SNPs) within the sequened samples.

ITS2 Analysis

Protocol Summary:

PCR Amplification of ITS2

DNA that was previously extracted from suspected Kumamoto oysters (n=5) was amplified using the Wang & Guo (2008) ITS2 primer set (ITS2_5_8SF TCTCGCCTGATCTGAGGTCG; ITS2_18SR GCAGGACACATTGAACATCG). A Pacific oyster (*Magallana gigas*) positive control originating from Hog Island was also included. Approximately 40ng of input DNA was amplified in 12.5ul reactions with 1x DreamTaq and 0.5uM of each primer under the following conditions: one cycle at 95°C for 5 minutes; 30 cycles of 95°C for 1 minute, 58°C for 1 minute, 72°C for 1 minute; and one cycle at 72°C for 5 minutes.

Gel Electrophoresis & Imaging

1ul of amplicon (post-PCR) DNA was loaded onto a 2% agarose gel stained with GelGreen and run for 1hr 15 min at 75V alongside E-gel 1kb plus ladder (New England Biolabs). The resulting gel was imaged using a UV light box and phone camera to generate Figure 2.

Results and Conclusions:

As demonstrated in the previous report, there are sufficient nucleotide differences within the COI region between *M. gigas* and *C. sikamea* to differentiate them at the species level. The suspected Kumamoto sequences aligned 99-100% with *C. sikamea* references, providing confident positive species-level identification. Several SNPs are present in the Test1-3 samples (Figure 1), which may be attributed to population and/or regional differences. The ITS2 region is slightly smaller in Kumamoto oysters compared to Pacific oysters. Therefore, Kumamoto ITS2 bands should appear slightly lower on the gel when compared to the Pacific oyster ITS2 band. As seen in Figure 2, all suspected Kumamoto samples appear lower than the Pacific oyster control. Combined, the data indicate that the tested oysters are indeed *C. sikamea*.

References:

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, A. R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.*, 294-9.

Wang, Y., & Guo, X. (2008). ITS length polymorphism in oysters and its use in species identification. *Journal of Shellfish Research*, *27*(3), 489-493.

Figure 1. Multiple Sequence Alignment of Susected *C. sikamea* COI Sequences and NCBI References

Figure 2. ITS2 Gel of Suspected Kumamoto Oysters (*C. sikamea*) in Reference to a Pacific Oyster (*M. gigas*) Control