FINAL PROJECT REPORT

Other Mariculture Species

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EXECUTIVE SUMMARY



To date, the Alaska oyster industry has solely relied on the Pacific oyster (Magallana gigas) in shellfish farming, however the sector's sustainability and long-term resilience requires diversification. The "Other Mariculture Species" project, conducted by Blue Starr Oyster Co., worked to build resilience and diversity in the sector by assessing the feasibility of growing novel oyster species in Alaska: Kumamoto oysters (Magallana sikamea), Virginica oysters (Crassostrea virginica), and Olympia oysters (Ostrea lurida). Blue Starr Oyster Co. has leveraged 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' cultivation.

A main goal of this project is to lay the foundations for increased resilience and competitiveness of the oyster industry in Alaska by introducing new species to the repertoire of farmers. Diversification of species can expand markets for farmers as new and specialty species can build interest from buyers and increase competitiveness of Alaskan oysters in the PNW market. Diverse species can provide a buffer in the event that anything happened to disrupt the current reliance on Pacific oysters, such as interruptions to seed supply or disease. Importantly, this project wanted to use all of the same growing gear that is currently used for Pacific oysters, ensuring that farmers do not need to invest in additional equipment in order to trial and grow these species. All three of these novel species have been successfully farmed in the broader Pacific Northwest but had not been grown in Alaska due to barriers in seed supply, previous interpretation of regulations, and financial risk to farmers.

To test the feasibility of each novel oyster species, the Blue Starr crew worked from April 2025 to September 2026 to acquire, cultivate and evaluate oyster seed of the three proposed species. A cohort of what was thought to be Kumamotos was purchased and planted on the farm in May 2025 but it was later discovered that this cohort was a hybridized seedstock due to accidental Pacific oysters in the broodstock. This cohort was still monitored throughout the project as a point of interest even though the seed was not true Kumamoto. A cohort of Virginica oysters were planted in August 2025 and unfortunately did not fare well though the project, with high mortality rates observed from this seedstock.

Over the winter of the project, the crew found that interstate regulations between Washington and Alaska would ultimately make sourcing Olympia oysters impossible for this project. Finally, “true” Kumamotos were sourced in April 2026 and grew well throughout the 2026 growing season, suggesting that Kumamoto oysters may potentially be a good fit for species diversification. Details of the cultivation experience with each species are outlined in this report.

This project sought to de-risk the process of understanding and trialing new oyster species in Alaska. Throughout the 18 months of the project the crew faced numerous setbacks that are common, challenging, and time consuming for farmers that we hope can be easier to navigate in the future with the knowledge gained from this project. The cost of this project in terms of effort and expense were higher than independent farmers would bear, when the prevailing assumption was that none of these species would perform well enough to cover the financial commitment. Despite several challenges, the crew had many successes that can be shared to inform ongoing innovation in oyster cultivation methods in Alaska.

PROJECT UPDATE

In the final quarter of the project (July - September 2026), the Other Mariculture Species project wrapped up its second growing season and completed the last of the data collection for the purposes of this project. The main project activities included receiving another cohort of Kumamoto oysters from Hog Island Oyster Co. in July, continuing to monitor the cohort of Kumamoto oysters received in April and Virginica oysters from 2025, market research for Kumamoto oysters, and a visit to Hog Island Oyster Co. hatchery and farm in Arcata, CA.

Throughout the project, the cohorts of oysters being monitored shifted as information about their genetics came to light. Some cohorts of oyster seed were discontinued due to issues with genetics (i.e., the second “false kumamoto” group - **K2**) or atypical mortality issues (i.e., first Pacific oyster control group). In the 2026 season, the project monitored the following cohorts of seed:

K1 → “False Kumamotos” (April 2025)
KC → Pacific oyster control group (May 2025)
K3 → larger “overwintered” Kumamoto seed (April 2026)
K4 → smaller (2026 hatched) Kumamoto seed (July 2026)
V → Virginica oyster seed (August 2025)

Oyster Growth Data



All cohorts of oysters were monitored approximately every two weeks during the growing season between April to October 2025 and April to September 2026. From late October to April, the FLUPSY (Floating Upwelling System / oyster nursery) is not in operation and the oyster seed overwinters in passive floating gear on the farm where it is not actively sampled.

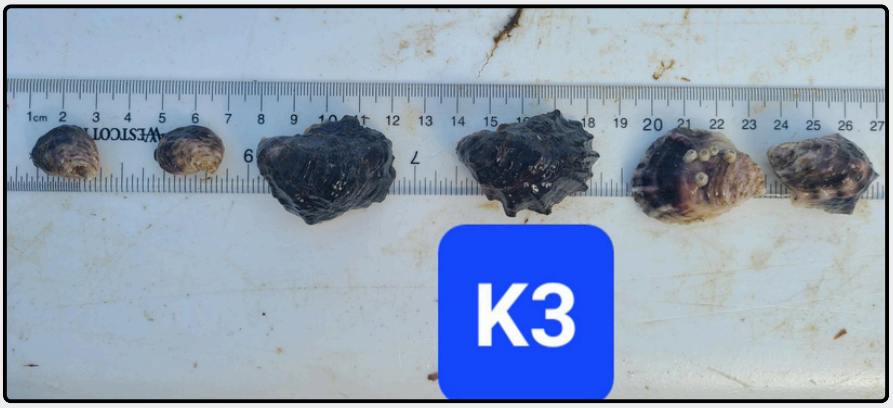
In July 2026, after ongoing high mortality rates, the crew decided to stop actively sampling the Virginica oysters. The V cohort remained in the farm under visual observation, however the remaining live cohort was too small to gain further data insight from. See section below for further details on the observations of growing Virginicas.

The following results follow cohorts **K1, K3, K4**, and **KC** over the course of the project. The full set of raw data observations can be found attached to this report.

The Pacific Control cohort (**KC**), followed an expected trend based on previous experience farming the species. Throughout the project, slower growth was observed in the early spring and more rapid growth occurred in the warmer summer months, with seed ultimately reaching a healthy size by the end of the growing season. The “false kumamotos” (**K1**) followed a similar trend, even seeming to grow faster than the Pacific Control at some points, which made sense once it was established that they were in fact genetically indistinguishable from Pacific Oysters due to hybridization during spawning. Once received, the “true” Kumaomotos (**K3** and **K4**) were found to grow very well in the 2026 season, especially **K3**, which was received at a slightly larger seed size in April 2026 and all the seed was > 16 mm screen by September. To ensure a thorough testing of true Kumamotos, another cohort of smaller seed, **K4**, was ordered from Hog Island Oyster Hatchery. **K4** was received at 6 mm in July 2026, smaller in size than the **K3** cohort, which allowed for testing the survivability of smaller, less expensive Kumamoto seed in the farm. **K4** was also found to grow very well, with most of the seed reaching > 9 mm by September.



a) The Virginica seed cohort in September, 2026, showing the small and inconsistent sizing after more than one year in the FLUPSY.



b) The “overwintered” Kumamoto seed (**K3**), spawned in late 2025, from Hog Island Oysters



c) The early 2026 spawned Kumamotos (**K4**) from Hog Island Oysters showing good growth from their 6 mm beginnings to > 9 mm pictured here.

The chart below (Figure 1) shows the growth trends of **KC, K1**, and **K3** over the 2026 season. 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 chart shows that all three cohorts grew well over the season and exhibited similar growth patterns. The **K3** cohort following a similar pattern to the KC cohort supports the feasibility of growing kumos in Alaska. **K4** is not included in this chart due to the significantly different scale of its seed numbers per liter and is shown below in Figure 2.

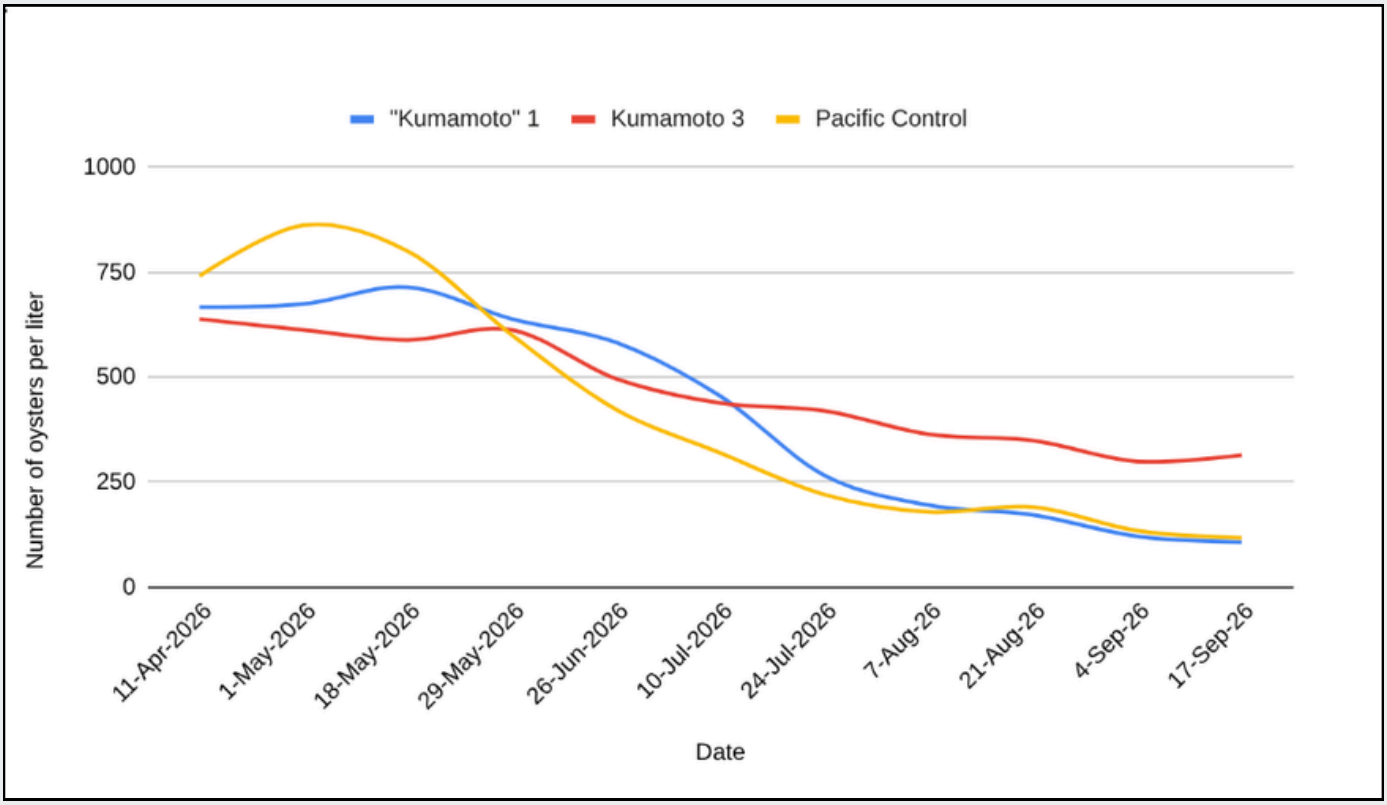


Figure 1: Growth trends of K1, K3, and KC over the 2026 growing season

The chart below (Figure 2) shows the growth trend for **K4** from July to September, 2026. Similar to **K3** and **KC**, **K4** experienced rapid growth during the warmer months of June, July and August before slowing down as waters cooled into September. Despite being introduced to the FLUPSY later in the season, the **K4** cohort performed well and is expected to survive through the winter well at the size it has grown to (~12 mm). Additionally, **K4** was a group at the leading edge of its full hatchery cohort, while **K3** was near the trailing edge of its cohort. It is worth noting that the **V** cohort was also received late in the season in August 2025 and yet did not experience this type of positive growth through the late summer.

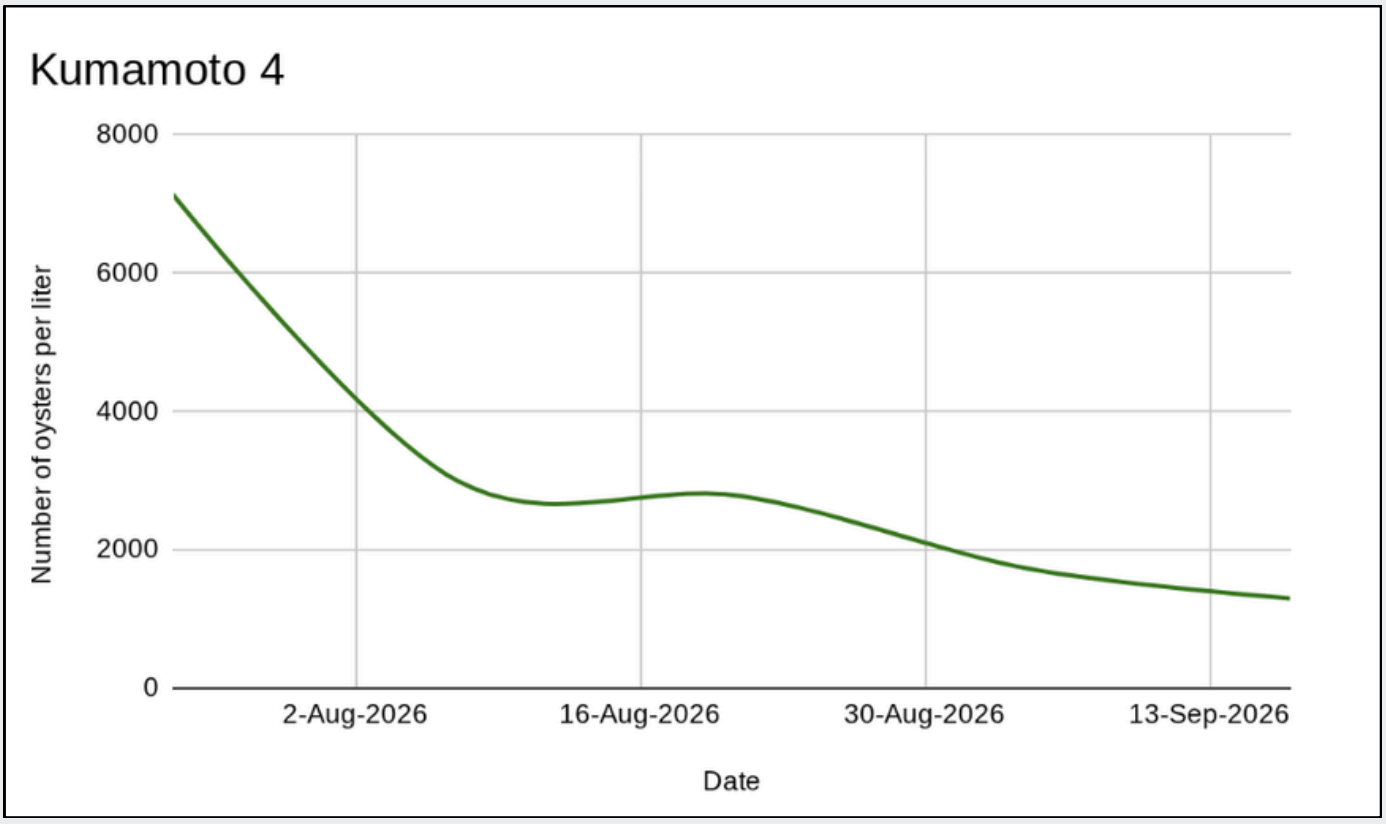


Figure 2: Growth trends of K4 over the 2026 growing season

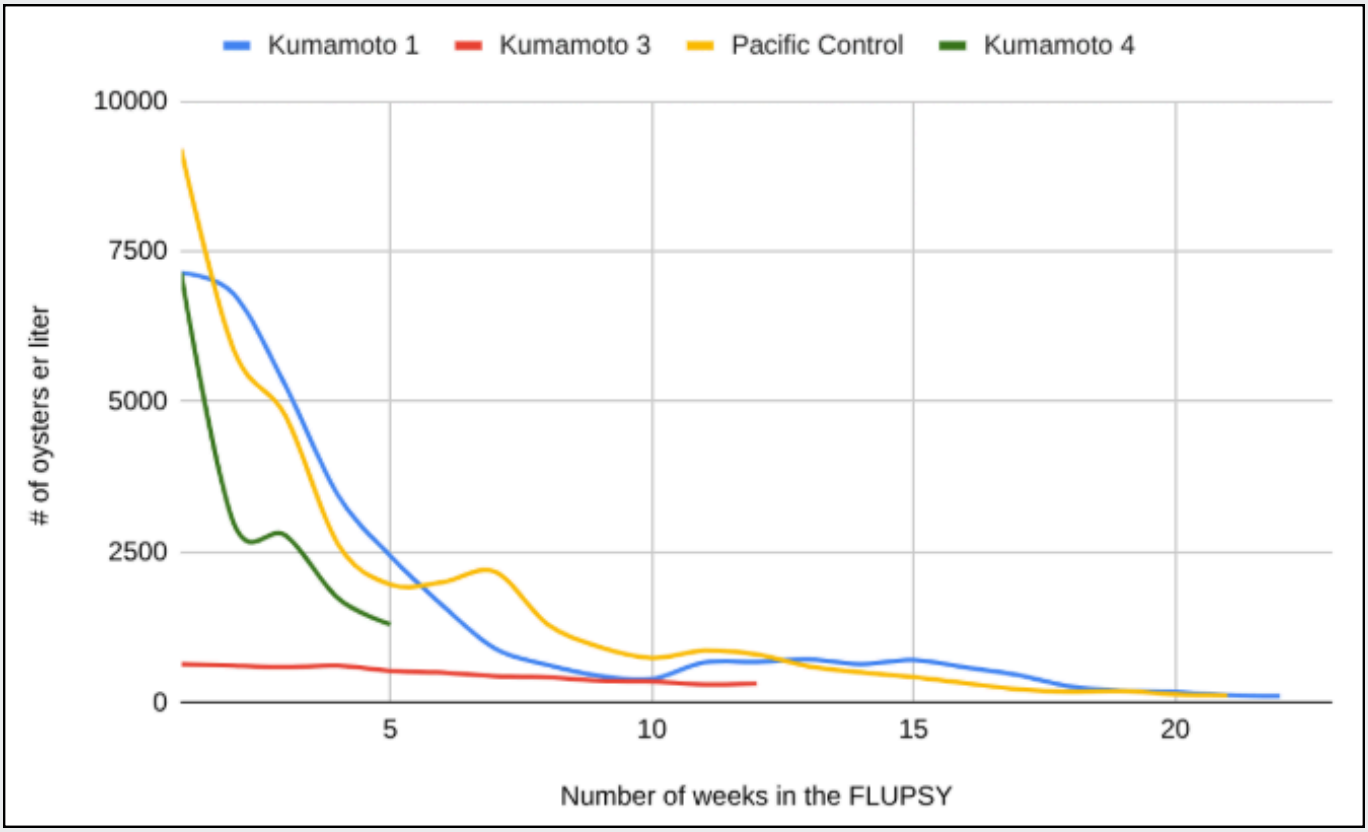
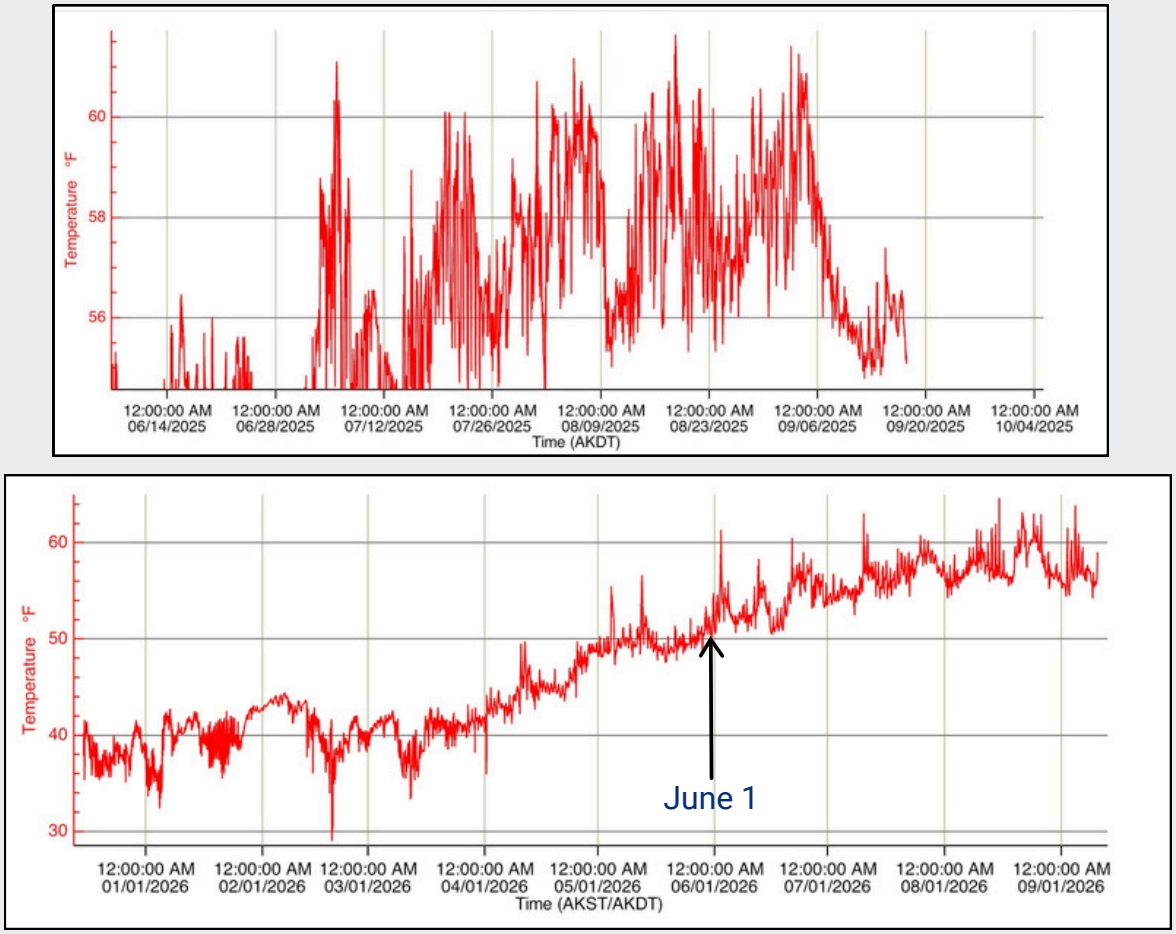


Figure 3: A comparative graph showing **K1, K3, K4,** and **KC** growth trends by number of weeks in the FLUPSY over both the 2025 and 2026 growing seasons.



Water temperatures at the farm. Top graph shows the 2025 season (June - September). Bottom graph shows the 2026 season (January - September).

Farm Observations

Beyond the data, the observations of the crew at Blue Starr Oyster Co. were important to understanding the feasibility and growth of the oyster cohorts. Close observation of the K1 cohort revealed that the seed did not have the expected Kumamoto shell qualities and that the growth rate was suspiciously rapid. Overall, the Kumamoto and Virginica oysters were easy to work with and did not require any additional efforts beyond the methods used for growing Pacific oysters.

The Virginica and Kumamoto seed were grown alongside Pacific seed in the nursery and farm setting throughout the project, providing the best comparative growth opportunities for the species. The summer of 2026 was another warm and sunny season (see 2025 and 2026 data in the image above) which resulted in above average growth of seed oysters, in general. Since the other species of oysters are cultivated in waters that are normally warmer than temperatures seen in Alaska, we have assumed that this growing season provided a best chance for success for the Kumamotos and Virginicas.

The poor performance of the Virginicas over an exceptionally good growing season compounds the negative possibility of future success of cultivation in Alaska. A small number of the Virginicas survived into the end of 2026, but grew very slowly. A much smaller number of the Virginicas grew faster, but were very misshapen. The overall performance saw an insignificant amount of survival over most of two growing seasons.

The high survival of the Kumamoto groups (**K3** and **K4**) is encouraging. In particular, the second cohort of Kumamoto seed (**K4**) grew at overall rates similar to cohorts of Pacific seed that we have seen in past seasons. Performance of Kumamoto seed over more typical temperature growing seasons remains to be seen, however, our data points toward acceptable survival and growth of Kumamoto oysters in Alaska.

Seed Challenges & Successes

In the early stages of the project, promising sources for all three novel oyster species had been identified. However, as the project began, a few challenges presented themselves, most of which the crew was able to address. When the project began, there were limited options for seed imports into Alaska from permitted hatcheries. Only Jamestown Shellfish (WA) was permitted to export Kumamoto (Magallana sikamea) seed and only Hawaiian Shellfish (HI) was permitted to export Virginica (Crassostrea virginica) seed. Both of these hatcheries supplied the initial cohorts for the 2025 season.



K3 cohort of Kumamoto (bottom row) compared to the **KC** seed (top row).



K4 cohort of Kumamoto seed.

It came to light at the end of the 2025 season, through observation and genetic testing, that the oyster seed purchased as Kumamoto in 2025 was not true Kumamoto seed due to genetic contamination from Pacific oyster broodstock in the hatchery process. Kumamotos can look very similar to Pacifics and the two species can interbreed, resulting in hybrids that seem to mostly present overwhelmingly as Pacifics visually and genetically when tested. In the second year of the project, ongoing conversations with Hog Island Oyster Co. (CA) resulted in the company going through the process of gaining permits to export oyster seed into Alaska. This collaborative approach opened up the opportunity for Blue Starr to secure Kumamoto seed from them and importantly, for another hatchery to be available to Alaskan oyster farmers moving forward. The Kumamoto seed grew well from the time it entered FLUPSY.

The crew was unable to acquire Olympia oyster seed due to opposing state regulations between Washington and Alaska. However, there were hatcheries that were willing and interested in providing seed and support in the process of investigating pathways to securing seed, including Hama Hama Oysters (WA). The details of the Olympia seed process are outlined in the section below.

The project was able to successfully monitor one and a half growing seasons of Virginicas and one full growing season of Kumamotos, as well as two full growing seasons of the Pacific oyster control group and the “false Kumamoto” hybrid group. The Pacific and hybrid seed did well as expected, the successful growth of the Kumamotos at a similar rate to Pacifics is very exciting. While the Virginica cohort did not fare well in this project, the species is now permitted in Alaska which could open doors to further innovation with this species.

It is important to note that there is always variability in the quality of oyster seed coming from a hatchery. Variability stems from broodstock genetics, broodstock conditioning, seasonal timing of the spawn, feeding and care of larvae and set seed, then feeding (or under nourishment) of the individual seed oysters until shipment to the farm. Because of this (and variability in farming conditions) the data generated from this project leads not to any definitive conclusions, but rather is information useful in directing future roads of exploration and potential development. Within the context of Alaska oyster farming, we believe that this information, however limited, is of high interest and useful to industry.

Olympia Oyster Seed Regulations Summary

During this project, we connected with state regulators in Alaska and Washington to better understand the barriers to acquiring seed for Olympia oysters, which are permitted to be grown in Alaska only if their seed meets the ADF&G regulations. We spoke extensively with the Olympia oyster program representative from the Washington Department of Fish and Wildlife (WDFW) to understand the program in place in Washington for hatcheries and for restoration. Olympia oysters, which are native to Washington State, have historically faced major population decline and the WDFW works in collaboration with restoration organizations and oyster farmers to rebuild their populations. The WDFW program's mission is as follows:

“The over-arching objective of WDFW guidance for native oyster restoration is the rebuilding of natural oyster bed habitat supported by self-sustaining populations of native oysters”.

Olympia oyster cultivation is under strict regulation to ensure genetic diversity in the ongoing restoration efforts throughout the state. All hatcheries producing seed, either for restoration or cultivation, must adhere to the WDFW protocols for “restoration-grade” Olympia seed. Some protocol highlights include:

- *Broodstock must be collected from approved wildstock sources that avoids use of hatchery-origin oysters and must be of local provenance to the sub-basin where restoration is planned.*[1]

- *A minimum of 1,200 wild broodstock should be used for each production cycle.*[1]

- *Annual genetic analyses of each year’s hatchery production are strongly recommended. For each sub-basin brood, acquire tissue samples (n=50) and analyze genetic diversity metrics of hatchery reproductive output destined for each population/area being restored. These metrics should be compared to those of a representative independent wild sample (n=50) from the same area.*[1]

- *Certify brood animals by a qualified histopathologist for disease-free status according to accepted OIE standards.*[1]

The full WDFW Protocols for Hatchery Production of Native Olympia oyster (Ostrea lurida) Seed for Restoration can be found attached to this report.

[1] *Brady, B., Martinelli, J., & Eardley, C. (2025). WDFW Protocols for Hatchery Production of Native Olympia oyster (Ostrea lurida) Seed for Restoration. Zenodo. https://doi.org/10.5281/zenodo.15932967*

The main takeaway is that restoration-grade Olympia seed must be sourced from wild broodstock every year. And, even though each Olympia broodstock cohort must be certified by a qualified histopathologist as disease-free, this protocol does not align with the requirements from the Alaska Department of Fish and Game (ADF&G) for oyster broodstock. ADF&G requires that the broodstock be derived from captive oysters commercially cultured on the Pacific Coast of North America through three or more generations so that a disease history can be established. This unfortunately creates a catch-22 between Alaska and Washington's regulatory framework, where if a hatchery in Washington is adhering to the State's regulations, the resulting seedstock is not allowed to enter Alaska, and if a hatchery produced seed that met the Alaskan regulations, it would not be allowed to be distributed in Washington. It is unlikely that a hatchery in Washington, or elsewhere, would begin an Alaska-centric, three-year broodstock program for Olympia oysters, especially given that their growth and end market have not been showcased in Alaska (something this project sought to accomplish). It is also unlikely that ADF&G will review and shift its regulations to align with Washington State's restoration-grade hatchery protocol.

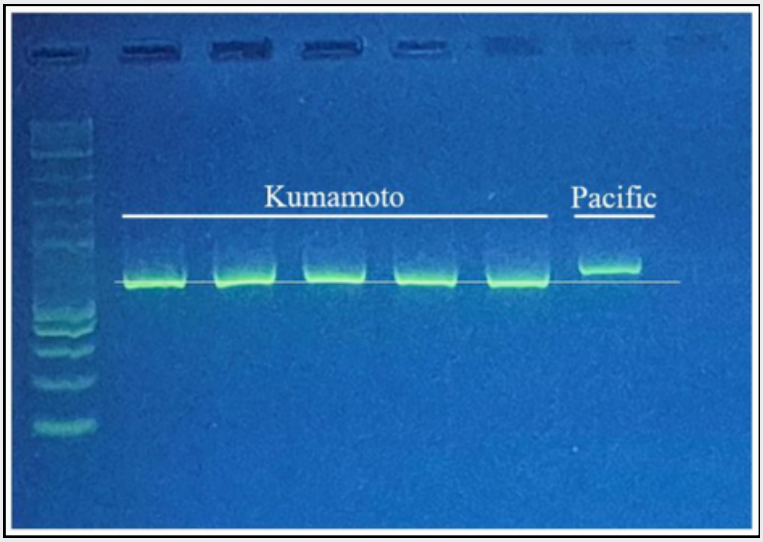
Virginica Mortality

The Virginica cohort was received in August 2025 from Hawaiian Shellfish, beginning as ~200,000 seed at < 6 mm in diameter. By July 2026, there were less than 100 individual seed left and the crew decided it was no longer producing significant data to continue to sample using the methods we had used throughout the project. The seed was kept in the same growing area throughout the remainder of the season for visual observation but was not sampled. Overall, the growth of the seed compared to the Pacifics and Kumamotos was very low and inconsistent throughout the seedstock, with some individuals growing larger than others and some taking on abnormal shapes.

There is large genetic variability in the broodstock and seed that is offered throughout the sector and the particular Virginica seedstock we received were the only ones we were permitted to get. They did not grow in the conditions we were able to provide, however different broodstock and conditions may have a different outcome. Significant time on the farm was put into their husbandry without positive results, even in an El Niño year when the water was well above 45°F (7°C), conditions when Virginicas should have been able to grow. Given the significant variability, we would not confidently encourage further trials with this broodstock.

University of Alaska Fairbanks (UAF)

We are grateful to have had the opportunity to collaborate with UAF on genetic work of the oyster seed throughout this project. When the K1 seed growth rate and physical appearance became suspiciously similar to Pacifics at the end of the 2025 growing season, we sent samples of our K1 Kumamoto oysters and KC Pacific oyster control to UAF to undergo DNA testing to assess the genetics of the Kumamoto oysters. Alorah D. Bliese, a Ph.D student at UAF, worked with our samples and put them through several tests to compare the DNA of each sample. Her careful research and analysis in 2025 suggested our K1 seed were not true Kumamotos, which was later confirmed upon contact with the hatchery. In 2026, when we received K3 seed from Hog Island Oysters, Alorah again tested our samples and through specific genetic testing, results showed a sufficient nucleotide difference between the samples of Pacific oysters (Magallana gigas) and Kumamoto oysters (Magallana sikamea) to differentiate them at the species level. This testing was an unexpected but vital contribution 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 COI process and report by Alorah can be found attached to this report.

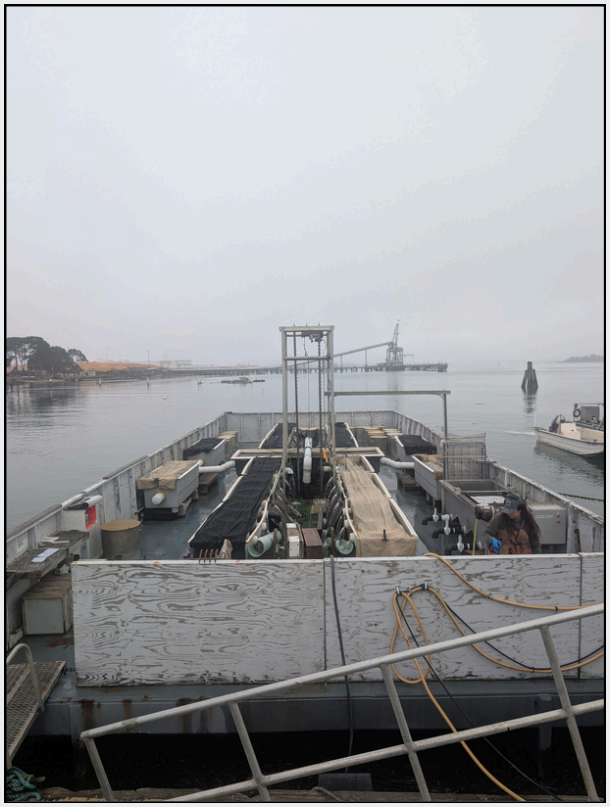


ITS2 Gel of suspected Kumamoto oysters (*M. sikamea*) in reference to a Pacific oyster (*M. gigas*) control. Image from attached report from Dr. Alorah D. Bliese.

This project has supported ongoing development of oyster genetic and life cycle work at UAF which was vital in the process of validating Kumamoto oyster seed. The team at UAF now has a more complete understanding of the unique genetics of Kumamoto and Pacific oysters for any potential future research or validation, helping to ensure quality and consistency of seed supply in Alaska. Continued relationships with UAF for oyster genetic studies could be beneficial to ongoing research and understanding of oyster culture in Alaska.

Hog Island Oyster Co. Visit

In September 2026, members of the Blue Starr Oyster Co. crew traveled to Arcata, CA to visit the Hog Island Oyster Co. operations which include their hatchery, nursery, and farm sites. The hatchery went through the process of becoming certified to export seed to Alaska in 2026 due to this project, opening up another pathway for seed in AK. During the visit, the crew toured the facilities and met with the hatchery manager and several other key staff at the facility, learning about their hatchery techniques and experience cultivating Kumamotos. The Hog Island Kumamotos are farmed in shallow intertidal waters in hanging baskets, which while different from the deep water gear common in Alaska, still allows for gentle tumbling and wave action that help shape and strengthen the oysters. During discussions, the hatchery manager shared insights and also heard from the Blue Starr crew about the needs of Alaskan farmers, such as having access to seed as early in the season as possible, and having access to robust, well fed seed that can transition well into Alaskan waters. Their receptiveness to industry needs in Alaska was a positive experience.



Hog Island Oyster Co. nursery barge, housing Kumamoto and Pacific oyster seed.



Fully grown Hog Island Pacific oyster (left) and Kumamoto oyster (right)



Hog Island Oyster Co. farm in Humboldt Bay, CA showing Pacific and Kumamoto oysters growing in shallow hanging baskets (left) and a freshly shucked Kumamoto oyster from the farm (right)



Kumamoto Oysters Feasibility and Markets

The Kumamoto oysters (Magallana sikamea) were the only novel species that was acquired as seed and saw successful growth in the FLUPSY. Given their initial success, Blue Starr will continue to grow the seed and observe the Kumamotos for their potential as a market-ready product.

Kumamotos are a close relative of the Pacific oyster (Magallana gigas), native to the Kumamoto prefecture in Japan and in the Western Pacific ocean including the shores of China and Korea. They are a well known shellfish aquaculture species on the west coast of North America, though much less widely farmed as compared to Pacific oysters. Kumamotos are a popular addition to raw bars and often marketed as a premium species favored for their sweet, melon-forward taste and unique shell, dark in colour and with distinct fluting. While they are smaller in size than a Pacific, they are known for forming a deep cup and having a mild flavor that most customers readily enjoy. It is more common in recent years for customers to have a preference for smaller oysters, while still being willing to pay typical prices, i.e., $2-$4 per oyster. This is seen in several companies, including larger oyster companies like Taylor Shellfish, offering “extra small” and “petite” oysters (2.5” - 3.25”) for $26-$30/dz, a slightly lower cost than small-medium (3”-4”) for $32/dz. This trend supports the idea that Kumamoto oysters would be a worthwhile product even though they are typically smaller in size (2” - 2.5”). The price per dozen of Kumamotos can vary across the Pacific Northwest as premium products can, typically with a higher value per dozen. In Washington's Puget Sound region, providers are charging retail values of $23/dz to $38/dz for Kumamotos while Pacific oysters are $16/dz to $26/dz. In California Kumamotos can fetch $43/dz while Pacifics are $31/dz from the same provider.

Kumamotos are typically farmed in regions with slightly warmer waters, including Puget Sound, WA, central to northern California, Oregon, and B.C.'s Salish Sea. However, our experience so far has suggested that they have potential to grow well in Alaskan waters. Their approximate growout time is three years to reach ~2.5" in Puget Sound, but it remains to be seen how long that may take in SE Alaska.

Detailed research on Kumamoto oyster cultivation and disease is more limited in the literature than for Pacific oysters. Additionally, disease resistance in oysters can depend heavily on the strain of pathogen, the genetics of a particular species, and geography, making it challenging to make broad sweeping conclusions about a specific species. However, some research indicates that Kumamotos seem to be more tolerant to Ostreid Herpesvirus-1 (also known as Pacific Oyster Mortality Syndrome (POMS)) than Pacific oysters (Liu et al. 2025). Additionally, Kumamotos have been known to flush Vibro parahaemolyticus from their systems faster than other species when put into depuration (wet storage) (Lunda, 2025).

Ultimately, species diversification that includes Kumamotos is likely to benefit farmers in Alaska that can provide a unique product, especially within the Alaskan market which currently offers exclusively Pacific oysters.

Workshops & Outreach

Throughout this project, there were multiple occurrences when the crew at Blue Starr was able to share information and experiences surrounding the innovative mariculture work being done at the farm. Outreach and education about Blue Starr Oysters and the methods used at the farm, including the Other Species Project, were shared at the following engagement opportunities:

- Blue Starr Oyster Co. took part in a three-day knowledge exchange organized by James Crimp and Arron Jones from Alaska Sea Grant. The Alaska Sea Grant Oyster Farming Operations Workshop was put together to connect those new to oyster farming in Alaska with those with significant experience in the sector. Participants toured the FLUPSY and FlipFarm setup at Blue Starr Oysters and were shown the details of the "Other Species" seed nursery process.

- Blue Starr hosted the 2025 University of Alaska Southeast Aquaculture Semester students at the farm, where they were shown the farm operations and taught about the Other Species Project and why this work is important to the sector. The local Naukati Bay and Whale Pass schools both visited the farm in 2026 where they also learned about basic oyster farming techniques and the species that are being cultivated.

- The Tastemakers FAM Tour made a stop at Blue Starr where the concept of novel species and how they can support a resilient market was shared. The tour was focused on the unique attributes and high quality of Alaskan oysters and how they are a piece of the exciting culinary story of Alaska. Unique and premium species can build on Alaska's already well known seafood offerings and could support finding and retaining new customers for seafood and oysters outside of Alaska as well.

- The Other Species project was discussed at the OceansAlaska 2025 and 2026 Hatchery Workshop, hosted in Ketchikan. Participants learned about the new species being trialed and the challenges that came with acquiring seed as well as how the new species could support sector resilience in Alaska.

- Future presentations about the project are planned for the Mariculture Conference of Alaska (March 2027) and the next Alaska Shellfish Growers Association meeting.

The reports put out by this project detail the processes and monitoring that have taken place in exploring the potential of "Other Species" in Alaskan mariculture. This includes the challenges and successes of acquiring Kumamoto seed, the interstate regulations that made Olympia seed impossible to import to Alaska, and the mortality issues faced with small Virginica seed. The reports are intended to be useful tools in ongoing outreach and education about oyster farming in Alaska and the processes of species diversification.



Alaska Sea Grant visit to Blue Starr Oyster Co.



UAS Aquaculture Semester 2025 cohort visit to the farm.

Next steps & ongoing monitoring

Given that the Kumamoto oysters are the species that have shown high potential to be a viable additional species grown in Alaska, Blue Starr will continue their growout into the next season and pursue further knowledge on their cultivation. Because of the Kumamoto seed challenges and time limitations of this project, this report does not include data or observations of the Kumamotos growing to full market size. We will be excited to see how the next season goes and how the Kumamotos grow in a year that is not influenced by El Niño. If they continue to grow well and reach market size, Blue Starr will introduce them to the market and purchase more seed for the FLUPSY for ongoing supply. From relationships with current customers, Blue Starr already has one interested buyer for market-sized Kumamotos and two interested buyers for nursery seed Kumamotos. With some additional marketing, it is likely that interest will grow. Ultimately, we will continue to assess if the Kumamotos are financially feasible in the long run for the farm.

Future work will also include ongoing relationship building with Hog Island Oysters as they have expressed interest in continuing to supply Kumamoto seed and supporting the Alaskan growing season with seed that has been spawned earlier. There is potential for more research to be done on Kumamoto oysters in Alaska through academic or other industry pathways.

FINANCIALS

The project has come to a conclusion and spent 87% of funds, with the remainder being primarily from unspent contractor fees. 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	$53,081.28	$16,332.92	$10,785.80	87%

State of Washington

DEPARTMENT OF FISH AND WILDLIFE

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Main Office Location: Natural Resources Building, 1111 Washington Street SE, Olympia, WA

Brady Blake, Julieta Martinelli, Christopher Eardley

WDFW Protocols for Hatchery Production of Native Olympia oyster (*Ostrea lurida)* Seed for Restoration

The over-arching objective of WDFW guidance for native oyster restoration is the rebuilding of natural oyster bed habitat supported by self-sustaining populations of native oysters.

This document contains:

1) Guidelines for when to use seed supplements in restoration.
2) A list of current requirements for the hatchery production of restoration-grade native oyster seed.
3) Background and requirements to minimize genetic risk by maintaining and protecting genetic integrity of populations within Puget Sound and the Washington coast.
4) Details on Composite Broodstock Zones in the five Puget Sound sub-basins identified by WDFW.
5) Recommendations for archiving tissue samples.
6) Requirements to minimize the introductions or spread of diseases and pests associated with using hatchery-produced animals for restoration.

1. When to use seed supplements

As some embayments hosting restoration sites support natural production and recruitment of Olympia oysters (and as others will eventually), not all sites are candidates for hatchery seed. WDFW emphasizes habitat enhancement (e.g. shell placement) over planting of hatchery-produced seed at such sites.

Restoration sites for the placement of hatchery seed vs. clean cultch must be approved by WDFW.

As a first approach to determine whether a site supports natural recruitment of Olympia oysters, we suggest conducting surveys to understand populations—including quantifying abundance, characterizing demographics, and characterizing habitat. We also suggest deploying spat collectors (recruitment stations) three or more years in advance of the start of a potential restoration project. Puget Sound Restoration Fund and partners have been monitoring larval recruitment Sound-wide since 2014. A map of the locations can be found here, and a manual to build and deploy your own spat collector can be found in the References section (available upon request).

Currently, small volumes of Olympia oyster seed are being produced in a limited number of hatcheries for aquaculture purposes. When possible, restoration-grade oysters or larvae are favored for this application. Otherwise, hatchery production of Olympia oysters for commercial purposes will be expected to abide by WDFW's genetic risk management requirements for aquaculture. Aquacultured Olympia oysters are intended for short-term deployment (grow-out) and removal and generally involve lower quantities of oysters than for restoration. Hatchery production for restoration purposes in commercial hatcheries must abide by the same requirements being utilized by NOAA's Kenneth K. Chew Center for Shellfish Research and Restoration operated by the Puget Sound Restoration Fund.

2. Requirements for production and use of hatchery seed for native oyster restoration:

- Proposals for hatchery-production and use of native oyster seed for restoration must be submitted to WDFW for review and approval.

- Sourcing of broodstock and planting of seed must be in accordance with WDFW's sub-basin approach, where broodstock must be sourced from the same basin intended for seed outplant (e.g. seed produced from South Sound broodstock can only be planted in South Sound).

- The Washington coast (Willapa Bay and Grays Harbor) has native oyster populations that are genetically distinct from Puget Sound. All native oyster restoration projects on the Washington coast should only use broodstock from the coast, and all the seed produced from this broodstock should only be outplanted on the coast.

- Broodstock must be collected from approved wildstock sources that avoids use of hatchery-origin oysters and must be of local provenance to the sub-basin where restoration is planned. Due to restoration outplants in some broodstock collection areas, the populations will trend initially towards being largely composed of $F_{1(H)}$ and $F_{2+(HxH,\ HxW)}$. Ceasing hatchery outplants into a location would allow a population to trend towards being largely composed of WxW composition.

- Minimize inbreeding by using a large geographically proximate broodstock population. Alternatively, composite broodstock collected from geographically proximate populations within a sub-basin can also be used.

- A minimum of 1,200 wild broodstock should be used for each production cycle. Note that the Puget Sound Restoration Fund attempts to collect 1,500 to account for any mortality prior to or during spawning. Broodstock may only be used to produce a single year's cohorts and must be refreshed annually.

- Use volitional mass-spawning methods to spawn all 1200 (or 1500) broodstock together.

- Once oysters spawn, collect larvae for a minimum of four weeks. Oysters may spawn multiple times during this time. Depending on production goals, larvae may need to be culled, sometimes up to 50-75% daily. Culling must occur evenly at the onset of larval release throughout the entire broodstock production period, to avoid introducing a bias by artificially skewing larval representation within a given cohort.

- For public tidelands, WDFW requires that broodstock are returned to a location adjacent to the site of collection (to avoid collecting same individuals in the future) after spawning to avoid broodstock mining. We define an 'adjacent location' as a location *within* the same beach proximal to where broodstock were collected. WDFW recommends returning broodstock collected from private tidelands in the same fashion as with public tidelands-derived broodstock.

- Collection of broodstock, planting of seed, and return of broodstock to source locations require WDFW transfer permits. Visit https://wdfw.wa.gov/licenses/fishing/shellfish-import-transfer for more information, or contact shellfishpermits@dfw.wa.gov. Collection also requires a Scientific Collection Permit https://wdfw.wa.gov/licenses/environmental/scientific-collection.

3. *Ostrea lurida* Population structure and Conservation Genetics Guidelines

The information below provides more detail on how to protect and maintain the genetic structure observed for *Ostrea lurida* populations in Puget Sound and the Washington coast. More details about genetics research on native oysters, and differences in populations between sub-basins can be found in Camara & Vadopalas 2009, Stick et al. 2009, Stick 2011, Heare et al. 2017, White et al. 2017, Silliman et al. 2018, Silliman 2019a, Silliman 2019b, Spencer et al. 2023, Silliman et al. 2023.

Minimize among-population effects
Use locally-derived broodstocks. Locally-derived is defined as the geographically closest and/or ecologically most similar and viable source population. WDFW further utilizes a "Sub-basin" or "Composite Broodstock Zone" approach to apply this principle (see below) – hatchery production must utilize broodstock from within the same sub-basin as intended seed out-plant.

Minimize within-population effects
Maximize the ratio of genetically effective numbers of breeders (**Nb**) to broodstock census size (**N**) and maximize genetic diversity. This Nb: N ratio can be maximized by using Volitional Mass Spawning. As mentioned above, the current

requirement is for a minimum of N_B=1200 oysters for broodstock. We have designated Composite Broodstock Zone (see details below) within the five sub-basins of Puget Sound identified by WDFW. Within these Composite Broodstock Zones (CBZ), broodstock should be rotated from year to year. That is, the same site within a zone/sub-basin should not be used for collections annually. The exception to this is Fidalgo Bay as it is the only site in North Sound with populations that can be used for broodstock collection.

WDFW does not currently permit selective breeding of Olympia oysters.

4. Composite Broodstock Zones in Puget Sound sub-basins (potential broodstock sites in parentheses):

- North Sound – (Fidalgo Bay).
- Strait/Admiralty Inlet complex – (Port Gamble Bay, Discovery Bay, Sequim Bay).
- Hood Canal – (Dabob Bay, Dosewallips State Park, Triton Cove State Park, Potlach State Park, DNR-48).
- Central Sound – (Liberty Inlet, Dyes Inlet, Sinclair Inlet).
- South Sound – (North Bay, Skookum Inlet, Totten Inlet, Eld Inlet).

5. Archiving of tissue samples

Annual genetic analyses of each year's hatchery production are strongly recommended. For each sub-basin brood, acquire tissue samples (n=50) and analyze genetic diversity metrics of hatchery reproductive output destined for each population/area being restored. These metrics should be compared to those of a representative independent wild sample (n=50) from the same area. Tissue samples can be archived if funding to conduct analyses is not currently available.

Tissue samples (ideally from the adductor muscle) should be collected immediately prior to field transfer and care should be taken to minimize contamination, and ensure the sampling is representative of the entire group. Tissue samples should be stored in 95% non-denatured ethanol and if possible, kept in a refrigerator. Make sure to label the samples with broodstock collection origin and date collected. Notes on other relevant details, such as number of broodstock used, mortality rates, total seed produced, etc. should also be maintained as supporting information for samples.

6. Requirements to avoid disease transfers

WDFW also seeks to minimize the risk of accidental introduction or spread of disease or pest organisms associated with restoration activities. The essential requirements of disease control and prevention related to native oyster restoration are:

- Certify brood animals by a qualified histopathologist for disease free status according to accepted OIE standards. WDFW requires a general histopathology exam as well as examination via PCR for *Bonamia* spp. on animals collected mid-summer. A Shellfish Transfer Permit from WDFW is required to ensure low risk of potential disease or pest spread or introduction.

- Certify hatchery-produced seed by a qualified histopathologist for disease-free status according to accepted OIE standards. A Shellfish Transfer Permit from WDFW is required to ensure low risk of potential disease or pest spread or introduction.

- Sample seed animals in numbers sufficient to provide 95% confidence of detecting a pathogen with prevalence as low as 5% (this is typically n=60 animals).

- Return brood animals to CBZ of origin after use when disease-free status of the broodstock population is known. A Shellfish Transfer Permit from WDFW is required to ensure low risk of potential disease or pest spread or introduction.

- WDFW shellfish transfer permits are required for transfers of broodstock to and from the hatchery, for transfers of seed from the hatchery, and for seed to restoration sites.

- Shell (cultch) used for habitat enhancement or for setting seed must come from WDFW inspected and approved (permitted) sources.

- WDFW does not permit "shell recycling," or using shell from sources not approved for use by WDFW (e.g. restaurants, other sources that have not been inspected and approved).

- Conditions to ensure broodstock and seed transfers do not carry unwanted hitchhikers such as oyster drills and European green crab will accompany transfer permits.

- Additional permit conditions may apply depending on specifics of facility or circumstance.

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

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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*.

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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

