CHUGACH REGIONAL RESOURCES COMMISSION

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FINAL REPORT

Gametophyte hatchery initiative: expanding research for gametophyte generation and direct seeding techniques for kelp mariculture in Alaska.

RFP #: 2024-06, Kelp Seed Quality Improvement

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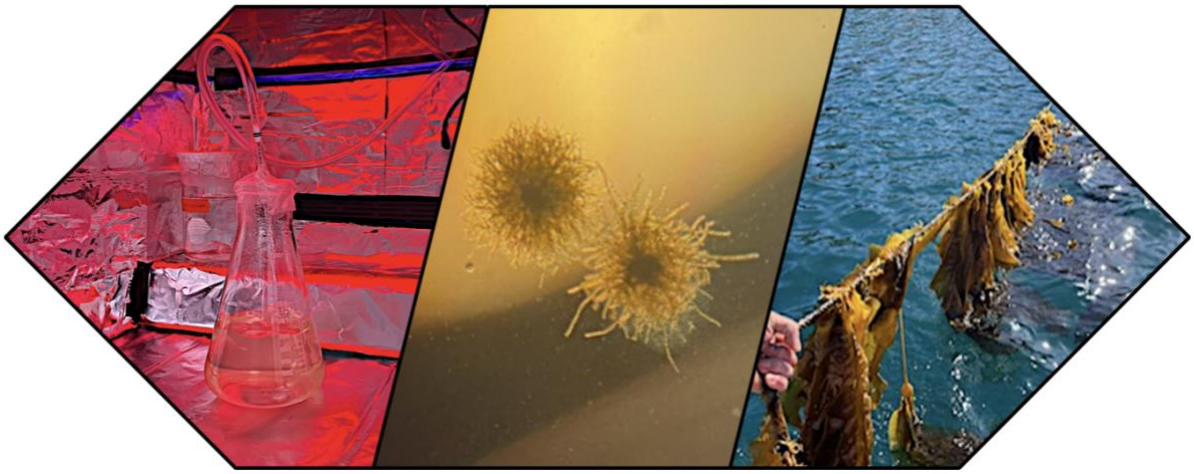


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Executive Summary

The nascent kelp farming industry in Alaska has struggled to secure reliable and financially sustainable seed, resulting in downstream impacts on crop yield and farmer profitability. Of significant interest to industry stakeholders is the transition to gametophyte based seeding, a method which propagates the haploid life stage in a vegetative state in order to provide year round seed with drastically reduced contamination and the ability to transfer seedlings directly to farm lines (i.e. direct seeding methods). While potentially advantageous to farmers, gametophyte hatcheries and their respective seeding techniques are less established than traditional spore and twine seeding. This project aimed to address key bottlenecks in understanding gametophytes hatchery methods, building off previous findings from the Alutiiq Pride Marine Institute Gametophyte lab. Here, we share our findings from an experiment comparing seeding techniques of the species *Saccharina latissima* (*S. latissima*, aka sugar kelp) at two test sites in Kachemak Bay, Alaska. The methods include spraying gametophytes directly onto grow-out lines, with and without a xanthan gum binding solution, as well as twine seeding with gametophyte inoculation. In addition, both braided and twisted ropes were tested for effectiveness, where the kelp was left to grow for ~4 months between January and May of 2026 before being collected and measured for biomass per unit line and sporophyte density. Findings indicated twine seeding maintains higher yields, however direct seeding produced good yields by industry standards (up to 5.55 kg/m) and was more efficient when a binding agent is used. In addition, we discuss other work under the project including challenges associated with an attempted fragmentation study, progress toward commercialization, and knowledge exchange travel.

Successes

This project successfully cultivated *S. latissima* (sugar kelp) via direct seeding techniques, being only the second reported instance in the State of Alaska (Stekoll et al., 2025). Using academic and industry connections we were able to use unpublished, experience based direction on the most promising techniques available, for instance the use of the common chemical xanthan gum as a binding agent, and testing specially made braided rope from Sirputis for gametophyte retention. Successful induction of reproduction using Industrial Plankton Seaweed bioreactors and validation of gametophyte inoculated spool techniques showed ease of adoption in Alaskan kelp hatcheries. Three types of seeding were tested, all using the same gametophyte stock for comparison. Meaningful results were found in this experiment and discussed.

An inter-lab exchange trip was conducted between March 13th and 17th following attendance and dissemination of the project at the 2026 Mariculture Conference of Alaska. Scott Lindell and Daniel Gossard joined Cameron Jardell in Seward to tour the Alutiiq Pride Marine Institute Facility and gave feedback on lab operations and set-up. The trio then traveled to the Kasitsna Bay lab to collect sori for spore release and later gametophyte isolations, as well as to check on the test lines of the seeding experiment.

Challenges

Several challenges faced the completion of objectives in this project, including stock contamination and individual gametophyte tracking under a microscope. Our original experimental gametophyte stock (*S. latissima*, mixed sex aka wildtype stock) grown in an Industrial Plankton Seaweed Bioreactor unit (SBR) was used in an attempt to inoculate twine-spools and induce suspended culture reproduction in October, 2025. When no sporophytes were found after 2 weeks, the stock cultures were sampled and photos were sent to our WHOI collaborators for review. While our lab was aware of contamination (occasional diatoms, green algae and unidentified bacteria) in the culture, the degree of contamination was unknown due to the presence of Ectocarpus spp. which morphologically looks very similar to the spindly filaments of male kelp gametophytes. Thus, the stocks were unusable and had to be destroyed. This was a major setback due to the amount of biomass needed to conduct the experiments in this project. The cryptic nature of Ectocarpus, it's presence as a common epiphyte, and its physiological similarity to kelp (shared Phaeophyceae lineage) make it difficult to exclude from gametophyte cultures, especially when starting with wild-type mixed sex stocks which expose the cultures to far more wild-sourced biological material. Presumably, the Ectocarpus was present in very small amounts and slowly grew to dominate the culture over the previous year the culture had been growing. While the seeding experiment was successfully carried out by starting a new stock in November of 2025, the experiment required every bit of biomass, preventing validation of propagation optimization in the SBR.

While the direct seeding experiment was ultimately successful, comparing the yield to the twine-seeded treatments is difficult as we were not able to standardize the number, or size of sporophytes before out planting. Thus, we cannot distinguish the relative yield gains from twine-seeded sections.

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Lessons learned

- Twine seeding consistently produces more biomass during complete annual harvests, primarily as a function of being larger at outplant when following standard nursery timelines.
- When direct-seeding, the use of a xanthan gum binder applied overtop the spray-applied sporophytes increases retention slightly, with significant differences in sporophyte density at harvest.
- No significant differences were found between twisted or braided rope types for any seeding method, where future research is needed to confirm the lack of substrate effects, while in the meantime farmers should feel comfortable trying direct seeding techniques on standard kelp grow-out-lines that are already in use.
- Direct-seeded juvenile sporophytes at high productivity sites can produce yields comparable to sheltered, lower productivity sites using twine seeding methods.
- Yield variability was fairly consistent between all seeding methods.
- No treatments showed density limited yields indicating seeding density in all methods is higher than is needed.
- Operationally, it was found that mixed-sex gametophyte cultures are difficult to maintain past 1 year due to residual contamination from spore release.
- Operationally, the Industrial Plankton Seaweed Bioreactor Units proved effective for inducing reproduction in gametophytes in suspension.

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Introduction

Economically successful cultivation of kelp depends on efficient and dependable seeding. Kelp seeding relies on two primary factors: the ability to control reproduction/grow juvenile kelp, and to transfer them to an in-ocean substrate for continued growth. As this process involves rearing kelp through multiple life stages in artificial conditions, there are many variables to be considered, and ultimately optimized. Emphasis lies in which life stage is the focus for growth, under what conditions are they grown, and how are they seeded onto a farm.

Traditionally, kelp hatcheries utilize the meiospore life stage which attach to seed-lines and cycle through a sexual gametophyte stage to produce harvestable sporophytes. This method is well established in the industry, although limitations including inconsistent sporophyte densities on seed lines, intensive resource demand, and potentially reduced growth performance, has prompted research into alternative methods. Rather than immediate succession among life stages in a hatchery, the gametophyte stage can be grown and multiplied in cultures in which conditions are manipulated to prevent sexual reproduction into sporophytes, and reversely, prompted to reproduce at any time. Gametophyte cultures have some distinctive advantages including an increased control over consistency and density on farms, the ability to be directly seeded onto farms without a period of growth on seed lines, the decoupling of out-plant timing from natural reproductive timing in wild kelps, and can allow for better control of genetics, even facilitating selective breeding if warranted (Umanzor et al., 2020; Kerrison et al., 2018: Li et al., 2022).

While gametophyte hatchery methods have been at the center of kelp domestication in East Asia for decades, research and implementation in this field has grown in burgeoning mariculture regions including Northern Europe and the east coast of the United States. While Alaska stands to become a major player in the industry — due to an expansive and undeveloped coastline, healthy stands of native kelp, and a strong maritime workforce — gametophyte hatchery and seeding research has been limited to small-scale laboratory based projects, with the exception being gametophyte seeding in Kodiak under the ARPA-e project (e.g., Umanzor et al., 2020). Although unique regulations designed to conserve wild kelp genetics in Alaska could reduce the efficiency of kelp gametophyte hatcheries, the utility of these methods are too great to ignore (Kerrison et al., 2018). While developments in other regions are insightful to gametophyte hatchery techniques in Alaska (e.g., Hortimare in the Netherlands; Woods Hole Oceanographic Institute [WHOI] and GreenWave in the US), investing in in-state efforts to build capacity for scaled, industry focused research for locally specific knowledge will enhance opportunities for our kelp farmers moving forward.

This project aimed to expand on the *Saccharina latissima* focused gametophyte research, specifically addressing seeding method performance through ocean trials, and further gametophyte culture optimization research. Leveraging collaborative efforts with WHOI and the NOAA Kasitsna Bay Laboratory, an experiment was conducted to assess farm performance in

two test sites in Kachemak Bay, Alaska, through seeding method and substrate type. We set out to test how seeding method (meiospore seed line, gametophyte seed line, juvenile sporophyte direct) affect biomass, density of sporophytes on an ocean farm, and if patterns differ between a sheltered and exposed site? This project is part of a greater effort by the Alutiiq Pride Marine Institute to establish commercial gametophyte hatchery services in Alaska, including procurement of high capacity propagation equipment, development of standard operating procedures, and farmer engagement with advanced seeding techniques. These goals address key challenges in the commercial viability of alternative strategies for securing consistent and robust seed to kelp farmers in Alaska.

Methods

Outplant Experiment

Experimental design

The experiment used a fully crossed block design to evaluate the effects of seeding method substrate and site on kelp establishment and growth. At each of the two sites (Jakalof Bay and Kasitsna Bay), six sections of 8 meters were established, with three sections assigned to each of the two substrate types. Each block contained eight experimental units, representing the three seeding methods with two replicates of the twine seeding method and 3 replicates of both the direct seeding methods per block. Thus, each site contained 48 experimental units, providing 6 + 9 + 9 replicates for each seeding-method × substrate combination per site. The experimental unit was a section of 0.75 m of grow line seeded using a single seeding method, while a smaller 0.5 m section within each treatment unit served as the sampling unit to minimize edge effects. The three predictor variables were seeding method (three levels: twine-spool and juvenile sporophyte sprayed directly onto lines, with and without xanthan gum binding solution applied over top), and sporophytes sprayed with xanthan binder without a setting period), substrate (Sirputis Braided 12 mm PES rope, and firm lay PolySteel/Dacron blend braided rope ½ “[Appendix A, Image 1] and site (Jakalof Bay and Kasitsna Bay, Figure 2). Response variables were average frond length (cm), sporophyte density (plants/m of line), and biomass (kg/m of line).

Seed acquisition

To create gametophyte stocks, wild sorus tissue was collected from mature *S. latissima* sporophytes from Jakalof Bay (59°27'25.87"N, 151°31'35.91"W) near Seldovia, Alaska. In October 2025, brown algae contamination of Ectocarpus spp. (Appendix A, Image 10) was discovered to have out-competed the gametophytes during the previous year of growth, having been a cryptic contamination due to a visibly close morphology to male kelp gametophytes. The contamination prevented reproductive success in cultures and rendered the stock useless for experimentation; therefore, a new stock was created by collecting 65 *S. latissima* sporophytes

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from the same location on November 6, 2025 (ADF&G P-26-016) via intertidal hand and snorkel collection. Sporophytes were collected from the substrate with holdfasts intact and placed in an insulated cooler with seawater. The sporophytes with visible sorus material were transported to Seward to APMI and placed in a race-way for live holding.

A one inch diameter aluminum pipe was used to separate 2 circular sorus-cuttings from each sporophyte, while preserving the health of the sporophytes themselves. The sori were cleaned with autoclaved seawater and chem-wipes and sterilized in 0.15% sodium hypochlorite solution following (GreenWave, 2024). The sori were desiccated on paper-towels and left in the dark overnight for 24 at 4° C. The desiccated sori discs were submerged in autoclaved seawater at 10° C for 30 minutes to induce spore release before the spore solution was vacuumed filtered through 5 micron nylon filter (UV sterilized). A hemocytometer was used to count spores from duplicate aliquots before inoculating at 10,000 spores/ml. A 1 L borosilicate glass flask was used to house the spore culture at a total volume of 800 ml of media (f/4 concentration using Procline f/2 liquid nutrient stock with 0.5 ml per L SSW of GeO2 (stock con. 0.1g per 200 ml DI water) and 5 ml per L SSW of Kanamycin sulfate (stock con. 1g/100 ml DI water)). The culture was aerated using an inline humidifier, a 5 ml glass serological pipette, and 0.2 micron sterile filter vent. The culture was incubated at 10° C under red light at ~19 ppfd (Mammoth lighting photometer) under a 12:12 L:D photoperiod. Media exchanges occurred every 14 days after an initial 30 day period, whereby aeration was ceased for 24 hours before the old media was removed within a laminar flow station to the visible point of maximum retention of gametophytes, then filled to approximately 800 ml total volume of new media.

Spool preparation

Spools for twine seeding treatments were prepared by soaking 3, 6 cm by 16.3 cm sections of PVC pipe wound with ~23 m of Holdfast brand nylon twine in 70% ethanol solution for 2 minutes. The sterilized spools were dried at 32° C in a lab oven wiped with ethanol. Individual beakers were used for each spool, where 3, 2 L glass beakers were autoclaved then wrapped in a layer of aluminum foil to increase light reflection to the middle. All aeration components (5 ml serological pipettes, silicone tubing and 0.2 micron double barbed inline air filters) were autoclaved as well (Appendix A, Image 3).

Spore-seeded spools

Sori – discs were collected from the same sporophytes as the gametophyte culture (held live), and were induced to release the spores following the same protocols described above. Two spools were inoculated with spores by adding spore solution directly into the grow-out beakers at concentrations of 2,500 and 5000 spores per ml, respectively. Both spools were left covered at ambient 9° C in their respective beakers for 24 with no aeration. GeO2 was used for the first 7 days at 0.11ml/L, while f/2 at 66.5 µL/L per part of Procline f/2 liquid media given daily, with a 24 hour exchange water exchange period using a flow-through system. The spools were

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abandoned, however, due to filamentous brown algae contamination and no visible sporophytes on the spools.

Gametophyte-seeded spools

To inoculate and culture gametophyte-seeded spools, gametophytes were harvested from the incubator by filtering the suspended media over a 50 micron nylon filter over a vacuum. The gametophytes were washed with then resuspended in SSW before being filtered again over a .2 micron filter over vacuum for 15 seconds. Gametophytes were scraped from the filter and weighed to 31.87 mg and ground in a sterile glass hand tissue-homogenizer. The ground gametophytes were re-suspended in SSW and left for 3 days in low light conditions (<15 ppfd) at 10° C. The gametophyte pellet was then very gently ground again to release fragments from each other without intentionally fragmenting the gametophytes themselves, then the suspension was brought to 12.5 mL with SSW. The gametophyte solution was agitated by pumping a pipette before taking a 0.4 mL sample, diluting to 1 mL then put on a grid slide and photographed 5 times randomly moving only from left to right to avoid double counting. All gametophyte fragments within the image were sexed. The suspension had an estimated density of 2.55 mg WW/mL, with a sex ratio of 1.6:1 F:M fragments, generally between 20 and 60 microns in length. Here, we used a one inch brush sterilized with ethanol to distribute the remaining gametophyte solution onto the dry twine-spool until the twine was saturated (following GreenWave protocols for painting gametophyte spools). The spool was then left in the dark at 10° C under a sterile beaker for 20 minutes to aid in gametophyte binding before being transferred to a 2 L sterile beaker and left in still, dark, cool water overnight (ambient air 9° C). The spool was introduced to light starting at 20 PPFD and working up to 100 over 12 days. Procline two part liquid algae food nutrient media was used in F/4 concentrations delivered once daily to the flow through system, while light aeration was added on day 5. No GeO2 was used in the gametophyte spool media. The sporophytes were cultivated for 34 days, with no added nutrients to the filtered flow-through seawater starting on January 4th to slow growth before out planting. On January 21st, a snippet between 2 and 5 cm was taken from the end of each 1.3 meter section of twine used in the outplant trial (Appendix A, Image 13). These were kept in filtered SSW in a sealed container in the dark at 4° C until analysis. 10 snippets were randomly selected, where one, 1 cm section was removed from one end of the snippets selected. These were then submerged in SSW, where a stereo dissection scope was used to count and photo graph the snippets. Each snippet was turned so that the sporophyte side (side facing out on the spool) was turned toward the lense of the scope. Two photos were taken from each snipped, where the first three snippets also included a photo of a slide micrometer which sat underneath the plastic petri dish with the snippet inside, so that the images could be calibrated for measuring. Sporophytes were counted both as 1 sporophyte per gametophyte group, and 1 sporophyte per blade. Counts were 12.9 (SD: 5.32), and 59 (SD: 22.51) respectively.

Direct seed fertilization induction

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For the direct-seeding treatment, gametophytes were induced to fertilize in an SBR unit starting on January 8th, 2026. 174.05 mg WW of gametophyte material was fragmented and suspended in 1800 mL of media with Procline liquid algae food nutrient media at F/8 concentration. No GeO2 was used during the fertilization process. Wet weight was determined by filtering the gametophytes over a 0.2 micron filter over a vacuum for 15 seconds then weighing. The sex ratio was not measured rather the stock was the same one as the gametophyte spool and was assumed to be the same. The suspended gametophytes were left in the dark overnight at 10° C with light aeration and a stir speed of 200 rpm (SBR internal vortex generator). After 24 hours, PPFD was raised to 20 with a photoperiod of 16:8 L:D with white light spectrum. After another 24 hours the irradiance was raised to 40 PPFD and the aeration was increased. On January 21, 14 days after induction began, the induced gametophyte/sporophyte mix was filtered over a 50 micron nylon filter then resuspended in 100 ML of SSW in a sealed glass container.

Three, .25 ml aliquots were pipetted out of the 100ml sporophyte solution after homogenizing the mixture by hand-shaking then diluted to 1 mL with SSW. 0.5 mL of the diluted mixture was then added to a 1ml welled grid slide, where a glass slide was placed over top. Every sporophyte was counted that was at more than the 8 cell stage (Appendix A, Image 9). Gametophytes with multiple sporophytes were counted as one sporophyte. The sporophyte concentration was estimated at 2506.67 / mL (SD: 476.34). 40 photos were assessed across two of the aliquots used for numerical assessment. Sporophytes were measured under a compound microscope using Moticonnect software at 4x. Only sporophytes that were entirely on the screen were counted. They were measured from the beginning of sporophyte cells to the end (not including the rhizoid). Only the longest sporophyte was measured if multiple sporophytes per gametophyte occurred. A straight line measurement was used even if the frond was bent. If bent more than 45 degrees, it was not counted. Mean sporophyte size was 542 μm (SD 304 μm).

Direct seeding standardization

To standardize the manual deployment of direct seeded gametophytes we had to determine the wettability of the ropes used, the rate of spray from the pressure bottle, and how many pumps are needed to maintain steady pressure (thus steady rate) inside the bottle as the volume of media (incompressible) is replaced with air (compressible). To achieve the first, we took 20 cm sections of each rope type then soaked each in DI water for 1 minute. The ropes were briefly shaken of excess water then weighed, where the difference between the dry weight and wet weight equated to the absorbed water mass. The braided rope was the limiting wettability of the two ropes at 0.7 ml/cm. To determine spray rate, the nozzle was fitted with a screw behind the trigger which allowed for fixed adjustment of the target rate of spray. The spray gun (Greenthumb brand 2 L) was filled with DI water, pumped, then discharged for 5 seconds into a graduated cylinder to determine rate of discharge. Both the gametophyte sprayer and xanthan gum sprayer were calibrated to maintain approximately consistent internal pressure as liquid volume decreased during sequential applications. For each sprayer, the number of pump strokes required to achieve the desired operating pressure was recorded at several liquid volumes. For the gametophyte

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sprayer, calibration measurements were collected at 2.0, 1.8, 1.6, 1.2, and 0.6 L, requiring 10, 14, 20, 32, and 48 pump strokes, respectively. For the xanthan sprayer, measurements were collected at 1.0, 0.8, and 0.5 L, requiring 10, 12, and 15 pump strokes, respectively.

A linear regression was fitted separately for each sprayer to describe the relationship between liquid volume and the number of pump strokes required:

$$\text{pump strokes} = \beta_0 + \beta_1(\text{liquid volume})$$

The fitted relationships were then used to predict the number of pump strokes required at each subsequent liquid volume. Predicted values were rounded up to the nearest whole pump stroke to ensure that the target pressure was maintained as liquid was depleted. This generated a treatment-specific pumping schedule designed to compensate for declining liquid volume and maintain relatively consistent sprayer pressure throughout the application sequence.

Sites and infrastructure deployment

Test-line deployment began by establishing the plot perimeters using GPS coordinates (Figure 2). At Kasitsna Bay, the plot corners were NW: 59.4720° N, 151.5575° W; NE: 59.4720° N, 151.5567° W; SE: 59.4698° N, 151.5567° W; and SW: 59.4698° N, 151.5575° W, with an approximate water depth of 60 ft. At Jakolof Bay, the plot corners were NW: 59°27.638′ N, 151°31.206′ W; NE: 59°27.640′ N, 151°31.077′ W; SE: 59°27.607′ N, 151°31.076′ W; and SW: 59°27.606′ N, 151°31.205′ W, with an approximate depth of 30 ft.

Following establishment of the plot boundaries, depth soundings were taken from the leeward side of each plot. A 3/8-inch Polydac line was cut to the measured depth and connected to the anchor and retrieval buoy system. At Kasitsna Bay, 1/2-inch Polydac anchor-line length was calculated as three times the water depth, less 30 ft to account for chain length, providing approximately a 3:1 scope. At Jakolof Bay, anchors were instead positioned at approximately equal ratios relative to existing farm gear. Anchor chains, 1/2-inch lines, seine rings, and A4 buoys were assembled and deployed from the vessel. The anchor was then deployed while line was paid out to the designated plot position, with remaining line length adjusted based on water

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depth and bottom contours. Retrieval lines were secured to the vessel cleat and tensioned to complete deployment.

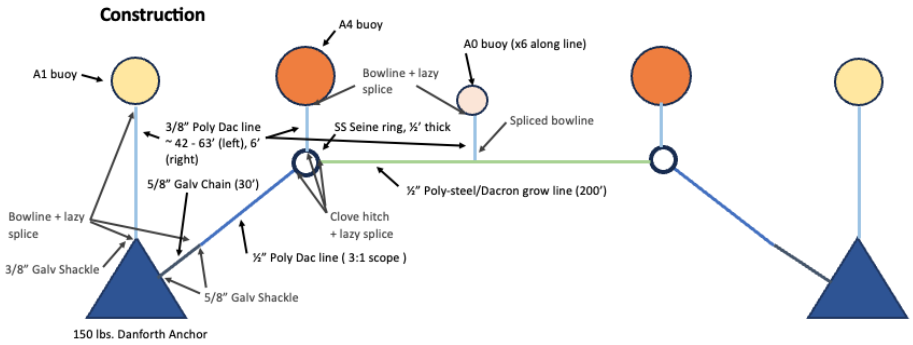


Figure 1. Test line array diagram (cross section).

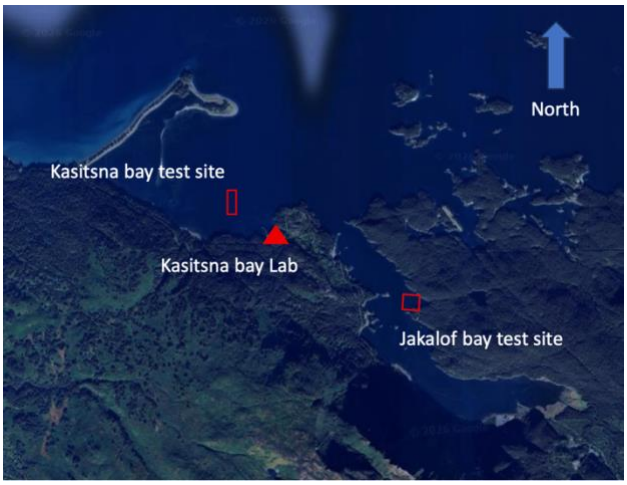


Figure 2. Map of kelp line test plots.

Data loggers

HOBO MX loggers were secured at the midpoint of each logger line. Conductivity was monitored using in situ conductivity loggers, with concurrent water samples collected at the logger depth using snorkel gear. Water samples were collected in plastic containers and allowed to equilibrate at depth for approximately 15 min before collection. The observation time was recorded, and samples were returned to the laboratory for conductivity measurements using a YSI meter. Conductivity measurements were repeated at the end of the season to assess logger

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performance. Lowell tilt meters were also deployed to monitor line movement, fixed to the test line in an inverted position.

Out planting

The seed spool was transported securely from Seward Alaska to the test locations in a cooled, moist, insulated container and kept in cool filtered seawater overnight at the Kasitsna Bay lab before deployment. Suspended juvenile sporophytes were transported in the same container in a sealed glass container. 8 meters of testing substrate was tied into the next with double fisherman's knots, and tagged with zip-tie labels every 75 cm. Twine was attached to the test substrates with an additional zip tie on each end, where 1 meter of twine was wrapped around the substrate forming a linear 0.75 m section of seeded substrate. For direct seeding, a 1" diameter PVC pip was cut in a half-pipe fashion to 75 cm in length, with markings dividing the distance in thirds. The pipe acted as a standardized application mechanism, where the sides of the sprayer nozzle rested on the pip at a regular distance from the substrate, and any overspray was collected, being absorbed into the other side of the rope-substrate. The markings aided in evenly distributing the seed whereby a 3 second metronome was used to keep pace, each second corresponding to 1/3 of the seeding distance. Test areas were sprayed on both sides, with a target application volume of 0.22 ml/cm per side. 0.5% W/V xanthan gum solution was applied to both sides for the binder treatment, using a separate spray gun with a target application of volume of 0.06 ml/cm per side, after both sides were sprayed with the seed solution. Xanthan gum application proved to be difficult due to the viscosity of the solution which formed a long bead rather than an even spray on the test substrate, thus after application the PVC pipe was rotated around the treatment section to spread the binder more evenly over the surface. The PVC pipe was washed with filtered salt water between each application. The seeded sections of treatment substrate were coiled in a figure 8 in an insulated cooler and misted with filtered salt water frequently to keep the seed moist (Appendix A, Image 6). Treatments were randomly allocated within substrate sections. Once the entire substrate was seeded, it was transported to pre-deployed anchors and set at the test sites, tensioned and positioned 2 m below the surface using mid-line buoys. The Kasitsna Bay site was seeded first, on January 21, followed by Jakolof Bay on January 22.

Table 1. Actual replicates per treatment combination.

Site	**Substrate**	**Binder treatment**	**Spray treatment**	**Twine treatment**
Jakolof	Braided	8	8	6
Jakolof	Twisted	9	9	6
Kasitsna	Braided	8	9	6
Kasitsna	Twisted	9	9	5
Total		34	35	23

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Table 2. Estimated biological metrics for seeding treatments. Gametophyte female to male ratio of 1.6:1 (fragment count) was used during fertilization. Sporophyte length average is unknown for twine treatment.

Treatment	**Gametophyte mg /m**		**Sporophytes /m seeded**		**Sporophyte length (µm)**	
	Mean	SD	Mean	SD	Mean	SD
Spray	3.36	NA	4795	911.3	542	304
Binder	3.36	NA	4795	911.3	542	304
Twine	1.79	NA	1290	532	--	--

Harvest and data collection

Cultivated sporophytes were harvested on May 12 at Kasitsna Bay, and May 13 at Jakolof Bay. Samples were harvested directly from the test lines using a 12 foot inflatable dingy, where the line was lifted over the bow and the treatment area to be sampled was placed over a rectangular funnel (Appendix A, Image 5). A 1" diameter 75 cm long PVC pipe cut into a half pipe shape and notched at 25 cm from the center on both sides was used to standardize the sporophyte samples being taken. The PVC sampler was placed over the test line treatment area before a knife was used to remove the sporophytes between the notches, where they fell into the funnel and were collected below in a 5 lbs plastic onion bag. The bagged kelp was labeled then placed into a cooler for transport to the lab.

Wet weight was determined by soaking the kelp-filled onion bags in filtered seawater then hanging them to drip-dry at ambient conditions (temperature: 11° C, RH: 59.3%) for 5 minutes (Appendix A, Image 7). The samples were then weighed using a hanging scale (Intelligent Weighing Technologies AHS-15, readability 5 g). Onion bag weight was assumed to be negligible. Sporophytes were counted in every sample by identifying stipes.

Data analysis

All statistical analyses were conducted in R. Biomass measurements were converted from grams to kilograms and standardized to a one-meter length of grow line for industry readability. Sporophyte counts were similarly standardized to counts per meter by multiplying observed counts by two. Site, substrate, treatment, and block were treated as categorical variables.

Biomass yield and sporophyte density were analyzed separately using linear mixed-effects models. For each response variable, the initial model included the fixed effects of site, substrate, and seeding treatment, as well as all two- and three-way interactions. Block was included as a random intercept nested within site to account for spatial variation among experimental blocks. The full model structure was therefore:

`Response ~ Site × Substrate × Treatment + (1 | Site/Block)`

For biomass yield, the full model was retained for inference because the experimental design was factorial and the interaction terms were used to evaluate whether treatment effects differed among sites or substrates. For sporophyte density, none of the interaction terms in the full model were significant; therefore, a reduced model containing site, substrate, and treatment as additive fixed effects and block nested within site as a random effect was used for final inference.

Model assumptions were evaluated using residual-versus-fitted-value plots, normal Q-Q plots, and histograms of model residuals. Random-effect variance components and model singularity were also examined. Sporophyte counts were initially evaluated for their distribution before model fitting; because counts were sufficiently large and residual diagnostics supported the assumptions of a Gaussian linear mixed-effects model, the response was analyzed using the same mixed-effects framework rather than a generalized count model.

When significant fixed effects were detected, estimated marginal means were used to estimate treatment and substrate means while accounting for the other factors in the model. Pairwise comparisons were conducted using Tukey-adjusted comparisons to control for multiple comparisons. Biomass comparisons among treatments were evaluated within each site × substrate combination, while substrate comparisons were evaluated within each site × treatment combination. Statistical significance was assessed at $\alpha = 0.05$.

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Results

Estimated biological efficiency of mg of gametophyte biomass and sporophytes per meter indicate higher yields for twine seeding than either of the direct seeding treatments. Despite having ~3.72 times the number of sporophytes / m during out planting, the spray treatment resulted in 8.3 times lower, while the binder treatment resulted in 5.16 times lower average sporophyte density at harvest compared to twine seeding.

Table 3. Input – output metrics of seeded material to sporophyte density at harvest. Harvest data was pooled between locations.

Treatment	Mean Observed sporophytes retained/m	Gametophyte biomass (mg) / seeded sporophyte	Retained sporophytes / mg gametophyte	Retention (%)
Spray	192	0.000701	57.1	4.00
Binder	308	0.000701	91.7	6.43
Twine	428	0.001388	239.1	33.18

Table 4. Mean, standard deviation, minimum and maximum values for sporophyte yield at harvest by site and seeding method treatment combination.

Site	Treatment	Yield n	Yield mean (kg/m)	Yield SD	Yield min (kg/m)	Yield max (kg/m)
Jakolof	Binder	17	3.38	0.86	1.90	5.49
Jakolof	Spray	17	2.63	1.00	0.93	4.47
Jakolof	Twine	12	6.38	1.06	4.89	8.69
Kasitsna	Binder	17	5.55	1.21	4.20	8.47
Kasitsna	Spray	18	5.15	0.90	3.93	6.84
Kasitsna	Twine	11	8.02	0.72	7.07	9.50

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Table 5. Mean, standard deviation, minimum and maximum values for sporophyte density at harvest by site and seeding method treatment combination.

Site	Treatment	Density n	Density mean (sporophytes/m)	Density SD	Density min (sporophytes/m)	Density max (sporophytes/m)
Jakolof	Binder	17	285.29	97.18	124	452
Jakolof	Spray	17	167.53	89.43	38	300
Jakolof	Twine	12	422.33	72.72	310	540
Kasitsna	Binder	17	324.47	69.35	220	458
Kasitsna	Spray	18	218.11	46.16	128	304
Kasitsna	Twine	11	427.09	112.75	248	614

Biomass yield differed significantly among sites and seeding treatments. Yield was significantly greater at Kasitsna than Jakalof ($F_{1,80} = 106.20$, $p < 0.001$) and differed significantly among seeding treatments ($F_{2,80} = 87.54$, $p < 0.001$). Model-estimated biomass averaged across sites and substrates was 3.2 kg/m for Spray, 4.7 kg/m for Binder, and 7.3 kg/m for Twine. Tukey-adjusted pairwise comparisons showed that Twine produced significantly greater biomass than both Binder and Spray (both $p < 0.001$), whereas Binder and Spray did not differ significantly ($p = 0.574$).

The overall effect of substrate was not statistically significant ($F_{1,80} = 2.96$, $p = 0.089$); however, estimated biomass was generally greater on Braided than Twisted substrate. The largest substrate difference occurred for the Spray treatment at Jakalof, where estimated yield was 3.09 kg/m on Braided substrate compared with 2.21 kg/m on Twisted substrate, representing a difference of approximately 0.89 kg/m or 40%. This contrast approached, but did not reach, conventional statistical significance after Tukey adjustment ($p = 0.063$). Substrate differences were smaller and less consistent for the other treatment × site combinations, and no site × substrate, site × treatment, substrate × treatment, or three-way interaction was significant (all $p > 0.20$).

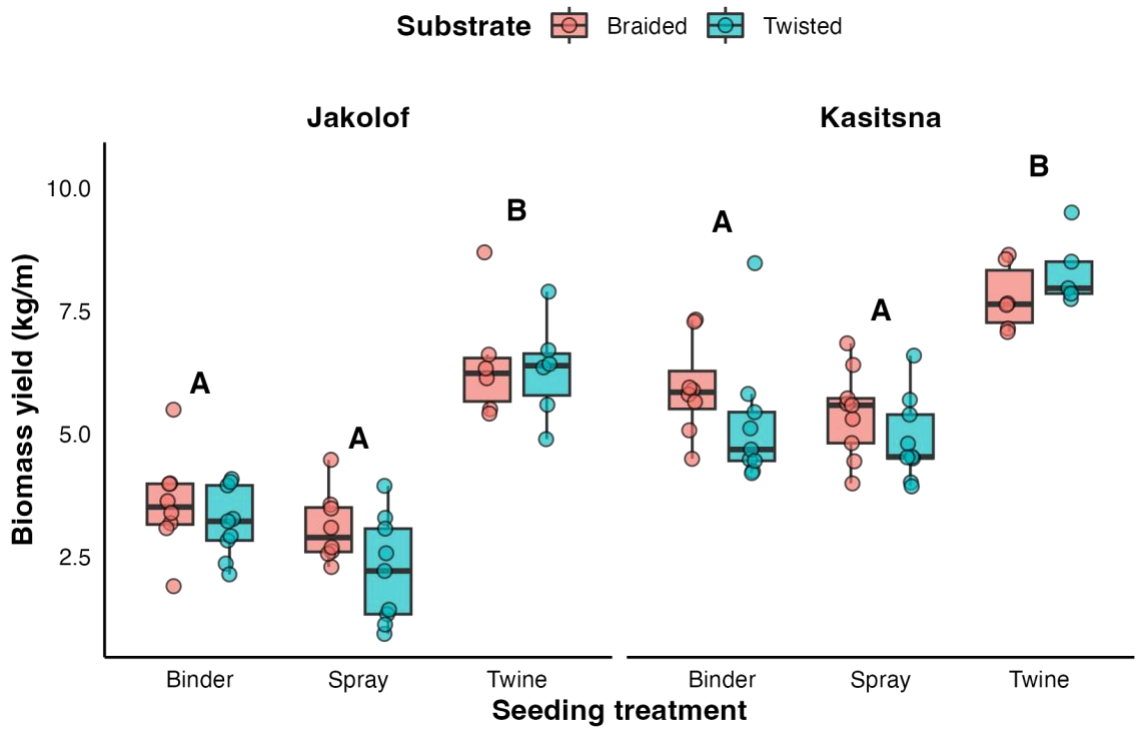


Figure 3. Biomass yield of kelp (*Saccharina latissima*) on two test lines at sites in Kachemak Bay Alaska (Jakolof and Kasitsna Bays), across seeding method (juvenile sporophyte spray plus xanthan gum binder [Binder], juvenile sporophyte spray [Spray], and gametophyte inoculated twine-spool [Twine], and between substrate types (PES 12 mm ∅ braided rope [Braided] and Poly Dacron ½" twisted rope [Twisted]). Kelp was seeded on January 21 and 22, and harvested on May 12 and 15, 2026.

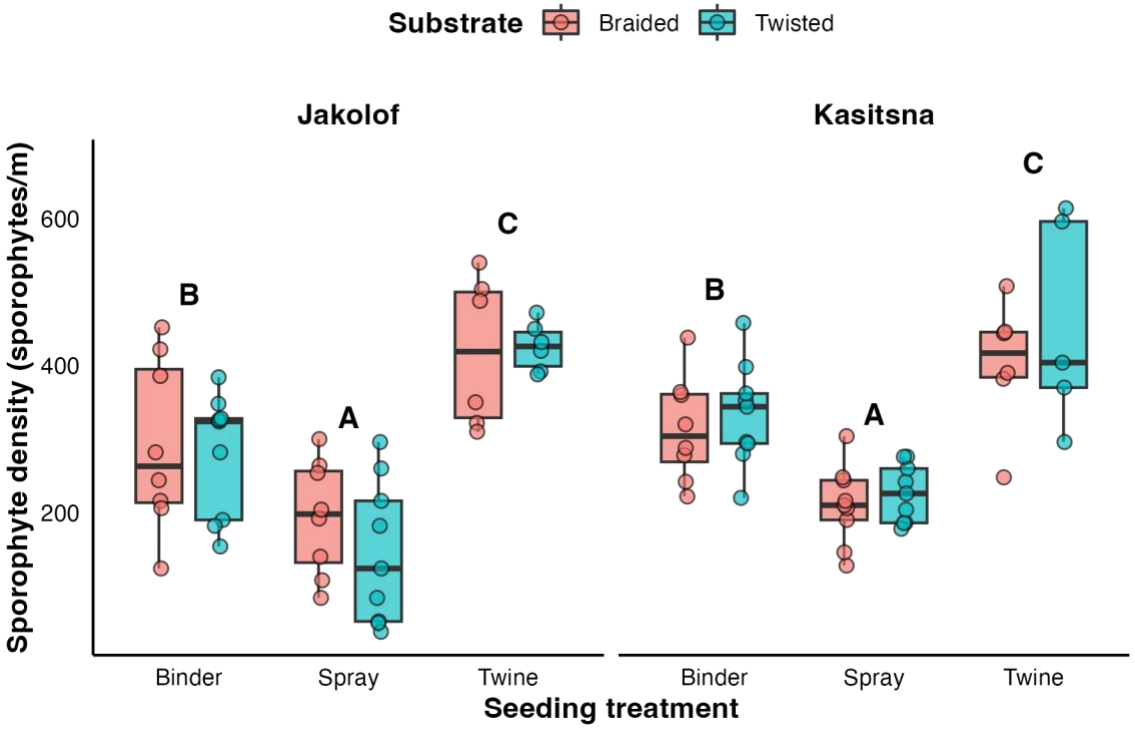


Figure 4. Sporophyte density of kelp (*Saccharina latissima*) on two test lines at sites in Kachemak Bay Alaska (Jakolof and Kasitsna Bays), across seeding method (juvenile sporophyte spray plus xanthan gum binder [Binder], juvenile sporophyte spray [Spray], and gametophyte inoculated twine-spool [Twine], and between substrate types (PES 12 mm ∅ braided rope [Braided] and Poly Dacron ½" twisted rope [Twisted]). Kelp was seeded on January 21 and 22, and harvested on May 12 and 15, 2026.

Sporophyte density differed significantly among seeding treatments ($F_{2,83.0} = 43.76$, $p < 0.001$), while neither site nor substrate had a significant effect on sporophyte density (site: $p = 0.394$; substrate: $p = 0.716$). Model-estimated sporophyte density averaged across sites and substrates was 192 sporophytes/m for Spray, 303 sporophytes/m for Binder, and 426 sporophytes/m for Twine. Tukey-adjusted pairwise comparisons showed that all three treatments differed significantly from one another (all $p < 0.001$), with Twine producing the greatest sporophyte density, followed by Binder and Spray. The site × block random effect accounted for approximately 25% of the combined random and residual variance, indicating meaningful spatial variability among blocks.

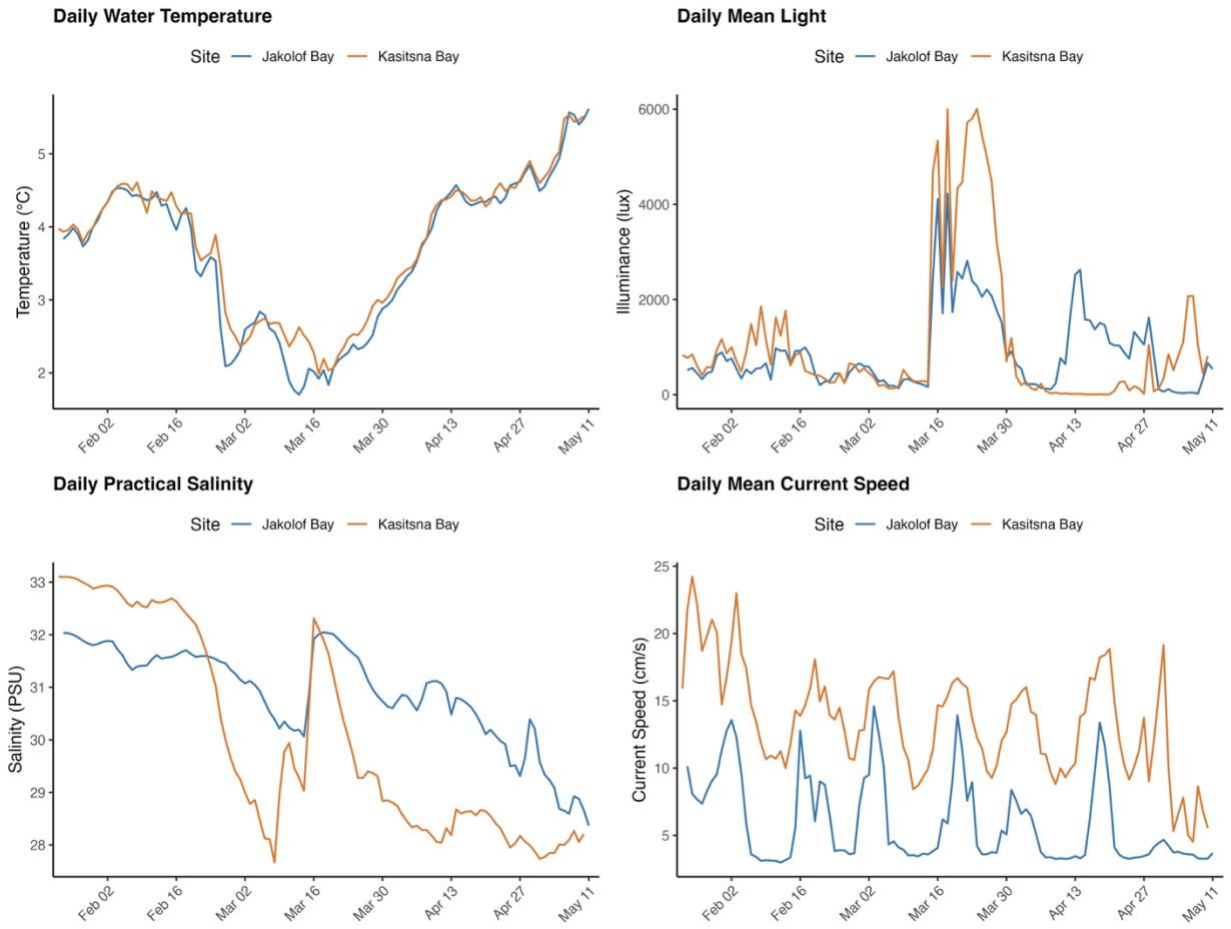


Figure 5. Seawater temperature, irradiance, salinity and current speed averaged daily between two test sites, Kasitsna Bay and Jakalof Bay between January 21 and 22 and May 11 and 12 for Kasitsna and Jakolof Bays, respectively. Plotted conditions represent relative comparisons and approximate values due to logger limitations (eg. Lowell Tilt Meter TCM-1 fixed to kelp line with known accelerometer sensitivities, irradiance spiking after biofouling cleaning).

Environmental conditions varied between sites, being most pronounced in the current speed with Kasitsna Bay appearing to have stronger currents. Salinity decreased at both sites, with Kasitsna Bay starting at a higher salinity and ending at a lower value compared to Jakolof Bay. Irradiance showed an erratic pattern at both sites, while seawater temperature was very similar between sites.

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Discussion

With the potential for gametophyte hatchery systems to provide brood biomass to commercial kelp farmers in Alaska in the near future, understanding the impacts of seeding method and the type of substrate farmers seed on is crucially important before adoption of these systems. All seeding methods tested in this study produced harvest yield averages within a comparable range of previously observed yields, with our highest observed average occurring in the Kasitsna bay twine seeded treatment at 8.02 kg/m which was just shy of the 8.2 kg/m harvest reported in a study from Kodiak Island looking at farmed *S. latissima* growth (Stekoll et al., 2025). Variability of yields were also notably consistent, with most standard deviations falling near ± 1 kg/m. These values should be taken in the context of single line arrays where multi-line density dependent growth restraints are absent, thus these numbers should be considered maximum values when comparing across a commercial size farm.

Efficiency of retention and sporophyte density

Assessing the biomass efficiency of seeding methods provides insights into optimization of hatchery resources. While not statistically analyzed, comparing estimated input means to observed average sporophyte densities we see possible biomass utilization differences between seeding methods where twine seeding with gametophytes resulted in almost twice the amount of gametophyte biomass used to produce the same number of sporophytes before out planting. This indicates an observed difference in fertilization efficiency in the induced gametophyte biomass between treatments, though most likely not as a product of the method itself since the experiment was not designed to test this instead using suggested industry best known practices for each method (Ebbing et al., 2021 and GreenWave protocols for painting gametophyte biomass onto spools). These results do suggest the possibility that seeding at ~2.55 mg WW/ mL is higher than the sporophyte capacity for the twine surface area, though it does not appear to be detrimental to fertilization as can happen in high density cultures (Ebbing et al., 2021).

The observed average sporophyte retention percents across seeding treatments saw twine seeding to maintain roughly a third of the sporophyte density between seeding and harvest, compared with just 4.00% and 6.43% for the spray and binder treatments, respectively. Indeed, the binder treatment showed significantly higher sporophyte densities than the spray treatment. These numbers reflect a discrepancy in the process of sporophyte thinning, where sporophyte loss in twine seeding is the result of competitive thinning based on variable growth, while direct seeded sporophytes which have not formed strong bonds to the substrate are susceptible to detachment (Chemello et al., 2025; Umanzor et al., 2020; Kerrison et al., 2019). Our results clearly support the effectiveness of the binder treatment in increasing sporophyte retention, a result that contradicts laboratory experiments but remains in popular support regardless (Umanzor et al., 2020; Visch et al., 2023; Tremblay-Gratton et al., 2023; Forbord et al., 2023). The rate of detachment in the direct seeding trials was lower than has been described in laboratory test systems (70 – 80% loss), but was closer to the 95% loss rate suggested in personal correspondence with WHOI advisors (Umanzor et al., 2020). Given these loss rates, it would

take ~5.16 times more sporophytes during out planting with binder mediated direct seeding to achieve the same average sporophyte density as twine, assuming detachment is not density dependent. This is an important consideration when calculating resource effort between seeding methods as it pertains to the cost of seed to farmers.

Yield

As a major revenue forcing variable, Biomass of yield of fresh kelp - a primary metric for determining farm performance – was found to be consistently highest in the twine seeding treatment, a result generally consistent with previous findings for annual kelp (Forbord et al., 2020; Boderskov et al., 2021). Contrary to our results, Boderskov et al., 2021 did observe an interaction with location where twine and direct sporophyte seeding achieved comparable results at their most exposed site. Given their much smaller observed yields across all treatments and sites, it appears growth was generally constrained and that the exposed site was the only site where the direct seeding succeeded in any rate, possibly as a result of the binder being washed away while the agent persisted at other sites, smothering the propagules (Boderskov et al., 2021). Site dependent growth among kelps is a widely described phenomenon, one that is well represented in our results where site had large effect on yield with proportional differences consistent across seeding methods between sites (Bruhn et al., 2016; Peteiro et al., 2013; Miller et al., 2011). The higher average yields seen at the Kasitsna Bay test site is likely the result of it being a more exposed site with faster currents and potentially more light availability, highlighting the importance of site selection on farm performance (Peteiro et al., 2013; Miller et al., 2011). In fact, when comparing yields between farms, the twine treatment in Jakolof bay performed very similarly in yield to the direct-seeded treatments at Kasitsna Bay. With average yields of 5.55 kg/m and 5.15 kg/m for the binder and spray treatments at Kasitsna Bay respectively, the performance of direct seeding – even if not the highest – are still considered good yields by contemporary standards, as the organization GreenWave recorded an average yield of 1.38 kg/m on Alaska kelp farms across three seasons (GreenWave, 2026). While sporophyte density differed significantly between the binder and spray treatments, yield did not. While sporophyte density and yield have been loosely positively correlated, the de-coupling of these two variables supports the hypothesis that the so called “lag phase” in direct seeded kelp is primarily the product of smaller sized sporophytes at out planting in the face of compounding growth, i.e. the larger they start in the ocean the larger they end up (Forbord et al., 2020; Stekoll et al., 2025). Additionally, initial resource allocation towards developing holdfasts may also delay growth in photosynthetic tissues, adding to the discrepancy in juvenile disadvantage when direct seeding. secondary biological impact of direct seeded sporophytes having resources allocated to holdfast development. Lastly, the comparable yields between direct-seeded treatments despite differences in sporophyte density indicate the seeding density used in this study is higher than is necessary, and no treatments experienced density limited yield.

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Substrate

Despite previous research suggesting the advantage of twisted ropes for direct seeding and personal correspondence suggesting braided ropes being superior (the braided rope used in this study made by the Norwegian Company Sirputis is marketed as a specially developed direct seeding rope), we found no significant difference in either yield or sporophyte density between the tested substrates (Kerrison et al., 2019). It may be that by allowing the seeded ropes to remain in a moist cooler for up to two hours before seeding, any mechanical adhesion advantageous if present would be superseded by the stronger primary polysaccharide gel adhesion phase (Cunningham et al., 2025). If so substrate may become more important if out planting the seeded line into the ocean more quickly. Observationally, the braided rope had non-significantly higher average yields in all direct seeding treatments at both sites indicating the possibility of a marginal effect that could not be described with our current sample size.

Conclusions

While gametophyte-inculcated twine seeding proved to produce the highest yield irrespective of site, direct seeding reliably produced fair yields, especially at the Kasitsna Bay site, and remains a potentially valuable method in certain situations, with room for optimization. Direct seeding has the potential to reduce seeding costs through efficient automation and use of hatchery resources, lending itself well to scaled farming practices. It's conceivable based on these findings that larger scale farms in more exposed waters could produce comparable yields with direct seeding methods to farms seeding with twine in protected locations. Furthermore, seeding perennial species can allow cropped harvests from the same sporophytes over multiple years, where averaged over several seasons yields may not be considerably different between seeding methods. The use of a binder, in this case using xanthan gum, is advantageous for direct seeding which combined with optimized target sporophyte densities would increase the efficiency of seed biomass. Reducing detachment also reduces escapement into wild systems, a concern for genetic maintenance in wild populations. While the type of rope (twisted vs braided) did not impact yield in this study, further research is needed, particularly if settlement time is reduced before out planting. Regardless of optimum substrate, farmers should not be afraid to test direct seeding on kelp lines already in use. Given the support for size-at-outplant forcing of differences on yield between seeding methods as opposed to detachment driven density limitation, it would be advantageous to test size classes of juvenile sporophytes to see how large sporophytes can be effectively seeded through spray and binder methods. This would be limited by increased drag forces, possibly decreased primary attachment strength as the ratio of female gametophyte rhizoid to sporophyte blade decreases, and the practicality of spraying where large sporophytes can clog spraying vessels.

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Additional work/future directions

WHOI travel knowledge exchange

An inter-lab exchange trip was conducted between March 13th and 17th following attendance and dissemination of the project at the 2026 Mariculture Conference of Alaska in the form of a lighting talk (5 minute presentation). Scott Lindell and Daniel Gossard joined Cameron Jardell in Seward to tour the Alutiiq Pride Marine Institute Facility and gave feedback on lab operations and set-up. Primary recommendations were to secure a lab grade laminar flow hood for sterility, as contamination was an issue in the lab, and to increase the number of clonal isolates under lab incubation. The latter being important for long-term contamination free cultures, better control of reproduction in experiments, and genetic biobanking. The trio then traveled to the Kasitsna Bay lab to collect sori for spore release and later gametophyte isolations, as well as to check on the test lines of the seeding experiment. They spent 3 days at the lab, using small vessels to access the test lines. The gear was checked for any structural damage, lines were visually assessed for kelp growth, and loggers were cleared of biofouling. Intertidal hand-and-snorkel collections were conducted in Jakolof bay, where fertility was notable low – typical for March – with very few ripe sori. However, 10 fertile *S. latissima* and 10 fertile *A. marginata* sorus pieces were collected under ADF&G permit P-26-016 and released following the methods of Inka Bartsch for field collection of gametophytes. Multiple releases were conducted from each parent sporophyte, with releases being kept at both APMI and WHOI for bio-bank redundancy. Ultimately, the knowledge exchange travel was an important part of this project where cutting edge phycologists were able to tour a partnering, applied mariculture facility to learn about capabilities for future projects, and dissiminate suggestions for lab and protocol upgrades from a well of experience. The trip capped off a reciprocal visit form APMI biologists to WHOI in November of 2024, facilitating greater inter-organizational collaboration.

Directions of the Gametophyte lab

In April of 2026 the Alaska Department of Fish and Game approved amendment #4 of the APMI Basic Hatchery Management Plan to allow commercial hatchery use of propagated, multi-annual gametophytes. APMI is not the only institution to be granted these privileges, but being one of the first the organization is currently in a position to expand the gametophyte program towards direct farmer support for commercial gain. New stocks are being developed during the writing of this report using clonal isolate techniques for improved contamination control and reproductive efficiency. Combined with additional funding from the Southeast Conference for equipment related to gametophyte production and seeding, the goals of the gametophyte lab are to build towards scaled production for commercial seed supply in the coming years. The ADF&G approved hatchery protocols represent a major step for commercial kelp seed supply in the state of Alaska, as regulatory hurdles used to pose a question mark for continued investment in gametophyte seeding. The biological and practical lessons learned during the execution of this project directly moved this process forward, answering a demand from kelp farmers for gametophyte based seeding.

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Attempted fragment experiment

As stated in the *Challenges* section of this report, the fragment experiment was not completed due to complications in practical study design related to gametophyte fragment tracking. The experiment was attempted three times without success. The experiment fell under objective 2, "assess further gametophyte culture optimization based on findings of the JIP project and validate effectiveness in seaweed bioreactors. The question posed was "how does gametophyte size class affect growth"? The goal was to look at individual growth rates in terms photograph based metrics, i.e. linear growth and % area change based on pixels. Originally, size class separation was attempted using systematic filtration between 100, 50 and 5 micron nylon filters in sequence using positive pressure filtration with a syringe after fragmenting the gametophytes. This resulted in in uneven size and biomass distributions, where smaller gametophytes were caught by the larger gametophytes on the first filter level. It was then decided to remove the filtering process and inoculate a 24 well plate with ground male or ground female gametophytes, being randomly allocated with 3 wells of each per 6 well row. The gametophytes were left for 48 hours at 10^{o} C with low light (~11 PPFD, 12:12 L:D) to let attach to the substrate. A TC coated sterile well plate was used for the substrate to enhance adhesion of the gametophytes. A sharpie was used to mark the approximate center of each well to use as a reference point for the photos, and to standardize photo location between wells. After the 48 period, images were taken using an inverted microscope (Am-Scope MU-1000-HS) at 4x, one per well. The irradiance was brought up to ~21 PPFD, and the well plate was situated on an orbital vertexing tray set at 60 rpms to stimulate growth through water flow. The gametophytes were meant to be left for 21 days before another photo of each well was to be taken and the gametophyte lengths compared using Image J software. However, on day 8 the vortexer was found to have shorted, increasing speed to >200 rpm. The intense water motion moved the position of the gametophytes so they could not tracked (Appendix A, Image 11). The experiment was attempted a second time without water motion stimulation, however even then the gametophytes could not be identified from the original images. In part this was due to bacterial contamination that made focusing on the gametophytes difficult, however they may have shifted from their original locations due to transfer from the incubator to the microscope and back. The sharpie-ink prints could be easily distinguished to find the exact photographic coverage from the first round of photographs. A fragment-frequency experiment was not attempted due to a lack of biomass.

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Costs

All funds were spent.

Appendix A – Photos



Image 1. Twisted kelp rope on left, braided kelp rope on right.



Image 2. Gametophyte-seeded twine spool used in direct seeding experiment.



Image 3. Spool nursery for direct seeding experiment.



Image 4. Syiera Clark preparing for farm line deployment in Kachemak Bay, Alaska.



Image 5. Harvesting of kelp samples. The funnel system can be seen for ease of collection.



Image 6. Syiera Clark seeding experimental line. Spray bottles can be seen on table.



Image 7. Weighing of kelp biomass.

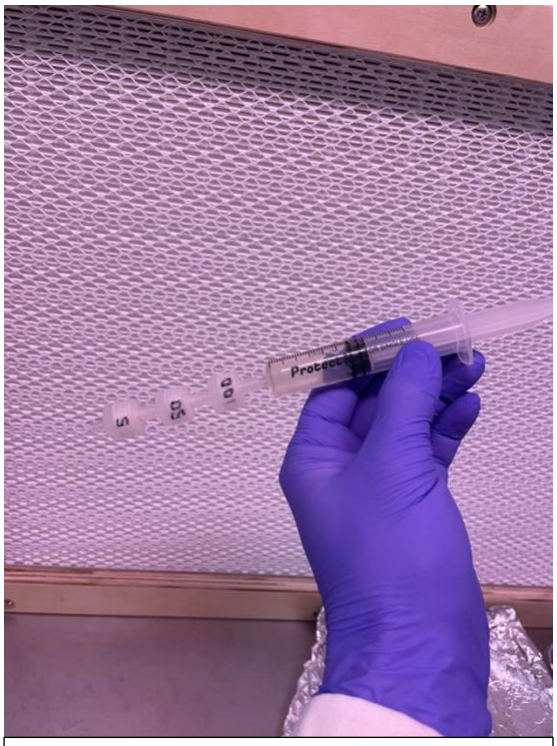


Image 8. Attempted step-wise gametophyte size isolation with filters.

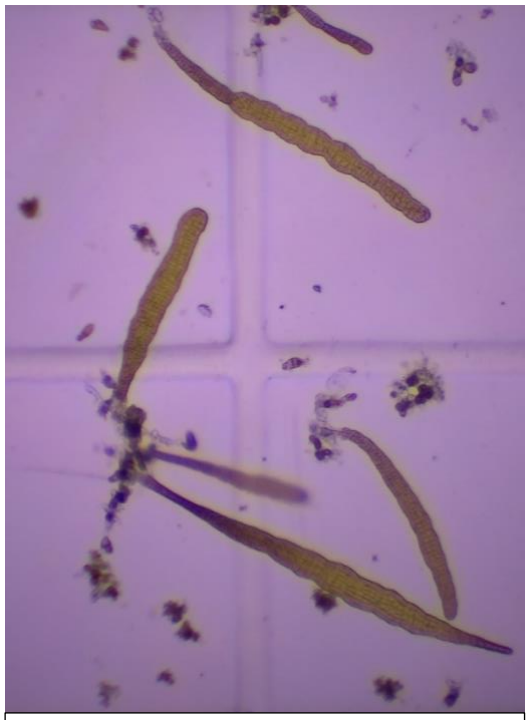


Image 9. Juvenile sporophytes used in direct-seeding treatments.



Image 10. Ectocarpus contamination in gametophyte culture.



Image 11. Surface buoys indicating experimental kelp line in Kasitsna Bay.

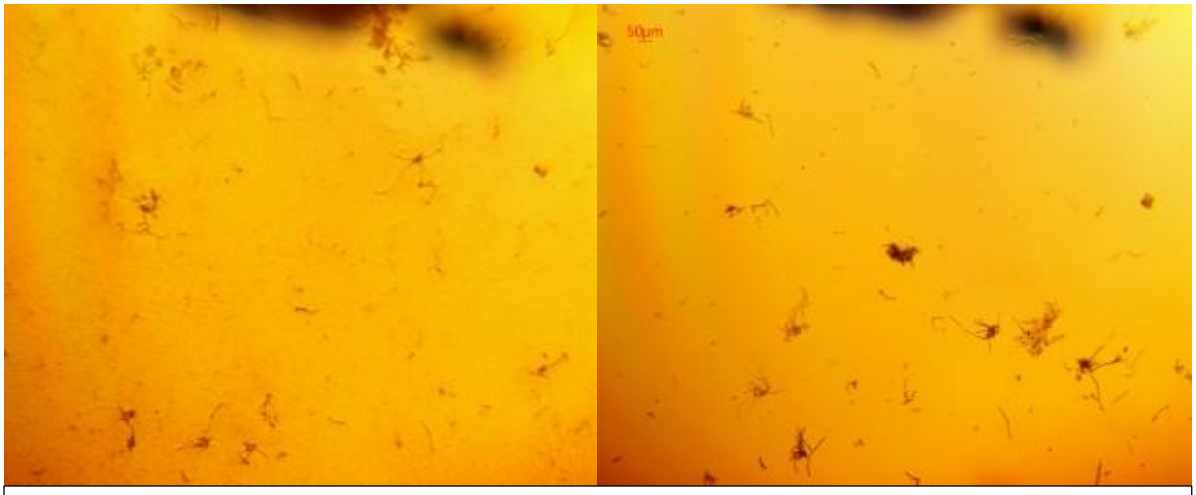


Image 12. Attempt at fragment experiment, before and after growth period. Black smudge is sharpie used for substrate mapping.



Image 13. Juvenile sporophytes on seed-twine.