

Exploration of *Nereocystis luetkeana* restoration in the Salish Sea: analysis of spore and gametophyte settled substrates to inform best techniques

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1.0 Abstract

Canopy-forming kelp forests are vital to the marine environment, providing habitat and essential ecosystem services. However, *Nereocystis luetkeana* (bull kelp), the primary canopy kelp in the Salish Sea, has experienced significant declines in recent decades. Restoration efforts are urgently needed, but in this developing field, best practices are still being refined. In collaboration with Vital Kelp, a restoration company on the Sunshine Coast, this project evaluated and compared cultivation and out planting techniques. Two seeding methods (spore-settled and fragmented gametophyte sprayed) were applied to four substrate types: twine, clay tiles, green gravel, and polypropylene grid mesh. Successful settlement did not occur on the green gravel or clay tiles for either method. Kelp successfully settled on polypropylene twine and grid mesh, which were subsequently out planted at Ole's Cove for further monitoring. Sporophyte recruitment was observed on the twine substrate for both seeding methods, but only the spore-settled grid mesh. This suggests that twine may be the most versatile and best substrate to work with. Additionally, performance of the seeding method may be influenced by the substrate type, with the gametophyte twine having a higher density and only the spore mesh exhibiting recruitment. However, none of these suggestions could be statistically concluded due to the limited dataset. Future kelp restoration efforts should prioritize exploring gametophyte-based approaches, as their potential for genetic preservation, selective breeding, and large-scale restoration, outweighs the benefits of traditional spore seeding methods.

2.0 Introduction

Kelp ecosystems, specifically those composed of the canopy forming kelp species *Nereocystis luetkeana* (bull kelp), are foundational habitat in the marine environment. Bull kelp has experienced a rapid decline in parts of the Salish Sea in recent decades (Berry et al., 2021; Hollarsmith et al., 2022). According to British Admiralty Nautical Charts from 1858 to 1956, bull kelp used to line more of the coasts of the inner Salish Sea than they do presently (Mora-Soto et al., 2024). Rising sea surface temperatures are a major driver of these declines (Karm, 2023). Innovative restoration efforts are needed to preserve the remaining kelp populations in the warmer parts of the Salish Sea. Kelp restoration is a novel discipline, and the best techniques have not been fully refined. Kelp restoration efforts on the Sunshine Coast are very limited and there is an absence of literature on the subject. Working to fill this gap in knowledge is Vital Kelp, a local research and restoration company founded by my mentor Lee-Ann Ennis. I have been helping Lee-Ann and learning from her for the past year.

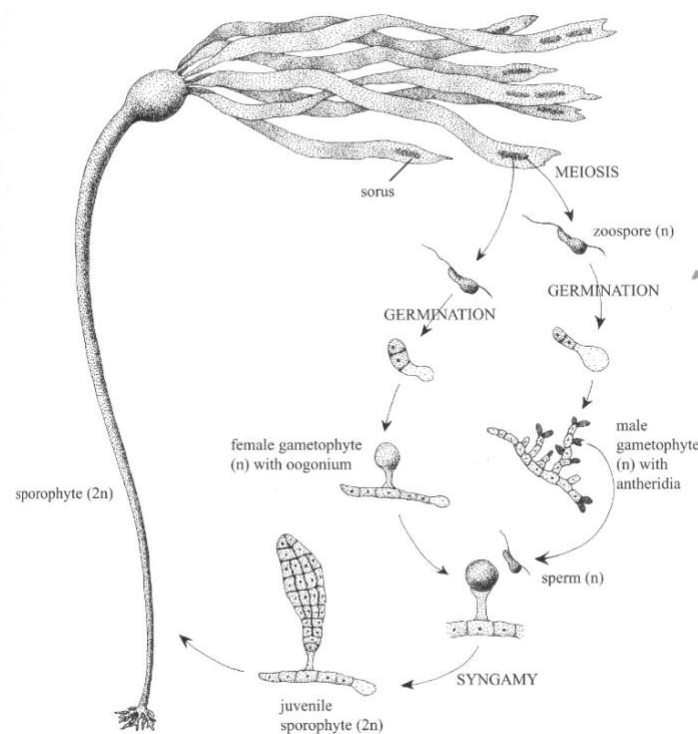


Figure 1. Diagram of bull kelp alternation of generations lifecycle. Illustration by Lisa (Scharf) Spitler (Mondragon & Mondragon, 2003).

To conduct kelp restoration, one must understand the kelp lifecycle. A diagram in figure 1 shows the different lifecycle stages. This project will be focused on cultivation using both spores and gametophytes. Gametophytes are in a vegetative state and can be easily stored under the correct light conditions (Lüning & Neushul, 1978) and propagated through fragmentation (Hadley et al., n.d). Once fragmented the gametophytes can be sprayed and applied to different substrates (Greenwave, n.d.; Visch et al., 2023). Historically, the more widely used and tested method in kelp aquaculture and restoration is the use of spores to inoculate substrate (Flavin et al., 2013). There are limited studies that make comparisons between methods of seeding with spores vs. gametophytes (Boderskov et al., 2021; Forbord et al., 2020) and none can be found for this region or specifically bull kelp. Refining the best methods for cultivation and out-planting of bull kelp will be crucial for restoration efforts within the Salish Sea.

The purpose of this project is to participate in every step of the kelp restoration process including planning, collection, cultivation, out-planting, and monitoring. As part of this effort, I have designed a project that will be specifically looking at the use of spore vs fragmented gametophyte seeding methods over different substrates. My role will be to analyze the methods and outcomes to inform best kelp restoration techniques. Based on the findings from this project and referring to other sources in the literature, I will make recommendations for the best seeding methods and substrates to use in future kelp restoration efforts.

3.0 Goals and Objectives

Goal 1: To gain experience in each step of the kelp restoration process including: planning, collection, cultivation, out planting, and monitoring.

Objectives:

- #1 Help fill out the required permits and paperwork to conduct restoration activities.
- #2 Help collect sori patches, process them, and induce spore release for inoculating the substrate.
- #3 Participate in some of the lab work that is required for growing kelp in the nursery (*e.g.* water changes, dealing with contamination, monitoring development of the microscopic kelp)
- #4 Help deploy seeded substrate from the surface in a boat at Ole's cove.
- #5 Try different monitoring techniques that can be done from the surface like using a go pro on a stick, analyzing drone images, etc.

Goal 2: Through analysis of the methods, findings, and literature, compare the use of spore vs. fragmented gametophyte settled substrate types to inform recommendations for best methods.

Objectives:

- #1 Create an experimental design that controls for factors to allow the comparison of spore vs. fragmented gametophyte settled substrates.
- #2 Observe methods both *in situ* (in the field) and *ex situ* (in the nursery).
- #3 Within this experiment test different substrates as well to see which do best.
- #4 Have divers actively monitor at least three times, collecting video and photos that have quantitative data.
- #5 Outplant enough replicates and use an experimental design that allows analysis of the statistical significance.
- #6 Analyze the data and run statistical tests to see which methods were most successful.
- #7 Find studies that have also investigated methods of spore and fragmented gametophyte settled substrates, compiling their findings to inform best techniques.
- #8 In general compare the pros and cons of the seeding methods and substrates

4.0 Restoration Site

Ole's cove is in Malaspina straight (central Salish Sea) just north of Secret Cove on the Sunshine Coast, B.C, in the traditional territory of the shíshálh Nation (Latitude: 49° 32' 37.51" N, Longitude: 123° 59' 04.64" W). Ole's cove meets many criteria as a suitable kelp restoration site including good current, large fetch, and wave exposure that brings in cold, nutrient rich upwelled water that aids in kelp growth (Weigel et al., 2023). This is a shallow reefed site (< 7m at chart Datum), with a mixed bottom composition of soft sediments, cobble, and shell hash, with bigger rocky boulders and bedrock, distributed throughout the cove (Fig. 2). This contributes to the site having a low grazer density, as urchins are observed to avoid soft sandy sediments (Kawamata et al., 2011). Berjie Shoals a reef close by historically hosted bull kelp, which suggests this site may also have favourable conditions (Fig. 3). Local divers, actively use this site and have observed many marine invertebrates and fish that would also benefit from the protection of a restored kelp

forest. Additionally, this site can be accessed from the shore, making it possible to visit safely during the stormy winter months when the kelp needs to be out planted.



Figure 2. Aerial drone photograph of Ole's Cove taken on February 6th, 2024, by Geoff Grognet. The bathymetry that can be seen through the clear water is the reef that extends approximately 30m out from shore. This is where the kelp out planting will take place.



Figure 3. Map of the central Salish Sea and Sunshine Coast. Ole's cove, the proposed restoration site is marked by a star. Pink polygons show historical kelp presence from nautical charts. Map provided by L. Ennis, created in March 2024 using QGIS software.

5.0 Methods

5.1 Collection of sorus and inducing of spore release

Sori was collected from a wild population in the Skookumchuck rapids, Egmont B.C. on October 17th, 2024. Blades with ripe sorus were handpicked from 30 plus individuals to maintain genetic diversity. Sori was prepared for induced spore release (Fig. 4), for full methods see Appendix B. To calculate the spore concentration a sample was taken and counted in a hemocytometer following methods from Flavin et al., (2013) (Appendix C).

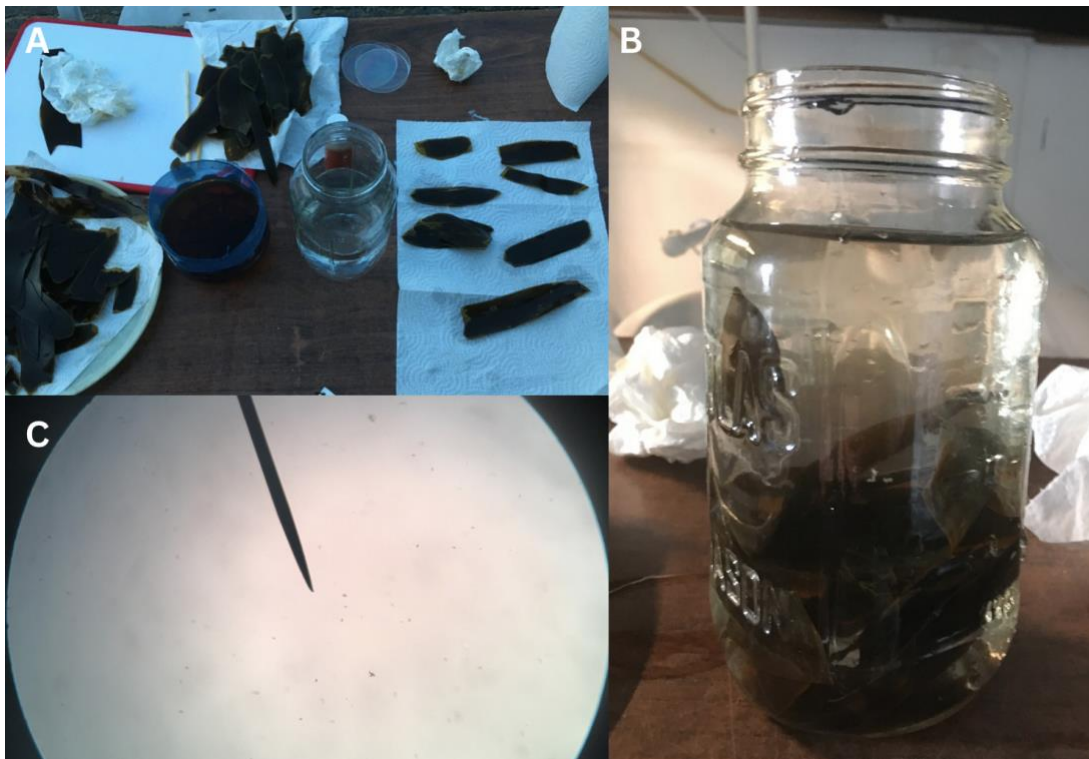


Figure 4. (A) Shows the sori processing station to properly clean them before inducing spore release. (B) Squares of sori material in sterilized seawater, preparing to release spores. (C) Spore sample under 100X magnification to check for actively swimming spores. Photos taken by Amelia Gray.

5.2 Cultivation in the Nursery

In the nursery four different substrates for each seeding method were used. These include (1) polypropylene twine, (2) clay tiles, (3) pea sized (~1 -2cm) gravel, and (4) polypropylene gridded mesh. The dimensions and quantities of each of these substrates is shown in Table 1.

Table 1. Summary of substrate type and quantities of each method and substrate.

Substrate type	Quantities
Seeded “green gravel” Pea sized gravel (1-2cm)	~ 2200 spore settled ~ 2200 fragmented gametophytes settled
Seeded tiles 6 x 12cm textures clay tiles	20 spore settled 20 fragmented gametophytes settled
Seeded line 2 mm twisted polypropylene twine wrapped on PVC tubes for ease of even settling and deployment.	40m line settled with spores. 40m line settled with fragmented gametophytes.
Seeded grid mesh 1cm x 1cm gridded polypropylene mesh	3000cm ² spore settled 3000cm ² fragmented gametophytes settled

Spore Settled Substrate

To seed the substrate, settling buckets had approximately 12 L of filtered sea water (enough to cover the substrate) and approximately 191.5 mL of spore solution was added to get a desired stocking density of 7,500 spores/mL (Fig 5. A) (calculation in Appendix C). Settling buckets were covered with a dark mesh screen for 24 hours to allow spores to settle and adhere. Following this, substrate was moved to the tanks and cultivated under specific conditions until ready for out planting (Fig. 5 B/C) (Appendix B for details).

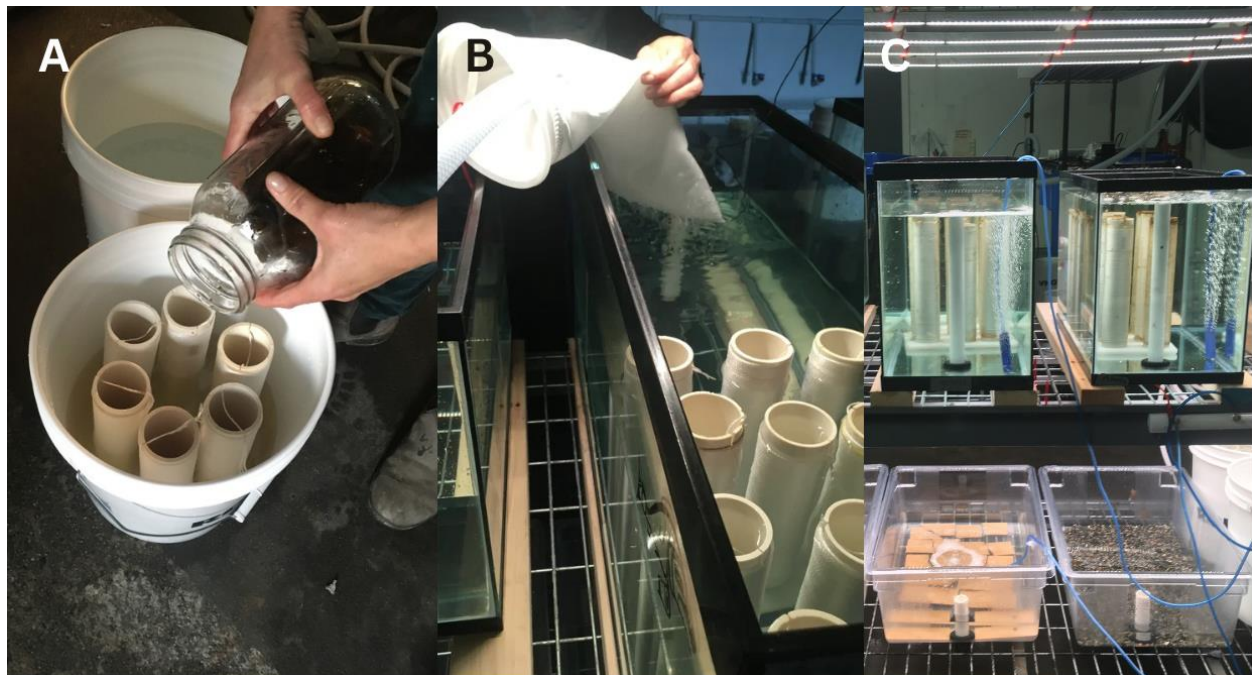


Figure 5. (A) Inoculating spools in spore settling buckets. (B) Filtering water through the last 1um filter before slowly filling tank with the newly settled spools. (C) Set up in nursery with the other spore settled substrates and air stones in tanks. Photos taken by Amelia Gray.

Fragmented Gametophyte Substrate

For the fragmented gametophyte technique, leftover gametophytes stuck to the sides of settling buckets were used. They were scraped off into 200mL of seawater to create a concentrated solution. A sterilized hand blender was used to chop the gametophytes for 10 seconds, until they were all a uniform size (Fig. 6 A/B). The fragmented solution was diluted to the same concentration as the spore solution (calculations in Appendix D). The solution was sprayed evenly onto all the substrate types (Fig. 6 C/D), which had been wetted with seawater prior. They were left sitting in the air for 45min-1hr to allow the gametophytes to properly stick before tanks were filled.

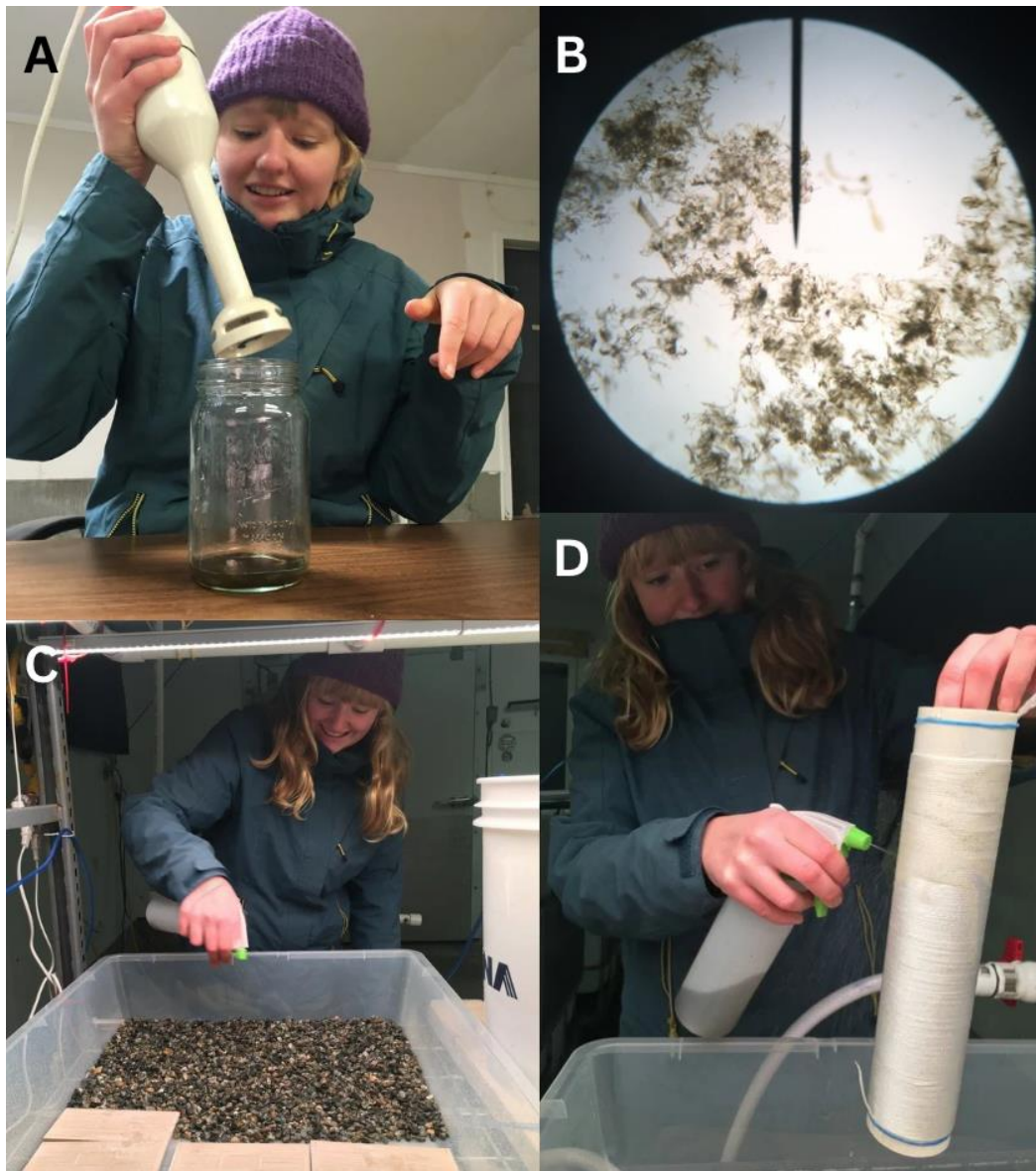


Figure 6. (A) Fragmenting the concentrated gametophyte solution with a hand blender. (B) Gametophyte fragments after chopping, observed under a microscope at 100x magnification. (C) Spraying green Gravel and clay tiles with the gametophyte solution diluted to 7,500 fragments/mL. (D) Spraying on fragmented gametophyte solution to spool with twine. Photos taken by Lee Ann Ennis

5.3 Nursery up-keep

Necessary activities carried out in the nursery included, water changes and cleaning of the tanks (every 1-2 weeks), filter changes (every 2-3 weeks), and addition of nutrients. Nutrients was added in the form of Fritz F/2 algae food part A and B (Fig. 7 C). Despite using a series of filters and UV sterilization for incoming seawater (Fig. 7 A), diatom contamination was still present (Fig. 7 B), which can inhibit kelp growth at microscopic stages (Merrill & Gillingham, 1991). To limit diatom contamination, a dose of germanium dioxide was used. To keep track of the development of the kelp material, samples were scraped off the substrate and observed under the microscope weekly. It took about two-three weeks to see fully grown gametophytes on the spore settled substrate. It took approximately six weeks for juvenile sporophytes to appear (Fig. 8). Further details on the nursery set up/upkeep are in Appendix B.

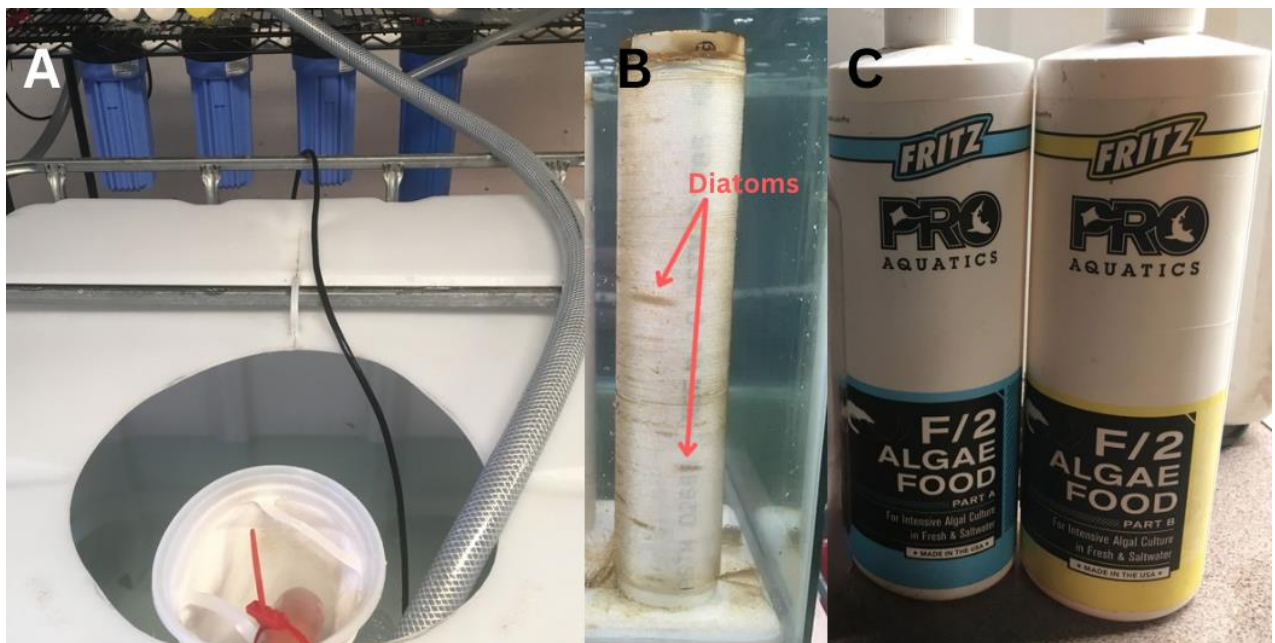


Figure 7. (A) Holding tank for sterilized seawater that has been mechanically filtered by the series of filters seen in behind. (B) Patchy diatom contamination on one of the seeded spools. (C) Fritz Pro aquatics F/2 algae food that was added to tanks weekly in small doses to keep up nutrients. Photos taken by Amelia Gray.

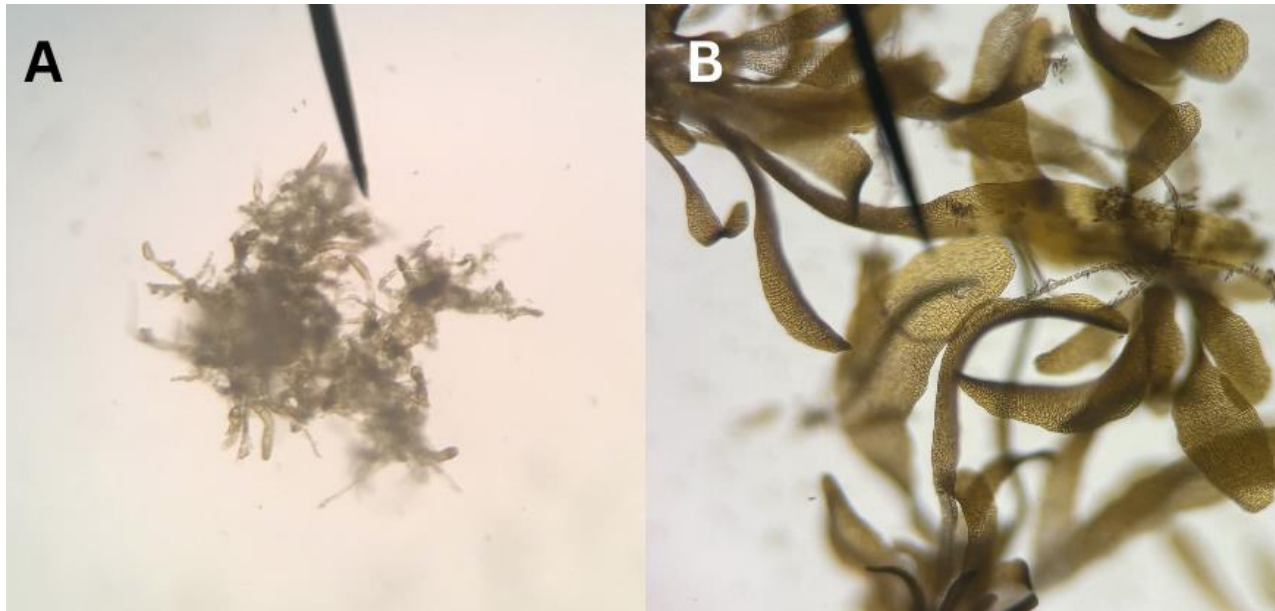


Figure 8. (A) Gametophyte from a spore seeded line after ~ 2 weeks of cultivation in the nursery, viewed under a microscope at 100x magnification. (B) Juvenile sporophytes from a spore seeded line after 6 weeks of cultivation in the nursery, viewed under a microscope at 100X magnification. Photos taken by Amelia Gray.

5.4 Out-planting techniques

On January 26th, 2025, successfully inoculated substrates were out-planted at Ole's cove. The mesh substrate was attached to two moorings that were built from available materials (Fig. 9 A). Both moorings consisted of weight bag to hold it in place, a float to hold the line normal to the seafloor and between them a frame that remained consistently flat between the two. One mooring was a wooden 1m² frame and held 3000cm² of fragmented gametophyte sprayed mesh and 1500cm² of spore settled mesh. The other frame was built from PVC piping and had a smaller dimension of 70cm by 30cm, this held the remaining 1500cm² of spore settled mesh (Fig. 9 B). These moorings were deployed from the side of the boat and positioned 1m above the seafloor to avoid herbivory from sea urchins at an overall depth of 7.5 m (at a ~4m tide) (Fig. 9 C). Two lines were deployed, one for fragmented gametophyte and the other for spore settled twine each measuring a total length of 40m. Seeded twine was deployed by letting it unravel from the PVC spool and wrap around floating Danline 3/8" polyline that was lowered to the bottom by anchored weight bags. Multiple weight bags were added along the line during the deployment, these held the rope taut to keep it at a consistent position about 30cm above the bottom. Lines were at an identical depth of 7.5 m and deployed parallel to the shore (Fig. 10). A diagram to show the deployed substrate set up underwater can be seen in Figure 11.

The green gravel and clay tile substrate did not show signs of settlement in the nursery, and hence were not deployed.

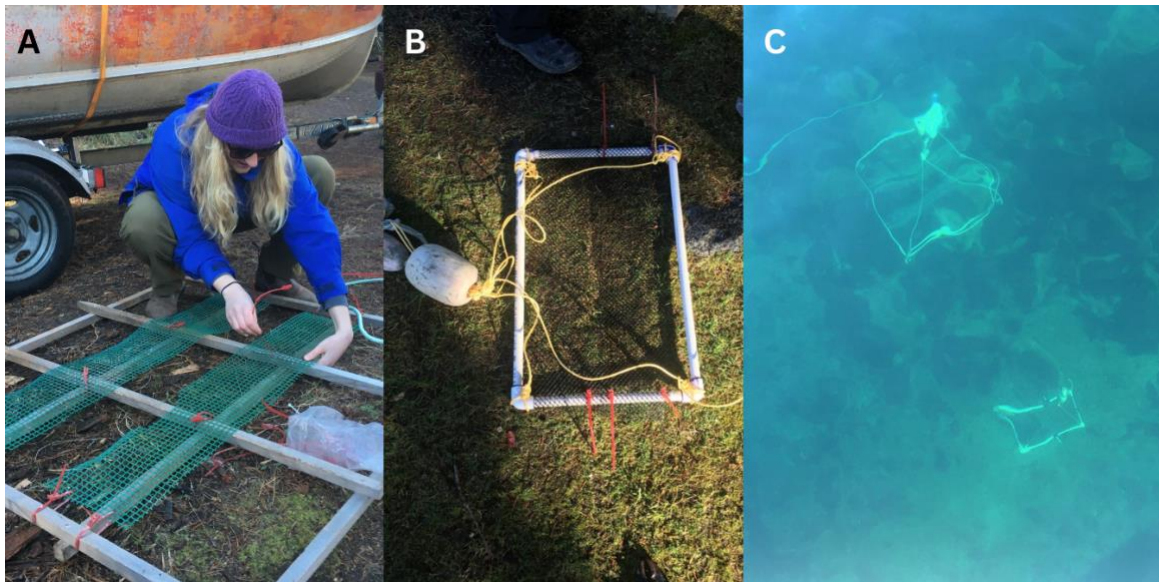


Figure 9. (A) Amelia building the wooden frame kelp mooring and attaching fragmented gametophyte seeded mesh. (B) The smaller kelp mooring made from PVC piping with 1500cm² of spore settled mesh, right before deployment. (C) Deployed kelp moorings side by side at 7.5m depth, part of one of the lines can also be seen in the left corner. Photos taken by Lee Ann Ennis and Amelia Gray.

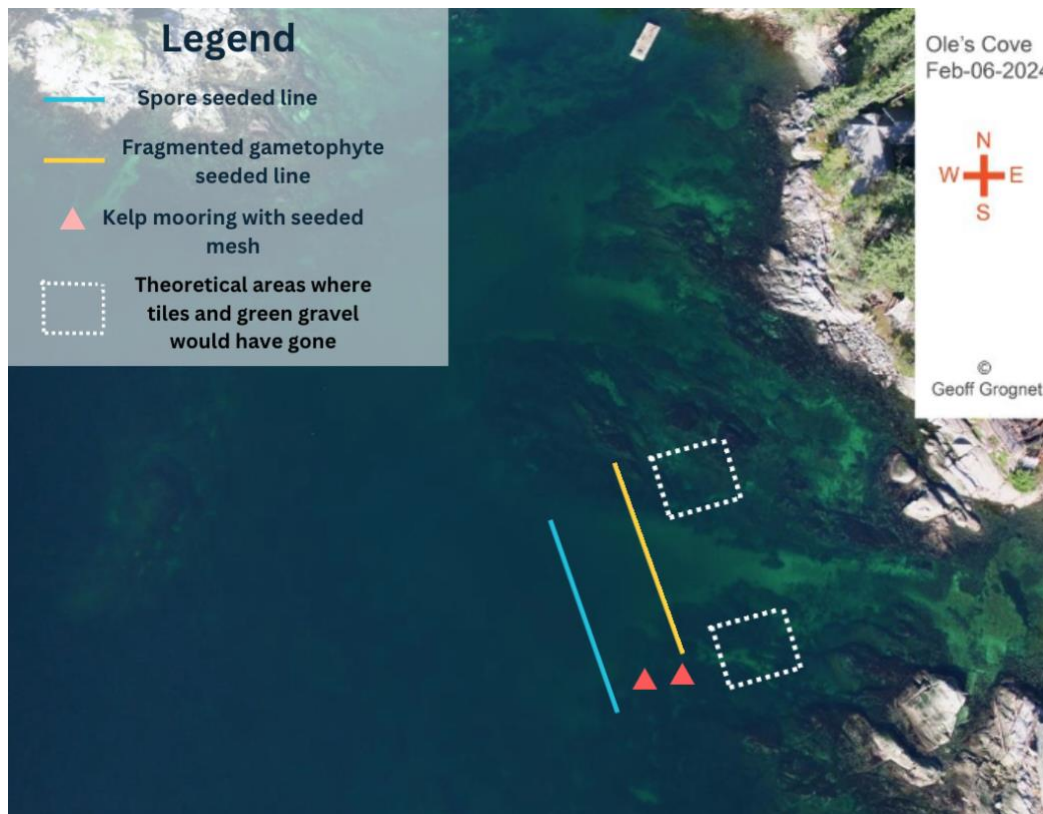


Figure 10. Overhead view of approximate location of out planted substrate. Seeded line types and kelp moorings denoted in legend. Proposed areas of where tiles and green gravel would have been deployed are denoted by dashed boxes. The aerial drone photograph of Ole's Cove taken on February 6th, 2024, by Geoff Grognet. Edited and added to by Amelia Gray using Canva.

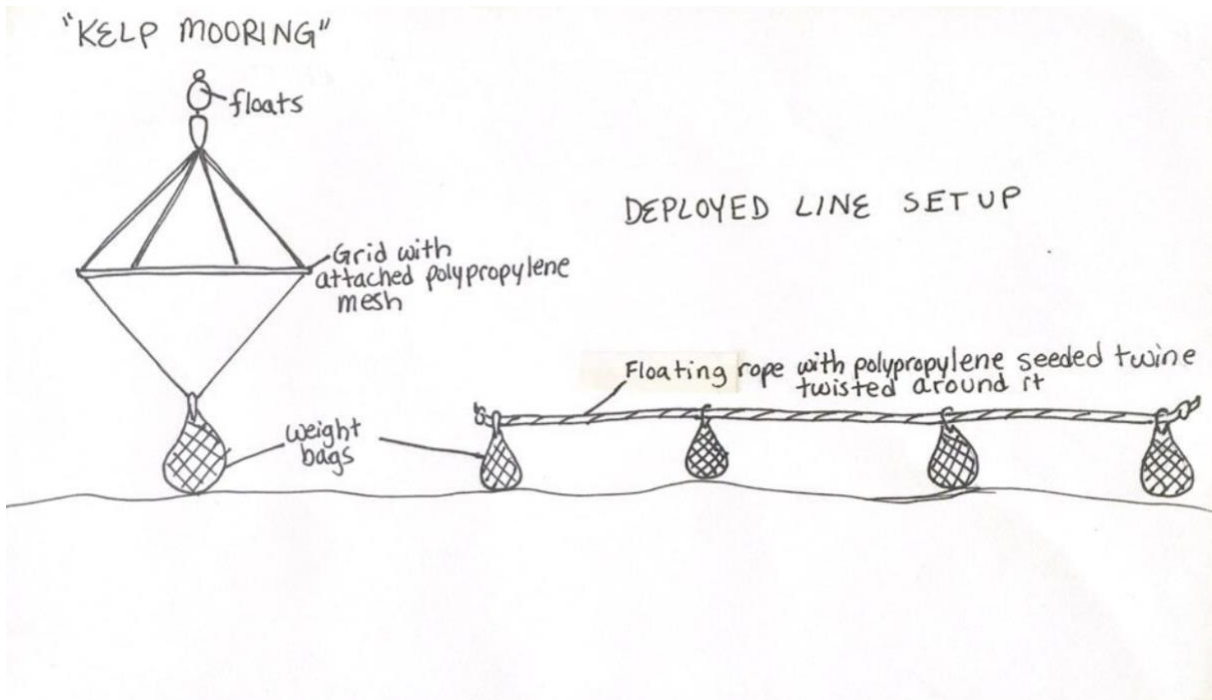


Figure 11. Diagram showing how the kelp moorings and line sit in the water after deployment. Side view drawing by Amelia Gray.

5.5 Monitoring and Analysis

Twine substrate

To quantify successful recruits on the twine substrates, divers were deployed to video and image sections of both fragmented and the spore settled lines on February 25, 2025. Unfortunately, the video quality and distance at which it was taken could not discern the kelp at this stage. However, 6 high-resolution images were collected of the spore-settled line, and 4 of the fragmented line using an Olympus TG series camera. Along 1 cm segments of the twine, the number of successful recruits was counted (Fig. 12). The presence of kelp along each segment of spore settled line and fragmented line was tabulated in Table 5. (Appendix E).

To determine if the proportion of segments containing kelp was significantly influenced by the seeding methods, a chi-square test was employed. Raw data was re-cast into a contingency table shown in Table. 2 using the R software and its ‘stats’ package (R Core Team, 2023).

Table 2. Contingency table for kelp presence in a 1cm unit area on the seeded lines for both seeding methods.

	ABSENCE (0)	PRESENCE (1)
FRAGMENTED (F)	25	22
SPORE-SETTLED (S)	100	41

Grid mesh substrate

Divers also monitored and imaged the kelp moorings with attached polypropylene grid mesh. Percent coverage of the mesh was calculated by counting the cm² grids that showed kelp presence, and dividing it by the total grid area. Limited photos and data for both seeding methods, did not allow for statistical analysis.

6.0 Results

Twine substrate

Data collected from the seeded twine and employed in the Chi-square test reported a value of 4.21 and a p-value of 0.0402. This is below a p-value of 0.05, meaning that the seeding method has a significant influence on probability that kelp is present on a 1cm unit of twine. The presence/absence data for the lines was also used to calculate density of sporophytes per cm. This came out to be 0.468 sporophytes per centimeter on the gametophyte fragmented lines, and 0.291 on the spore-settled lines. In addition to this, qualitative information was gathered by the divers who reported most sporophyte blades to be ¼ - ½ inch long with several measuring 2-3 inches in length. Most of these longer blades were located on the spore-settled line (Fig. 12 B).

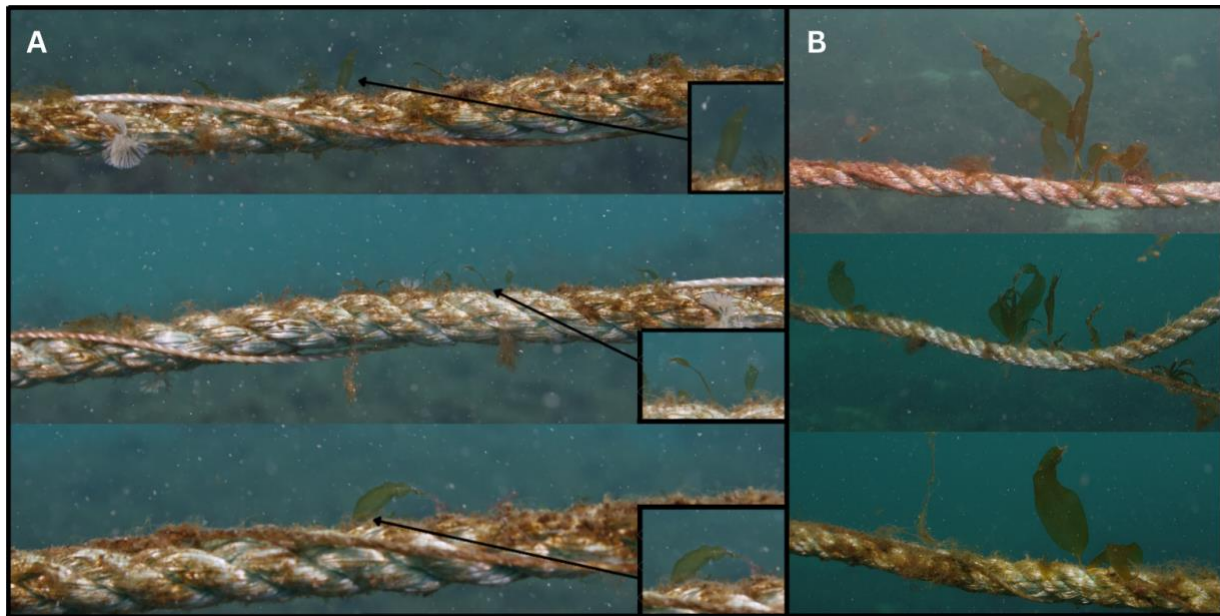


Figure 12. (A) Shows a panel of three photos from the fragmented gametophyte seeded line that were collected by the divers and used to quantify presence of kelp on any given 1cm unit of area. At this time sporophytes were still very small, inlayed photos show a zoomed in look of sporophytes that were counted. (B) shows a panel of three photos from the spore seeded line that were collected by the divers and used to quantify presence of kelp on any given unit of area. Sporophytes on this line were noticeably longer in the photos that were collected. Photos taken by scuba diver Douglas Swanston.

Grid mesh substrate

Only the spore seeded mesh on the smaller mooring exhibited kelp recruitment (Fig 13). The spore seeded mesh had 212cm² grids covered in sporophytes out of 1500cm². A percent coverage of 14% was calculated.

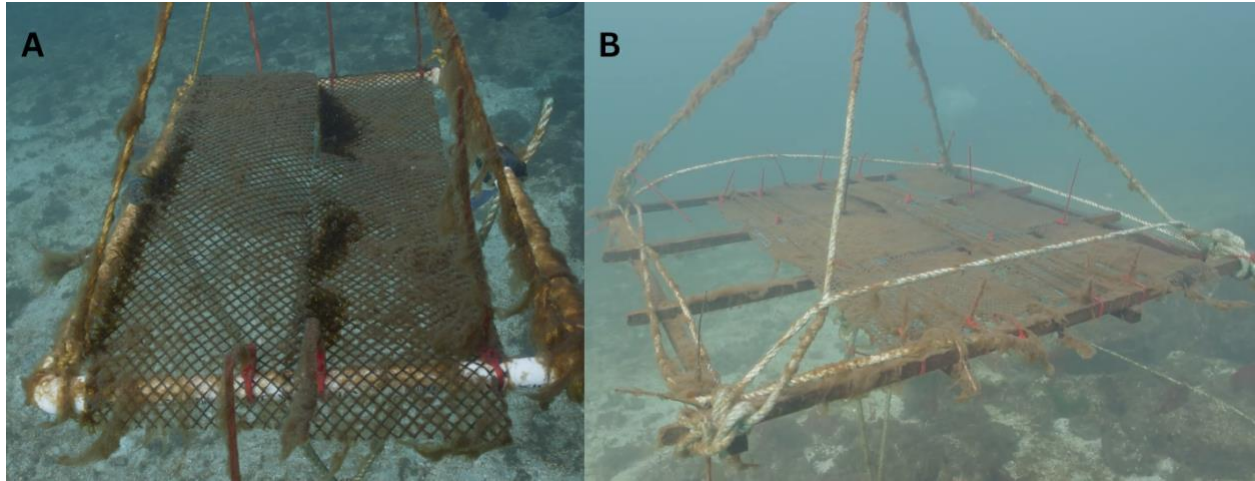


Figure 13. (A) Smaller kelp mooring containing 1500cm² of spore seeded mesh. After one month of deployment at Ole's Cove, small sporophytes were recruited in dense, dark patches along the edges. (B) The larger 1m² kelp mooring containing 3000cm² of fragmented gametophyte seeded grid mesh and remaining 1500cm² of spore seeded mesh exhibited no sporophyte recruitment that could be seen. Only brown epiphyte growth was observed on this kelp mooring. Photos taken by scuba diver Douglas Swanston.

8.0 Discussion

8.1 Summary of results

This project tested two seeding methods (fragmented gametophyte and spore settled) across four different substrates (twine, green gravel, clay tiles, and grid mesh). Kelp recruits were found on both seeding methods of twine substrate, but only the spore settled mesh. Kelp failed to recruit on the inoculated green gravel and the clay tiles at the nursery stage, and therefore were never out planted. These results suggest that polypropylene twine may be the best substrate to work with out of the four types used in this project. The overall density of sporophytes per centimeter along each line was 0.468 on the gametophyte fragmented lines, and 0.291 on the spore-settled lines. This suggests that the fragmented gametophyte seeding method may be more effective on the twine. Percent coverage on the spore seeded mesh was calculated to be 14%. No kelp recruits on the gametophyte mesh were observed, suggesting that spore settled mesh may be more effective than gametophyte sprayed mesh. It appears that performance of the seeding method may be dependent on the substrate type. Although, given the very limited dataset, these suggestions cannot be statistically concluded. Table 3 below shows the overall success of substrate and seeding methods.

Table 3. Shows the overall success that was observed for the spore and fragmented gametophyte (Frag. Gam.) seeding methods across the four substrate types of green gravel, clay tiles, Polypropylene twine (Poly. Twine), and polypropylene grid mesh (Poly. Grid Mesh). Green indicates successful kelp presence that was quantifiable. Red indicates the substrate that was unsuccessful, with no kelp presence.

Substrate	Seeding method	Kelp presence	Metric
Green gravel	Spore	X	NA
	Frag. Gam.	X	NA
Clay tiles	Spore	X	NA
	Frag. Gam.	X	NA
Poly. Twine	Spore	Yes	0.291 sporophytes/cm
	Frag. Gam.	Yes	0.468 sporophytes/cm
Poly. Grid Mesh	Spore	Yes	14% coverage
	Frag. Gam.	X	NA

The results of the Chi-square test suggest a significant relationship between the presence of kelp on a given 1 cm unit of twine and the inoculation method. However, a Chi-square test alone cannot suggest the direction of this relationship, or determine which seeding method is associated with the effect. Despite this, a comparison of the benefits and drawbacks of each method can be made from referring to the literature and personal communications.

8.2 Comparison of seeding methods

Direct seeding with gametophyte cultures presents numerous advantages to kelp restoration efforts. Selective breeding is possible where male and female gametophytes are separated within a culture and used to propagate clones (Lüning & Neushul, 1978). This controlled approach facilitates genetic selection for desirable traits (Hwang et al., 2019). This enables the use of a precise number of parent gametophytes to maintain diversity. Additionally, fertilization success through optimized male-to-female ratios is improved (Hadley et al., n.d.). Selective breeding will be particularly valuable for developing resilient kelp strains that are able to adapt to climate change and rising sea surface temperatures (Greenwave, n.d.). Using these strategies may be necessary for successful kelp restoration in the warmer waters of the Salish Sea.

Currently, collection of wild sori cannot be increased to an industrial scale because of outright cost and the stressors it would place on natural populations. Culturing gametophytes eliminates the need for annual wild sori collection. Additionally, this allows more control over the out-planting time. As such, gametophyte cultures will also pave the way for upscaling of restoration efforts. Companies like Industrial Plankton have created bioreactors that produce high-quality gametophytes and sporophytes under optimized controlled conditions. This use of gametophyte culturing reduces labor and enhances efficiency (Industrial Plankton, n.d.). Similar technology such as the SeaCoRe System (A. P. J. Ebbing et al., 2022) and BioSeedeX (Ocean Seeders Collective, 2025), also demonstrate the potential of bioreactor technology becoming a widely adopted tool in kelp restoration. To prepare for this, Lee-Ann Ennis recommends saving gametophytes from known populations to build up a biobank before they disappear (L. Ennis, personal communication, 2025).

The largest drawback of gametophyte seeding is the lack of a robust application method. Conventional methods of applying seed to a substrate include fragmenting and spraying (Le François et al., 2023), binding agents (Visch et al., 2023), and painting gametophytes onto substrates (Hwang et al., 2006). Some studies have found success by combining techniques for improved adhesion (Umanzor et al., 2021). Industrial paint sprayers have been used effectively to apply fragmented gametophytes without the need of binders (Greenwave, n.d.). Perhaps a more powerful sprayer could have improved recruitment outcomes in this project.

Historically, inoculating substrates with spores was standard practice in the kelp restoration and aquaculture disciplines. Spore inoculation offers reliable attachment and widespread literature support (Flavin et al., 2013; Fletcher & Callow, 1992; Gaylord et al., 2006; Goecke et al., 2020). A study by Forbord et al. (2020), found that seeding with spores gave significantly longer fronds and a higher biomass yield compared to the gametophyte seeded treatments. Based on observing the few photos from the lines on this project it did appear that the spore seeded line had more longer fronds. Forbord et al. (2020), also found prior to deployment spore spools had 84% coverage, while the gametophyte treatment only had 43% coverage. This may be due to how they attach to the substrate, as gametophytes are found to have poorer adhesion properties (Xu et al., 2009). Additionally, collection of new sori and spores from the wild every season would ensure genetic diversity from current surviving kelp populations, and may be more adapted to current environmental conditions (L. Ennis, personal communication, 2025). Although this method provides some benefits, its dependence on wild sori, cost and time in the nursery, and the inability for selective breeding (Flavin et al., 2013) should lead to a transition in methods. Spores alone will not be able to support the upscaling of kelp restoration efforts, this is where culturing gametophytes becomes very valuable.

8.3 Comparison of substrates

The results of this project showed varied kelp recruitment success across the substrates. Several factors may have led to a lack of recruitment on the green gravel and clay tiles which limits the statistical conclusions that can be made. However, results of both seeding methods working on the seeded twine, suggests this may be the most versatile and best substrate to work with. Further recommendations regarding the ideal substrate type can be informed by existing literature.

Seeded twine is the primary substrate used in kelp aquaculture and restoration (Flavin et al., 2013; Forbord et al., 2020). This project used twisted polypropylene twine, which exhibited recruitment across both seeding methods, but overall had sparse growth. Research suggests that twisted polyvinyl alcohol (PVA) twine offers 50% higher bio adhesion, and 11–24% greater biomass yield after out planting, than polypropylene, polyester, or polyamide (Kerrison et al., 2019). PVA twine, such as Kuralon, is widely used in Southeast Asia and may enhance future kelp restoration efforts (Werner & Dring, 2011).

Literature regarding polypropylene mesh as a substrate for seeding kelp was not found. However, it has been used successfully in other projects by bolting it to the seafloor to use as an anchoring point for transplants (Correa et al., 2006). The nature of the 1cm² grid makes for an easy unit of measurement to collect quantifiable data. The grid is also suspected to support

holdfasts of the larger sporophytes quite well, this will hopefully be observed in future monitoring on this project.

Other studies have found success in both seeding green gravel with fragmented gametophytes (Alsuwaiyan et al., 2022) and seeding with spores (Fredriksen et al., 2020). However, in general, successful out planting and monitoring of green gravel has been limited. It is often reported that green gravel is too small and drifts away when the kelp becomes larger and positively buoyant (Earp et al., 2024; Good, 2024). More success has been found with using larger cobble or rocks and out planting in areas with low wave action (Earp et al., 2024).

This project observed no kelp recruitment for either method of seeded clay tiles, suggesting they were an unsuitable substrate. However, clay tiles have proven to be successful in other past restoration conducted by Vital Kelp (L. Ennis, personal communication, 2025). A new tile type was used this year at Vital Kelp, perhaps the chemical composition or surface texture was not conducive for settling. Terracotta tiles have been widely adopted, due to their surface roughness and microstructure resembling real reef substrate (Kennedy et al., 2017). However, materials with increased surface roughness such as concrete may aid in better kelp adhesion (Fong et al., 2024; Kerrison et al., 2016). Furthermore, local rock types along the Sunshine Coast resemble concrete surface rugosity more than clay, suggesting they may be a better attachment for local kelp.

8.4 Sources of error and other challenges

It is unknown why the clay tiles and green gravel did not show kelp recruitment, but it was likely due to complications at the settling phase, when the kelp material was being applied to the substrate. Seeding density may have played a factor in this, as it is found to significantly influence gametophyte growth (A. Ebbing et al., 2020). Seeding green gravel with higher densities can promote early growth, but may reduce long-term recruitment, so low densities are recommended for highest kelp abundance in the long-term (Chemello et al., 2024). Perhaps the use of a lower seeding density for the green gravel and clay tiles would have yielded kelp recruitment, however, this does not explain the lack of successful settlement all together. A step that was not fully implemented in this experiment, was soaking the tiles and green gravel in seawater for 24 hours prior to seeding (Good, 2024). Immersing surfaces in seawater allows for the absorption of macromolecules, proteins, and polysaccharides, this creates a complex chemical topography which increases the settlement of organisms (Lejars et al., 2012; Thome et al., 2012). The pre-conditioning of these substrates for longer may have resulted in settlement success.

Kelp growth and development are highly dependent on site suitability and environmental conditions (Kerrison et al., 2015). In previous years, Vital Kelp observed larger sporophytes by late February at sites nearby, with limited growth occurring by April as waters warm (L. Ennis, personal communication, 2025). This project aimed to align with that timeline, however, a delayed out planting time at Ole's cove paired with uncontrollable poor conditions did not allow for this. Late deployments in February often fail due to competition with spring phytoplankton blooms and nutrient depletion by April (Boderskov et al., 2021). Similarly, by mid-March, phytoplankton blooms in Ole's Cove reduced light availability at depth, and created poor

visibility, preventing data collection at that time. Epiphyte growth across the substrates also contributed to smaller kelp and challenges in quantifying kelp sporophytes.

To address these monitoring challenges, out planted substrate should be made more accessible. One potential solution is a pulley system that allows lines to be adjusted in depth. This would enable surface-level monitoring while also controlling light exposure. Early in the season, kelp could be grown closer to the surface for optimal light conditions, then lowered as they grow larger to avoid rising sea surface temperatures.

8.5 Recommendations for future restoration and research

Future kelp restoration efforts should prioritize investigating the use of direct seeding with gametophyte cultures. Although, significant conclusions on the efficacy of this technique could not be made in this project, referring to the literature revealed many of its benefits. Other recommendations from the literature and findings of this project are provided in Table 4.

Table 4. Recommendations for best seeding methods, substrate type and monitoring/ data collection for future kelp restoration efforts. These recommendations are informed by findings from the project and best practices found in the literature.

Recommendations	Rationale
Seeding Method	
More investigation into the best methods for applying gametophyte cultures to substrate.	There is lots of knowledge around the storing and cloning of gametophytes, however, application of them to substrate is still being explored.
Exploring the use of binders with gametophyte seeding methods.	-Gametophytes have weaker adhesion than spores (Xu et al., 2009), the use of binders may lower the chance of detachment. -Binders also allow for possibilities of seeding onto different types of substrates (Wilding et al., 2025) or even directly into rocky crevices in the seafloor (e.g. “gametophyte glues”) (L. Ennis, personal communications, 2025).
Start creating a biobank of healthy gametophytes from known populations.	To scale up kelp restoration efforts, the use of gametophytes cultures will be the primary method. Bioreactors have already been created to aid in this process. Gametophyte cultures will also allow for selective breeding of desirable traits (Hwang et al., 2019).
Substrate Type	
Use twisted twine composed of Polyvinyl Alcohol (PVA) (e.g., Kuralon twine)	-This twine had the highest kelp recruitment when tested against other polymer types, 50% higher bio adhesion than polypropylene (Kerrison et al., 2019). Twine in general may be the best substrate for seeding kelp.
Try other tile types like concrete.	The substrate roughness and texture more closely resemble the local rocks in the Salish Sea, and may

	increase settlement of kelp material (Kerrison et al., 2016).
Pre- conditioning the substrate in seawater for 24 hours before inoculation.	Immersing surfaces in seawater creates a complex chemical topography which increases the settlement of organisms (Lejars et al., 2012; Thome et al., 2012).
Monitoring/ Data Collection	
Create lines or out planting mechanisms that can change depth and come to the surface.	This would make monitoring much more accessible; it would also allow control over the amount of light and conditions the kelp was receiving.
If sporophytes are small (<7cm) divers should collect more close-up high quality photos at random points along the lines.	More data is needed to make statistically sound conclusions and run models. If kelp is small, it is not feasible to capture close-up video of all the substrate on a project. Numerous photos taken randomly is recommended.

9.0 Conclusion

The overall goal of this project was achieved by gaining experience in every step of the kelp restoration process. This project was also designed to make comparisons between seeding methods using fragmented gametophytes and traditional spore settling methods across different substrate types. Successful kelp recruitment was observed for both seeding methods on the twine, but only on the spore settled mesh. Kelp failed to settle on the clay tiles and green gravel. The seeding method statistically influenced the probability of observing kelp on a given cm unit of the twine substrate. Additional data is required to comment in any robust way about which of the two methods, if not both, produced this result. Density of kelp on the gametophyte line was higher than the spore line, and spores did better on the mesh, suggesting that the performance of the seeding method may be influenced by the substrate type. Additionally, the success of both seeding methods on the twine, and recommendations in the literature suggests this may be the best substrate for seeding. It is also recommended that gametophyte cultures are used for seeding, as benefits include selective breeding, increased fertilization success, and the upscaling of efforts in conjunction with new bioreactor technology. The future of kelp restoration efforts should prioritize determining best practices for gametophyte culture application, as this is still under refinement.

10.0 Appendices

10.1 Appendix A: Acknowledgments

I would like to give a huge thank you to my mentor Lee-Ann Ennis, for all the knowledge and experience she shared with me through the process of this project. This project would not have been possible without her. I would also like to thank Scott Hodges for being our emergency check in and being a huge support. I'd like to acknowledge and thank Douglas Swanston and

Geoff Grognet the divers on this project for monitoring and taking photos, it is not easy collecting data underwater. Finally, I'd like to thank Nancy Shackleford for approval of this project and all the support along the way.

10.2 Appendix B: Detailed Methods

Preparation of the sori and spore release

To prepare for spore release sorus patches were cut out of blades and cleaned by scraping the surface with a blade to remove diatoms and other fowling organisms. To further sanitize, they were dipped in 10% Iodine, wiped, and then dipped in mechanically filtered UV sterilized seawater (sterilized seawater). They were kept between moist but not wet paper towel, in a fridge at 10°C, for ~ 24hrs. To induce spore release, cleaned sorus patches were cut into approximately 5x5 cm squares and submersed into a jar full of sterilized sea water. The mixture was stirred every few minutes to keep any released spores suspended. After about 15 minutes spores started to release and appeared as a milky cloud. A sample was taken and checked under a microscope at 100x magnification to observe actively swimming spores shaped approximately like almonds. The spore solution was sieved through cheesecloth before adding to settling buckets.

Light and tank conditions for cultivation

Once confident spores and gametophytes had stuck to the substrate they were gently moved to the tanks. Green gravel and clay tiles that were already in the tanks were filled on low flow. They were put under 5-10 $\mu\text{mol m}^{-2} \text{s}^{-1}$ lighting for the first few days. Every week the lighting was increased by about 10 $\mu\text{mol m}^{-2} \text{s}^{-1}$, until a maximum intensity of 80-100 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Barhina LED full spectrum grow lights were used and set to a photoperiod of 16:8 light to dark (Supratya & Martone, 2024). A few days in, air stones were introduced gradually to tanks to increase aeration. The aeration serves two functions 1) to keep the water moving, delivering nutrients to the gametophytes and 2) to mix more CO_2 into the water which the kelp sequesters, balancing pH at an optimal range between 7-9 (L. Ennis, personal communication, 2025). The temperature of the water in the tanks hovered around $10^\circ\text{C} \pm 2^\circ\text{C}$, falling within the thermal tolerance of *N. luetkeana* (Supratya et al., 2020).

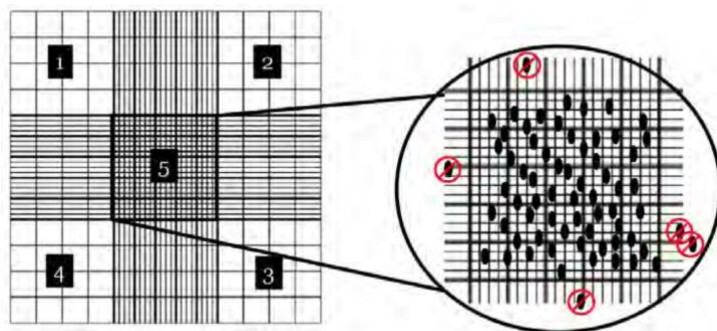
Nursery set up and upkeep

The nursery was set up with a Hayward 1 Horsepower Magnetic pump to bring water in directly from the ocean. Before using the water for anything, it first went through a series of filters that decreased in micron size (30 μm , 5 μm , 1 μm , and 0.2 μm). It would then go through a UV filter and one more 1 μm filter before getting used in the tanks and nursery activities. Spools and grid mesh were kept in ~100L tanks and the tiles and green gravel were in shallower ~50L tanks. The first water change in the individual tanks occurred 10 days after the substrate was settled. Following this, when substrate was still in the gametophyte stage water changes were done every 1-2 weeks or if water was cloudy. When sporophytes started to appear, water changes were done weekly, to ensure more nutrients in the water. Water changes consisted of dumping the tanks

completely, scrubbing the sides, and refilling with fresh sterilized seawater. At this time a 1mL/1000mL dose of Fritz F/2 algae food was added weekly to each tank, coinciding with the water change. At 5 weeks a small dose of 5mL of each nitrogen, phosphorus, and potassium (NPK) was also added. This was needed for the heavy feeding sporophytes. When dealing with diatom contamination a germanium dioxide stock solution of 1g/250mL was used, and a dose of 3mL/100,000mL was added to the tanks. All activities in the nursery were done using the clean technique and 70% alcohol was the primary cleaning agent

10.3 Appendix C: Counting zoospores and calculating stock density

Methods from Flavin et al., (2013)



Using a hemocytometer, spores in the center square were counted.

Square 5 Count = 47 spores

Spores per mL = 47 x 10,000

Spore concentration = Spores per mL = 470, 000 spores

$$\text{Volume of Release Water (mL) to Inoculate Settling Tubes} = \frac{\text{Desired Stocking Density (Spores/mL in Settling Tubes)}}{\left(\frac{\text{Number of Spores/mL in Release Water}}{\text{Volume of Seawater (mL) in Settling Tubes}} \right)}$$

This is the formula used by Flavin et al. (2013) to calculate the stock density.

A recommended stock density falls within 5000-10,000 spores/mL.

An average stock density of 7500 spores/ mL was chosen to use.

12,000 mL of filtered seawater was used in the settling buckets.

Using the known count of 470,000 spores/mL, the following calculation can tell us how much of the spore solution should go into each settling bucket to have stocking density of 7,500 spores/mL.

$$\text{Volume of Release Water (mL) to Inoculate Settling Tubes} = \frac{7500 \text{ Spores/mL}}{\left(\frac{470,000 \text{ Spores/mL}}{12,000 \text{ mL/Seawater}} \right)} = 191.5\text{mL}$$

10.4 Appendix D: Counting gametophyte fragments and calculating a known concentration

Starting volume = 200mL of highly concentrated gametophyte fragmented solution

Five samples of 5 μ l volume was taken, and fragments were counted under the microscope, to get an average fragment count:

Sample	Vol μ l	# fragments
1	5	62
2	5	45
3	5	39
4	5	52
5	5	48

Average number of fragments from 5 samples = 49.2 fragments/ 5 μ l

1ml = 1000 μ l 1000 μ l/ 5 μ l = 200

49.2 fragments/ 5 μ l x 200 = 9,840 fragments/ 1000 μ l or 1 mL

A starting solution of 200mL with 9,840 fragments/ mL

To get a diluted solution of 7,500 fragments/ mL a volume of water must be added.

Using this calculation: $C_1V_1 = C_2V_2$ the unknown V_2 can be calculated

$$\begin{array}{ccc} \text{C1} & \text{V1} & \text{C2} \\ 9840 \text{ fragments/mL} \times 200\text{mL} & = & 7500 \text{ fragments/mL} \times V_2? \\ \hline & \nearrow & \\ \frac{9840 \text{ fragments/mL} \times 200\text{mL}}{7500 \text{ fragments/mL}} & & V_2 = 262.4\text{mL} \end{array}$$

To make a 7500 fragment/mL concentrated solution, 262.4 mL of filtered seawater was added to the stock solution of fragmented gametophytes.

10.5 Appendix E: Kelp presence data frame

Table 5. Data frame for determining presence of sporophytes on any given 1cm unit of twine.

Image ID	Unit cm with kelp	Total unit cm Segments	Seeding Method
F 1	9	11	Fragmented
F 2	7	13	Fragmented
F 3	2	10	Fragmented
F 4	4	13	Fragmented
S 1	4	12	Spore-Settled
S 2	7	32	Spore-Settled
S 3	2	16	Spore-Settled
S 4	4	19	Spore-Settled
S 5	13	33	Spore-Settled
S 6	11	29	Spore-Settled

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