

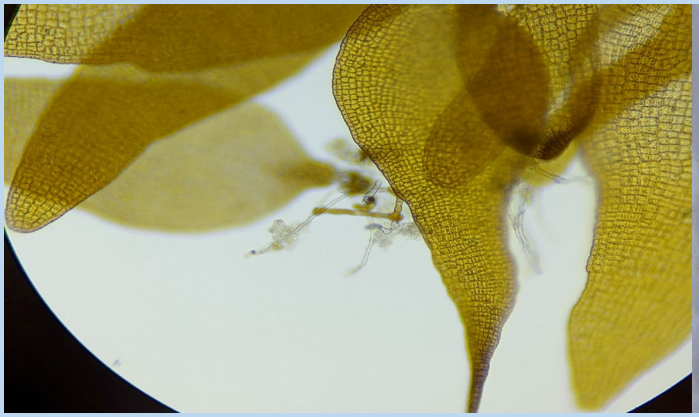


Seaweed Nursery Production and Collection

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ACKNOWLEDGEMENTS

The Centre for Aquaculture and Seafood Development (CASD) would like to thank the Canadian Centre for Fisheries Innovation (CCFI) for their financial support of this project. CCFI is a conduit between industry, researchers, and academic institutions, providing guidance to project development and execution through its relationship and expertise team building skills, securing/leveraging funding to support innovative projects, as well as project management. CCFI collaborates directly with industry to facilitate access to scientific knowledge and expertise of Memorial University, the Fisheries and Marine Institute and other Canadian institutions, providing access to new technologies and methodologies that lead to operational improvements, industry-wide growth, and prosperity.

EXECUTIVE SUMMARY

The scope of this project was twofold: 1) overwinter experimental collectors, determine biofouling rates in local sites, as well as seaweed maturation, and 2) develop a nursery for local seed spool production. The project was Phase 2 of the previous project: Fogo Island Seaweed Aquaculture Development. The current work was performed in conjunction with the clients Shorefast Foundation (SF) and Fogo Island Co-operative Society Ltd. (FICSL) who plan to build a small on-island nursery set-up to produce seed spools locally.

Two of the intermediate research opportunities included:

1. Seed collection, sampling, and overwintering of test collectors in the key locations identified in the previous project.
2. Nursery sporophyte production at the Marine Institute of Memorial University of Newfoundland (MI), which entailed collecting sporophytes with mature sorus tissue, releasing spores, inoculating spools, and growing gametophytes.

In terms of collector deployment and biofouling during the summer, it was determined that fouling by marine invertebrates became heaviest at about two meters depth in August and September. Ongrowing of seaweed on longlines should take place before August for successful harvest of a clean product. Monthly sample collections by Shorefast of wild seaweed showed the sporophytes were essentially mature in late August. Other producers in Maine, New Brunswick, and Quebec noticed sugar kelp were mature in early August and began their nursery cultivation then.

Nursery production for this pilot project was based on the Flavin et al. (2013) manual for kelp nursery culture in Maine, with minor modifications as determined by the team. The pilot project showed that Newfoundland strains take longer to grow to farm deployment size compared to that is recorded in the literature by others farming in the Northwest Atlantic, Alaska, or Europe.

Seeded seaweed collected at Fogo Island started to show visible growth at four weeks in the nursery, where the others reported major growth within 2-3 weeks of seeding. According to the manual, seaweed should stay in the nursery for six to seven weeks on spools, and then be deployed. However, the spools produced in the MI nursery from Fogo Island sporophytes

required 19 weeks. Logistics and planning delays prevented the deployment of spools in the field before the winter period set in. The extension of the nursery phase of this project was transferred to Project P5573 on March 16, 2023.

The growth of Fogo Island's seaweed in the nursery compared to the production cycle in the manual differed by approximately three to four weeks.

However, the CASD team concluded that the structure of the nursery and the protocols for water quality, light regimen, biofouling control and nutrients ensured the successful production of sporophytes from the adult seaweed collected at Fogo Island. Future attempts should be conducted earlier in the year when mature sporophytes are available, typically in August or September.

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1 BACKGROUND AND INTRODUCTION

Shorefast Foundation is a registered Canadian charity that serves the local community to promote economic, cultural, and environmental resiliency on Fogo. Employing a holistic approach to community development, their suite of businesses serves as economic engines for the community and are operated for the exclusive benefit of strengthening the cultural heritage, supporting ecological sustainability, and investing in the economic well-being of Fogo Island for generations to come.

Seaweed cultivation is an environmentally restorative and accessible economic opportunity that builds upon existing cultural skills and infrastructure and is tailored to small, outport communities. By supporting seaweed cultivation initiatives through early research and development and through end-of-chain market development, Shorefast Foundation can underwrite the risk for small farmers who wish to start their own seaweed farms. Although seaweeds were not historically cultivated on Fogo Island, their harvest for various uses (particularly for fertilizer) was common, and the targeted cultivation of high-value species that are native to the region offers a unique and innovative means of diversifying our marine economy and supporting existing seafood enterprises on Fogo Island. In the face of warming oceans and fluctuating quotas and market prices for traditional fisheries, Fogo Island may be in a unique position to diversify the seafood economy in the face of changing oceans.

In August and September 2021, Shorefast Foundation (SF) requested assistance from the CASD to conduct a feasibility study to determine if there were suitable locations for seaweed farming, what species were available locally and community engagement on the concept of seaweed aquaculture. Several seaweed types were identified around Fogo Island through shoreline and subtidal observations. There was evidence of forming sori in several species including *Saccharina latissima*. There were varying quantities of the seaweeds of interest depending on the location.

The current project is the first step in developing potential seaweed aquaculture sites around the Fogo Island area. The idea is to promote sustainability, the diversification of the economy, and to support existing seafood enterprises on Fogo Island.

2 SCOPE AND PURPOSE

2.1 Scope of Project

The scope of this project was: 1) to evaluate biofouling on collectors and seaweed maturity, and 2) to build and develop a nursery for seed spool production with Shorefast (SF). Work was performed in the Centre for Aquaculture and Seafood Development's (CASD) aquaculture facility located at Marine Institute's Ridge Road campus.

2.2 Purpose of Project

The seaweed nursery was built as a pilot project to produce sporophytes using the methods outlined in the Kelp Farming Manual by Flavin *et al*. (2013), with adaptations required for Newfoundland's *Saccharina latissima* (sugar kelp) strain. Over 19 weeks, sporophyte development was closely monitored to understand how sugar kelp, can be produced here in Newfoundland.

3 OBJECTIVES

Four main research opportunities included:

1. Seed collection, sampling, and overwintering,
2. Nursery development,
3. Nursery sporophyte production, and,
4. Sporophyte monitoring and maintenance.

Each of these objectives are discussed in more detail below.

3.1 Seed Collection, Sampling, and Overwintering

The Centre for Aquaculture and Seafood Development assisted the Fogo Island team in collecting and assessing wild seaweed. This assessment was necessary to collect specific information regarding wild seaweed in the Fogo Island region. Areas of interest for wild collection were Oliver's Cove, Shoal Bay, Deep Bay, and Cobb's Cove. The quality and value of seaweed can vary throughout the year, so it was important to identify species and biofoulers and seaweed maturity

to ensure seaweed is harvested in an optimal timeframe, which would be helpful in determining the farmed seaweed harvest. Lastly, the assessment of wild seaweed determined when seaweed was mature and showing reproductive tissue. This helped with preparing the nursery and the seaweed production process.

3.2 Nursery Development

Using the Marine Institute's aquaculture facilities and technical assistance, a pilot seaweed nursery, with reference to the Kelp Farming Manual (Flavin *et al*., 2013), was constructed.

3.3 Nursery Sporophyte Production

With the assistance of CASD technical personnel, sugar kelp was produced from gametophyte to mature sporophyte stage, conducting a 10-week seaweed nursery study to observe and understand *Saccharina latissima* (sugar kelp) development in Newfoundland. By using the Kelp Farming Manual (Flavin *et al*., 2013), as a guideline, technical staff developed a plan for the pilot nursery.

The life cycle of *Saccharina latissima* (Figure 1) is characterized by a heteromorphic alternation of generation between adult macroscopic sporophytes and microscopic gametophytes. Adult sporophytes release spores that settle into the substrate and differentiate between female and male gametophytes. The female gametophytes produce eggs that are fertilized by the sperm produced by the male gametophytes. The fertilized eggs develop into a zygote that will turn into sporophytes and develop into adult seaweed. The process of developing the sporophytes included nursery construction, mature sorus tissue collection, spore release, spool inoculation, sporophyte growth observation, and maintenance. The seaweed was closely monitored to observe the growth and methodological adaptations were necessary for successful sugar kelp growth here in Newfoundland.

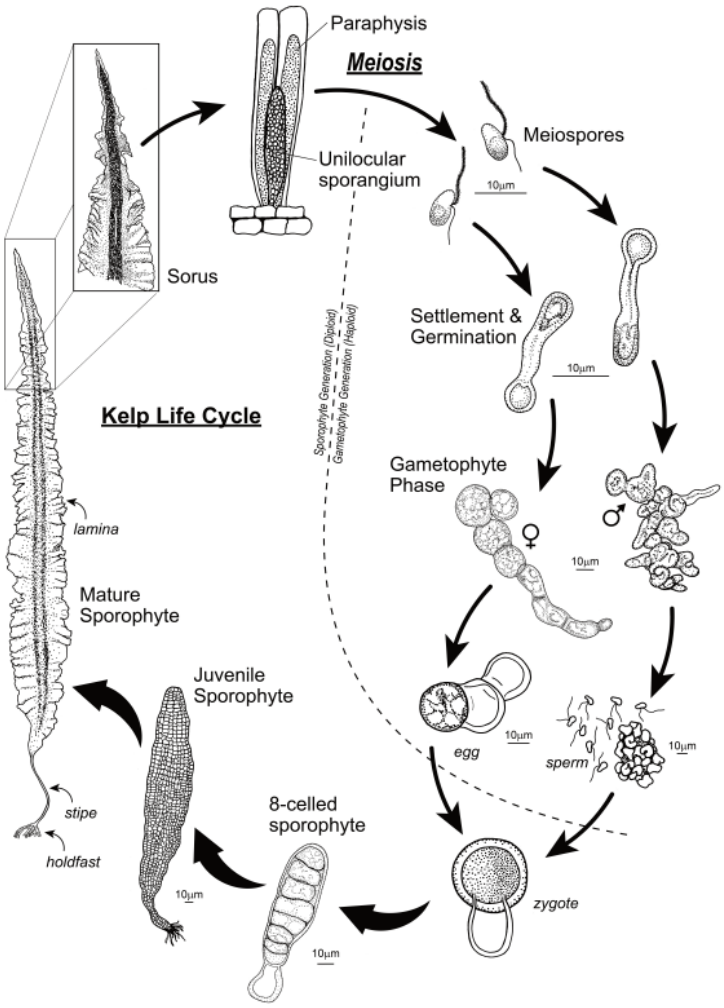

Figure 1 - The Kelp life cycle, Courtesy of C. Yarish (Illustration by Virge kask, 2012 ©Charles Yarish)

3.4 Sporophyte Monitoring and Maintenance

Daily monitoring and weekly maintenance were completed during the seaweed nursery process. These tasks helped to keep close observation on seaweed development and to adapt methods to Newfoundland's strain of sugar kelp (Appendix 8.1).

4 METHODS

4.1 Seed Collection, Sampling and Overwintering

4.1.1 Test Collector Construction

Eight test collectors were constructed at the Marine Institute for deployment to four sites with two collectors per site. The four sites were Shoal Bay, Deep Bay, Cobb's Cove, and Oliver's Cove. Each collector was positioned so that it collected information from the front and back of each potential farm site. Each test collector had a cement block mooring system attached to chain and rope. The rope drop contained six vertical PVC plates on the top and bottom of a six-rope collector. Each collector was approximately 6-7 m long. Figure 2 shows the test collector design

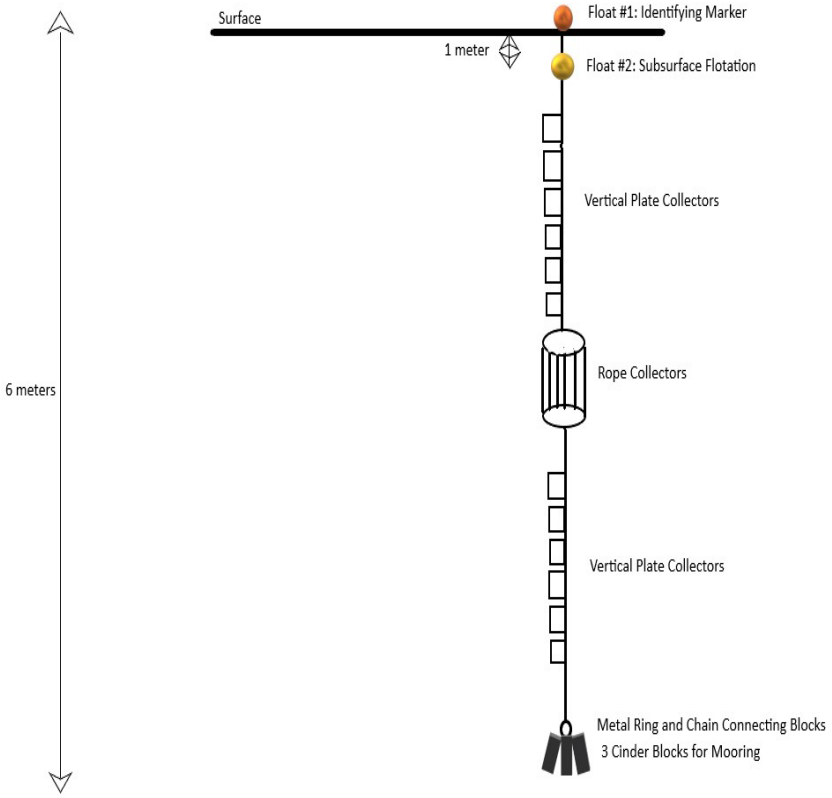

Figure 2 - Design of the test collector.

4.1.2 Test Collector Deployment and Overwintering

A CASD technician travelled to Fogo Island in December 2021 to meet with a boat operator to deploy the test collectors in Oliver's Cove and Cobb's Cove. In January 2022, test collectors were deployed in Shoal Bay and Deep Bay. Approximate locations are identified in Table 1.

Table 1: Guideline for the location of test collectors.

Collectors	Latitude	Longitude	Description
Front of Site	49.6619541	-54.2601986	Deep Bay Collector/Overwintering Site
Back of Site	49.6623851	-54.2630385	Deep Bay Collector/Overwintering Site
Front of Site	49.5803995	-54.2610492	Cobbs Cove Collector/Overwintering Site
Back of Site	49.580182	-54.261821	Cobbs Cove Collector/Overwintering Site
Front of Site	49.6875681	-54.1961787	Shoal Bay Collector/Overwintering Site
Back of Site	49.6866202	-54.1779211	Shoal Bay Collector/Overwintering Site
Front of Site	49.6981081	-540567347	Oliver's Cove Collector/Overwintering Site
Back of Site	49.697011	-54.054671	Oliver's Cove Collector/Overwintering Site

4.1.3 Test Collector Sampling

In April 2022, a CASD technician returned to sample the test collectors in each deployment area. Each collector was raised by hand and the top three vertical plates, the bottom three vertical plates, and three rope pieces were removed. Samples were placed in plastic bags and transported back to MI in coolers. Once in the laboratory, the samples were thoroughly assessed to observe if there were any sporophytes or other settlers present and to identify them if possible. Collectors and seaweed samples were collected again in June, August, and October by Shorefast and picked up by CASD personnel at the ferry to bring back to the lab for analysis. Pictures were taken of all samples to record the findings. In October 2022, the test collectors were retrieved.

4.2 Nursery Development

Nursery construction began by collecting all the materials and equipment needed for setup. Using Flavin *et al*. (2013) as a guide, tanks, chillers, pumps, tubing, lights, polyvinyl chloride,

twine, mesh, filters, and nutrients were all purchased. Two baker's racks were setup in the aquaculture facility, isolated from the facility tank systems with a large curtain. All four systems were thoroughly cleaned prior to use. This process was completed by mimicking the final tank design. Tanks were filled with freshwater and 12% bleach (380ml for 20-gallon tanks and 285ml for 15-gallon tanks) and attached to a chiller and pumps. All four systems sat with bleach for 24 hours and then neutralized with Sodium Thiosulfate (16.72g for 20-gallon tanks and 12.54g for 15-gallon tanks). Once neutralized, tanks were drained, thoroughly rinsed, and refilled with city water and left to filter for another 24 hours to ensure no bleach or sodium thiosulfate residue was left inside the tanks, tubing, chillers, and pumps. After the 24-hour waiting period, the nursery was fully assembled to its operational state (Figure 3).

Figure 3 - Assembled seaweed nursery. Top: Front view of nursery setup with rack, chillers, tanks, and lighting panel. Bottom: Over head view of spools in tank.

Next, in the nursery phase, the spools and settling tubes were constructed. The spools were cut from 2-inch pipe into 15.25” lengths (qty = 16) and 11.25” lengths (qty = 16). The settling tubes were cut from 4-inch pipe into 16” lengths (qty = 16) and 12” lengths (qty = 16) (Figure 4). Bases were cut from 6” x 6” squares and glued on the bottom of all 32 settling tubes with Oatey, medium gray, PVC cement. When all settling tube bases were glued, tubes sat for 24 hours to cure. After curing, settling tubes and spools were thoroughly washed with dish soap and water, rinsed thoroughly then placed in deionized water for 72 hours, to further sanitize the tubes prior to use. Once fully sanitized, spools and settling tubes were air dried in a clean, sanitized area for 24 hours.

Figure 4 - Settling tube crafting. Top L to R: PVC piping being cut to size and prepped for base assembly. Bottom L to R: Cement being applied to settling tube and base, assembled settling tubes left to dry.

Dry spools and tubes were put into clean bags and brought to the Marine Institute’s machine shop where 32 pipes were wrapped in 2 mm white braided nylon twine. A lathe was used to wrap the twine tightly and evenly in a single layer around the pipe and secured with elastic bands on each end. Completed spools were placed in clean garbage bags and stored in the freezer until ready to use (Figure 5).

Figure 5- Spools crafting.

Next, filtration was set up for 8000L of seawater that was received from the Department of Ocean Sciences and stored in CASD's storage tank. Seawater was filtered through three bag filters (5.0µm, 1.0µm, 0.5µm), UV sterilized at 3 gal/min, then filtered through two more 1.0µm filters (ceramic and carbon) before being pumped into the nursery tanks (Figure 6).

Figure 6 - Seawater filtration system.

The nursery setup (Figure 3) was composed of a baker's rack with two shelves. The bottom shelf held two chillers that were connected to a power bar and tubing connected to the inlet and outlet ports. The chillers were covered by a corrugated plastic sheet to prevent any water damage. The top shelf held two 20-gallon tanks placed side by side. Tubes for the inflow/ outflow of water from the chiller were secured into the tanks, the inflow tube had a small pump attached and placed at the bottom of the tank. A hose was attached to the facility air supply bank with a 20µm filter and pipette. This was placed inside the tank to provide aeration. Acrylic (Plexiglas™) lids were placed on top of the tanks to protect the seaweed from potential contamination.

Finally, lighting and timers were installed because they are crucial to the seaweed growth cycle. Each setup had two 4' T12 LED lights, with one located on each side of the rack. Light strength was preset and changed throughout the growth process. Three lighting intensities (20 $\mu mol\ m^{-2}\ s^{-1}$, 55 $\mu mol\ m^{-2}\ s^{-1}$, 100 $\mu mol\ m^{-2}\ s^{-1}$) were employed throughout the production cycle by varying the layers and sizes of fly screen (fine and wide screen). Light intensity was confirmed using an apogee instruments underwater quantum flux light meter.

4.3 Nursery Sporophyte Production

Prior to seaweed spore release, the wet lab in the Marine Institute's aquaculture facility was cleaned and prepared for the release. In reference to Flavin *et al*. (2013), necessary materials for sorus prep and spore release were obtained and setup (Figure 7). A CASD technician travelled to Fogo Island in October 2022 to meet with divers and a boat operator to collect sporophytes from *Saccharina latissima* (Sugar kelp). Sites where species were numerous and accessible, were revisited, such as Turpin's Beach, Cobb's Cove Point and Oliver's Cove. Mature sorus tissue was transported immediately back to the Marine Institute and stored using the techniques detailed in Flavin *et al*. (2013).

Figure 7 - Materials for sorus prep and spore release

Following the procedures specified by Flavin *et al*. (2013), the mature sori were removed from the mature kelp collected in Fogo, Newfoundland. Sori was separated from the non-reproductive kelp tissue with a clean razor blade (Figure 8).

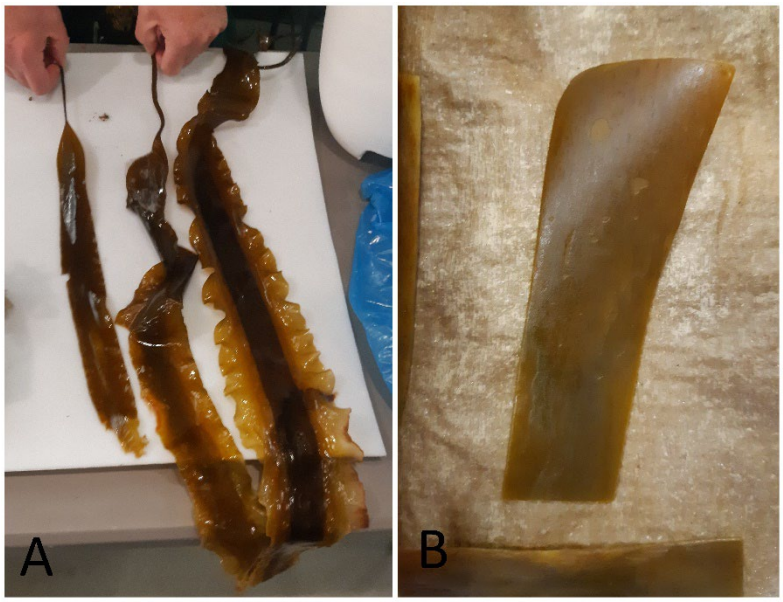

Figure 8 - Mature kelp collected at St. Mary's (A) and sorus tissue removed (B).

Excess biofouling was removed by gently scraping the surface of the sorus using the razor blade. Next, the sori were cleaned with paper towel on both sides to remove any mucilage. The sorus tissue was disinfected by being dipped in a 3% iodine solution for 30 seconds, then thoroughly rinsed with 10°C filtered seawater. The sori were dried with paper towel and carefully placed in single layers between paper towels to be prepared for overnight storage. The sorus container was placed in a laboratory refrigerator for 24 hours at 10°C for drying and induction of spore release (Figure 9).

Figure 9 - Dried sorus stored at for 24 hours at 10°C.

Prior to spore release, tanks were filled with filtered seawater and chillers turned on to get water to 10°C. Settling tubes were filled with approximately two liters of filtered seawater, covered with aluminum foil, and placed in the tanks overnight to reach proper temperature. The spore release occurred 24 hours after the sorus was desiccated in the 10°C refrigerator. The spools were removed from the freezer to thaw, dried sorus was placed into 1-liter beakers containing cultured nutrients and 10°C filtered seawater for 30 min to 1 hour, and each beaker's contents was stirred every few minutes (Figure 10). The temperature and time were recorded every 5 minutes, while checking a sample of water from the release beakers using a hemocytometer to calculate stocking density of the zoospores (Figure 11). A calculation was used to determine spore concentration and how much was needed to inoculate the settling tubes (Appendix 8.2).

After approximately one-hour, culture nutrients and spools were added into the settling tubes. The zoospores were poured into the settling tubes at the calculated stocking density allowing the

spores to settle and attach to the twine (Figure 12A). Settling tubes were re-covered with tin foil to avoid any contamination, and the tank was covered with a plexiglass™ lid. After 24 hours, spools were transferred from the settling tubes to a 20-gallon tank containing filtered (UV and mechanical filtration) seawater and culture nutrients (Figure 12B)

Figure 10 - Sorus placed in saltwater with nutrients for spore release.

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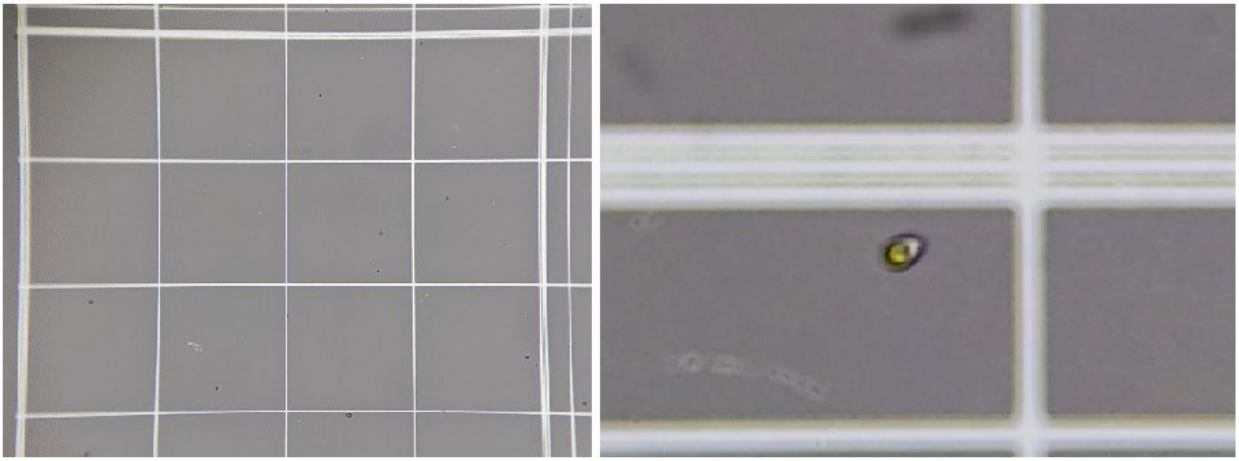

Figure 11 - Microscopic images of the zoospores on the hemocytometer.

Figure 12 - Spools in settling tubes with zoospore suspension (A). Spools in the nursery tank after zoospore settling (B).

The tank water was recirculated employing constant aeration, and a 12 h dark / 12 h light photoperiod using a 1200 K lightbulb. An Active Aqua Hydro Culture chiller system kept the water temperature at 10°C (±0.58) by following the methodology specified in Flavin *et al*. (2013):

- The solutions containing the nutrients needed for gametophyte and sporophyte development were prepared and added to the tank (Appendix 8.3).
- The spools were transferred weekly to a new tank with clean seawater and new nutrient solution.
- The light intensity was increased (Appendix 8.4).

4.4 Sporophyte Monitoring and Maintenance

Sporophytes monitoring was performed by daily observation of the spool's appearance (colour and aspect) and weekly, microscopic observation of a piece of twine collected randomly from one of the spools. Results were recorded on the spool's aspects, sporophyte development, pictures, and measurements of sporophytes using ImageJ image analysis software (https://imagej.net/ij/).

The water quality was checked daily by measuring the temperature, pH and visual evaluation of water turbidity. Using a colorimeter, a small water sample was taken from the tank and pH was analyzed.

The pH measurements through nursery weeks ranged from 7.6 to 9.1. As the lighting was increased to 100 µmol m^{-2} s^{-1}, the sporophytes grew at a faster rate which elevated the pH. To maintain the pH within the range recommended by the literature (7.0 to 9.0), CO_2 was injected. During week 11, the first CO_2 injection was performed, and CO_2 injections were repeated when needed after the daily water quality measurement.

During weekly water changes, a small piece of twine was removed from a spool and placed on a microscope slide to observe gametophytes and sporophyte settlement and development. Then, using a pair of tweezers, the surface of the twine was scraped on to a new microscope slide along with a drop of seawater and covered with a slip for detailed observation of the organisms in a higher augmentation. At the end of the 19-week nursery period, even with the possibility of observing the sporophytes without magnification, the twine scraping procedure was still performed so that it was possible to observe the dynamics of gametophyte reproduction and new sporophyte development under the developed sporophyte layer.

5 RESULTS and DISCUSSION

5.1 Seed Collection, Sampling and Overwintering

5.1.1 Test Collector Deployment and Overwintering

Test collectors were successfully deployed in December 2021 and January 2022 and left to overwinter until sample collector plate retrieval began. The collectors for Shoal Bay were deployed in late January by a local fish harvester, and anchors were used instead of concrete blocks. The Deep Bay collectors were also deployed in late January and anchors were used instead of concrete blocks, unfortunately, these collectors disappeared by spring. In hindsight, they were deployed too far into the inlet and frozen freshwater at the head of the inlet likely tore the floats away. Collectors in Oliver's Cove used anchors instead of concrete blocks and moved

slightly once deployed. Lastly, Cobb's Cove was successfully installed with concrete blocks and stayed in place.

5.1.2 Test Collector and Seed Sampling

Samples of wild seaweed were collected from four regions of Fogo Island between May 2022 and October 2022 (Appendix 8.5) and assessed. The primary findings from Deep Bay showed little to no biofouling on all seaweed samples until late August when coffin box and tube worms were seen on *Laminaria longicruris* seaweed samples (Figure 13). Due to the loss of the collector lines in Deep Bay, there were no collector plates to observe. No seaweed samples were taken from Deep Bay after August as it was decided not to pursue this area for deployment of sporophytes by the client.

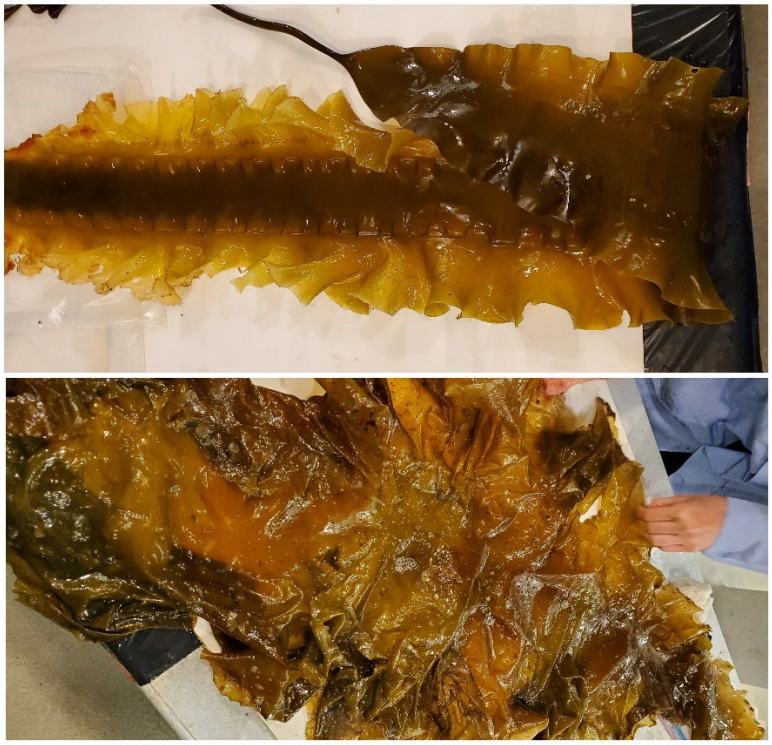

Figure 13 - Top: Sugar kelp sample showing no biofouling Bottom: Laminaria heavily biofouled.

Samples from Oliver's Cove showed very little biofouling overall on all sugar kelp samples, there were some gastropods and mussels present throughout. The sugar kelp was starting to show signs of maturity in May with some darkening and small bumps (Figure 14A). By July, some sugar kelp samples were showing moderate maturity (Figure 14B) and by August there was kelp that looked mature and ready to release spores., with darkening seen on the sorus tissue and other

samples that were still developing (Figure 14C). No samples were assessed in September, but the October seaweed samples continued to show little to no biofouling, mature sorus and some had sign of spore release.

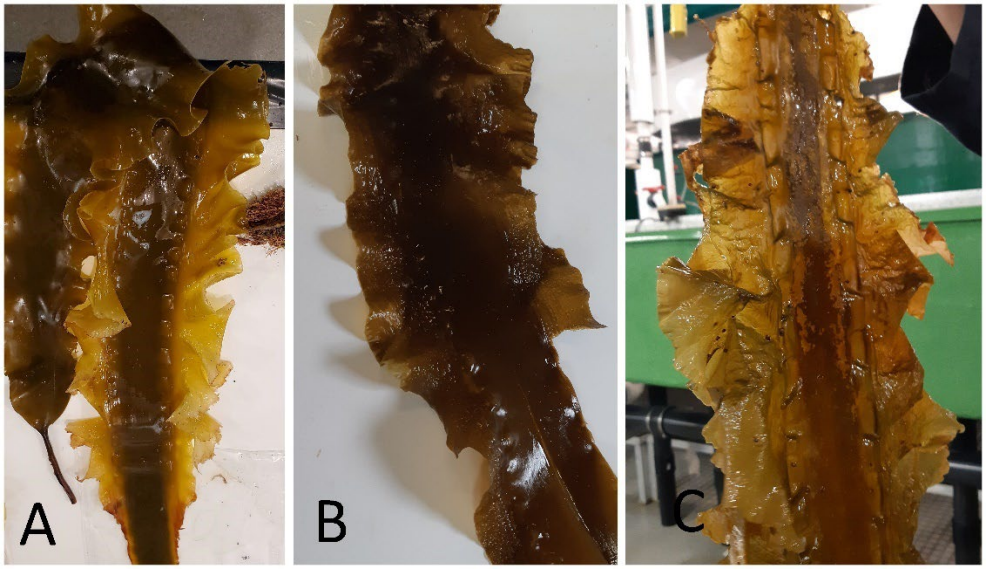

Figure 14 - Seaweed Development in Oliver's Cove during sampling period. A: Maturing sugar kelp frond. B: Moderately mature sugar kelp frond. C: Maturing seaweed sample showing signs of spawning.

Collector plates in Oliver's Cove showed different results over the sampling period. The first sampling indicated no signs of fouling on plates. However, in June there was growth present on the collector plates which looked like early algae growth, as well as small seaweed fronds (Figure 15A). After observing evidence of *Ectocarpus* present in the nursery, the algae growth on the plate was suspected to be *Ectocarpus*. Later in the sampling season, plates showed more growth of algae, along with larger fronds (Figure 15B). Red algae began growing on the plates in August, and in September, there was heavy biofouling on the plates with a small amount of seaweed growth. Coffin Box was present on the collector plates and encased the fronds that were attached (Figure 15C&D).

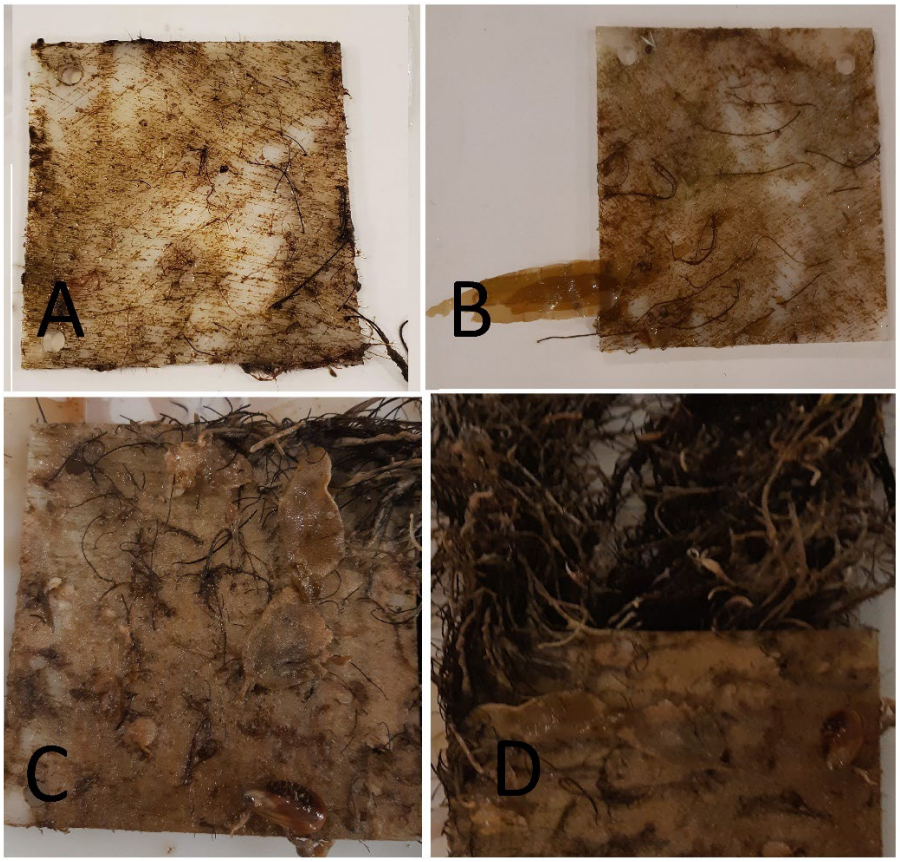

Figure 15 - Plate collector sampling progression. A: Early algae growth. B: Suspected Ectocarpus growth and seaweed fronds. C: Coffin Box biofouling. D: Ectocarpus growth.

Collector plates in Cobb's Cove showed very little biofouling on seaweed fronds in May and June, except for gastropods and mussels. In August, Cobb's Cove seaweed samples indicated more biofouling, including coffin box, tube worms, red algae and starfish, and little reproductive development. There were portions of the seaweed that were bleached out, with reddening on the edges, typical of dead and decaying seaweeds (Figure 16). The last observation for Cobb's Cove, an increased amount of coffin box was observed with reddening edges and little reproductive maturity.

Figure 16 -Seaweed normal (top) and with portions bleached (Bottom).

Collector plates from the top and bottom showed algae growth which was seen on the nursery spools. Throughout the observation process, signs of algae growth was observed in May and continued to increase throughout the sampling period until the last collection in September (Figure 17A&B).

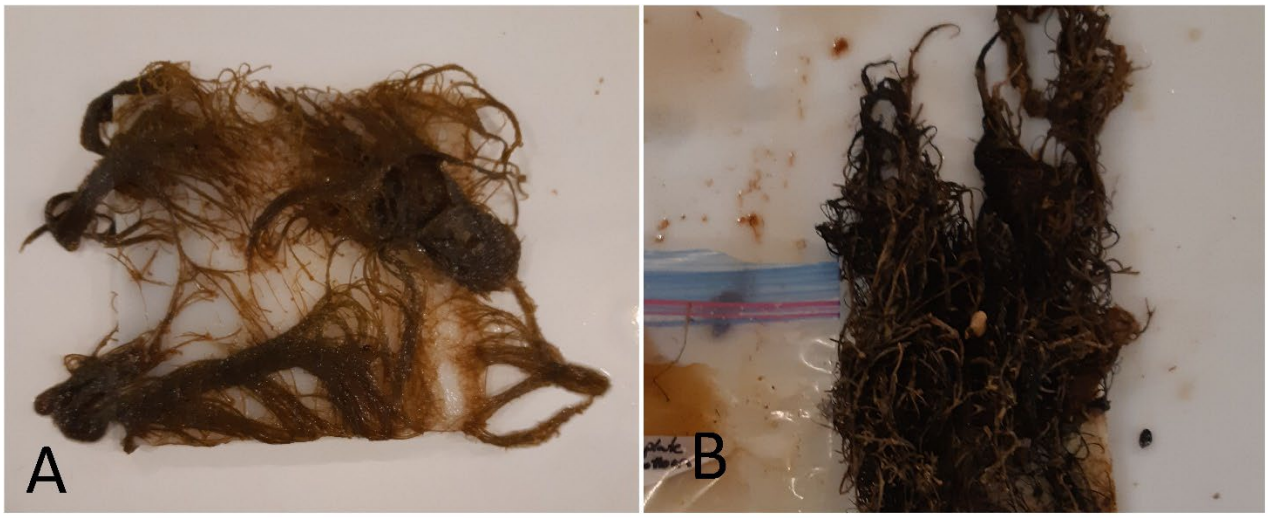

Figure 17 - Ectocarpus growth on plates from May (A) to September (B).

No biofouling was observed in the wild seaweed samples collected at Shoal Bay except for barnacles. However, seaweed showed very little reproductive development and an increase in biofouling, and low pigmentation (Figure 18) beginning in late summer.

Figure 18 - Fouling and low pigmentation seen on seaweed sample.

Collector plates in Shoal Bay had no signs of fouling until late Spring. Algae growth was seen on sample plate 1 and 2 with less on sample plate 2 which had small fronds present. The next set of plate samples assessed in July showed less fouling than the spring samples, but still displayed algae growth. By August, top collector plates were 100% fouled with *Ectocarpus* (Figure 19). The bottom collector plates were fouled with branched macroalgae, mussels, red algae, and green algae. After further analyzing collector plate samples, this confirmed the *Ectocarpus* contamination seen in the nursery came from our wild sorus samples.

Figure 19 - Plate with Ectocarpus growth.

5.2 Seaweed Development

Seaweed development was observed and recorded by regular observation of the spool's appearance by microscope. Samples were taken randomly from one of the two twine loose ends left on the spools for sampling. After observing the piece of twine, the material was scraped using two tweezers, a drop of sample water added, and a cover slip was placed for observation in higher augment. The development of the seaweed attached to the spools was observed for 19 weeks.

At the end of the first week, technologists observed the gametophyte cells settling on the twine fibers (Figure 20). At this point, the cells were round, and it was not possible yet to differentiate between female and male gametophytes. In week two the cells started to elongate, and some sporophytes were observed (Figure 21).

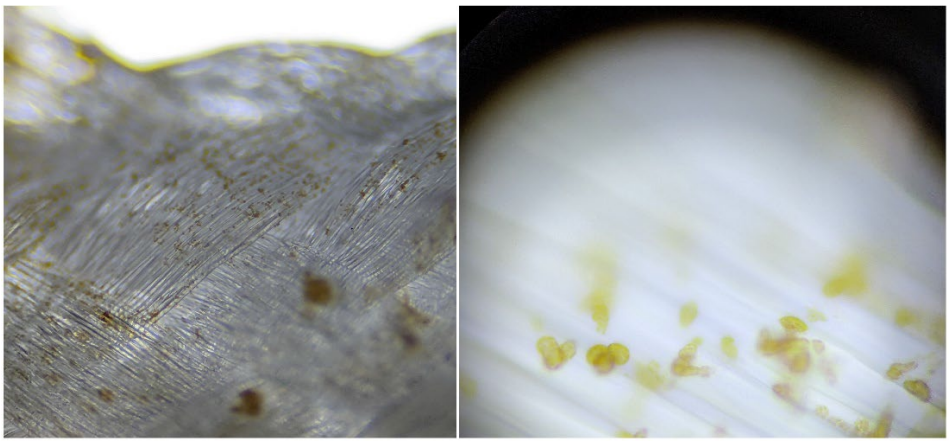

Figure 20 - Gametophyte cells settled at the twine fibers.

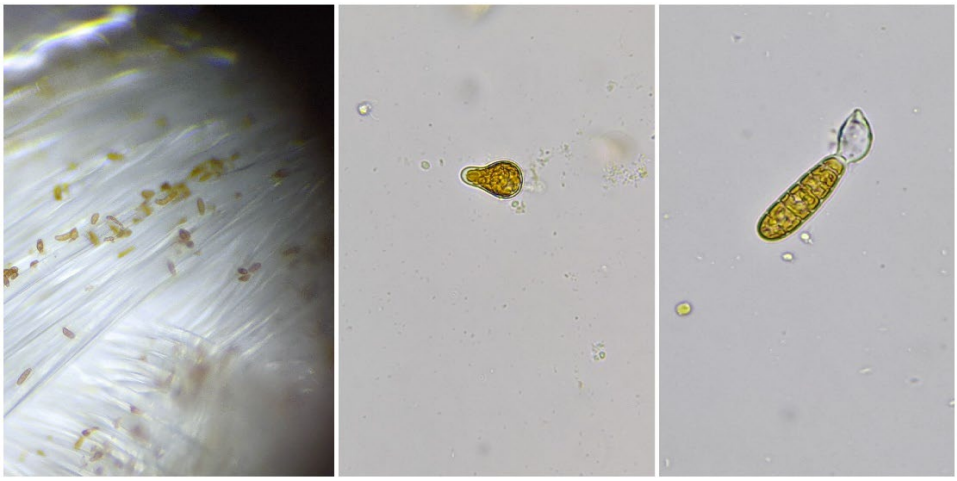

Figure 21 - Elongate shape cells and sporophyte.

During the third week there was an increase in the amount of cells and it was possible to observe the differentiated female and male gametophytes (Figure 22A) and it was also possible to observe the growth of the young sporophytes (Figure 22B). During the time of microscopic observation, the technicians regularly identified gametophytes, eggs, and sporophytes, at different stages of development, at the same location. This indicates that once the reproductive cells settle, they start and continue reproducing and their offspring start settling around them. (Figure 22A).

Figure 22 - (A), FG, Female gametophyte, MG, Male gametophyte, SP sporophyte. (B) Growing sporophytes.

From the fourth week on, the spools gradually changed from white to brown. Microscopically, the technologists observed sporophytes measuring from 100 to 200 µm (Figure 23). On the sixth week the technologists observed sporophytes measuring from 100 to 300µm attached to the twine (Figure 24A) and, after scraping the twine content, they observed a sporophyte with developed rhizome after cytoplasm absorption (Figure 24B). The rhizome is a root-like structure that will develop in the future into the holdfast that anchors the kelp to the substrate.

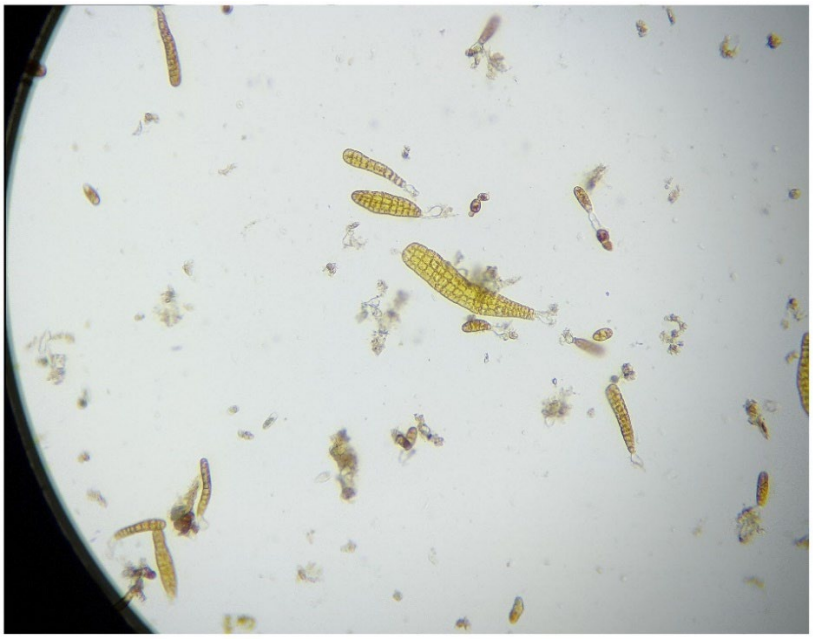

Figure 23 - Week 4, Sporophytes measuring from 100 to 200µ.

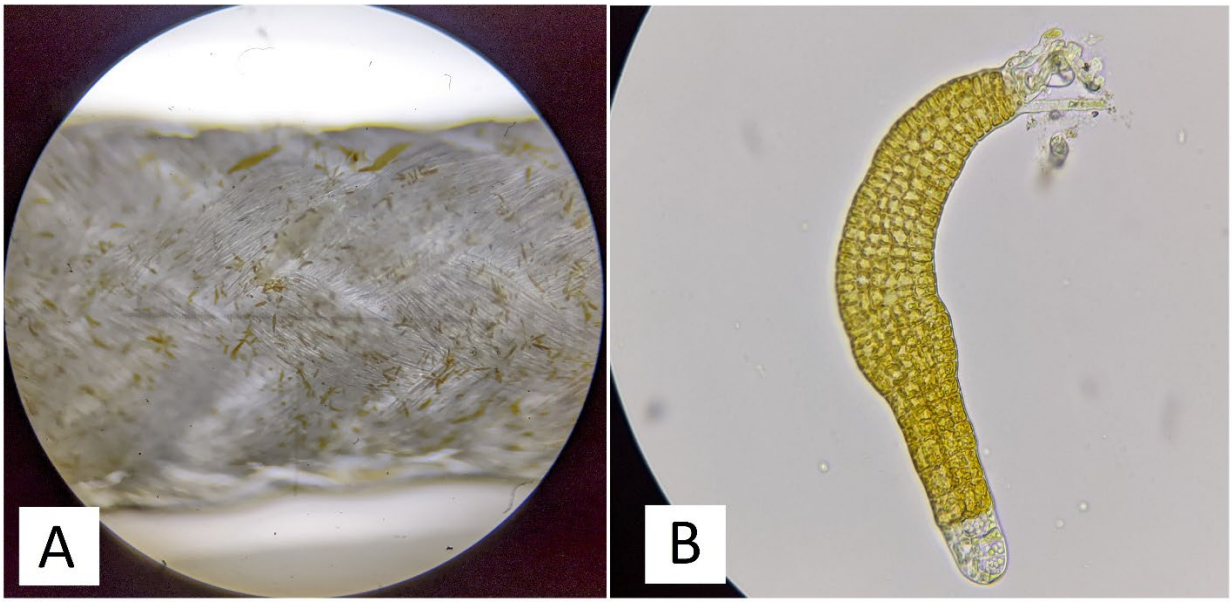

Figure 24 – (A), Sporophytes measuring from 100 to 300µm, (B) Scraped sporophyte with developed rhizome.

Within seven weeks after seeding, the sporophytes sizes ranged from 100 to 400µm, and it was possible to observe their presence and distribution macroscopically (Figure 25). Twine samples were taken weekly until week 10 when the size of the sporophytes ranged from 3mm to 4mm. At this point, microscopy was not necessary to observe the seaweed development. However, during the entire 19-week period, it was possible to find gametophytes and very young sporophytes along with the juvenile sporophytes. CASD technologists assume that the gametophytes settled on the spools, remained alive, kept reproducing, and producing new sporophytes.

Figure 25 - Sporophytes macroscopic observation, week 7.

During the ninth week, the sporophytes were measuring from 200µm to 1mm (Figure 26). CASD technologists observed *Ectocarpus* growing within the sample (Figure 27), an epiphyte filamentous brown alga. The alga growing can inhibit the kelp development by reducing the light while covering the cells or sporophytes and competing for nutrients.

The technicians performed research about possible treatments and procedures that could be used to control the epiphyte growth. Unfortunately, the information about biofouling control in seaweed nurseries is scarce. Some basic information was found in the two available kelp farming manuals (Flavin *et al*., 2013; Redmond *et al*., 2014). Other information was obtained from the seaweed nursery and production community, like "greenwave.org" by the "Ocean Farming Hub" forum and through the shared experience dealing with seaweed nursery routines.

The first corrective measure taken was to reduce the light intensity from full light, 100 µmol-s, to 55µmol-s. However, the *Ectocarpus* continued to spread. Next, it was decided to use a local application of 1 $mL.L^{-1}$ of Germanium dioxide (GeO_2) or 3% hydrogen peroxide (H_2O_2). The procedure was made using a 3mL syringe with a 23-gauge needle (Figure 28A). The spools were removed from the tank (Figure 28B), the *Ectocarpus* was removed using tweezers and scissors, and the treatment applied at the spot where the alga were attached.

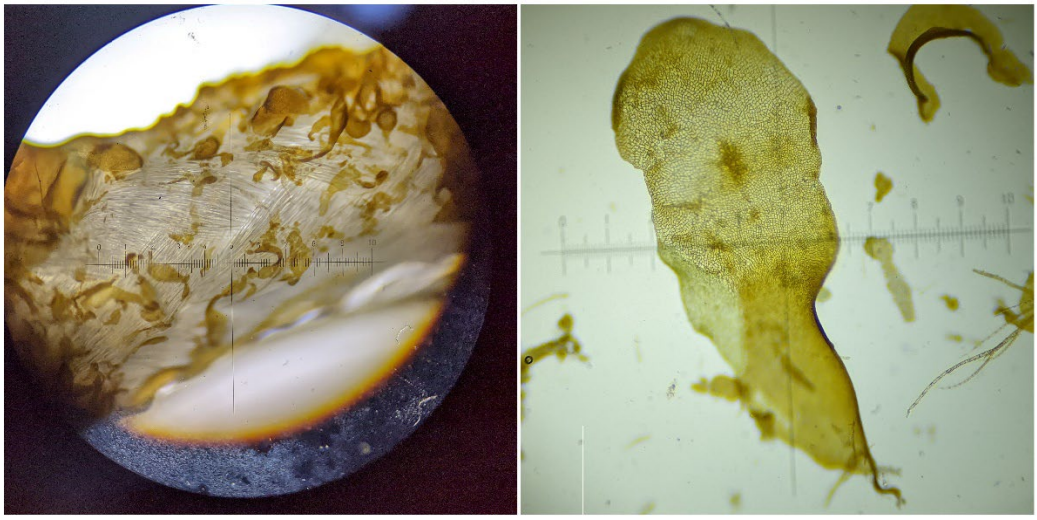

Figure 26 - Sporophytes measuring 200µm to 1mm.

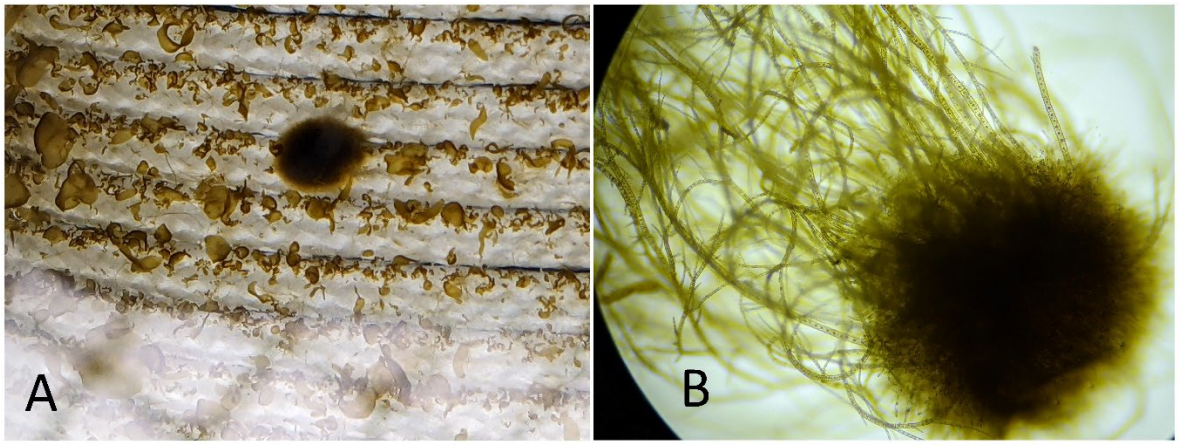

Figure 27 - Ectocarpus, (A) Macroscopic and (B) Microscopic.

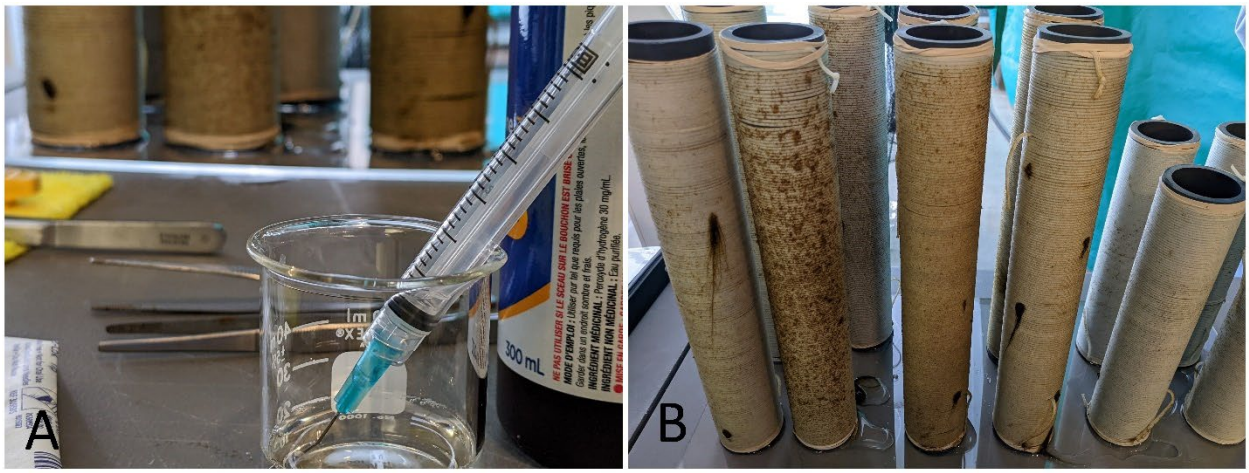

Figure 28 - Local treatment for Ectocarpus.

During week 11, technologists observed that the spools treated with hydrogen peroxide had blank spots where the chemical was applied (Figure 29). However, these spots filled back in with sporophytes during subsequent weeks. After these treatments, and until the end of the nursery period, the *Ectocarpus* had their growth impeded but they still appeared to spread along the spools (Figure 30). To reduce the *Ectocarpus* development, each week, during the water change and nutrient renewing procedure, the spools were taken out of water for 15 minutes. The *Ectocarpus* is sensitive to desiccation, so this procedure slowed the algae growth and helped the sporophytes to overcome the epiphyte in the spools. As the seawater used by the nursery is UV treated and mechanically filtered, CASD technologists believe that the *Ectocarpus* was introduced in the nursery by the sorus tissue used for spore release and started to grow as a result of the delay in the sporophyte development.

Figure 29 - Blank spot after Hydrogen peroxide treatment.

Figure 30 - Ectocarpus spread along the spools.

By the end of the nursery period, the mass of developed juvenile sporophytes did not allow for the proper measure and observation of other phases present on spools (Figure 31). To reduce the kelp's growth and to preserve it for a possible deployment, the light intensity was decreased to 20µmol-s during week 14 and nutrients were added at half strength beginning week 16. At this time, sporophytes ranged in size between 2cm and 5cm. As the deployment of the kelps at the test plot sites were delayed, CASD technologists observed signs of deterioration on some of the

kelp spools (Figure 32). This may have occurred because of the attempt to slow down the *Ectocarpus* growth or because of the extension in the nursery time.

Figure 31 - Kelp growth in spools on week 19.

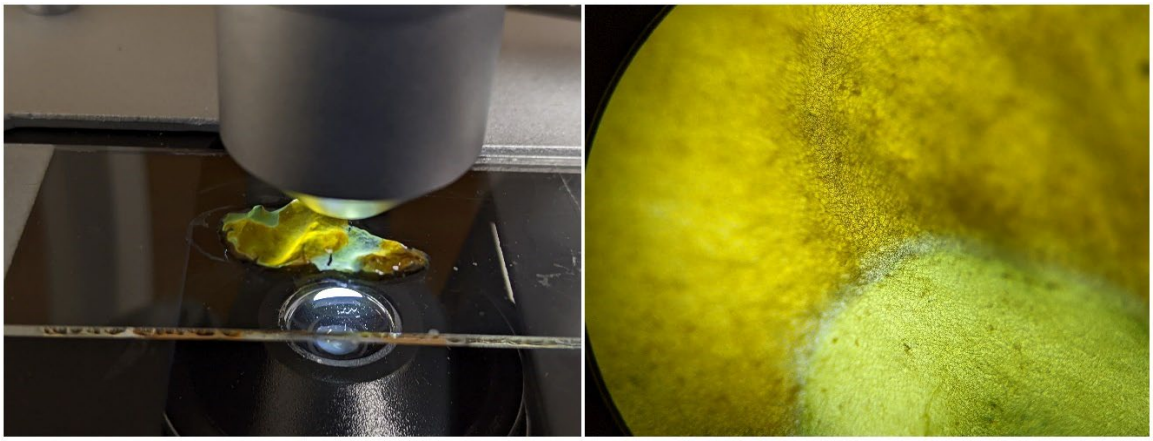

Figure 32 - Microscopy viewing of kelp deterioration.

CASD technologists observed several differences between this strain of sugar kelp and those reported in the Kelp Farming Manual (Flavin *et al*., 2013) and the New England Seaweed Culture Handbook (Redmond *et al*., 2014). The timeline for development needed to be extended three

to four weeks compared to strains reported in the literature. This is assumed to be due to a combination of factors including:

- The strain of sugar kelp used for spore release,
- The temperature of the tanks, and
- The light intensity and regimen which was not possible to reproduce identical to the literature.

However, these procedures produced spools containing well-developed and healthy sugar kelp sporophytes for deployment on the test lines.

6 CONCLUSIONS AND RECOMMENDATIONS

CASD technologists concluded that regular monitoring of the light intensity, water quality, and the addition of nutrients resulted in the proper development of seaweed and the identification of biofouling. The quality of the sorus collection, as well as the disinfection procedures prior to spore release, ensured the control of protozoan and other organisms until week 8. The developmental stages of the seaweed proceeded as expected based on the literature with the exception of needing a longer timeline (likely due to nutrient competition by *Ectocarpus* and over-ripe sori in late October). CASD technologists assume this to be a result of the strain of sugar kelp collected in addition to the contamination of *Ectocarpus* slowing seaweed growth. The nursery lighting protocols, and the water temperature, may have also impacted our growth progression, however, these procedures produced 12 spools containing well developed sugar kelp sporophytes from seaweed collected in Fogo Island.

For future experiments, CASD technologists recommend following the lessons learned in the MI nursery. Some recommendations include:

- ❖ It is important to keep track of each spool by numbering them. This will help researchers/producers observe sporophyte growth progression and react to issues that may impact the spool's health. It would also be beneficial to easily track the development of the seaweed after deployment.

- During the execution of this project, CASD technologists adapted a method for estimating the length of young sporophytes using ImageJ software. This methodology will be beneficial to future projects by providing clients with sporophyte growth data.
- Prior to spore release, clean and disinfect the sorus tissue and autoclave the UV and mechanically filtered seawater to further decrease the potential of contamination during the spore release process.
- Collect mature sori earlier in the season to enable sporophyte production in August or September for deployment in October or November when the kelp will grow best.
- Cleaning off as much biofouling as possible prior to transporting wild collected seaweed may help reduce the possibility of contamination in the nursery.
- Prior to wrapping the twine onto spools, soak the twine in deionized water and let it dry thoroughly to ensure no twine residue leaches into the tanks.
- Standardize the sample collection method by recording which numbered spools are sampled each week.
- In addition to observing each spool's appearance, CASD technologists recommend weekly microscopic observations of the gametophytes and sporophytes attached to the twine to collect measurements and photographic records. These records are necessary to understand the seaweed's development, assess the health of the sporophytes, and identify possible biofouling.
- Do more research to become more familiar with *Ectocarpus* and identify better ways of eradicating it from the nursery.

The experience gained from the seaweed nursery and collector plate analysis, provides more insight on how to streamline seaweed nursery practices and the development of future research involving seaweed seed collection, replication, stocking, and grow out on Fogo Island.

7 REFERENCES

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Yarish, C., J.K. Kim, S. Redmond, C.D. Neefus and L. Green. 2014. Part 1-6. Seaweed Culture in New England. https://seagrant.uconn.edu/2014/01/01/new-england-seaweed-culture-handbook-nursery-systems/ 93 pp

8 APPENDICIES

8.1 Daily and Weekly Seaweed Maintenance Charts

H. Nursery Daily Maintenance Checklist

Nursery Daily Maintenance Checklist							
Task	**Sun**	**Mon**	**Tues**	**Wed**	**Thurs**	**Fri**	**Sat**
Check nursery air temperature							
Check aquaria water temperature							
Overall sound inspection							
Overall smell inspection							
Check for leaks in plumbing							
Visual health inspection of spools							
Visual inspection of aquaria water visibility							
Check all lights and timers							
Rotate spools							
pH readings							
Clean/disinfect nursery equipment and aquaria							
Clean plexi glass aquaria lids							
Notes:							

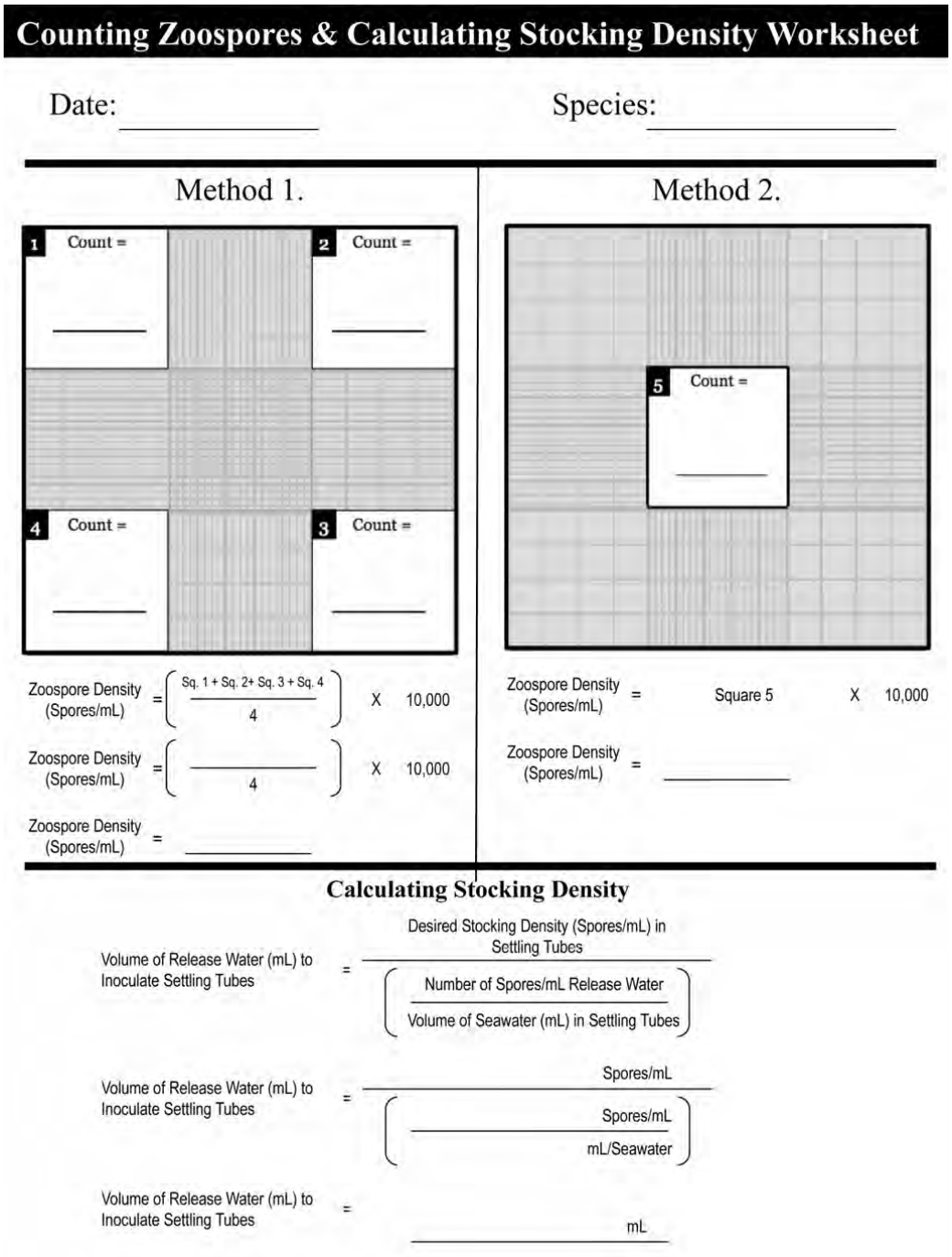
Daily Nursery Maintenance Checklist from Flavin *et al*. (2013)

Shorefast
Fogo Island
Oct. 29-30/22: Spores released. Oct. 31/22: Spools transferred

Nursery Weekly Maintenance Checklist										
Task	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
Date										
Daily Nursery Checklist Completed										
Aquaria Nutrients Added										
Spools Transferred										
Twine Sample Collected										
Twine Sample Observed										
Data Record Updated										
Spool photos taken										
Light Wattage										
Water Change Complete										
Equipment/ Aquaria Cleaned										
Disinfect Holding Tank (ClO2)										
Notes:										

Example template of weekly nursery maintenance checklist.

8.2 Zoospore Counting and Stocking Density Calculations



Counting Zoospores & Calculating Stocking Density Worksheet

Date: _____ Species: _____

Method 1.

1 Count = _____ 2 Count = _____

4 Count = _____ 3 Count = _____

Zoospore Density (Spores/mL) = ((Sq. 1 + Sq. 2+ Sq. 3 + Sq. 4) / 4) X 10,000

Zoospore Density (Spores/mL) = (_____ / 4) X 10,000

Zoospore Density (Spores/mL) = _____

Method 2.

5 Count = _____

Zoospore Density (Spores/mL) = Square 5 X 10,000

Zoospore Density (Spores/mL) = _____

Calculating Stocking Density

Volume of Release Water (mL) to Inoculate Settling Tubes = Desired Stocking Density (Spores/mL) in Settling Tubes / (Number of Spores/mL Release Water / Volume of Seawater (mL) in Settling Tubes)

Volume of Release Water (mL) to Inoculate Settling Tubes = Spores/mL / (Spores/mL / mL/Seawater)

Volume of Release Water (mL) to Inoculate Settling Tubes = _____ mL

Zoospore Count and Stocking Density Calculation Worksheet (Flavin *et al*. 2013).

8.3 Nutrient Formulations and Concentrations

Provasoli's Enriched Seawater (PES) Culture Media	
Solution I: Base Solution Deionized Water $NaNO_3$ Na_2glycerophosphate Thiamine-HCl (Vit. B1) Tris buffer	**1000mL Quantity** 1000mL 2800mg (2.8g) 400mg (0.4g) 4mg (.004g) 4000mg (4g)
Solution II: Fe (as EDTA complex; 1:1 molar) **Deionized water** **$Fe(NH_4)_2(SO4)_2 \cdot 6H_2O$** **$Na_2EDTA$**	 250mL 175mg 150mg
Solution III: Metals Deionized Water $MnSO_4H_2O$ (manganese sulfate monohydrate)	 200mL 26.0mg
Solution IV: Vitamins Deionized Water Vitamin B12 Biotin	 1000mL 2.0mg 1.0mg
PES Culture Media **Solution I: Base Solution** **Solution II: Fe (as EDTA complex 1:1 molar)** **Solution III: P II Metals** **Solution IV: Vitamins**	 1000mL 200mL 200mL 1mL

Nutrient formulations used throughout the culturing process (Flavin *et al*. 2013).

Table 3.4 Summary of Nutrient Concentrations Used in the Ocean Approved Nursery

Release Beaker	Settling Tubes	Aquarium
1000 mL seawater	2300 mL seawater	20 gal (76,000 mL) seawater
9 mL PES	21 mL PES	700 mL PES
0.9 mL vitamins	2 mL vitamins	70 mL vitamins
0.8 mL GeO_2	2 mL GeO_2	60 mL GeO_2

PES: Provasoli's Enriched Seawater; GeO_2: germanium dioxide;

The nutrient concentrations used in the seaweed nursery process, Flavin *et al*. 2013

8.4 Light Intensity Schedule

Light Intensity		
Days 1-14	~20 µmol-m^{-2} S^{-1}	Fine Mesh Screen
Days 15-28	~55 µmol-m^{-2} S^{-1}	Wide Mesh Screen
Day 29+	100 µmol-m^{-2} S^{-1} full light	No Screen

Table: Light regime schedule used during St. Mary's seaweed culturing. Flavin *et al*. 2013).

8.5 Seed and Plate Collection Observations

Date	Location	Observations
May 30th, 2022 Seaweed	Deep Bay	- No obvious signs of fouling - Both pieces of seaweed were *Laminaria longicruris* - Seeing signs of maturity
	Oliver's Cove	Sugar kelp: -no obvious signs of fouling -No full fronds collected -Noticed some bumps on mid portion -Some darkening
	Shoal Bay	Seaweed not in great shape (holes, tears, missing pieces)
May 30th, 2022 Collector Plates	Cobb's Cove #1	- A lot of collection on the collectors from Cobbs Cove #1. - A lot on the bottom of the bucket as well. - 60% covered, slight fouling - hard to determine what fouling is on collectors
	Cobb's Cove #2	- Some collection on #2 collectors but not as much as the #1 collectors - Looks like early *Ectocarpus* growth
	Oliver's Cove	-No obvious collection on plates, no signs of fouling
	Shoal Cove #1 and #2	-No obvious collection on rope upon gross observation. -Very little collection. Saved in jar. -Plates: No obvious gross observations.
June 17th, 2022 Seaweed	Deep Bay	- *Alaria* starting to produce spores/sori - Sugar Kelp: No biofouling or development of spores - Solid stipe and no biofouling
	Oliver's Cove	- Sugar kelp: very early start of development, no biofouling, one tube worm - *Alaria*: starting to produce spores - Mussels and gastropods starting to settle (late Fall set or secondary set) - All *Alaria* collected from here had no to little fouling (early mussel fouling)
	Cobb's Cove	-Sugar Kelp: little biofouling, little set of mussels and gastropods -Deformity: dark line on edge of one side of the sugar kelp.

		-Early development of maturity, just starting to produce spores on all the pieces of *Alaria*. -This *Alaria* has broader leaf than other sites.
	Shoal Bay	-Sugar kelp: red epiphyte on two pieces -No biofouling -Early development -*Laminaria longicruris*: hollow stipe and the stipe is longer, no biofouling, a deformity where the kelp was split
June 17th, 2022 Collector Plates	Oliver's Cove	Top #1: Little fouling on plates, looks like early-stage growth of algae, most likely *Ectocarpus*. -small fronds growing on plate Bottom #1: Little biofouling on plate, small gastropods and tube worms. -Small fronds growing on plate
	Cobb's Cove	Top #1: similar looking to bottom plate, so mussels or gastropods present Bottom #1: Plate looks slimy, growth of algae -gastropods present Top #2: Little biofouling on plate, ~20% covered, looks like *Ectocarpus* growth Bottom #2: ~50% covered in *Ectocarpus* -Some gastropods present
	Shoal Bay	Top#1: Longer fibers seen, *Ectocarpus* growth ~80% coverage Bottom #1: Fibers present, *Ectocarpus*. Less than plate #2 Top #2: Lightly fouled with fibrous texture, likely *Ectocarpus* Bottom #2: Lightly fouled, less than top, small fronds present
July 15th, 2022 Seaweed	Deep Bay	-No collector plates -*Laminaria longicruris* 1: Has hold fast attached to rocks, sea star, little biofouling (barnacles), no maturity -*Laminaria longicruris* 2: Has holdfast attached to mussel and rock, long thick stipe, no maturity -*Laminaria longicruris* 3: No maturity, little biofouling (barnacles)
	Oliver's Cove	-Sugar kelp 1: minimal biofouling, mostly gastropods and mussels, moderate maturity -Sugar kelp 2: minimal biofouling, mostly gastropods and mussels, moderate maturity -Sugar kelp 3: No biofouling, no maturing

		-Sugar kelp 4: little fouling with gastropods, low maturity, deformed with 3 frills -Sugar kelp 5: small number of gastropods, maturing -Alaria: No biofouling, starting to develop spores
	Cobb's Cove	-*Alaria* 1: no biofouling, very little maturity -*Alaria* 2: A lot of holes in seaweed, reddish tint around holes, not mature -Sugar kelp very little maturity, a little of a hairy type of biofouling
	Shoal Bay	-Sugar Kelp 1: Some biofouling, large number of barnacles, dirty, not maturing -Sugar Kelp 2: Some biofouling, large number of barnacles, dirty, not maturing. -Sugar Kelp 3: Some biofouling, large number of barnacles, dirty, not maturing -unknown species (long flat) -*Alaria*: no signs of maturity
	Wild Cove Tilting	-*Alaria* 1: Little biofouling (gastropods), Maturing -Sugar kelp 1: Screw like end, deformed with one side not frilly, no maturity -Sugar kelp 2: maturing
July 15th, 2022 Collector Plates	Oliver's Cove	Top: Looks heavily fouled in *Ectocarpus* ~90% Bottom: Large frond coming from the plate along with some smaller fronds. Small *Ectocarpus* growth, not as heavily fouled as top plate ~60%
	Cobb's Cove	Top and Bottom#1: Unknown algae (maybe a type of sea lettuce), no biofouling -2nd look, could be *Ectocarpus* growth, plates ~60% fouled Top #2: Looks gooey and small amount if algae growth ~70% covered Bottom #2: ~40% fouled with algae growth and light fouling on plate
	Shoal bay	Top #1: Fouled and looks like *Ectocarpus* ~60% Bottom #1: Lightly fouled, ~30% but growth present, could be *Ectocarpus* Top #2: Plate is heavily fouled in algae, looks like *Ectocarpus*, ~80% Bottom #2: Algae growth but less than top, looks like *Ectocarpus,* ~40% coverage

August 23rd, 2022 Seaweed	Deep Bay	-No Collector Plates -*Laminaria longicruris* – Large frond, fat, hollow stipe, biofouling was coffin box, old and dirty frond. -*Laminaria longicruris* – no stipe, old frond, lots of biofouling, coffin box, tube worms.
	Oliver's Cove	- Sugar Kelp: mature, developed, looks ready to spawn, lots of darkening, little to no fouling, 2m length. -Sugar Kelp: Not as dark as bag 1, partially developed, patchy dark spots, no obvious reproduction signs, little to no fouling, 2m length. - Sugar Kelp: Developing, no signs of reproduction, 2m length, little to no fouling. -*Alaria*: 3 fronds, not seeing and sporangia, no signs of reproduction, no fouling.
	Cobb's Cove	-Sugar Kelp: 1 large and 1 small frond, bleached (dying), biofouling, narrow small frond had tube worm, little reproductive development. -Sugar Kelp: 2 fronds, wider frond had lots of coffin box, bleached out, lack of nutrients, little reproductive development, red algae biofouling. -Sugar Kelp: half meter in length, red biofouling on edges of seaweed, bleached out, brown and red seaweeds, looks unhealthy. -*Alaria*: 2 small fronds, hard to observe anything. -Sugar Kelp: Short stipe, not hollow, coffin box, tubeworms, red algae, starfish, partially developed for reproduction.
	Shoal Bay	-Sugar Kelp: Some encrusting, Some biofouling (small amount of coffin box, tube worm shells, no reproduction) Sugar Kelp: Broader leaf, very little reproductive development. -Sugar Kelp: Lots of biofouling (incrusting worm), beat up, low pigmentation, no development. Sugar kelp: Lots of coffin box biofouling, encrusting tube worms, some gastropods and bivalves, large frond and small (2 fronds), small amount of reproductive development.
August 23rd, 2022 Collector Plates	Oliver's Cove	-Bottom Plate: *Laminaria* settling, gastropods and mussels settling, red algae (looks like *Polysiphonia sp*) growth -Top Plate: Less settlement than the bottom plate, gastropods and mussels settling, red algae growth

	Cobb's cove	-Bottom Plate 1: Mussels, lots of algae. 90-100% coverage. -Top Plate 1: Lots of mussels, filamentous branched algae. 90-100% coverage. -Bottom Plate 2: No Mussels, brown algae. 50% coverage. -Top Plate 2: Polychaete, green and brown algae, fragrant. 80% coverage.
	Shoal Bay	Top Plate 1: Lot of biofouling with 100% coverage. Filamentous algae, small frond of *Laminaria.* -Bottom Plate 1: More biofouling than top plates, red filamentous algae are one species. 100% coverage. -Top Plate 2: Lot of biofouling with 70-80% coverage. Green algae on one side of plate, mussels, green tubular algae (possibly *Polysiphonia sp*) -Bottom Plate 2: Biofouling with green algae, red algae, tubular, branched macroalgae and mussels. 70-80% coverage.
September 2022 Collector Plates	Oliver's Cove	Top Plate: Lots of heavy biofouling from algae growth, *Ectocarpus*? -There is a small amount of seaweed growth that is attached to the plate -Coffin box present on the collector plate and on attached fronds -Some mussels present Bottom Plate: -Much less biofouling on plate than top -Small red leaves attached -Looks like algae growth on plate, could be the beginning of *Ectocarpus* growth.
	Cobb's Cove	Top Plate#2: Lots of bio fouling, mussels are easily seen -Brown algae (*Ectocarpus*) -*Polycaete* Bottom Plate#2: Biofouling seen, less than top but mussels are present Brown algae present Top Plate#1: Very heavy biofouling, mussels, sea stars, *Polychaetes* can be seen on the plates, etc -Lots of algae growth Bottom Plate#1: Biofouling less than top plate -*Ectocarpus* growth on 80% of plate -*Polychaetes*, small mussels present

October 12th, 2022 Seaweed	Oliver's cove	-Sugar Kelp: 4 fronds, very little biofouling, mature, younger fronds ~1-2 yrs, ready for nursery culture. -Sugar Kelp: Both mature, nice-looking fronds, very little biofouling (gastropods and coffin box on one), ready for nursery culture. -Sugar Kelp: Nice fronds (4), 3 mature sori, little biofouling (some coffin box on tips). -Sugar Kelp: 2 fronds showing signs of sori release, very little biofouling. -Sugar kelp: 1 frond, little biofouling (*Polychaete*), mature sorus and ready to release sori. -Sugar kelp: 2 fronds, distal portion has coffin box, little biofouling, indication of maturity. -Sugar kelp: 2 fronds, one with coffin box mid frond, full maturity and ready to release spores. -Sugar Kelp: 2 fronds, one very healthy looking and the other not well developed (early release or degenerating).
	Cobb's Cove	Sugar Kelp: 2 fronds, lots of biofouling (coffin box), not so great samples with reddening on the edges (environmental conditions), little maturity near bottom of second frond
October 12th, 2022 Collector Plates	Oliver's Cove	Collector Line and Plates: -A lot of fouling on ropes -Bottom Plate: *filamentis* seaweed growing, mussels present, *Polysiphonia* and small *laminaria*/ kelp -Top Plate: very little biofouling, some small seaweed fronds (*Polysiphonia*)