Seaweed Nursery Research to Support a St. Mary's Seaweed Farm

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MEMORIAL UNIVERSITY

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EXECUTIVE SUMMARY

The scope of this research was to establish a seaweed nursery for one species of sugar kelp (*Saccharina latissima*) and to produce viable lines for deployment in St. Mary's Bay. This work was performed in conjunction with HoldfastNL (the client) who are assembling a seaweed aquaculture farm in St. Mary's Bay, NL.

Two of the intermediate research opportunities included:

1. Nursery sporophyte production at the Marine Institute, Memorial University of Newfoundland, which entailed collecting sporophytes with mature sorus tissue, releasing the spores, inoculating the spools, and growing the gametophytes to for deployment by the client at the St. Mary's Bay farm site.
2. A seaweed nursery procedures manual, including lessons learned and adapted methods for commercial production of sea kelp in Newfoundland.

It was shown through nursery production for this pilot project that Newfoundland strains take longer to grow to farm ready size compared to what was observed in the literature. St. Mary's seaweed started to show visible growth at 4 weeks in the nursery, which was later than the 3 weeks which the manual stated. According to the manual, seaweed would stay in the nursery for six to seven weeks before it was ready for on growing deployment. The spools produced at the Marine Institute nursery were ready for deployment at week 10. However, due to logistics and planning delays, the spools were deployed during week 13.

The growth of St. Mary's seaweed compared to the production cycle in the manual, differed by approximately three to four weeks. However, CASD technologists can conclude that the structure of the nursery and the protocols for water quality, light regime and nutrients ensured the successful production of healthy sporophytes for deployment at the St. Mary's Bay farm site.

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1 BACKGROUND AND INTRODUCTION

HoldFastNL Seaweed Farm Inc. is a company formed in 2022 exclusively for developing seaweed farming in Newfoundland. The company founder, Michael Teasdale, M. Sc., is a senior marine scientist with more than 20 years of experience with a focus on marine ecology and carbon foot printing. He has worked as a graduate student and technician in three separate marine laboratories (Friday Harbor, Chesapeake Biological Laboratory, and Turkey Point Labs) on three coasts of North America on field, and wet laboratory flume and tank studies on copepods, corals, algae, and polychaetes. He also has extensive experience in Newfoundland on coastal environmental and habitat monitoring leading the marine environmental components in many of the recent megaprojects on the island in the last fifteen years. He is currently leading work for DFO on the carbon footprints of salmonid aquaculture. He also is certified in ISO 14064:1-3. His most recent and relevant experience has been the analysis and comparison of carbon footprints related to various aquaculture technologies for Fisheries and Oceans Canada (DFO). He is also first author on a recent essay for the Journal of Ocean Technology (for current Spring 2022 issue) on using seaweed as a carbon sink and the constraints and opportunities associated with.

Seaweed farming produces 27 million metric tonnes annually and is the largest marine aquaculture market by weight with a large majority (99.5 % of the farmed seaweed) of seaweed production from Asia. The seaweed market is growing in North America and Newfoundland and Labrador (NL) is ideally situated to play a large role in that growth, as there are the right people, the right infrastructure, and the right environment.

The people of NL have generations of experience on the water with many of the skills already in place for seaweed farming. Like other areas in North America developing seaweed operations (i.e., Alaska and Maine), there are numerous small rural fishing communities with limited economic opportunity where supplemental farming in the offseason could prop up the local economies. In addition, community members already have skillsets and equipment (i.e., working on the water using moorings and fishing gear) that could easily be adapted to seaweed farming.

NL also has the existing infrastructure and resources including marine research centres focused on marine technology and aquaculture developments (Marine Institute, Ocean Science Centre) as well as fish plants that are only seasonally active that could potentially be used for processing and nursery activities. With over 17,542 km of coastline, most of which is relatively untouched and sparsely developed, and native populations of commercial seaweed species, NL has the requirements necessary to develop a commercial seaweed industry.

A common theme of all the commercially viable operations in North America is investment by governments and industry in the local marine research to help with the methods of growing as well as commercialization of the products. NL invested in seaweed harvesting in the early 1990s (as well as currently heavily invested in fish farming) but a similar initiative needs to be launched now for seaweed farming so this province can take advantage of the current and expanding markets of seaweed for human consumption and the developing blue technologies.

2 SCOPE AND PURPOSE

2.1 Scope of Project

The scope of this project was to collaborate with HoldfastNL to build and develop a nursery for sugar kelp (*Saccharina latissima*) seed spool production. Work was performed in the Centre for Aquaculture and Seafood Development's (CASD) aquaculture facility located at the 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 8-10 weeks, sporophyte development was closely monitored to understand how sugar kelp can be produced here in Newfoundland.

3 OBJECTIVES

The immediate research objectives for this project included the following.

3.1 Nursery Development

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

3.2 Nursery Sporophyte Production

With the assistance of CASD technical personnel, sugar kelp was produced successfully, and observations were made from the gametophyte to the mature sporophyte stage.

The life cycle of *Saccharina latissima* (Figure 1) is characterized by a heteromorphic alternation of generations between adult macroscopic sporophytes and microscopic gametophytes. The adult sporophytes release spores that settle into the substrate and differentiates on female and male gametophytes. The female gametophyte produces eggs that are fertilized by the sperm produced by the male gametophytes. The fertilized eggs develop into a zygote that will develop into sporophytes which then grow into adult seaweed.

The process of developing the sporophytes at the Marine Institute included nursery construction, mature sorus tissue collection, spore release, spool inoculation, sporophyte growth observation and maintenance, and preparation for ocean deployment into St. Mary's Bay. The seaweed was closely monitored to observe the growth and learn methodological adaptations necessary for successful sugar kelp growth here in Newfoundland.

3.3 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 adapt methods to Newfoundland's strain of Sugar kelp (Appendix 8.1).

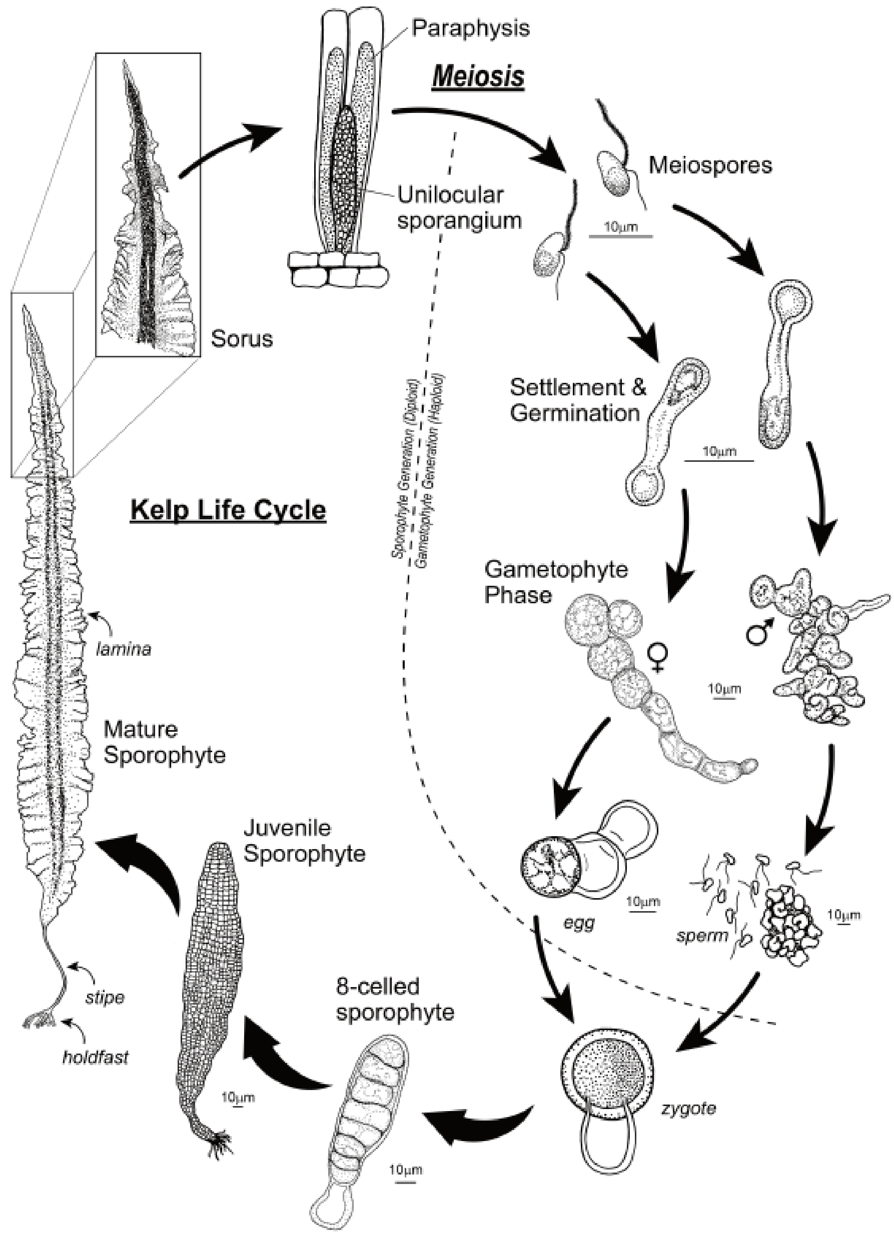

Figure 1 - The Kelp life cycle, Courtesy of C. Yarish (Illustration by Virge Kask, 2012 ©Charles Yarish)

4 METHODS

4.1 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, PVC pipe, 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 and disinfected prior to use. This process was completed by mimicking the final tank design setup. 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. The chlorinated water was run through the entire systems. All four systems sat with bleach for 24 hours and was 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 run 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 2).

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 3). 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 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.

Dry spools and tubes were put into clean bags and brought to Marine Institute's machine shop where 32 pipes were wrapped in 2mm 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 wrapped in clean bags and kept in the freezer until ready to use (Figure 4).

Figure 2 – Assembled seaweed nursery. Top: Front view of nursery setup with rack, chillers, tanks, and lighting panel. Bottom: Over head view of spools in tank.

Figure 3 - 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.

Figure 4 - Spool crafting.

Filtration was set up for 8000L of seawater that was delivered by 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), ultraviolet (UV) filtered at 3 gal/min, then filtered through two more 1.0µm filters (ceramic and carbon) before being pumped into the nursery tanks (Figure 5).

Figure 5 - Seawater filtration system. Left: Ultraviolet and mechanical filtration of seawater. Right: Mechanical filtration.

The nursery setup (Figure 2) had a baker's rack with two shelves. The bottom shelf had two chillers that were connected to a power bar and tubing connected to the inlet and outlet ports. Chillers were covered by a corrugated plastic sheet to prevent any water damage. The top shelf had 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 with a 20 µm filter and a pipette was placed inside the tank to provide aeration through the attachment to the facility air supply bank. Plexiglass lids were placed on top of the tanks to protect the seaweed from potential contamination.

Lastly, the lighting and timers were put in place, as 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 µmol m^{-2} s^{-1}, 55 µmol m^{-2} s^{-1}, 100 µmol 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.

4.2 Nursery Sporophyte Production

Prior to seaweed spore release, the wet lab in the Marine Institute's aquaculture facility was cleaned, disinfected, and prepped for the release. In reference to Flavin *et al*. (2013), necessary materials for sorus preparation and spore release were obtained and set up (Figure 6).

Figure 6 - Materials for sorus prep and spore release

Following the procedures specified by Flavin *et al*. (2013), the mature sorus were removed from the mature kelp collected in St. Mary's, Newfoundland. Sorus was separated from the non-reproductive kelp with a clean razor blade (Figure 7).

Excess biofouling was removed by gently scraping the surface of the sorus using the razor blade. The sorus was cleaned with paper towel on both sides to remove any mucilage. The sorus tissue was disinfected by dipping it in a 3% iodine solution for 30 seconds, then thoroughly rinsing with 10°C filtered seawater. The sorus was dried with paper towel and carefully placed in single layers between paper towels 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 8).

Figure 7 – Mature kelp collected at St. Mary's (A) and sorus tissue removed (B).

Figure 8 - 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 set the water temperature to 10°C. Settling tubes were filled with approximately 2 liters of filtered seawater, covered with tin foil, and placed in the tanks overnight to reach proper temperature.

The spore release occurred 24 hours post sorus desiccation in the 10°C refrigerator. The spools were removed from the freezer to thaw, and the dried sorus were placed into 1-liter beakers containing culture nutrients and 10°C filtered seawater for 30 min to 1 hour, stirring every few minutes (Figure 9). The temperature was recorded every 5 minutes and a sample of water from the release beakers was checked using a hemocytometer to calculate stocking density of the zoospores (Figure 10). A calculation was used to determine spore concentration and how much was needed to inoculate the settling tubes (Appendix 8.2).

Figure 9 -Sorus placed in saltwater with nutrients for Spore release.

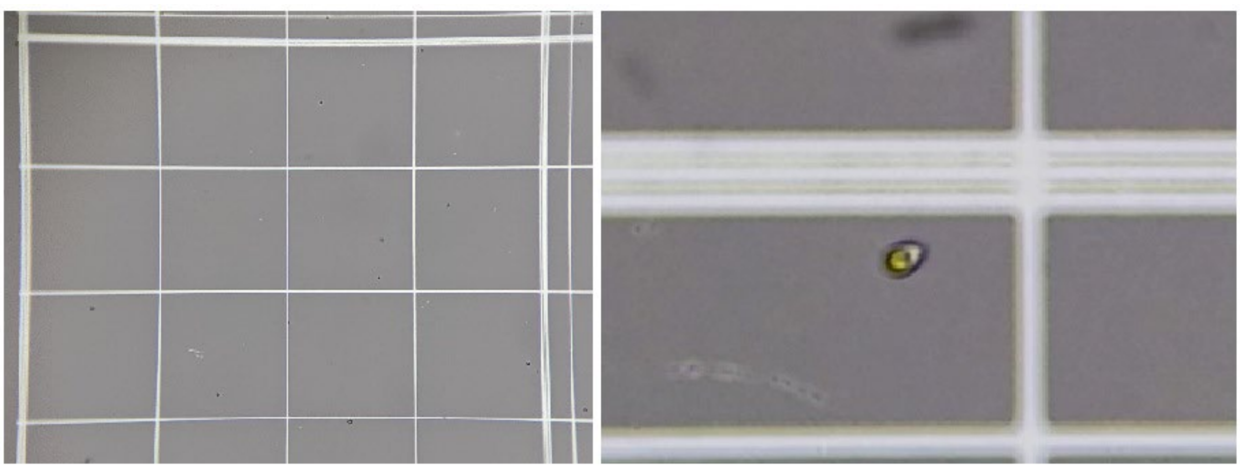

Figure 10 – Microscopic images of the zoospores at the hemocytometer. Left: 10x magnification. Right: 40x magnification.

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 11A). Settling tubes were then 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 11B).

Figure 11 - Spools in settling tunes with zoospore suspension (A). Spools in the nursery tank after zoospore settling (B).

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). The weekly maintenance schedule followed 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).

4.3 Sporophyte Monitoring and Maintenance

Sporophyte monitoring was performed by daily observation of the spool's appearance (color and aspect) and weekly, there was a 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 water sample was taken from the tank and pH was analyzed.

The pH measurements through the nursery stage ranged from 7.6 to 9.0. As the lighting was increased to 100 µmol m^{-2} s^{-1}, the sporophytes grew at a higher rate and elevated the pH. To maintain the pH within the range recommended by the literature (7.0 to 9.0) (Flavin *et al*. 2013). $C0_2$ was injected. During week 9, 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. Using a pair of tweezers, the surface of the twine was scraped onto a new microscope slide along with a drop of seawater and covered with a coverslip for detailed observation of the organisms at a higher augmentation. At the end of the 13-week nursery period, even though the sporophytes could be observed without magnification, the twine scraping procedure was still performed. In this case 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 Seaweed development

Seaweed development was observed and recorded by regular observation of the spool's apparency by compound light microscopy. Samples were taken randomly from one of the two twine loose ends left on the spools. After the observation of the piece of twine, the material was scraped using two tweezers and a cover slip was placed for observation in higher augment. The development of the seaweed attached to the spools was observed for 13 weeks, ending with the delivery of the spools to deployment to the test lines.

During the two first weeks, technologists observed the gametophyte cells settling on the twine fibers (Figure 12). The cells were round, and it was not possible to differentiate between female (Figure 13A) and male (Figure 13B) gametophytes.

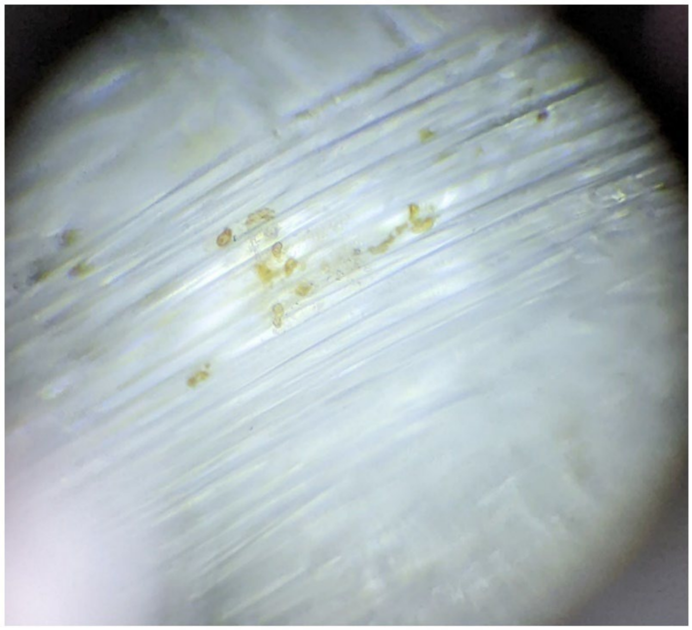

Figure 12 - Gametophyte cells settled at the twine fibers.

During the third week, there was an increase in the amount of gametophytes observed, and it was possible to differentiate between males and females. It was also possible to observe the presence of oogonium and young sporophytes (Figure 14A). The young sporophytes measured approximately 70 to 100µm. During the time of microscopic observation, we noticed that frequently we could find gametophytes, eggs, and sporophyte at different stages of development, at the same location, which makes us believe that once the reproductive cells settle, they start and keep reproducing and their offspring start settling around them. (Figure 14B).

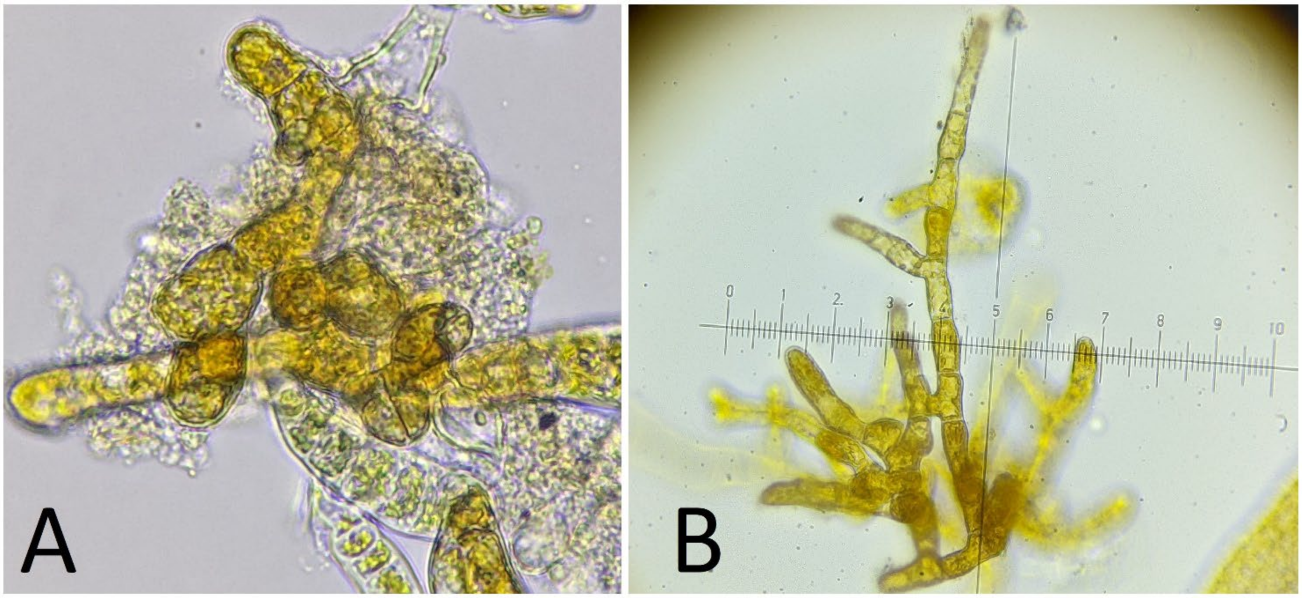

Figure 13 - (A) Female and (B) male gametophytes.

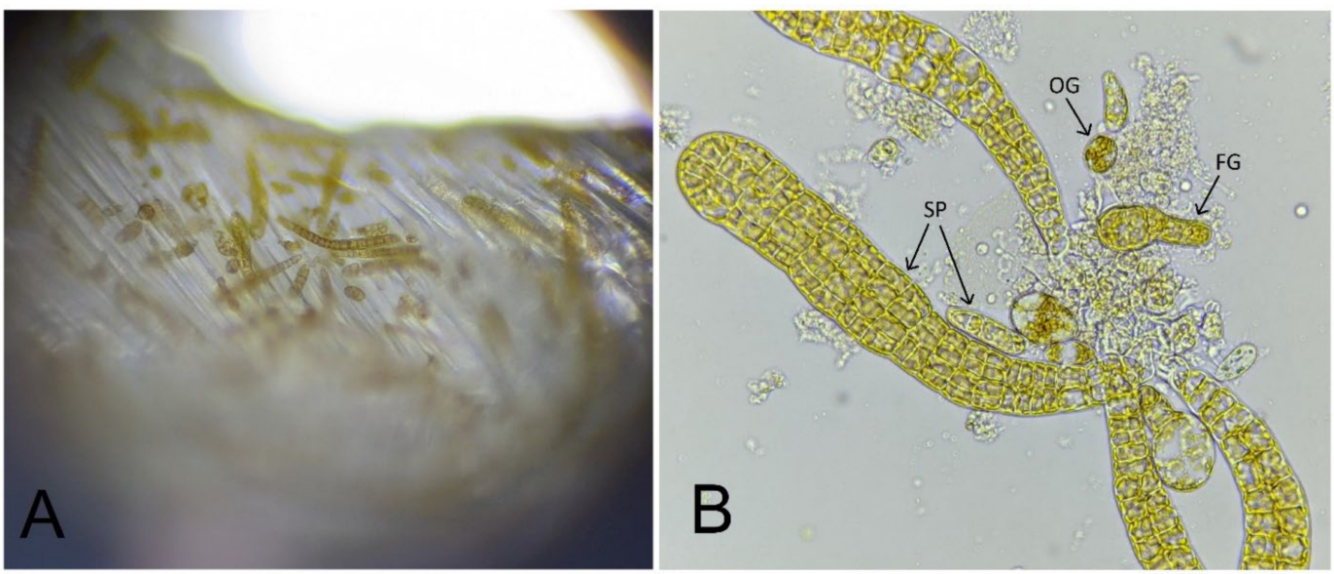

Figure 14 - (A), Young sporophytes. (B) Gametophyte and sporophytes organization. (FG) female gametophyte, (OG), oogonium and (SP), sporophytes.

From the fourth week on, the spools gradually changed in colour from white to brown. Microscopically, the technologists observed the absorption of the cytoplasm at the base of the sporophyte and the differentiation of the cells on small rhizoids (Figure 15A) that developed onto the holdfast during week 13 (Figure 15B).

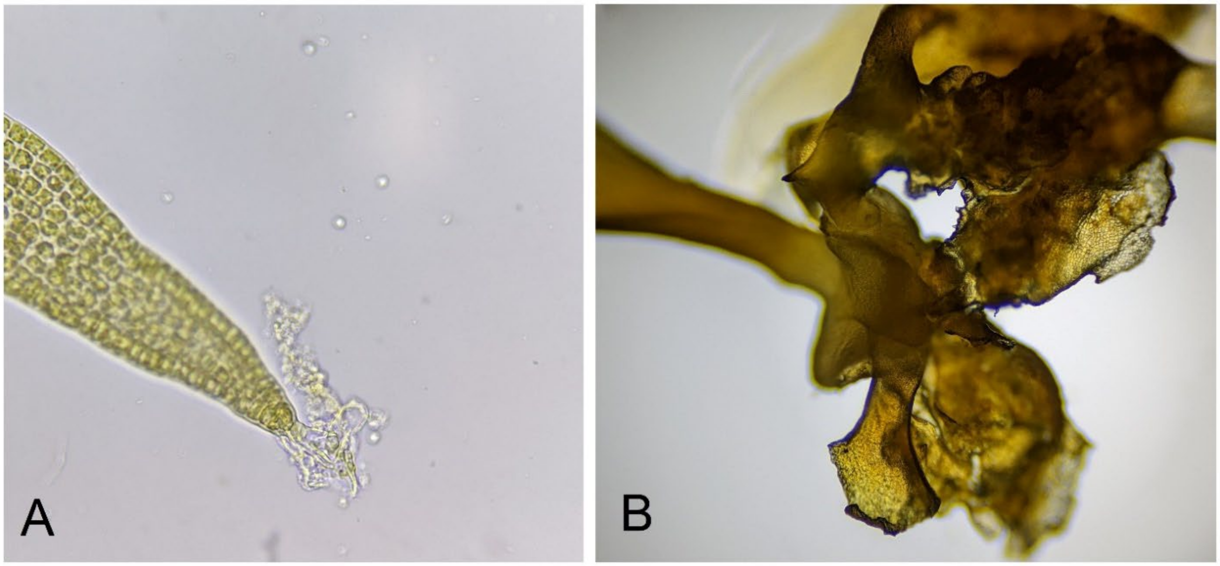

Figure 15 - (A) Sporophyte rhizoid, week 4. (B) Holdfast development, week 13.

Within five weeks post seeding, the sporophyte sizes ranged from 200µm to 1mm, and it was possible to observe its presence and distribution macroscopically (Figure 16). Twine samples were taken weekly until week 11 when the size of the sporophytes ranged from 2mm to 9.2mm. At this point, microscopy was not necessary to observe the seaweed development. However, during the entire 13-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.

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. At this time, sporophytes ranged in size between 1cm and 3cm (Figure 17.)

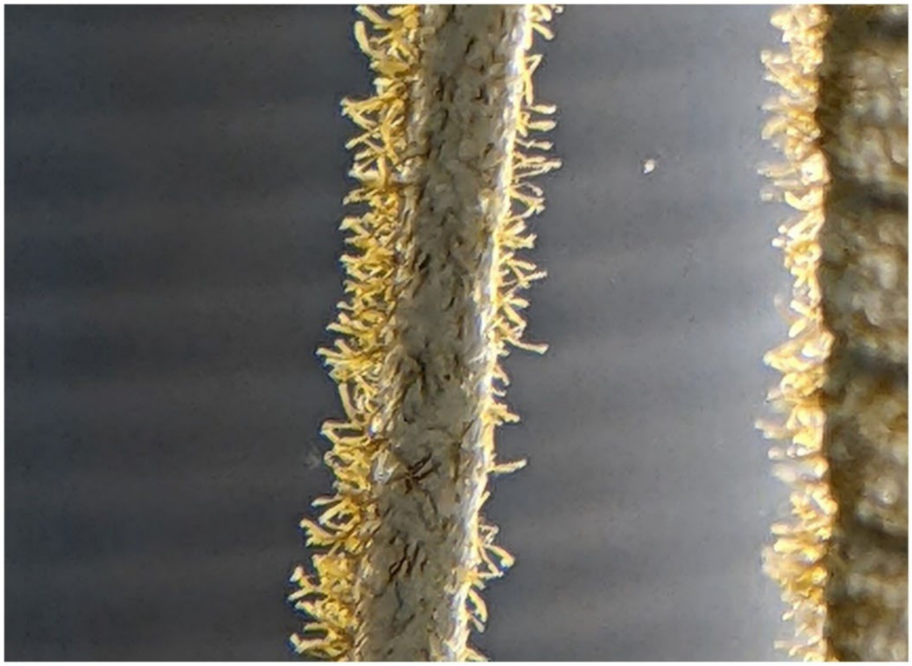

Figure 16 - Sporophytes macroscopic observation, week 5.

Figure 17 - Sporophytes macroscopic observation, week 13.

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 (Raymond *et al*., 2014). The timeline for development in the NL nursery was extended three to four weeks compared to strains reported in the literature. This is assumed to be due to a combination of factors including:

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

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

6 CONCLUSIONS AND RECOMMENDATIONS

Regular monitoring of light intensity, water quality, and the addition of nutrients resulted in the proper development of seaweed. The quality of the sorus collection, as well as the disinfection procedures prior to spore release, ensured the control of protozoan and other organisms that could have interfered in the seaweed development. The development of the seaweed proceeded as expected based on the literature except it required a longer timeline. This may be a result of the strain of sugar kelp collected, nursery lighting protocols, and the water temperature. However, these procedures produced eight spools containing well-developed sugar kelp sporophytes that were successfully transferred to St. Mary's Bay.

CASD technologists learned several lessons adapting the method proposed by Flavin *et al.* (2013) to the local *Saccharina latissima* strain which should be incorporated into future projects. These include:

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

- ❖ Measurement of young sporophytes. 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.
- ❖ 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.
- ❖ Prior to spore release, CASD technologists recommend autoclaving the mechanically and UV filtered seawater to further decrease the potential of contamination during the spore release. This process is of the utmost importance.
- ❖ Prior to wrapping the twine onto spools, CASD technologists recommend soaking the twine in deionized water and letting it dry thoroughly to ensure that no twine residue leaches into the tanks.

The client is excited to continue doing work with CASD now that the seaweed has been deployed into St. Mary's Bay. Future projects include grow out, harvesting and processing of seaweed. Monitoring and water quality are important to better understand seaweed development at sea in NL. The experience gained with the seaweed nursery project will allow the development of future research involving seaweed seed collection, replication and stock, improvements on seaweed nursery and deployment. The potential for a variety of processing techniques in seaweed farming opens opportunities for Newfoundland to be at the forefront of seaweed aquaculture.

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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:							

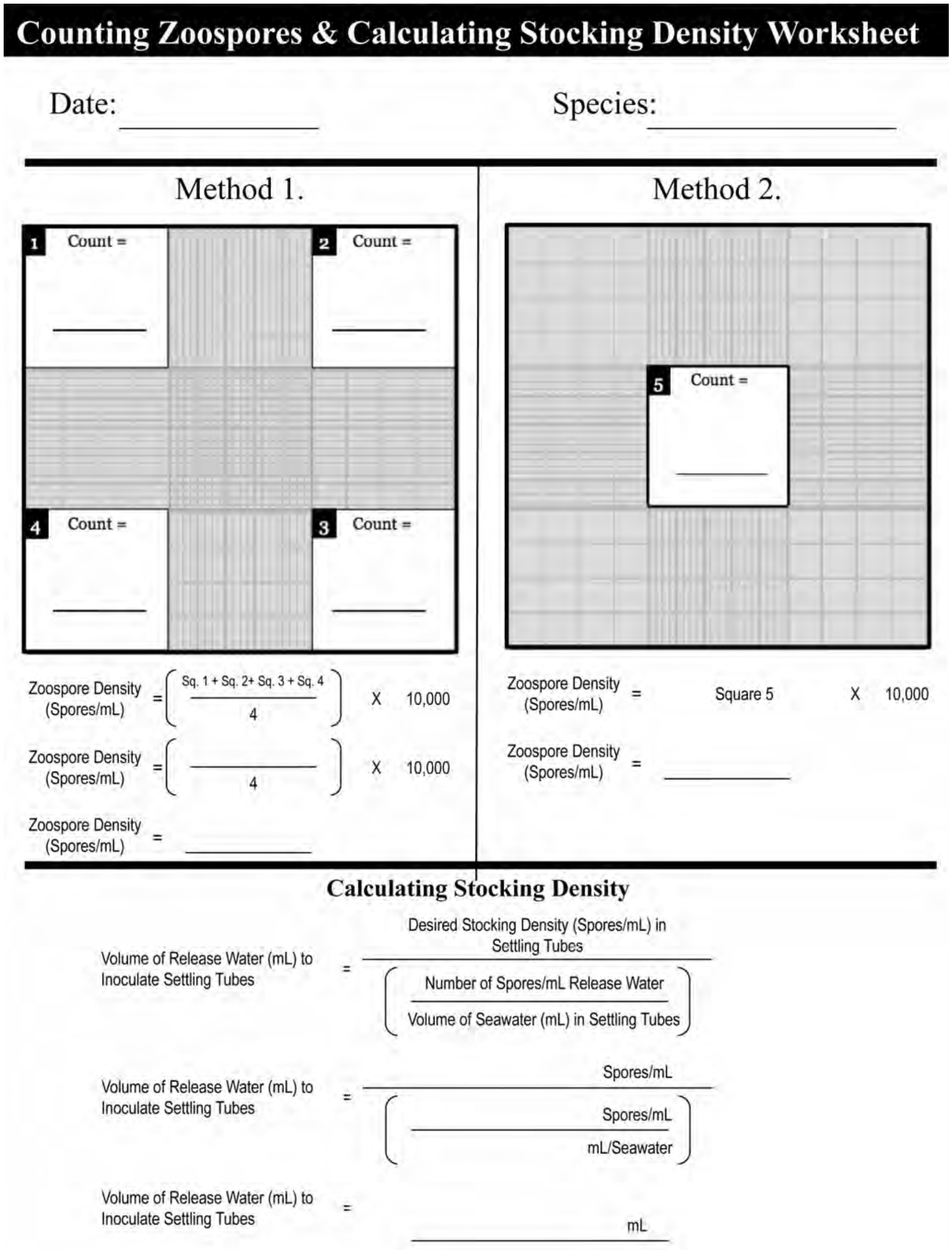
Daily Nursery Maintenance Checklist from Flavin et al. (2013)

Holdfast
St. Marty's
Oct. 19-20/22: Spores released. Oct. 21/22: Spools transferred

Nursery Weekly Maintenance Checklist								
Task	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Ocean Transfer	Additional Observations
	Gametophyte		Sporophyte Stage					
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	**1000mL Quantity**
Deionized Water	1000mL
$NaNO_3$	2800mg (2.8g)
Na_2glycerophosphate	400mg (0.4g)
Thiamine-HCl (Vit. B1)	4mg (.004g)
Tris buffer	4000mg (4g)
Solution II: Fe (as EDTA complex; 1:1 molar)	
Deionized water	250mL
$Fe(NH_4)_2(SO4)_2{\cdot}6H_2O$	175mg
Na_2EDTA	150mg
Solution III: Metals	
Deionized Water	200mL
$MnSO_4H_2O$ (manganese sulfate monohydrate)	26.0mg
Solution IV: Vitamins	
Deionized Water	1000mL
Vitamin B12	2.0mg
Biotin	1.0mg
PES Culture Media	
Solution I: Base Solution	1000mL
Solution II: Fe (as EDTA complex 1:1 molar)	200mL
Solution III: P II Metals	200mL
Solution IV: Vitamins	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

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