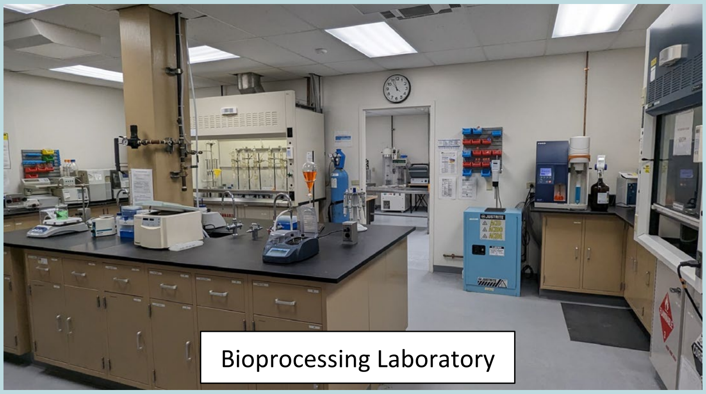

Seafood Pilot Plant


Aquaculture Facility


Bioprocessing Pilot Plant


Bioprocessing Laboratory

Seaweed Test Plot Development and Growth Study

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EXECUTIVE SUMMARY

In August and September 2021, Shorefast Social Enterprises Inc. (SSEI) and Fogo Island Co-operative Society Ltd. (FICSL), requested assistance from the Centre for Aquaculture and Seafood Development (CASD) to conduct a feasibility study to determine if there are suitable locations for seaweed farming, what species was available locally and promote community engagement on the concept of seaweed aquaculture.

Out of several sites that were assessed for the possibility of seaweed aquaculture around Fogo Island, four sites of interest have been identified at present. These sites were selected using assessments based on limited data collection over a short time period (3 days). A more extensive investigation has been conducted, including data collection over an extended time to take into consideration seasonality, changing environmental conditions and user conflicts at the sites of interest. Collectors were deployed at four sites and overwintered. Pack ice studies were conducted, and the initial collector plates were recovered and studied in the lab at CASD. Some sea plants were found, but very little biofouling was observed. By the end of the site selection research and with the execution of the test plot deployment it was determined that the complexity and novelty of the operation added to the costs attached to perimeter markers and moorings, therefore it was decided to set three 60-meter lines at Cobbs cove.

Concurrently, CASD settled a nursery to produce seeds from carefully selected samples of *Saccharina latissima* from Fogo Island. Initially it was planned to also produce *Alaria esculenta* seeds, but it was not possible to find mature samples of that species at that time. CASD kept researching seed collection and nursery production of sporophytes through to the fall of 2022, however due to delays on the research and execution of the test plot, the seeds produced by CASD couldn't be seeded in time and were lost due to degradation and algae activity. The seaweed nursery was executed as another project and the lessons learned and recommendations were presented in a report in March 2023.

With the advance of the deployment of the lines at Cobbs Cove, a new *Saccharina latissima* nursery was set at CASD and the seaweed was seeded on the first week of December 2023, on the lines deployed earlier in August 2023. During the process of seeding the lines the whole plot was sunk to protect the gear from possible pack ice and brought up to the surface in May 2024. In that occasion the lines were monitored, and it was noticed that the seaweed growth was limited to a couple of sections of the lines. Several factors likely contributed to this result for example: The spools didn't have good coverage which highlights the need for alternate methods for settling the spores; The seeds were transferred to the aquaculture site earlier than the ideal (5 to 7 weeks); As the lines were on the water since August 2023, they were covered by biofouling that may have overcome the seaweed in growth when seeded in December 2023.

The harvest was performed in July 2024 by Shorefast and FICSL team. Approximately 30 kg was harvested in total and, considering one of the best covered sections of the line, the seaweed seed growth was of 1.9 kg/meter of line. 15kg of seaweed was brought to CASD for processing trials and lab analysis. The samples were processes by blanching with steam, freshwater or salt water followed by either air drying or freeze drying. A sample of raw seaweed was also dried using both methods.

The samples were analyzed for proximate composition, salt content, trace metals and iodine content. For proximate analysis, the highest range was observed to ash content, with the freshwater blanched displaying the lowest value as part of the mineral salts may leach during the process with fresh water. The type of process also promoted differences in salt and mineral content. Through heavy metal analysis and in comparison, with the literature, all samples had values well below those considered to be toxic concentrations. While the boiling process (freshwater and salt water) reduced the Iodine content, the same result wasn't observed for steaming.

The main goal of this project was to assess if high quality value driven seaweed could be cultivated successfully in the waters around Fogo Island. Although it was not possible to settle lines in all planned locations, and despite the limitation faced in relation to the seed quality and performance, based on the growth that was observed in some sections of the line and on the analysis of the harvested seaweed, there is potential to grow *Saccharina latissima* in Cobbs cove by improving the process based on the lessons learned in this trial.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1 BACKGROUND AND INTRODUCTION

Seaweed aquaculture is gaining attention among aquaculture and bioprocessing related companies due to its potential for generation of biomass and high value compounds with low carbon footprint. Shorefast Social Enterprises Inc. (SSEI) and Fogo Island Cooperative Society Ltd. (FICSL) requested assistance from CASD to investigate possible sites suitable for seaweed aquaculture in Fogo Island and to establish an experimental seaweed farm with seeds produced at MI.

Seaweed farming is conventionally setup in horizontal lines in which the substrate containing young sporophytes (seeds) will be seeded. When it comes to kelp species, one of the main types of seaweed cultured, the seeds are prepared by harvesting mature kelp samples, isolating their reproductive tissue (sorus) for spore release in a controlled environment including sterilized salt water, utensils, and consumables. The spores are placed to settle on nylon thread spools, a substrate that can be effectively transferred to the culture lines (Flavin etal., 2013).

In Atlantic Canada, mature kelp can be found mostly during late spring and late fall. The nurseries are established within this period and are usually seeded early to mid-winter. For aquaculture sites, depending on the region they are situated, the occurrence of pack ice may require the lines, floaters, and gear to be sunk to avoid damage (Mallet and Myrand, 2000). The lines and buoys are brought to surface by the end of the ice season. The harvest of kelp will occur in mid-summer and the timing is dependent on the occurrence of fouling organisms and kelp degradation. Therefore, the monitoring of the lines is important to determine the harvest time before biofouling occurs, ensuring a high-quality product.

A broad range of applications, including various industry sectors, are currently being identified and developed for the harvested cultured seaweed. Different species of kelp are currently being processed for feed, food and nutrition (animal feed, human consumption, and supplements), health (cosmetics and therapeutics) and agriculture (fertilizers). Additionally, researchers are investigating the use of kelp in the fabrication of bioplastics as a sustainable and eco-friendly alternative to conventional sources (Sæther *et al.*, 2024)

The harvested kelp is processed according to each intended application. Processing includes grinding and/or drying. For human consumption there is a concern with the iodine content that is usually high in a variety of kelp. One solution to reduce the iodine levels is to blanche it by steaming or boiling before drying and stabilizing (Nielsen *et al*., 2020). The general quality and composition of the harvested kelp can be assessed by proximate analysis, minerals, trace metals, moisture, and water activity (Sæther *et al.*, 2024). The results of the laboratory analysis can help determine potential uses for the product.

Kelp aquaculture is new in North America when compared to Asia. While seed production and sea outgrowing are processes that are currently in place in Canada, there remains a significant gap in processing technologies, product stabilization and marketing strategies that must be developed to support sustainable economic growth in the industry (Kim *et al*., 2019; Kotowicz *et al*., 2024).

2 SCOPE AND PURPOSE

2.1 Scope of Project

The scope of this project was identifying aquaculture sites suitable for kelp farms in Fogo Island waters and determine the effectiveness and quality of lab produced seaweed seeds outgrowing on experimental lines.

2.2 Purpose of Project

The purpose of this project was to help Shorefast Social Enterprises Inc. and the Fogo Island Cooperative Society to develop seaweed farms as an aquaculture activity on Fogo Island and engage the local fisheries industry and community.

3 OBJECTIVES

The objectives of this study included:

1. Design and engineer a small-scale seaweed aquaculture test plot for the specific conditions at the chosen Cobbs Cove site on Fogo Island.
1.1 Study, design and execution of small (60 m X 10 m) test plot, including rigging and mooring systems for the specific conditions at the selected aquaculture site.

2. To deploy seeds produced at the Centre for Aquaculture and Seafood Development (CASD) aquaculture facility on the lines of the test plot in Cobbs Cove, conduct site monitoring, harvest timing identification and harvest the cultured kelp.

3. Processing and Analysis of the harvested kelp
3.1 To assess processing methods including grinding, three types of blanching and two types of drying methods
3.2 To analyse the proximate composition, minerals, metals, and Iodine content of the harvested and processed kelp

4 METHODS

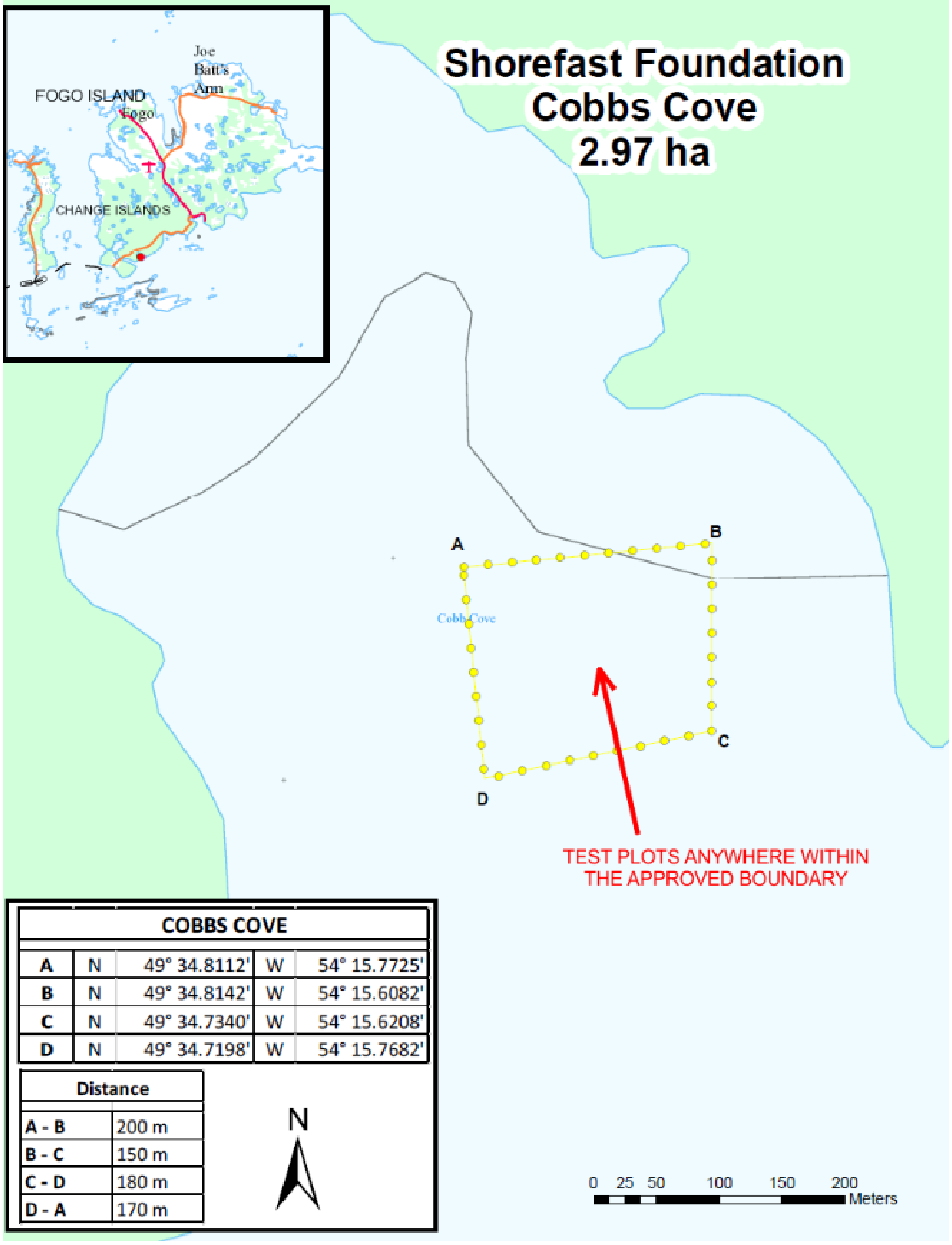
To develop the design of the test plot, a third part company, Silk Stevens, was hired to work on the design and materials selection based on CASD input and later reviewed and adjusted by CASD's engineer and School of Fisheries (SOF) scientific advisor. The CASD team and the clients worked to acquire the licenses required by provincial and federal organizations to delineate the aquaculture site in Cobbs Cove, deploy the test plot gear, harvest the mature seaweed for the seed production and finally transfer the seeds back to the aquaculture site. The execution of the project started with the deployment of the gear (perimeter markers and the test plot) and the harvest of mature Sugar kelp samples for the preparation of the seeds at CASD's aquaculture facility. The seeds were transferred to the site in early winter and during this operation the gear (lines and buoys) were sunk to prevent any damage due to pack ice. In mid-spring the gear was brought to the surface and the seaweed growing on the lines was monitored. The harvest was made in mid-summer at the first sign of biofouling deposit on the Kelp's blade. Part of the harvested kelp was brought to CASD's seafood pilot plant for processing and to CASD's marine bioprocessing lab for analysis. Samples were also sent to an external lab for elemental analysis.

4.1 Site selection and test plot design

The site selection was made based on two factors: 1) An assessment from a previous project that took into consideration the biofouling accumulation on test plates; 2) The operational and material cost for execution.

Federal and provincial applications for establishing the aquaculture site (DFO/FFA) and perimeter markers were submitted by the client for Cobbs Cove (Figure 1), where the experimental test plot was deployed after approval.

The final design for the test plot (Figure 2) made by Silk Stevens, (based on "GreenWave" (Figure 3), a nonprofit organization that replicates and scales regenerative kelp ocean farms) included perimeter markers, deployed with 500 lb concrete blocks, and three 60-meter lines (proflex sinking rope 5/8''), two of them set with two 500 lb conventional plow anchors (Figure 4) and one with two 400 lb repurposed anchors made of rail bar and rebar (Figure 5).



COBBS COVE				
A	N	49° 34.8112'	W	54° 15.7725'
B	N	49° 34.8142'	W	54° 15.6082'
C	N	49° 34.7340'	W	54° 15.6208'
D	N	49° 34.7198'	W	54° 15.7682'

Distance	
A - B	200 m
B - C	150 m
C - D	180 m
D - A	170 m

Figure 1 - Licensed site at Cobbs Cove.

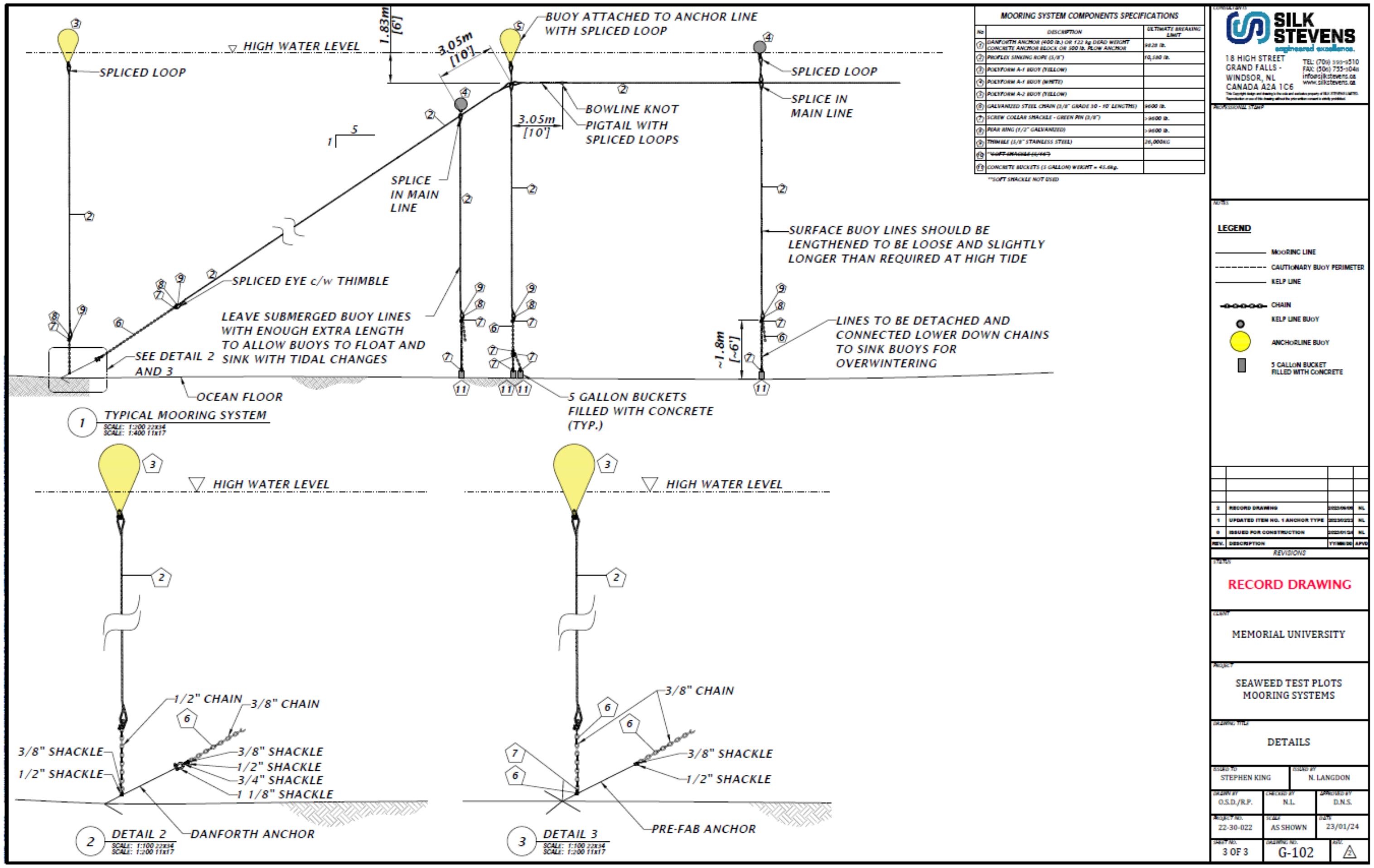


Figure 2 - Lines and Moorings - Silk Stevens final design.

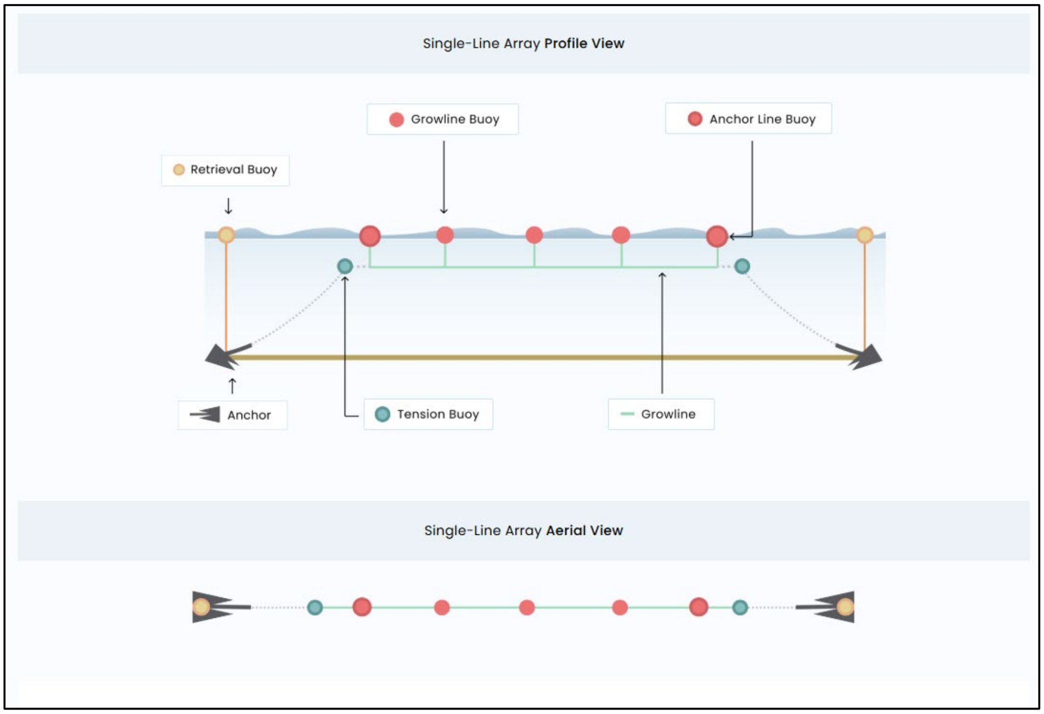


Figure 3 - Greenwave lines and moorings design (Source, https://www.greenwave.org/).

Figure 4 - Conventional anchor deployment.

Figure 5 - Repurposed anchors

4.2 Test plot deployment

The perimeter markers and test lines were deployed in August 2023 in a two-day operation. The work started with the deployment of the markers, following the GPS coordinates according to the license, setting the area for the site and followed by setting the anchors buoys and ballasts for each of the three horizontal lines. As part of the experiment, the ballast attached to the main line weren’t set in one of the three lines, changing the test plot design.

4.3 Nursery and site seeding

The harvest of mature Sugar kelp samples and spore release were performed in late October 2023. The samples were brought to CASD’s aquaculture facility wrapped in double plastic bags in a cooler with ice packs for processing and spore release according to Flavin *et al.*, 2013. The mature portion of the kelp blades were removed, disinfected and dried at 10°C for 24 hours (Figure 6). The processed samples were placed in salt water at 10°C for spore release and checked in 15-minute intervals for number of spores for approximately one hour, the time that the release reached its maximum point (i.e., no increase in numbers between two counting). A concentration of $50x10^3$ spores were inoculated on each of 10 spools placed in individual settling tubes with salt water at 10°C in the dark for 24 hours. The inoculated spools were transferred to 20 gal aquariums with chilled recirculated water (10°C), aeration and 12/12 photoperiod at ≈ 22 µmol m^{-2} s^{-1} (Figure 7). 700 ml of Provasoli’s enriched media (Flavin *et al.*, 2013) was prepared and added to the aquariums weekly, along with total water change. As part of the nursery weekly routine, a piece of the sample twine was removed for observation of the gametophyte and sporophyte development under the microscope (Figure 8). The total nursery time was four weeks, and the light intensity was increased to ≈ 55 µmol m^{-2} s^{-1} after two weeks and to ≈ 100 µmol m^{-2} s^{-1} for the remaining time.

After four weeks of nursery time (early December 2023), eight spools containing Sugar kelp sporophytes were transferred to the aquaculture site at Cobb’s Cove. The main line was untied from the mooring line and passed trough the spool carrying one end of the nylon thread, unspooling the ≈ 40m thread with sporophytes along each of the three 60-metre culture lines (Figure 9).

After seeding, the test plot and perimeter markers, lines and buoys were sunk by shortening the distance between the ballasts and buoys and/or additional weight (cinder blocks) (Figure 10), in anticipation of pack ice occurrence during the winter. The gear was brought to the surface after five months (mid-May 2024) along with a first observation of the seaweed growth along the lines. During this process, the ballast tied to the seaweed lines were removed to facilitate hauling the lines in anticipation of the harvesting execution.

After bringing the lines to the surface, three observations (May 16, June 11 and July 3, 2024) were conducted to evaluate the seaweed growth and to monitor the first biofouling occurrence, so the seaweed harvesting time could be planned to avoid biofouling accumulation and degradation, ensuring the best quality.

Figure 6 - Processed mature kelp.

Figure 7- Seeded spools transferred to the nursery.

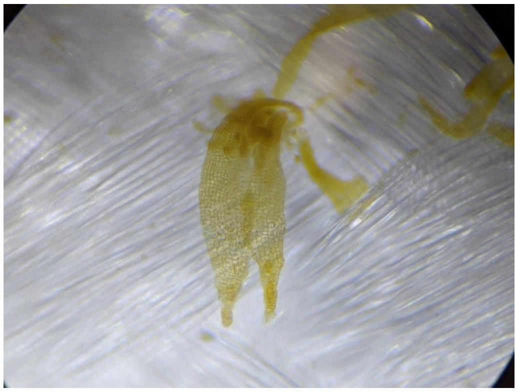


Figure 8- Seeded spools observation at the microscope.

Figure 9 - Line seeding.

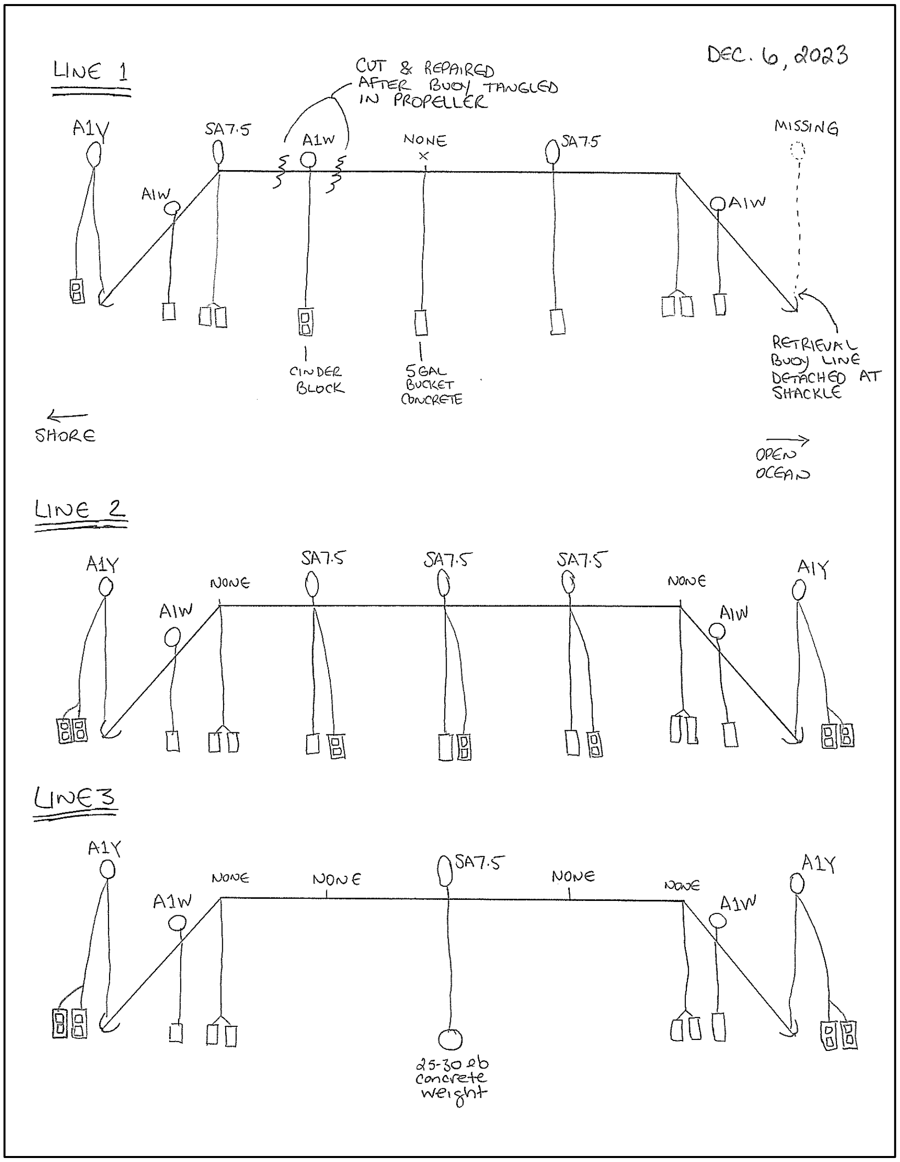


Figure 10 - Sketch of the lines as sunk.

4.4 Harvesting

Based on the results of the last observation (July 3, 2024), the harvest was executed on July 18, 2024. The lines were hauled to the boat and all the seaweed content of the three lines were pulled out and placed in fish totes. Part of the harvested seaweed were destinated to Professor Christina Smeaton's team at Memorial University, Grenfell Campus and the remaining (15 kg) was brought to CASD's pilot plant for processing.

4.5 Processing

The raw seaweed was chopped by hand in ~5mm pieces (Figure 11) and split into four samples that were blanched by steam (2 min), freshwater or saltwater immersion (100°C/1 min) or kept raw (Figure 12). The processed samples were then split into two subsamples for air drying (75°C/ 3 days) or freeze drying (equipment preset program). The samples were all weighed on the start and finish of each blanching and drying process and, once dried (Figure 13), the samples were tested for water activity and moisture using an Ohaus Moisture Meter.

Figure 11 – ~5mm Chopped seaweed.

Figure 12 - Seaweed blanched by steam (A) or freshwater/saltwater (B).

Figure 13 - Dried kelp samples.

4.6 Analysis

Due to limitations in sample quantity and available resources, some analyses were performed only on either freeze-dried or air-dried samples. The results of these analyses are presented below.

4.6.1 Crude Proximate Analysis

Crude proximate analysis was performed on four samples of air-dried seaweed (*Saccharina latissima*) including raw, steam blanched, fresh water blanched, and salt water blanched. The crude proximate analysis included moisture (%), ash (%), fat (%) and protein (%) analyses. Carbohydrates (%) were calculated by difference. Crude proximates were performed using Official Methods of Analysis of Association of Official Analytical Chemists (AOAC International) (AOAC, 2012) as per Table 1.

Table 1: AOAC methods performed for laboratory analyses.

	Method	AOAC Method #'s
1	Moisture Content	950.46B
2	Ash	938.08
3	Fat (Soxhlet method)	920.39
4	Protein (Kjeldahl method)	954.01/988.05

4.6.2 Moisture

The moisture content of the samples was determined by AOAC Method 950.46B for Moisture Content (AOAC, 2012). Approximately 5-10 g of homogenized samples was placed into pre-weighed aluminum moisture pans and dried in a 105°C oven overnight. The samples were then cooled in a desiccator and the weights of the samples were measured. The moisture content of the samples was calculated as follows:

$$\%\ Moisture = \frac{W1 - W2}{W1 - W0} \times 100$$

Where:

W_1 = weight of dish and sample before drying
W_2 = weight of dish and sample after drying
W_0 = weight of empty dish

4.6.3 Ash

The ash content of the samples was determined by AOAC Method 938.08 for Ash Content (AOAC, 2012). Approximately 2g of sample was placed in pre-weighed crucibles and heated at 550°C until completely ashed. The crucibles and samples were weighed to the nearest 0.0001g. The ash content was calculated as follows:

$$\% \, Ash = \left(\frac{W_3 - W_1}{W_2 - W_1}\right) \times 100$$

Where:

W_1 = weight of empty crucible
W_2 = Weight of crucible and wet sample
W_3 = Weight of crucible and ash

4.6.4 Fat

The crude fat content of the samples was determined by the semi-continuous solvent extraction method: The Soxhlet method, Crude Fat Method 920.39 AOAC (AOAC, 2012). Approximately 2g of dried sample was placed in a thimble and solvent was heated and built up in the extraction chamber until it surrounded the sample and then was siphoned back to the boiling flask. This was repeated until all fat was extracted from the sample and collected in the boiling flask. The solvent was removed, and the fat content was determined by the weight of the fat that was collected. The crude fat content was calculated as follows:

$$\% \, Fat = \frac{(g \, fat \, in \, smp)}{(g \, dried \, smp)} * 100$$

4.6.5 Protein

The crude protein content of the samples was determined by the Kjeldahl method, AOAC Method 954.01/988.05 (AOAC, 2012). The dried sample was first digested with sulphuric acid and catalysts and the total organic nitrogen in the sample was converted to ammonium sulphate. Next, the digested sample was neutralized with alkali and distilled into a boric acid solution and then titrated with hydrochloric acid solution. The nitrogen and protein contents were calculated as follows:

$$\%N = \frac{(Vol_{HCl \, sample} - Vol_{HCl \, blank}) \times Normality \, of \, HCL \times 14.0067}{weight \, of \, sample \, [mg]} * 100$$

$$\% \, protein = \%N * 6.25$$

4.6.6 Carbohydrate

The crude carbohydrate content of the samples was calculated by difference. The carbohydrate content was calculated as follows:

$$\% \, Carbohydrates = 100 - (\%Moisture + \%Ash + Fat + \%Protein)$$

4.6.7 Salt content

Salt content analysis testing was performed on four samples of air-dried seaweed (*Saccharina latissima*) including raw, steam blanched, fresh water blanched, and salt water blanched.

Approximately 5 g of each seaweed sample were accurately weighed and transferred to a beaker with 200 ml of distilled H_2O and mixed on a magnetic stirrer. Before measuring conductivity, the samples were given at least 5 minutes to equilibrate. Conductivity was performed with a HQ series pH/DO/Conductivity meter.

The conductivity probe was calibrated using a 1000 µS/cm ±10 µS/cm at 25°C (491 mg/L ±2.5 mg/L NaCl) standard solution.

The salt content was calculated as follows:

$$C = \frac{R}{W}(\mathrm{V} + \frac{(M \times W)}{100})$$

where: C = concentration of NaCl in sample expressed as percent on a wet weight basis
M = Moisture as percent by weight
R = % NaCl (converted from ‰ NaCl reading on meter)
V = volume (ml) distilled water added
W = weight (g) of sample used

4.6.8 Trace metal analysis

Trace metal analysis, including mercury, was performed on four samples of freeze-dried seaweed (*Saccharina latissima*) at RPC in Fredericton, N.B. The four samples were raw, steam blanched, fresh water blanched, and salt water blanched.

Portions of the samples were prepared by Microwave Assisted Digestion in nitric acid (RPC method: SOP IAS-M26). The resulting solutions were analyzed for trace elements by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (RPC method: SOP IAS-M01).

Mercury was analyzed by Cold Vapour Atomic Absorption Spectroscopy (AAS) (RPC: SOP IAS-M52 & SOP IAS-M53).

4.6.9 Iodine analysis

Iodine was performed on four samples of freeze-dried seaweed (*Saccharina latissima*) at SGS Canada, Health & Nutrition Division, Burnaby, BC. The four samples were raw, steam blanched, fresh water blanched, and salt water blanched.

The freeze-dried seaweed samples were analyzed by the Iodine in Food method using ICP-MS.

5 RESULTS

5.1 Test plot

The operation to deploy the test plot lines was successful and executed as designed and planned, however, by the time the lines deployed in August 2023 were seeded, in December 2023, they were found to be covered in biofouling. During the seeding operation, line number 1 was caught by the boat propeller and needed to be reconnected, this event also caused the removal of one of line 1 ballasts. The perimeter mark and test plot lines and buoys were successfully sunk after seeding and brought back to the surface late May 2024. During this process, and while monitoring the seaweed growth, it was observed that the central ballasts from lines 1 and 2 was preventing the lines to be hauled for observation, so they were removed from both lines.

5.2 Nursery and Lines seeding

The samples obtained for spore release were healthy and mature enough to release spores and the total concentration of the spore suspension was 1,067,500 spores x mL^{-1}. The first gametophytes were observed under the microscope after the first week of nursery while the first sporophytes were observed within three weeks after the nursery was set (Figure 14). The overall coverage of the spools by the time of seeding (four weeks) wasn’t satisfactory. Patches with no seaweed along all the spools were observed, and the average size of the blades was 0.5mm.

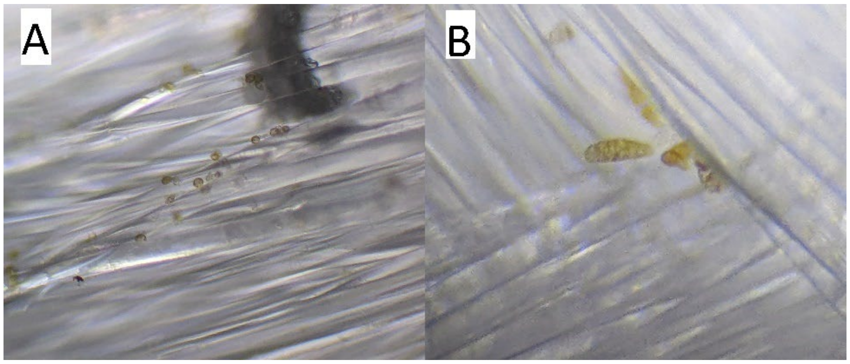


Figure 14 - First gametophytes (A) and sporophytes (B) observed.

5.3 Monitoring, Harvesting

According to the monitoring data realized by the Shorefast team on May 16, June 11 and July 3rd, 2024, there was growth on only some portions of the lines (Figure 15). The growth was sparse and mainly concentrated in two sections of line 1, one section of line 2, and one section of line 3, with an average of 70 blades x ft^{-1} and 40 in of maximum length (Table 2), while just a few blades were observed on line 3.

All of the seaweed was harvested from the three lines at the end of the growing season. The total harvested was 39.41 kg. The density observed in the best covered section of the line was 1.90 kg x ft^{-1}.

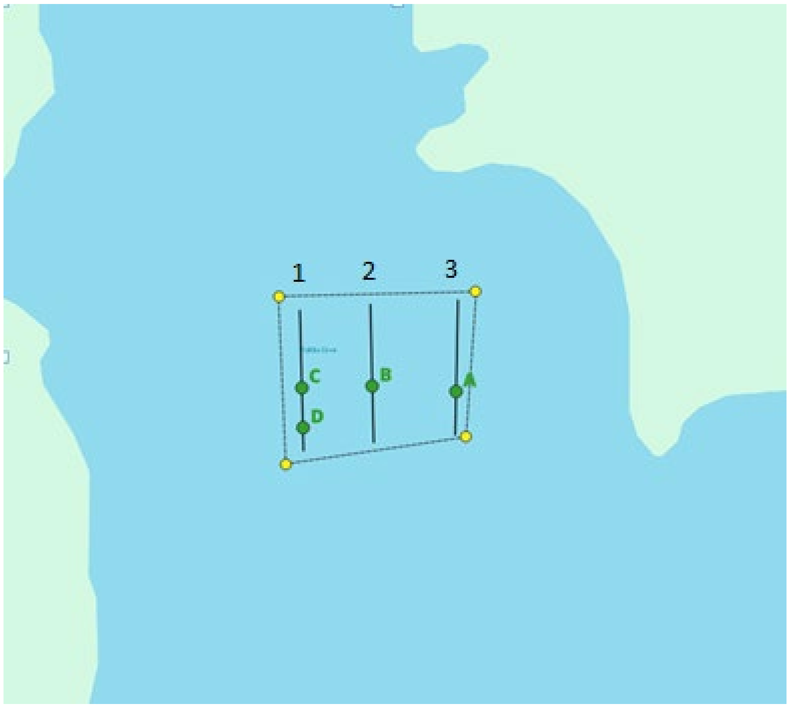


Figure 15 - Seaweed measure data points.

Table 2: Seaweed monitoring data, 2024

Observation	Line	Location	Blades (n)	Maximum Lenght
May 16	Line 3	A	-	26 in
May 16	Line 2	B	-	27 in
May 16	Line 1	C	58/ft	30 in
May 16	Line 1	D	-	-
June 11	Line 3	A	4	24 in
June 11	Line 2	B	-	45 in
June 11	Line 1	C	73/ft	39 in
June 11	Line 1	D	-	-
July 3	Line 3	A	-	32 in
July 3	Line 2	B	-	41 in
July 3	Line 1	C	71 / ft	45 in
July 3	Line 1	D	-	40 in

5.4 Processing

While processing the seaweed, it was observed that the color of the samples changed from brown to light green during all blanching methods. This occur because heat will denaturise red and brown colored pigments, but not the green-coloured chlorophylls (Yamanaka & Akiyama, 1993). It was also observed that the raw samples retained the most vibrant green color after drying, whereas the blanched samples became brown. A significant weight reduction was observed due to blanching, with the greatest loss occurring in samples treated with fresh water (Table 3). Water activity and moisture levels were lower for the freeze-dried samples with the lowest values observed for the one blanched by saltwater immersion (Table 3). Freeze-drying methods were developed as an alterative to hot air drying to mitigate the loss of volatile compounds in food or samples, preserving its quality for consumption or extractions, and removing more water due to direct sublimation of ice at reduced pressure (Chan at al., 1997; Wong & Cheung, 2001).

Table 3: Seaweed processing treatments and results

Blanching Method	Drying method	Weight before blanching (g)	Weight after blanching and draining (g)	Dry weight (g)	Water activity	Moisture (rapid test)
Steam, 2 min	Air-dried, 74C	800	962.5	95.4	0.444	9.16
Fresh water immersion, 1 min	Air-dried, 74C	800	914.5	58	0.456	11.52
Saltwater immersion, 1 min	Air-dried, 74C	800	927.5	89.2	0.469	11.86
None	Air-dried, 74C	800	800	124.8	0.443	10.7
Steam, 2 min	Freeze-dried	800	962.5	87.1	0.224	4.88
Fresh water immersion, 1 min	Freeze-dried	800	914.5	52.3	0.138	5.13
Saltwater immersion, 1 min	Freeze-dried	800	927.5	74.7	0.123	3.54
None	Freeze-dried	800	800	123.1	0.248	4.32

5.5 Lab analysis

5.5.1 Crude Proximate Analysis

The proximate analysis of the four types (raw, steam blanched, fresh water blanched, and salt water blanched) of air-dried seaweed were conducted and recorded on a wet weight basis (WWB) and a dry weight basis (DWB). Excel 2016 was used to analyze the data; the means and standard deviations (s.d.) are presented for the four types of seaweed in Table 4 (WWB) and Table 5 (DWB). The results are presented on a WWB in Figure 16 and on a DWB in Figure 17.

Table 4: Proximate analysis results on a wet weight basis (WWB) for four samples of air-dried seaweed (<u>Saccharina latissima</u>): raw, steam blanched, fresh water blanched and salt water blanched.

Test/ Sample	Moisture (%)		Ash (%)		Fat (%)		Protein (%)		Carbs by difference (%)
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	
Raw- Air-dried	9.9772	0.0131	22.2240	0.0589	0.1301	0.0152	2.3873	0.0625	65.2814
Steam blanched - Air-dried	10.4695	0.0283	16.5596	0.8789	0.2034	0.0619	2.9714	0.0159	69.7961
Fresh water blanched - Air-dried	12.7439	0.0735	11.7406	0.0634	0.2818	0.0369	3.7702	0.0135	71.4635
Salt water blanched - Air-dried	12.2244	0.0880	22.9376	0.4771	0.1808	0.0007	3.1625	0.0086	61.4946

Table 5: Proximate analysis results on a dry weight basis (DWB) for four samples of air-dried seaweed (<u>Saccharina latissima</u>): raw, steam blanched, fresh water blanched and salt water blanched.

Test / Sample	Ash (%)		Fat (%)		Protein (%)		Carbs by difference (%)
	mean	s.d.	mean	s.d.	mean	s.d.	
Raw- Air-dried	24.6871	0.0655	0.1445	0.0169	2.6519	0.0695	72.5166
Steam blanched - Air-dried	18.4960	0.9817	0.2272	0.0692	3.3189	0.0178	77.9579
Fresh Water blanched - Air-dried	13.4554	0.0727	0.3229	0.0423	4.3209	0.0155	81.9008
Salt water blanched - Air-dried	26.1321	0.5435	0.2060	0.0008	3.6029	0.0098	70.0589

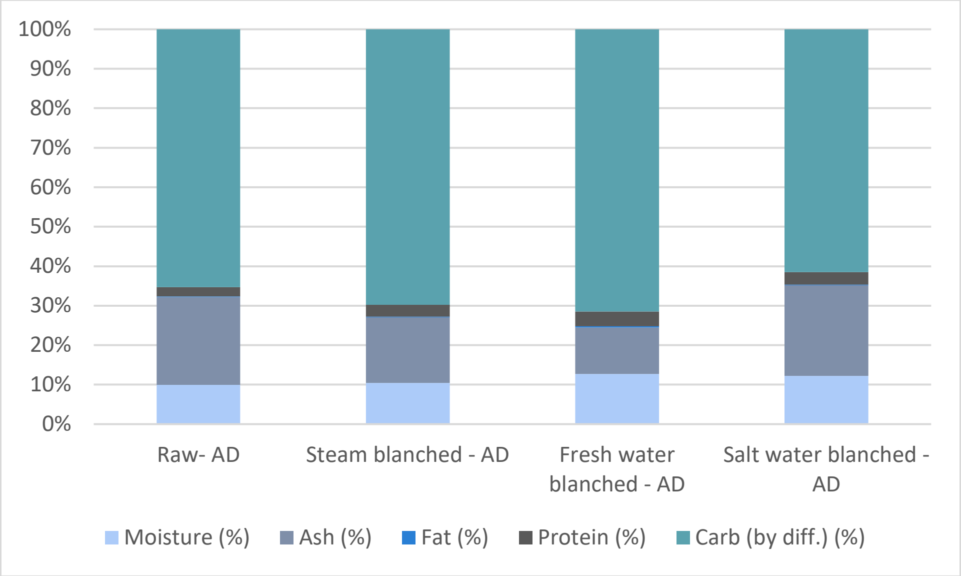


Figure 16 - Comparison of the proximate results (WWB) for four samples of air-dried seaweed (<u>Saccharina latissima</u>): raw, steam blanched, fresh water blanched and salt water blanched.

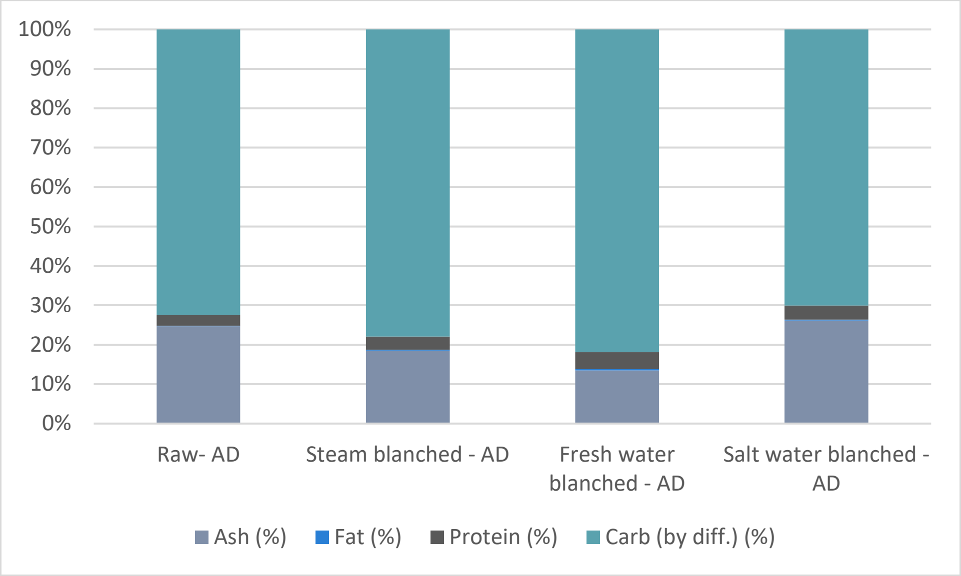


Figure 17 - Comparison of the proximate results (DWB) for four samples of air-dried seaweed (Saccharina latissima): raw, steam blanched, fresh water blanched and salt water blanched.

5.5.2 Salt Content

Salt content analysis of the four samples (raw, steam blanched, fresh water blanched, and salt water blanched) of air-dried seaweed was conducted and recorded. Excel 2016 was used to analyze the data; the means and standard deviations are presented for the four samples of seaweed in Table 6. The Salt content results are presented in Figure 18.

Table 6: Salt content of four samples of air-dried seaweed (Saccharina latissima): raw, steam blanched, fresh water blanched and salt water blanched.

Test /Sample	Moisture (%)	
	mean	s.d.
Raw- Air-dried	27.3277	0.1250
Steam blanched - Air-dried	15.4675	0.0080
Fresh water blanched - Air-dried	2.7761	0.0024
Salt water blanched - Air-dried	23.9135	1.2329

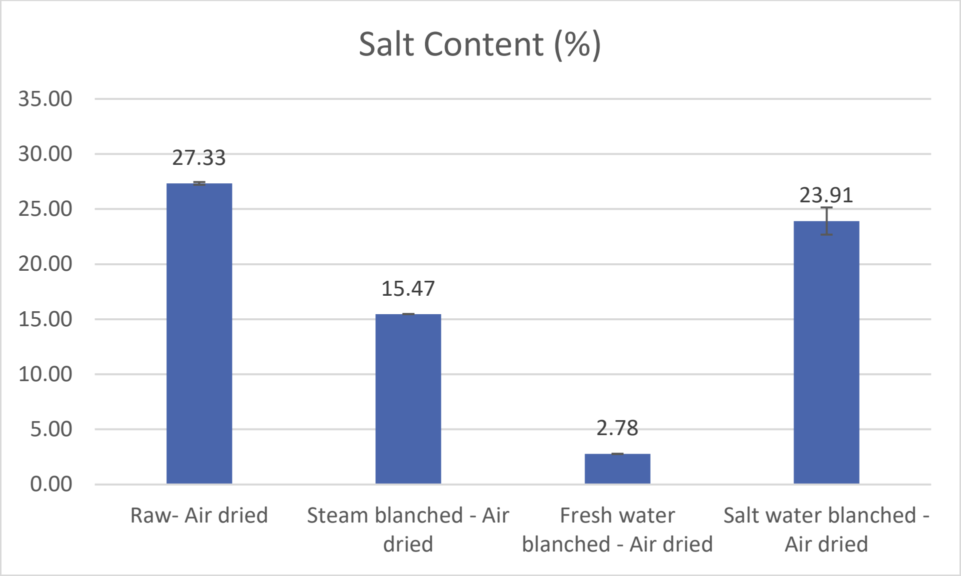


Figure 18 - Comparison of the salt content (%) of samples types of air-dried seaweed (Saccharina latissima): raw, steam blanched, fresh water blanched and salt water blanched.

5.5.3 Trace Metals Analysis

Trace metal analysis, including mercury, of the four samples (raw, steam blanched, fresh water blanched, and salt water blanched) of freeze-dried seaweed was conducted and recorded. Excel 2016 was used to analyze the data; the means and standard deviations (s.d.) were calculated for raw, freeze-dried seaweed only; and all results are presented for the four samples of seaweed in Table 7. As noted in Table 5, the Raw seaweed results are the average of duplicate analysis (N=2), whereas the blanched treatment results are the results of single analysis.

5.5.4 Iodine Analysis

Iodine analysis of the four samples (raw, steam blanched, fresh water blanched and salt water blanched) of freeze-dried seaweed was conducted and recorded. All results are presented for the four types of seaweed in Table 8.

Table 7: Trace metal analysis results for four samples of freeze-dried seaweed (Saccharina latissima): raw, steam blanched, fresh water blanched and salt water blanched.

Metals	Raw	Steam Blanched,	Fresh Water blanched	Salt Water Blanched,
	Freeze-Dried *	Freeze-Dried	Freeze -Dried	Freeze -Dried
Aluminum	14.0 ± 0.57	13.7	11.4	16.2
Antimony	< 0.02	< 0.02	< 0.02	< 0.02
Arsenic	35.4 ± 0	30.2	21.1	18.8
Barium	14.05 ± 0.07	18.6	27.0	20.5
Beryllium	< 0.02	< 0.02	< 0.02	< 0.02
Bismuth	< 0.2	< 0.2	< 0.2	< 0.2
Boron	136.0	106.	19.9	34.5
Cadmium	0.588	0.683	0.948	0.295
Calcium	11400 ± 141.42	14200	20600	11200
Chromium	< 0.2	0.4	0.2	0.3
Cobalt	0.07 ± 0.01	0.07	0.08	0.03
Copper	0.6 ± 0	1.1	1.2	8.9
Iron	51.5 ± 0.71	63	68	55
Lead	0.41 ± 0.01	0.36	0.70	1.36
Lithium	0.51	0.37	0.23	0.13
Magnesium	6450 ± 14.14	6440	9230	2540
Manganese	3.75 ± 0.07	4.1	5.5	2.5
Mercury	0.03	0.05	0.05	0.05
Molybdenum	0.12	0.10	0.03	0.03
Nickel	0.2	2.4	3.0	0.3
Potassium	53100 ± 141.42	37500	18000	9600
Rubidium	19.75 ± 0.07	13.8	6.50	3.45
Selenium	< 0.2	< 0.2	< 0.2	< 0.2
Silver	< 0.02	< 0.02	< 0.02	< 0.02
Sodium	32350 ± 70.71	22800	12000	85100
Strontium	996.5 ± 0.71	1310	1880	1270
Tellurium	< 0.02	< 0.02	< 0.02	< 0.02
Thallium	< 0.02	< 0.02	< 0.02	< 0.02
Tin	< 0.02	< 0.02	< 0.02	0.10
Uranium	0.08 ± 0.01	0.09	0.10	0.10
Vanadium	0.85 ± 0.07	0.9	0.6	0.7
Zinc	8.7± 0.14	10.5	14.6	8.9

Note: For Raw (N=2), other types N=1. For Values recorded as <0.02 the observed values are below the detection limit of the equipment.

Table 8: Iodine analysis results for four types of freeze-dried seaweed (Saccharina latissima): raw, steam blanched, fresh water blanched and salt water blanched.

Metals	Raw	Steam Blanched,	Fresh Water blanched	Salt Water Blanched,
	Freeze-Dried	Freeze-Dried	Freeze-Dried	Freeze-Dried
Iodine (mg/Kg)	2148.5	2100.7	655.9	469.5

6 DISCUSSION

6.1 Test Plot

The process for design, licensing and execution of the test plot lines took more time than expected and we can attribute this mostly to the novelty of this research. Considering the experience while executing the plans for the test plot and the outcomes observed, from sourcing materials to handling the lines for harvesting, it is safe to state that a simpler design can be beneficial. A simpler design may reduce operational costs and facilitate the monitoring and harvesting of the lines without compromising the results.

The ballasts along the culture lines (experimentally removed from line number three) when deployed, for comparison, were designed to prevent the lines from moving sideways due to the sea current. These where proven to be unnecessary as line number three remained in place throughout the growing period. A decision from both the Shorefast and CASD team was made to remove the ballasts from lines one and two during the operation to sink the lines for the winter, due to the difficulty encountered in hauling the lines for monitoring and in anticipation for the harvesting operation. This change didn't affect the lines position, as observed for line three.

Regarding the timeline for deployment, the fact that the lines were deployed in August, four months before seeding, allowed biofouling and organisms to accumulate over this period (Figure 19 - Fouled line). Despite the attempt to clean the lines while seeding, it was not possible to totally remove micro algae, slime, mussels and other organisms that probably competed with the seaweed seeds for nutrients.

Sinking the lines in anticipation of pack ice occurrence was a laborious and time-consuming operation and the effectiveness of this activity couldn't be evaluated as no ice formation or movement was observed in the Cobb's Cove area for that season. In the future it would be more beneficial to monitor the weather conditions and plan to sink the lines only if necessary.

Overall, the test plot lines deployed were effective and allowed the seeding of the lines and outgrowing of kelp, however, adjustments for future operations can be beneficial as this may reduce materials and labour costs and increase the system's productivity.

Figure 19 - Fouled line

6.2 Nursery and Site Seeding

Harvesting mature seaweed for spore release is one of the critical points for establishing a seaweed nursery. Not only the quantity, but the quality of the samples available for spore release and seed production play an important role on seed quality and harvest output. The samples harvested from Fogo Island and brought to CASD's aquaculture facility by the Shorefast team were mature and had good quality however, desirable seaweed coverage on the spools was not achieved. Seaweed spores are motile and depend on this motility to move to a proper surface and settle. So, using the method described by Flavin *et al*. 2013, in which the solution is poured and mixed with saltwater on a settling tube may not be effective depending on the spore concentration per liter or on the spore activity after release. This could be one of the reasons why good coverage on spools was not observed. Additional research regarding changes in spore concentration and spool seeding methods is recommended for future nurseries.

Despite the lack of coverage in some portions of the spools, the nursery activities and seed development happened as expected. According to the literature available (Flavin *et al*., 2013; Redmond *et al*., 2014) the average nursery time for sugar kelp is four to six weeks, however, based on previous experience at CASD's seaweed nursery, it was observed that the desired size of the kelp blades (2-3 mm) for deployment will be achieved within five to seven weeks. Because of logistics, vessel and crew availability, the spools were transferred to the aquaculture site at the start of the fourth week of nursery. The spools were unwrapped onto the growing lines from a longliner (Figure 20) but demanded too much handling on both spool surface and seeded lines adding to the lack of coverage of the spools. It is possible that the premature seeding and seeding method contributed to the poor growth performance at the aquaculture site and, taking all these factors into consideration, it will be possible to improve the methods for seed preparation, nursery time and site seeding for better results in the future.

Figure 20 - Seaweed lines seeding

6.3 Monitoring, Harvesting

The monitoring process is important to keep track of kelp growth along the season and to observe signs of degradation and biofouling deposition. Ideally, harvesting should occur at first signs of deterioration and fouling to ensure a good quality of the harvested kelp. The monitoring was well conducted by the Shorefast team and, despite the low coverage of the lines, the measurements for length and number of blades per length happened as expected considering the time passed from seeding. Considering that 1.9 Kg per foot was harvested from the best section of the lines, it is safe to assume that, with adjustments on seed production and seeding methods it will be possible to harvest around one ton of seaweed from the three experimental lines.

6.4 Processing

The processing of the seaweed samples was designed to simulate practical methods for future product development. Sugar kelp has a high Iodine content, so blanching is performed when the intention is to remove part of this mineral (Nielsen *et al*., 2020). Visually, all the blanching methods caused the samples to change color from light brown to light green. The two drying methods, hot air and freeze drying were performed to prepare the samples for lab analysis in a way to detect the influence the drying methods on moisture and water activity. It was observed that the freeze-drying method promoted lower values of moisture and water activity in the samples. The results of this preliminary processing assessment will be useful for improving stabilization and product development of kelp.

6.5 Lab Analysis

6.5.1 Crude Proximate Analysis

Proximate compositions of the four samples (raw, steam blanched, fresh water blanched, and salt water blanched) of air-dried seaweed displayed significant differences between the treatment types (Table 4 & Table 5).

The highest range of proximate values was seen in the average ash contents which ranged from 11.74-22.94% wet weight basis (WWB). The fresh water blanched seaweed had the lowest ash content at 11.74% (WWB). Both Raw and salt water blanched seaweed had ash content values over 22% with 22.22% and 22.94% (WWB) respectively. The lower ash values of 16.56% and 11.74% (WWB) seen in the steam blanched and fresh water blanched, respectively, were likely due to the removal of various types of salts during the blanching process. A study by Nielsen *et al.*, 2020 on the effect of fresh water blanching on *Saccharina latissima* found that blanching at temperatures from 30-80°C and from 2-120 seconds gave a significant reduction in the ash content). They suggested that both the low salinity of the fresh water and increased temperature caused a shock to cells which caused the cell walls to rupture leading to minerals washing away even at the lowest blanching time of 2 seconds. Some water-soluble components can leach out during the blanching process (Xiao *et al.*, 2017). As some mineral salts such as sodium chloride, are highly soluble in fresh water the reduction in ash values for steam and fresh water blanched seaweed are likely due to fresh water in the blanching process. The content of mineral salts in the saltwater blanching water may have prevented some mineral salts from leaching out during blanching as both raw and saltwater blanched seaweed had similar ash contents.

The average carbohydrate content values also displayed significant differences between the treatment types with a range of 61.49-71.46% (WWB). The saltwater blanched seaweed had the lowest observed level of carbohydrates at 61.49% (WWB). The highest of carbohydrates was observed in the freshwater blanched seaweed at 71.46% (WWB).

Percent average moisture and protein displayed much smaller variation between observed values. Moisture ranged from 9.98%-12.74% (WWB) and protein ranged from 2.39-3.77% (WWB). Raw seaweed had both the lowest moisture at 9.98% and protein at 2.39%. Freshwater blanched seaweed had both the highest moisture at 12.74% and highest protein at 3.77%. The small increases in moisture in contrast to the Raw seaweed samples are likely due to picking up moisture during the blanching process.

Very little fat was found in all seaweed types tested, with a range of 0.13% (Raw)-0.28% (Freshwater blanched).

6.5.2 Salt Content

The salt content values of the four samples (raw, steam blanched, fresh water blanched, and salt water blanched) of air-dried seaweed displayed significant differences between the treatment types (Table 6).

The salt content values ranged from 2.78% (Freshwater blanched) to27.33% (Raw). The salt content values were similar to the average ash content values. The salt content of the steam blanched seaweed was between these values at 15.47%.

6.5.3 Trace Metals

Trace metal analysis of the four samples (raw, steam blanched, fresh water blanched, and salt water blanched) of freeze-dried seaweed displayed significant differences between the treatment types (Table 7). The trace metal analysis included results for some macro-minerals and trace minerals.

Macro-minerals are minerals which are typically needed at levels over 100mg/Kg per day and are required by the body to sustain basic functions (Farag *et al*., 2023). Health Canada provides dietary reference intakes for minerals (Health Canada, 2023). Health Canada daily intake macro-mineral reference values for males and females aged 19-50 for sodium, potassium, magnesium and calcium are listed in Table 9

The essential macro-minerals: calcium, magnesium, potassium and sodium, were found to be present in the raw seaweed (dwb) in significant amounts: 11400mg/Kg, 6450mg/Kg, 53100mg/Kg and 32350mg/Kg, respectively. Blanching caused an increase in calcium and magnesium 20600mg/Kg and 9230 mg/Kg, respectively, but a loss of potassium and sodium as both dropped to 18000mg/Kg and 12000 mg/Kg, respectively, for the fresh water blanched treatment. For the salt water blanched treatment, blanching caused a large increase in sodium which jumped to 85100mg/Kg, more than double the amount in raw seaweed. This increase is likely due to sodium uptake from the salt water. The salt water blanched treatment also saw a decrease in magnesium and potassium to 2540 mg/kg and 9600mg/kg, respectively. This decrease was likely due to the leaching of these minerals into the blanching water. Steam blanching did not result in significant changes from raw seaweed macro-mineral values, except for potassium which dropped to 37500 mg/Kg.

Table 9: Health Canada daily intake macromineral reference values for males and females aged 19-50 for sodium, potassium, magnesium and calcium.

Macromineral	Male, 19-50 years old (mg/day)	Female, 19-50 years old (mg/day)
Sodium	1500 (AI[1])	1500 (AI)
Potassium	3400	2600
Magnesium	400-420 (UL[2]=350)	310-320 (UL=350)
Calcium	1000-1200 (UL=2500)	1000 (UL=2500)

Note: [1] AI=Adequate intake; [2] UL = Upper Limit

Some trace minerals, which can play an important role in the prevention of some diseases and facilitate healing (Shirin *et al*., 2010) were found in the raw *S. latissima*. The minerals that were identified at trace levels in all the raw and treated seaweed were cadmium, chromium, copper, iron, lithium, manganese, molybdenum, nickel and zinc (Table 7). None of the treatments significantly changed the trace mineral levels except for nickel. Blanching in steam and freshwater lead to a ten-fold increase in nickel with an increase to 2.4mg/Kg and 3.0 mg/Kg, respectively, from raw which was identified as having 0.2 mg/Kg. A study on the blanching of *S. latissima* harvested off the coast of Sweden also found that blanching increased the levels of nickel (Trigo *et al*., 2023). In the Swedish study they found that blanching at 45°C for 2 minutes increased nickel levels from 0.73mg/kg (dwb) in unprocessed *S. latissima* to 1.45mg/Kg, and a higher temperature of 80°C increased that level to 4.31mg/kg (dwb). The increase in nickel after blanching is likely due to an increase in alginates in the blanched seaweed. Alginates, which are very abundant in brown seaweeds, such as *S. latissima* (Ordóñez *et al*., 2023; Sheng *et al*., 2004) can affect the bioabsorption of heavy metals. Alginates contain many carboxylic groups that can bind light metals, such as sodium, or exchange them for other elements/heavy metals, such as nickel (Davis et al., 2003). Trigo *et al.* (2023) confirmed that an up-concentration of heavy metals (e.g. nickel) occurred as the alginate residue increased after blanching. They did this by quantifying the principal alginate residues (mannuronic and guluronic acid) in their *S. latissima* samples. Their testing showed that the alginate residues increased from 21.2% (unprocessed seaweed) to 38.7% and 45.3% after blanching at 45°C and 80°C, respectively.

The levels of potentially toxic elements (PTEs): arsenic, cadmium, mercury and lead are listed for the raw freeze-dried seaweed and the blanched treatments in Table 10. The values of these PTEs in the raw and blanched seaweeds were within the range of reported literature values for fresh *Saccharina latissima* (Afonso *et al*., 2021)*,* (Bruhn *et al*., 2019)(Krook *et al*., 2024) *and* (Ometto *et al*., 2018) except for lead in the saltwater blanched treatment. Blanching caused a slight decrease in arsenic levels from raw seaweed at 35.4mg/kg dwb to 18.8-30.2mg/Kg dwb for the blanched treatments. Mercury had a very slight increase in all blanching treatments rising from 0.03 mg/kg dwb to 0.05mg/kg dwb.

A targeted survey of ready-to-eat foods & seaweed products by the Canadian Food Inspection Agency (CFIA) was carried out to determine baseline surveillance data on the level of metals in those foods (Canada, 2023). The survey included 183 ready-to-eat meals (fish/meat/pasta meals, pizza products and complete infant/toddler meals) and 9 seaweed products (fresh, roasted, dried/dehydrated, soups and snacks). The survey found that cadmium had the highest detection rate of the PTEs, with the highest levels coming from the seaweed products tested. The seaweed products had an average level of 1.48ppm (1.48ppm=1.48mg/kg) and a range from the limit of detection (LOD) to 4.99ppm. Although Canada does not have a regulation for metal levels in seaweed products, Health Canada evaluated the levels of metals found in the survey products and deemed those levels as safe for consumption by Canadians.

Although steam and freshwater blanching did increase the cadmium level it is still below the high levels reported in the CFIA study at 0.683 and 0.948 mg/kg dwb, respectively. Saltwater blanching caused a slight decrease in cadmium to 0.295 mg/kg dwb.

Table 10: Comparison of the concentration (mg/kg dry weight) of potentially toxic trace elements: arsenic, cadmium, mercury, and lead in literature values and test samples.

Seaweed Sample	Arsenic (mg/Kg dwb)	Cadmium (mg/Kg dwb)	Mercury (mg/Kg dwb)	Lead (mg/Kg dwb)
Literature values for raw *S. latissima*[1]	28-120	0.2-4.6	0.01-0.06	0.1-1.1
Raw FD[2]	35.4 ± 0	0.587 ± 0	0.03 ± 0	0.40 ± 0.01
Steam Blanched FD	30.2	0.683	0.05	0.36
Freshwater blanched FD	21.1	0.948	0.05	0.70
Saltwater blanched FD	18.8	0.295	0.05	1.36

Note: [1]Literature values are from (Afonso et al., 2021), (Bruhn et al., 2019)(Krook et al., 2024) and (Ometto et al., 2018). [2] Abbreviation: FD = Freeze Dried

6.5.4 Iodine Analysis

Iodine analysis of the four samples (raw, steam blanched, fresh water blanched, and salt water blanched) of freeze-dried seaweed displayed large differences between raw and the water blanched treatment types (Table 8). The iodine value dropped from 2148.5 mg/Kg for the raw seaweed to 655.9 mg/Kg and 469.5 mg/Kg for fresh water and salt water blanched freeze dried seaweed, respectively. A Norwegian study (Nielsen *et al*., 2020) found that various conditions of fresh water blanching significantly reduced iodine content. Of the conditions tested in the Norwegian study it was found that fresh water blanching at 60°C & 300 seconds and 80°C & 120 seconds gave the greatest reduction in iodine content to 321 mg/Kg and 293 mg/Kg (dwb), respectively from 4605 mg/Kg (dwb) for raw seaweed.

Table 11 compares the level of iodine for the raw freeze-dried seaweed and the blanched treatments with some literature values. The iodine values detected in the raw and blanched seaweeds were all below the range of reported literature values for fresh *Saccharina latissima* (Afonso *et al*., 2021), (Bruhn *et al*., 2019)(Krook *et al*., 2024) *and* (Ometto *et al*., 2018).

For Algal products, Australia has set an upper limit of 1000mg/Kg (dwb), and the US Food &Drug Administration (FDA) has set an upper limit of 1000mg/Kg (dwb) (Yeh *et al*., 2014), whereas France recommends less than 2000 mg/Kg (dwb) (ANSES, 2018).

Krook et al. (2024) studied the potential of various blanching treatments to reduce the iodine content of *S. latissima*. They reported that warm freshwater or seawater blanching reduced the dry iodine content by 73% and 59%, respectively, whereas the steam treatment was less efficient and reduced the iodine content by 26%. Similarly, the test samples had a slight reduction in the iodine content; the value dropped from the raw value by only 47.8 mg/Kg to a value of 2100.7 mg/Kg for the steam blanched seaweed (Table 11).

Table 11: Comparison of the concentration (mg/kg dry weight) of iodine in literature values and test samples.

Seaweed Sample	Iodine Conc. (mg/Kg)
Literature values for raw *S. latissima*[1]	2630-7977
Raw FD	2148.5
Steam Blanched FD	2100.7
Freshwater blanched FD	655.9
Saltwater blanched FD	469.5

Note: [1]Literature values are from (Afonso et al., 2021), (Bruhn et al., 2019)(Krook et al., 2024) and (Ometto et al., 2018).[2] Abbreviation: FD = Freeze Dried

7 CONCLUSIONS

The following conclusions were drawn from this project:

1. The test plot designed and deployed was effective for seaweed growth, but the design can be simplified to reduce costs and optimize the work, and the operation can also be improved.
    - 1.1 The biofouling that accumulated on the lines may have interfered with the seaweed development.
    - 1.2 Handling while seeding the lines from a longliner may have interfered with the number of seeds on the lines.
    - 1.3 Removing the ballasts in the growing lines didn't affect their positioning and facilitated the monitoring and harvesting work.

2. The method used for the preparation of the spools at the nursery phase wasn't effective to produce good quality seeds and the nursery time (starting of fourth week) wasn't enough to ensure that the seeds were transferred to the experimental aquaculture site with the proper initial condition.

3. The seaweed grew as expected, which makes Cobb's Cove a potential site for the operations, and an estimated average of one ton of sugar kelp could be produced at that site considering the current test lines and the growth observed in its best section.

4. Blanching reduced iodine content of sugar kelp samples. The process with freshwater also reduced the salt content, and freeze drying ensured lower moisture and water activity.

5. The proximate composition and trace metals were affected by all blanching methods and the potentially toxic elements detected were compatible with the literature for Sugar kelp and below the range considered harmful for human health.

8 RECOMMENDATIONS

The following recommendations are presented based on the study results:

1. The test plot design needs to be revisited and adjusted to facilitate deployment, monitoring and harvesting operations. A good source for updated information and experience sharing is https://www.greenwave.org/.

2. The seed production method needs to be improved for better spool coverage and the nursery time needs to be extended to the point in which the blades are stronger and ready to overcome the environmental challenges and grow.

3. It is recommended to use new clean lines while seeding the site.

4. A tech-transfer project to develop a seaweed nursery at Fogo Island would be the next step to improve the client's capability and autonomy to sustain and grow their seaweed operations.

5. After the preliminary processing assessment and lab analysis performed for this project, the next step for developing products using cultivated sugar kelp will require in-depth applied research focusing on possibilities for both clients and the Fogo Island community.

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