USING MACROALGAE, DISSOLVED NUTRIENT CONCENTRATIONS, AND
NATURAL ABUNDANCE STABLE ISOTOPES TO TRACK SOURCES OF
NITROGEN IN AQUACULTURE FARMS

by

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DEDICATION PAGE

To my friends and family, near and far, who helped and supported me from across the globe.

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ABSTRACT

Seaweed aquaculture is a growing market in the North Atlantic which has sparked the need for finding areas that can naturally support their growth. In this thesis six farms growing *Saccharina latissima,* or sugar kelp*,* were evaluated*.* At these farms, kelp growth had no relationship with nitrate or phosphate, but had an inverse relationship with ammonium and temperature. This suggests that turbidity, irradiance or salinity is affecting the growth at a few sites. Not only are kelp marketable products but they are also used as tools to trace and track anthropogenic nutrients in coastal bays. In the second study, *Chondrus crispus*, *Ulva lactuca* and dissolved natural abundance isotopes of nitrate and ammonium were used to assess nutrient pollution in Shelburne, NS. This study showed that nitrogen is in a constant state of flux which makes monitoring anthropogenic source in coastal harbours difficult.

LIST OF ABBREVIATIONS AND SYMBOLS USED

Abbreviations	Description
δ	Delta-notation
$\delta^{15}N$	Nitrogen isotope ratio
$\delta^{18}O$	Oxygen isotope ratio
°C	Degrees Celsius
μM	Micromoles per liter
%	Percent
‰	Per mil
^{14}N	Isotope of Nitrogen with mass of 14
^{15}N	Isotope of Nitrogen with a mass of 15
^{16}O	Isotope of Oxygen with a mass of 16
^{17}O	Isotope of Oxygen with a mass of 17
^{18}O	Isotope of Oxygen with a mass of 18
AH	Arichat Harbour
BB	Bounty Bay Shellfish
CB	Cape Breton
CBB	Cape Breton Bivalves
CERC	Canadian Excellence Research Chair
CHN	Carbon Hydrogen Nitrogen
cm	Centimeters
COVE	Centre of Ocean Ventures and Entrepreneurship
CT	Connecticut
CTD	Conductivity, Temperature & Depth
FMQ	Fermes Marines du Québec
GC-IRMS	Gas chromatography Isotope-ratio Mass Spectrometer
hr	Hour
Hypobromite	BrO^-
km	Kilometers

Abbreviations	Description
LP	Lennox Passage
M	Moles per liter
m	Meter
MA	Massachusetts
mg	Milligrams
mg/L	Milligrams per Liter
min	Minute
mL	Milliliter
mm	Millimeter
MVCO	Martha's Vineyard Coastal Observatory
N	Nitrogen
N_2	Atmospheric Nitrogen
N_2O	Nitrous Oxide
NH	New Hampshire
NH_4^+	Ammonium
NO_2^-	Nitrite
NO_3^-	Nitrate
NO_3+NO_2	Nitrate+Nitrite
NS	Nova Scotia
O	Oxygen
OFI	Ocean Frontier Institute
OPA	Orthophthaldialdehyde
PCA	Principal Component analysis
PO_4^{3-}	Phosphate
POS	Provasoli's solution
PS	Premium seafoods
R^2	Coefficient of determination
SAB	St. Ann's Bay
SD	Standard deviation

Abbreviations	Description
SE	Standard Error
UCONN	University of Connecticut
UNH	University of New Hampshire
VSMOW	Vienna Standard Mean Ocean Water
WHOI	Woods Hole Oceanographic Institute

ACKNOWLEDGEMENTS

CHAPTER 1 INTRODUCTION

1.1 MOTIVATION

The uses of macroalgae in the aquaculture industry is becoming more important worldwide for both commercial and environmental purposes. Commercially, seaweeds are used in many products including fertilizers, cosmetics, fish feeds, pharmaceuticals and as a food source. Between 2000 and 2018, the global production of seaweeds tripled from 10.6 million tonnes to 32.4 million tonnes (*FAO*, 2020). In North America, seaweed farms represent approximately 0.1% of the total global production (Piconi et al., 2020). This increased interest in seaweed aquaculture has sparked the need for finding areas, such as Atlantic Canada and the Northeastern United States, that can naturally support their growth in the water column.

Aquaculture has the potential to increase food supply and strengthening local economies (Frankic & Hershner, 2003). The aquaculture industry in Nova Scotia provides a small but significant economic impact to the province by providing jobs and money to rural communities. In 2020, Nova Scotia's Aquaculture production, which included kelp for the first time, generated over $90 million in revenues (*NSDFA*, 2021). Since there are currently three producers of kelp in Nova Scotia, the estimated value is protected in accordance with the freedom of Information and Protection of the privacy Act (NSDFA, 2021). In the United States, aquaculture is a $1.5 billion industry with thriving finfish and shellfish industries on every coast (Raimondo et al., 2021).There are risks associated with aquaculture including creating reduced levels of oxygen, enrichment of nutrients such as nitrogen and phosphorus, and changes in food web interactions (Frankic & Hershner, 2003).

In Nova Scotia, there have been a couple of studies that look at the effects of anthropogenic nitrogen in various harbours but prior only one other study has used macroalgae to attempt to trace and identify pollutants (Howarth et al., 2019). Using macroalgae as a tool to trace nutrients is one way to understand how increased amounts of anthropogenic nitrogen in the environment affects the health of the coastal ocean.

Anthropogenic nitrogen can come from effluents produced at finfish farms, wastewater treatment plants, agriculture runoff, and other indirect sources (García-Sanz et al., 2010; García-Seoane et al., 2018). These sources of excess nitrogen are leading to adverse side effects including, but not limited to, the reduction in desirable fish catches, depletion of deep-water oxygen, and shifts in phytoplankton composition (Smith, 2003; Eddy, 2005). With a surplus of various aquaculture ventures, there is the potential for an increase in waste produced from the finfish farms which could in turn affect aquaculture production. By looking at macroalgae from both a commercial and environmental perspective, the goal is to find ways to not only expand the commercial use of macroalgae but to also potentially use macroalgae as a way to trace pollutants.

1.2 BACKGROUND

Seaweed cultivation is a relatively new industry that is starting to take root in North America. Atlantic Canada and the northeastern United States are two regions expected to increase aquaculture production over the next several years. In 2013, 46% of Canada's national aquaculture production output came from the Atlantic provinces, with Nova Scotia making up roughly 5% of the total (ACOA, 2013) with the majority coming from finfish and bivalve aquaculture. In the United States, the commercial seaweed market was valued at approximately $311.4 million in 2019 (*Grandview Report*, 2020) with the largest producer of seaweeds coming from Maine and Alaska (Piconi et al., 2020). Approximately 58% of the edible seaweeds production in the US is conducted in the northeast with only 3% coming from Massachusetts, Connecticut, New York, New Hampshire and Rhode Island (Piconi et al., 2020). Currently there are more than 27 commercially operated sea farms growing *Saccharina latissima* between the New England region and New York (Kim et al., 2019).

Nova Scotia, Connecticut, Massachusetts and New Hampshire have the potential to become bigger players in the cold-water seaweed aquaculture industry due to many of the harbours and bays having optimal growth. Macroalgae growth is controlled by environmental factors including light, temperature, salinity, carbon dioxide, currents, and

pH (Handå et al., 2013; Roleda & Hurd, 2019; Sharma et al., 2018); as well as, biological factors such as the life stages and age class of the macroalgae (Roleda & Hurd, 2019). Together, these factors can affect the ability of macroalgae to take up nutrients and grow. It is important to note, that the optimal conditions and factors that affect macroalgae vary between species.

A common type of macroalgae grown in aquaculture farms is *S. latissima. S. latissima,* common name sugar kelp, is a large brown macroalgae that has a life span of two to four years (White & Marshall, 2007). Generally, *S. latissima* grows well in waters between 10-15˚C, but can withstand temperatures up to 23˚C for short periods of time before degrading (Bolton & Lüning, 1982; White & Marshall, 2007). This is an important factor because in Nova Scotia, the water temperatures tend to stay below 19.5˚C at the surface whereas water temperatures in the New England area can exceed 21˚C in the late summer ("Buzzards Bay water temperature", n.d.; "Cape Breton water temperature", n.d.). Generally, in late winter and early spring, *S. latissima* grows quickly, with growth rates between 1.1 cm/day to 4.8 cm/day, (White & Marshall, 2007). In the summer, growth is slowed likely due to nitrogen limitation (Gagné et al., 1982)

Nutrients, like temperature, affect growth. Typically, during the winters in Nova Scotia cold nutrient-rich water is upwelled into these harbors, supplying nutrients to seaweeds and other sessile organisms (*Natural History of Nova Scotia, Volume 1*, n.d.). Dissolved nutrient concentrations in harbours and bays around Nova Scotia are highest in winter and early spring and decrease in the late spring. In St. Margaret's Bay (Nova Scotia), Chapman et al., (1977) found that nutrients were highest in the winter during periods of upwelling and decreased in the summer months, following the expected seasonal patterns for temperate regions. Nutrient cycling in the Northeastern US exhibits similar seasonal patterns to those seen in Nova Scotia but due to increased urbanization in this area, some areas may exhibit higher concentrations than would naturally occur.

Nitrogen, which is a major nutrient required for biological productivity, can limit primary production on both spatial and temporal scales (Casciotti, 2016). Macroalgae,

like *S. latissima*, require the bioavailable form of nitrogen, nitrate, nitrite, or ammonium (fixed nitrogen) to grow since they can only assimilate nutrients that are dissolved in the water. They can deplete the nitrogen and phosphorus in the water and store it to continue growing once nutrients are gone. Kelp use these nutrients and accumulate them in their tissues for later use (Kerrison et al., 2015). Young et al (2009), saw that to maximize growth and for the collection of internal storage, nitrate concentrations of at least 10 μM are necessary (Chapman et al.,1978). Nutrients are taken up by macroalgae in either through the passive transport of nutrients down a concentration gradient or the active transport of nutrients against the concentration gradient (Hurd et al., 2014; Harrison & Hurd, 2001). In areas that have low nitrogen concentrations, it would be necessary for the tidal flow rates to be faster as that helps increase nutrient uptake by the kelp (Kerrison et al., 2015; Wheeler & North, 1980). Chapman and Craigie (1977) conducted one of the first studies in Nova Scotia that showed that low growth rates of seaweeds correlated with low nitrogen concentrations. Additionally, Gagné (1982) showed that depending on the year round availability of nitrogen, the growth rates of *Laminaria longicruris* were affected. Understanding the seasonal patterns of nutrients and how it affects growth of kelp in coastal waterways is important when choosing sites for aquaculture farms.

Photosynthetically active radiation (PAR), or the availability of light can inhibit or threaten the survival of the macroalgae (Airoldi & Beck, 2007; Kerrison et al., 2015). To much or too little light can limit photosynthesis. Typically, *S. latissima* is naturally found at a depth of less than 30 meters (White and Marshall, 2007; Boden, 1979). In Maine, Boden (1979), reported that the best depth for growth occurred between 9 and 12 meters. Depending on the life stage of the kelp, the amount of light available can either positively or negatively impact the growth.

If salinity levels are low, the photosynthetic ability of macroalgae is also affected. When *S. latissima* is exposed to water with salinities lower than 6, a 95% reduction in their ability to photosynthesis has been shown to occur (Karsten, 2007; Peteiro & Sánchez, 2012). *S. latissima*, is more tolerant to low salinities, and has been shown to

survive in a salinity of a ~11 for up to 4 days before showing signs of degradation (Peteiro & Sánchez, 2012).

While seaweeds are cultivated in aquaculture for fertilizers and other products, they can also be used as a tool to trace nutrient sources. Shelburne, NS is currently home of a large-scale finfish aquaculture industry. To monitor the flux of nitrogen in and out of the bay, two types of macroalgae, *Chondrus crispus* and *Ulva lactuca*, were deployed. *C. crispus*, commonly referred to as Irish moss, is a red macroalgae that is found in the rocky shores of the Northern Atlantic. Typically found within the upper 7 meters of the water column attached to rocks, they can also be found floating around in groups (Collén et al., 2014). *U. lactuca* is a green macroalgae that can be grown attached to substrate or free floating (Dominguez & Loret, 2019). Similar to *S. latissima*, *C. crispus* and *U. lactuca* are cold-water seaweeds that can withstand temperatures as low as 4˚C and even freezing conditions for brief periods of time (Collén et al., 2014; Dudgeon et al., 1990).

C. crispus and *U. lactuca* vary in their growth patterns. *C. crispus*, grows apically, meaning new growth forms at the tips of the blade (Chopin, Hourmant, Floc'h, & Penot, 1990). Whereas, *U. Lactuca* grows new tissue around the entire fond, this is known as uniform growth and can assimilate dissolved nutrients after 48 hours (Orlandi et al., 2014). By using macroalgae as bioassays, long term fluctuations can be observed as the plants take up the nitrogen and store it in their tissues (García-Sanz et al., 2010). Bioassays are helpful since dissolved nutrients, which are useful in understanding pollution in harbors, can be diluted quickly due to hydrodynamic conditions like tides, currents, and flushing time (García-Sanz et al., 2010). *U. lactuca* is one of the most commonly used species for bioindicator because of its fast growth rates (García-Seoane et al., 2018).

Nitrogen (N) is a major nutrient required by phytoplankton and macroalgae. Two bioavailable forms that macroalgae use are nitrate (NO_3^-) and ammonium (NH_4^+), which are present in the ocean as dissolved nutrients. N has two stable isotopes with atomic

masses of 14 (^{14}N) and 15 (^{15}N). To measure the heavy and light isotopes in a sample, δ-notation where R is ^{15}N/^{14}N with the units as per mil (‰)(Peterson & Fry, 1987):

$$\delta^{15}N\ (‰) = \left\{\frac{R_{sample}}{R_{standard}} - 1\right\} \ x\ 1000$$

The universally accepted standards for N is atmospheric N_2 (0‰). δ^{15}N values vary depending on the material and types of processes- chemical, physical, or biological occurring at the time (Casciotti, 2016; García-Seoane et al., 2018; García-Sanz et al., 2010). During processes where nutrients are assimilated, organisms fractionate the isotopes by preferentially taking up the lighter isotopes ^{14}N. Dissolved natural abundance stable isotopes of nitrate and ammonium allow for the examination of the conditions in that region at one specific point in time.

1.3 RESEARCH OBJECTIVES

This thesis is two pronged. The first goal is to monitor the effects of nutrients on kelp growth. By doing this, we will be assisting seaweed aquaculture farmers in locating waterways with adequate nutrient supply that can support the growth of macroalgae in the water column. The seaweed aquaculture study is a pilot project being used to determine the feasibility of increasing the commercial kelp farming industry. In Nova Scotia, this project was the first attempt to grow kelp in an aquaculture setting, specifically on small scale farms that have the potential to create more jobs in rural communities. The second goal was to examine how nitrogen loading from multiple effluent sources was affecting the water quality of a local harbour.

To address these two goals, the thesis will focus on the following objectives:
1. Determine which sites are ideal for the installation of aquaculture farms to support growth of *S. latissima* and other kelp. Identify how nutrient concentrations affect the growth of seaweeds in multiple coastal bays in Cape Breton (Canada), Massachusetts (USA), New Hampshire (USA) and Connecticut (USA).

2. Investigate how the natural abundance of ^{15}N and ^{18}O isotopes in nitrate and ammonium and ^{15}N in macroalgae tissues can be used to trace nutrient pollution in Shelburne Harbour, Nova Scotia.

1.4 APPROACH

In the first study, *survey of seaweed aquaculture farms in the Northwestern Atlantic,* water samples were analyzed for nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), and phosphate (PO_4^{3-}) concentrations to determine if the ambient nutrient concentrations were high enough to support kelp growth. *S. latissima* grown on the farms was characterized for multiple biomass characteristics such as blade length, width, and thickness to examine how well the kelp grew at each site.

In the second study, *using bioindicators to trace pollutants in Shelburne Harbour,* water samples were analyzed for ammonium, nitrate and nitrite concentrations to monitor the flux of nitrogen in and out of Shelburne Harbour. Dissolved natural abundance stable isotopes of nitrate and ammonium were measured in water samples to pinpoint the sources of nitrogen in the bays and to understand how the microbial biogeochemical processes such as anammox, denitrification, nitrification and dissimilatory nitrate reduction produce and consume nitrogen over time (Buchwald et al., 2015; Sigman et al., 2009). $\delta^{15}N$ and $\delta^{18}O$ in nitrate and $\delta^{15}N$ in ammonium allow us to examine the conditions in those regions at one specific point in time. Tissue samples of two macroalgae, *C. crispus* and *U. lactuca*, were analyzed for $\delta^{15}N$ to trace sources of nitrogen in Shelburne Harbour. Algal $\delta^{15}N$ values reflect the $\delta^{15}N$ of the nitrogen source and were used as tracers of anthropogenic and natural N inputs (Dailer et al., 2010; Lemesle et al., 2016; Orlandi et al., 2014). By combining dissolved natural abundance stable isotopes and algal tissue $\delta^{15}N$ values, long term data can be more effectively collected to see what is occurring at different time scales.

1.5 THESIS/MANUSCRIPT ORGANIZATION

This thesis is organized as follows. Chapter 2 presents the methods and materials used in the field as well as laboratory procedures, statistical and spatial analyses. Chapter 3 combines the results and discussion for the first study, *survey of seaweed aquaculture farms in the Northwestern Atlantic*. Chapter 4 focuses on the second study, *using bioindicators to trace pollutants in Shelburne Harbour*. Chapter 5 presents the concluding ideas to this thesis. Appendix A focuses on the methods used to produce the seed lines used in the first study. Appendix B presents biomass characteristic results not discussed in chapter 3.

CHAPTER 2 METHODS & MATERIALS

2.1 FIELD SAMPLING

2.1.1 Survey of seaweed aquaculture farms in the Northwestern Atlantic

This study was conducted at six different farms. Three were Cape Breton, Nova Scotia in Arichat Harbour, Lennox Passage, and St. Ann's Bay. Three were in the Northeastern United States. One farm was in Buzzards Bay, Massachusetts, one in the Piscataqua River, New Hampshire and one in Long Island Sound, Connecticut (Figure 2.1). The sites were chosen, where leases were already approved. A lease is an area in which the respective governments have granted and approved companies and organizations to conduct seaweed aquaculture ventures.

Arichat harbour, is approximately 3 km long, and has two entrances to Chedabucto Bay. The deepest point on the lease is approximately 15 m. The small town of Arichat, with a population roughly of 4300 people, is located next to the harbour. Lennox Passage is located on the other side of Isle Madame and is approximately 22 km long and roughly 14 m deep. This passage has two openings as well: one into Chedabucto Bay and the large one into the Atlantic Ocean. Lennox Passage is not heavily populated but has a few towns including River Bourgeois and St. Peters along its coastline. Most of the coastline is sparsely or unpopulated. Similar to Lennox Passage, a majority of the St. Ann's Bay coastline is sparsely inhabited, with the largest population located in Englishtown on the northeastern side of the bay. St. Ann's Bay is approximately 20 km long with a small passage, approximately 150 meters splitting the bay into two sections, more inland as St. Ann's harbour and the outer potion as St. Ann's Bay which opens to the Atlantic Ocean near the Cabot strait. The deepest point on the lease is approximately 19 m.

Buzzards Bay is 45 km long and 12 km wide. The lease is located southeast of the northernmost Weepecket Island and is approximately 9 meters deep at low tide. Long Island Sound is tidal estuary situated between New York and Connecticut. It is roughly

180 km long and 34 km wide at its widest point. The GreenWave lease, is located near Stony creek, Connecticut on the North side of the estuary and is located in waters between 3 and 6 meters deep. The third lease in the United States, is located at the mouth of the Piscataqua river known as Portsmouth Harbor which is approximately 10 km in length. The University of New Hampshire (UNH) farm is located close to New Castle in waters between 6 and 9 meters deep. All three farms in the United States, are located in urban areas. Further physical characteristics of each site can be found in Table 2.1.

Table 2.1: Physical characteristics of farms

Location	Latitude	Longitude	Max depth (m)	Temperature Range (° C)	Salinity	Ice coverage
Arichat Harbour	45.5055	-61.01980	15	-0.2-5.0	30	no
Buzzards Bay	41.5203	-70.73166	9	4.0-16.6	35	no
Lennox Passage	45.5881	-61.00916	14	-0.3-11.6	30	no
Long Island Sound	41.2572	-72.76581	6	3.8-18.3	n/a	no
Piscataqua River	43.0689	-70.70849	9	4.2-13.9	n/a	no
St. Ann's Bay	46.2625	-60.57320	19	n/a	17-28	yes
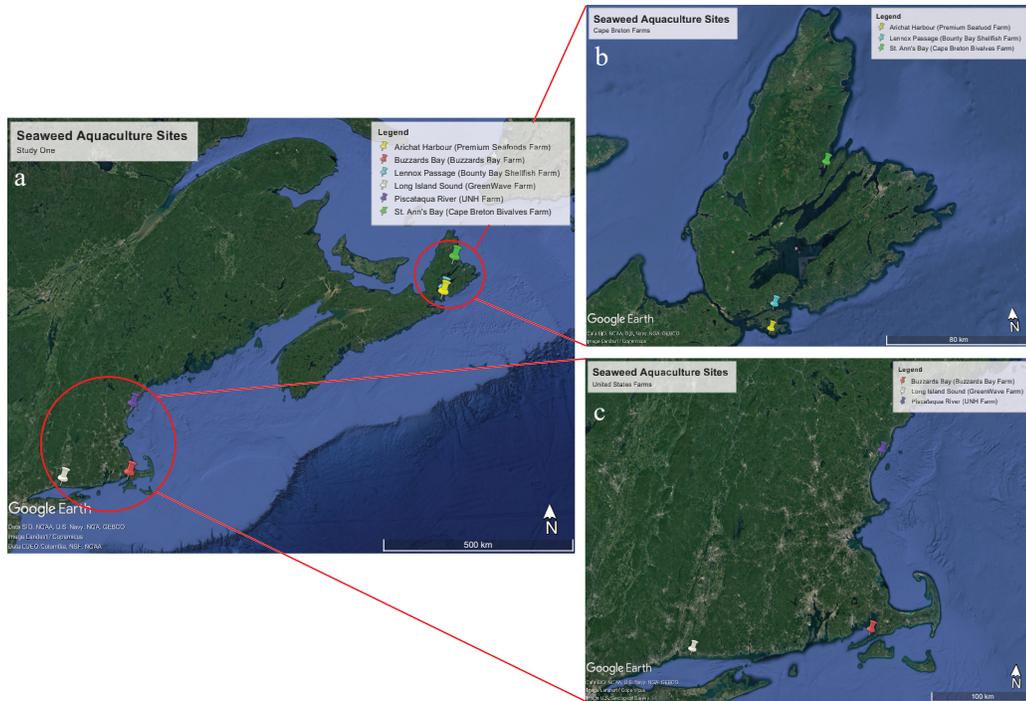
Figure 2.1: (a) Map of all kelp farms along the east coast of North America. Three sites in the United States, three sites in Canada. (b) Zoomed in section of the Cape Breton kelp farms. (c) Zoomed in section of the kelp farms in the United States

2.1.1.1 Methods for seaweed cultivation at three farms in Cape Breton

Three bays were studied in Cape Breton. At each location, three stations were sampled to test for nutrient variability within the harbour (Figure 2.2). One station was at the entrance to the bay, one at the center of the lease where the kelp would be housed, and the last located further into the bays.
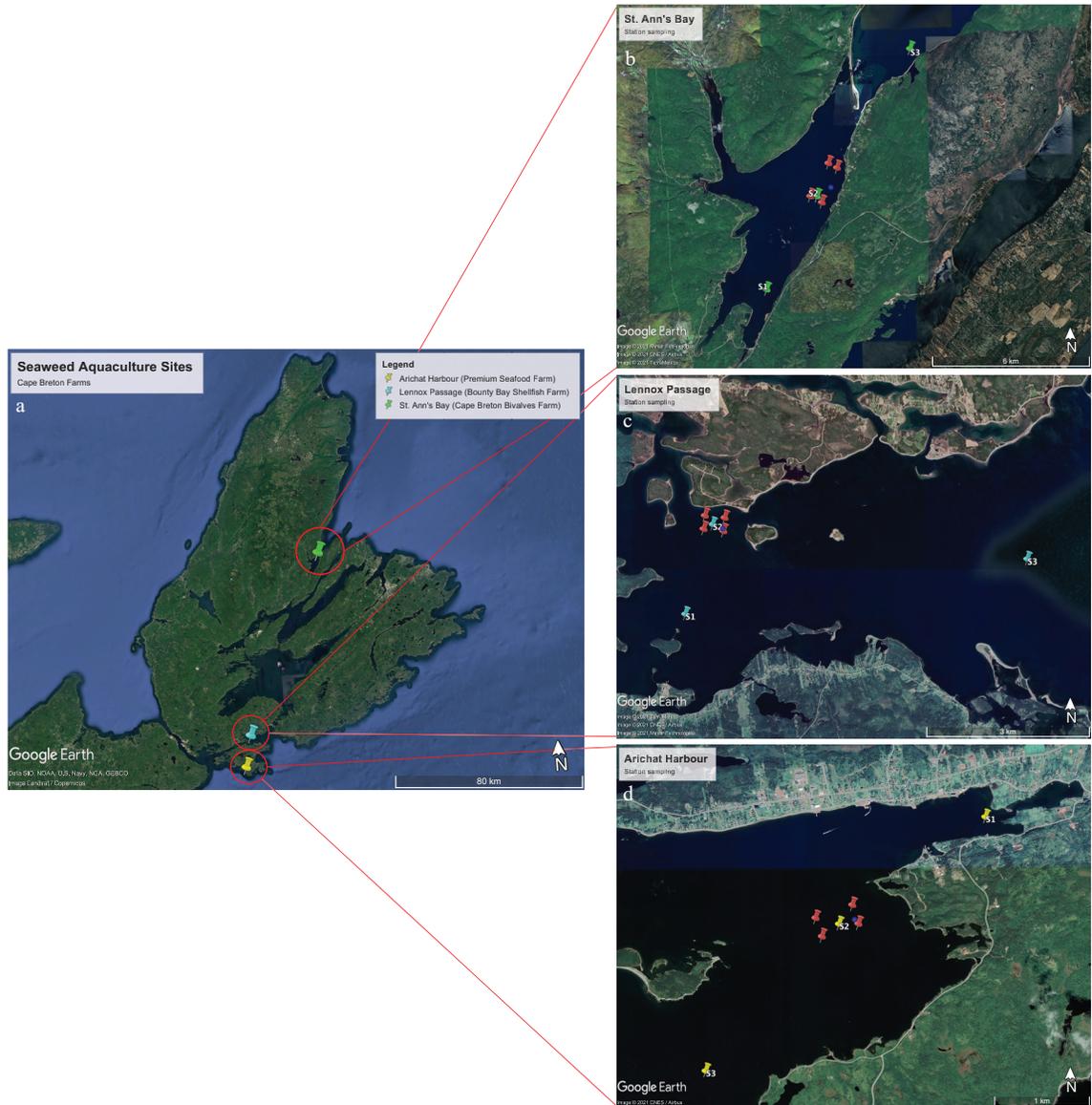
Figure 2.2: (a) Map of Cape Breton kelp farms. (b) Zoomed in view of St. Ann's Bay. (c) Zoomed in view of Lennox Passage. (d) Zoomed in view of Arichat Harbour. The orange pins represent the border of lease in which the seeded lines were grown. The green, blue and yellow pins in maps a, b and c represent Stations 1, 2 and 3 at each site. The blue dot in the lease are the Hobo loggers.

Hobo U24 temperature loggers were deployed between 2018-2020 at all three sites at depths of 2 meters and recorded data every 30 minutes. Initial logger deployment dates for each site can be found in Table 2.2. In November 2019, the loggers were temporarily removed to allow data extraction and then put back in between seven and ten days later when the seeded lines were installed on all three farms. The Hobo U24 salinity

loggers were also deployed but not used due to technical issues and were not reinstalled after the first time they were put in the water.

Table 2.2: Field deployment and collection information from the Cape Breton seaweed farms

Location	Farm name	Logger deployment date	Logger data collection rate	Logger depth (m)	Seed line deployment date	Line depths (m)	Harvest date	Harvest processing date
Arichat Harbour	Premium Seafoods Farm	January 4, 2019	30 minutes	2	November 20, 2019	2 & 4	June 14, 2020	June 15, 2020
Lennox Passage	Bounty Bay Shellfish Farm	April 2, 2019	30 minutes	2	November 19, 2019	2 & 4	June 14, 2020	June 15, 2020
St. Ann's Bay	Cape Breton Bivalves Farm	November 2, 2018	30 minutes	2	November 22, 2019	2 & 4	June 24, 2020	June 25, 2020

In July, September and October 2018, divers collected mature *S. latissima* near each lease. Wild sporophytes were harvested to use as spawners to produce seed lines. Details on the production of the seed lines can be found in Appendix A. Seed lines were installed over the course of one-week, specific dates can be found in Table 2.2. For each farm, four single 100-meter lines of seaweeds were deployed. Two of the lines were located at depths of 2 m and the other two at 4 m depth. The ends of each line were held down by weights with a buoy at the surface and at least one buoy was placed in the center of the line to assist in keeping the line at the proper depth as *S. latissima* grew and became heavier (Figure 2.3).
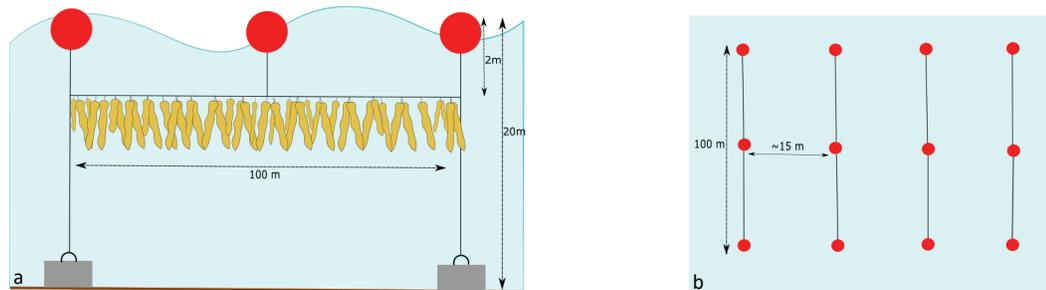
Figure 2.3: Diagram of farm layout at Cape Breton sites: (a) side view and (b) aerial view, where the red circles are the buoys (Lionard, 2019).

Lines were installed by either manually winding the seed line around the main rope (Figure 2.4a) or passing the rope through the inside of a collector and unwinding (Figure 2.4b). The method was selected based on the farm's ability and at the discretion of each farmer.
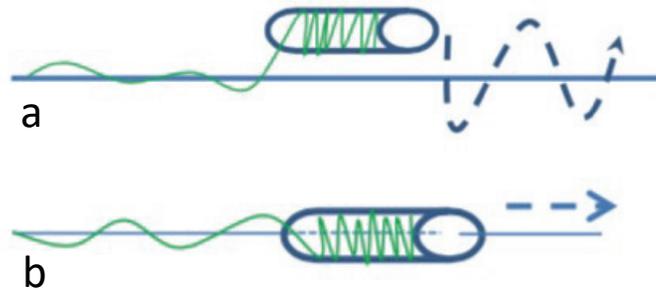
Figure 2.4: Diagram of seed line installation methods. (a) Manually winding the seed line around the main rope. (b) passing the rope through the inside of the collector and unwinding (Lionard, 2019).

Surface water samples at depths of 0 m, 2 m, 4 m, and 6 m were collected monthly from the Cape Breton sites between May and November 2019. Samples were collected at three stations during this time. Starting in December 2019, samples were only collected at 0 m from the farm, Station 2. Surface water from Arichat Harbor was collected between December 2019 to May 2020. Water was not collected between this time at Lennox Passage and St. Ann's Bay due to ice coverage and the removal of boats from the water for the winter. Due to the Covid-19 pandemic restrictions, spring sampling did not occur at any site. In June 2020, a surface water sample was taken at the time of harvest. All sample bottles were pre-rinsed three times with seawater and then rinsed three more times with filtered seawater. Using 0.45 μm sterile syringe filters, 60 mL samples were filtered into the collection bottles. Samples were then stored at cold temperatures until they could be placed in a -20°C freezer.

Originally, seaweed growth was also supposed to be tracked in January and March 2020. At Arichat the farmers were able to check on the growth of the seaweeds in January but were unable to measure the growth at St. Ann's Bay and Lennox Passage. Due to the Covid-19 pandemic restrictions, all sampling trips between March and June 2020 were canceled.

In June 2020, *S. latissima* was harvested from the lines by the farmers for biomass characterization and tissue analysis. The lines at Arichat Harbour and Lennox Passage were harvested on June 14, 2020 and in St. Ann's Bay on June 24, 2020 (Table 2.2). Three 50 cm plots were collected from the 2-meter and 4-meter lines from each site. Seaweeds from every plot were counted and the longest 20 were characterized for wet fresh weight, blade length, stipe length, and blade width. Due to Covid-19 restrictions, the team was unable to harvest the seaweeds with the farmers. Instead, the seaweeds were harvested in the morning and then transported to Halifax on the same day so that characterization could occur at the Centre of Ocean Ventures and Entrepreneurship (COVE). Five seaweeds from each plot were collected and transported back to the laboratory over ice to be stored in a -20°C freezer. These samples were then dried and used for determining nitrogen and carbon content (described in Section 2.2.2).

2.1.1.2 Methods for seaweed cultivation at three farms in the Northeastern United States

Three farms in the Northeastern United States; Piscataqua River in New Hampshire, Long Island Sound in Connecticut, and Buzzards Bay in Massachusetts (Figure 2.1c), were examined to compare to the farms in Nova Scotia. Hobo U24 temperature loggers were installed at GreenWave farms in Long Island Sound and at the UNH farm in the Piscataqua River. Table 2.3 presents the dates loggers were deployed at each site and their data collection rates.

Table 2.3: Field deployment and collection information from the United States farms.

Location	Farm name	Logger deployment date	Logger data collection rate	Logger depth (m)	Seed line deployment date	Line depths (m)	Harvest date	Harvest processing date
Buzzards Bay	Buzzards Bay Farm	N/A	N/A	N/A	December 13, 2019	3	No harvest	N/A
Long Island Sound	GreenWave Farm	October 1, 2019	6 minutes	2	December 4, 2019	2	June 1, 2020	June 1-2, 2020
Piscataqua River	UNH Farm	December 6, 2019	10 minutes	2	December 6, 2019	2	June 9, 2020	June 9-10, 2020

Temperature loggers were not installed at Buzzards Bay instead data from Martha's Vineyard Coastal Observatory, MVCO, were used. The CTD, conductivity, temperature, and depth instrument, was deployed at 4 meters below the air-sea interaction tower. The tower was approximately 56 km away from the site. No salinity loggers were installed at any of these locations.

In the United States water and seaweed samples were collected from all three sites. These samples were collected between installing the seed lines in November and harvesting in June. Sampling frequency was determined by how often the U.S team was able to visit the site. Table 2.4 depicts the dates and frequency at which the sites in the United States were sampled.

Table 2.4: Water sampling dates and data collection frequency

Location	Farm name	Water collection dates	Did winter sampling occur?	Data collection rate
Buzzards Bay	Buzzards Bay Farm	November 2018-June 2019; December 2019-February 2020; June 2020	yes	monthly
Long Island Sound	GreenWave Farm	January-March 2020; April 2020	yes	weekly
Piscataqua River	UNH Farm	December 2019- March 2020	yes	weekly

Water sampling for these sites was unable to occur at full capacity or at all between March and May 2020 due to the Covid-19 pandemic. Like the Cape Breton sites, bottles were pre-rinsed three times with seawater and rinsed three more times with filtered seawater. Using 0.45 μm sterile syringe filters, 30 mL of surface water were filtered into the bottles. Upon collection, the samples were stored in a -20 °C freezer until they could be shipped to Dalhousie University.

At UNH and GreenWave farms, 5-line arrays were deployed (Figure 2.5). Each of the systems was designed to keep the seeded lines at 2 meters depth (Lindell, 2020). The seed lines for UNH were 67 meters long while the GreenWave farm lines were 48.7 meters long. The UNH array was deployed on November 19, 2019 using the R/V Gulf Challenger and the GreenWave array was deployed using the GreenWave farm vessel, the "Mookie", on November 21, 2019 (Lindell, 2020).
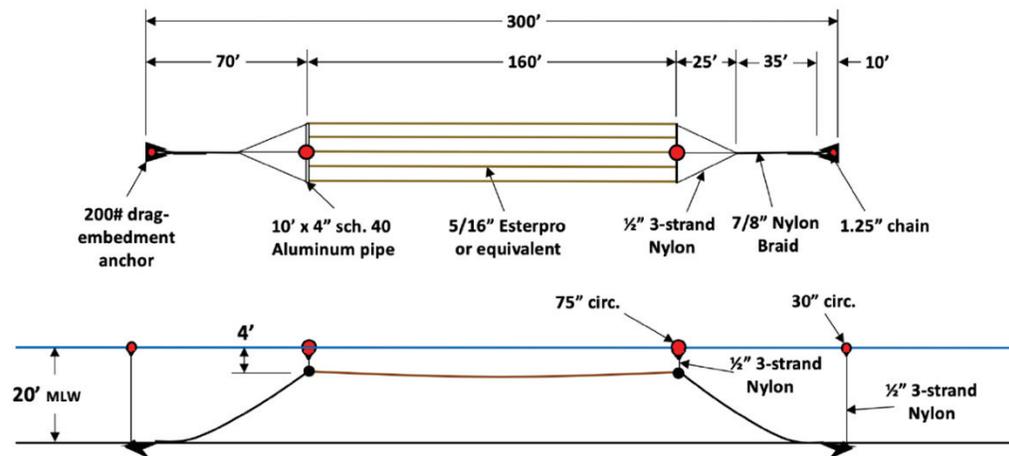
Figure 2.5: Diagram of 5-line array used at GreenWave farm site. The set up at UNH was similar except that the lines were longer (Lindell, 2020).

On December 3, 2019, collaborators at Woods Hole Oceanographic Institute (WHOI) and the University of Connecticut (UCONN) attached the seed lines of the various phenotypic crosses to the ropes inside the GreenWave facility in New Haven, Connecticut and stored them in coolers overnight. Each cross was marked and labeled as its own plot. On December 4th, the lines were put in the water in Long Island Sound and attached to the array making sure all five lines were taut (Lindell, 2020) (Table 2.3). The same process for the farm in the Piscataqua river occurred on December 5th and 6th at the UNH Marine lab in Newcastle, New Hampshire (Lindell, 2020) (Table 2.3).
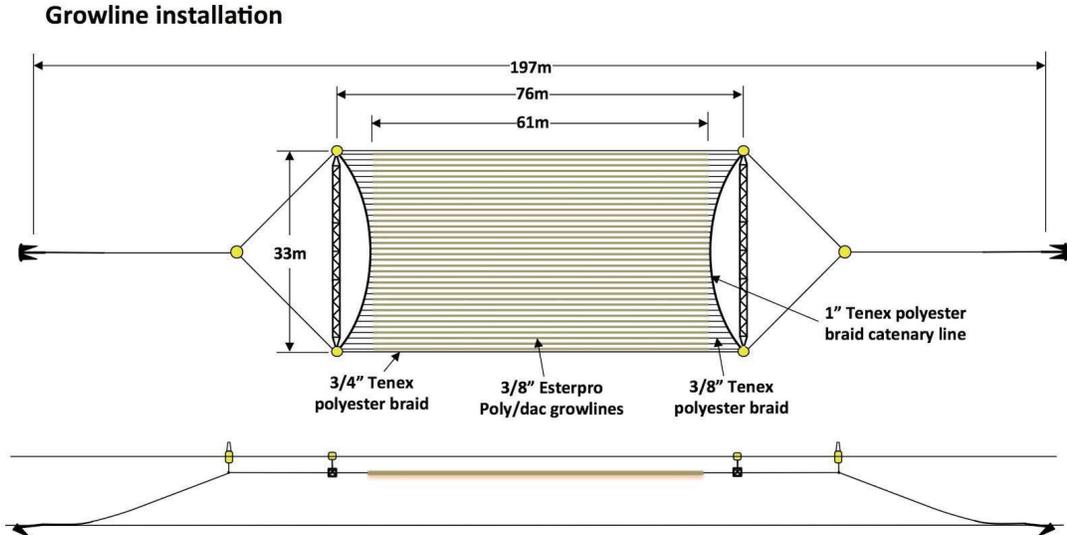
Figure 2.6: Diagram of 33-line array used at the Buzzards Bay farm site(Lindell, 2018).

Buzzards Bay farm had the same array set up as UNH and GreenWave but on a larger scale. Submerged at a depth of 3 meters, 33 seeded lines were supported by this array (Figure 2.6-(Lindell, 2018)). Due to lack of growth observed during the 2018-2019 season, a small amount of seed of locally-sourced seed stock was deployed on December 13, 2019 (Table 2.3), in hopes that the local seed stock would perform better (Lindell, 2020). All three sites were monitored biweekly from December through the beginning of March 2020 until the Covid pandemic began. Details on how the seed lines were created can be found in Appendix A.

S. latissima was harvested from the lines in June 2020 (Table 2.3). From every plot on the lines, 10 cm sections were counted, and the longest ten seaweeds were characterized for biomass. All seaweeds were dried in the greenhouse overnight and split between collaborators for various analyses.

2.1.2 Using bioindicators to trace pollutants in Shelburne Harbour

Shelburne Harbour, a coastal inlet located along the southern shore of Nova Scotia was selected as the location for the second study of this thesis. It is approximately 16 km long, and between 1-4 km wide and has a flushing time of 53 hours, with the longest flushing time occurring within the harbour (Avendaño, 2017). This site was selected due

to the prominent aquaculture industry, the proximity to the town of Shelburne, the wastewater treatment facility, and industrial and urban developments such as the large industrial shipyard (Figure 2.7).
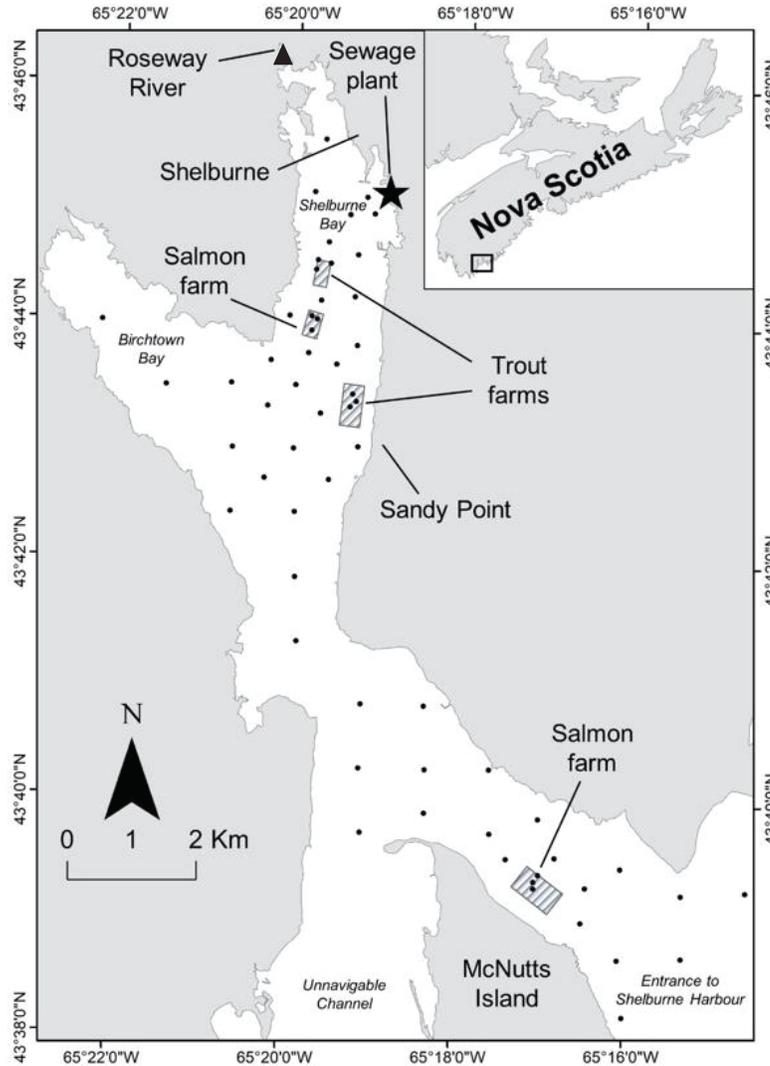
Figure 2.7: Map of Shelburne Harbour indicating the location of towns, key geographical features, and probable effluent sources. Points indicate the locations of macroalgae deployments and collection of water samples.

Field sampling occurred on November 8, 2019 and November 22, 2019. On November 8, 20 grams of *C. crispus* and *U. lactuca* were suspended in perforated clear containers to a depth of 2 meters using a combination of buoys, rope, weights, and anchors at 48 stations (Costanzo et al., 2001; Howarth et al., 2019; Howarth et al., 2020). Three more containers were deployed around each of the 4 fish cages in the harbour to

maximize the resolution, close to potential point sources of anthropogenic nitrogen, i.e., the sewage treatment plant and around the fish farms. All seaweed samples were left in the water to incubate for two weeks. This was done to assess the spatial variability of nutrient concentrations and their isotopic signatures.

On November 22, tissue samples of *C. crispus* and *U. lactuca* deployed two weeks prior were removed from the water and stored in the dark at 5°C for 12 hours until samples were relocated to a -20 °C freezer. Only 51 *C. crispus* and 41 *U. lactuca* were retrieved from the original 60 deployed. These samples were lost as a result of either rough weather conditions and/or interference by local boaters. Water samples were taken to assess whether the nutrient concentrations of the water changed during the two-week period. Tissue samples were analyzed for nitrogen isotopes outlined in section 2.2.2.

60 surface water samples were collected on each field day; 12 of which were directly around the salmon and trout cages. 125 mL of sample was collected at each station and filtered with 0.45 μm sterile syringe filters. Upon collection, samples were stored at -10°C for 12 hours until they could be placed in a -20 °C freezer. Dissolved nutrient concentrations and dissolved natural abundance stable isotopes were analyzed using methods described in section 2.2.1 and 2.2.2.

2.2 LABORATORY ANALYSIS

2.2.1 Dissolved nutrient concentrations

Water samples were analyzed for PO_4^{3-}, NO_2^-, NH_4^+ and NO_3^-. Table 2.4 lists the detection limits, bracketing standards and instruments used for each analysis.

Table 2.5: Dissolved Nutrient methodology information

Nutrient	Instrument	Detection limit (μM)	Bracketing Standards (μM)	Citations
Ammonium (NH_4^+)	*Turner designs AquaFluor Handheld Fluorometer*	*0.19*	*0 - 10*	(Holmes et al., 1999; Taylor et al., 2007)
Nitrate (NO_3^-)	*Analytical Sciences NOx 5100 Thermalox detector and a Teledyne 200 coupled with peak simple*	*0.1*	*0 - 50*	(Hendrix & Braman, 1995; Schnetger & Lehners, 2014)
Nitrite (NO_2^-)	*Thermo Scientific Evolution 260 Bio UV-Visible Spectrophotometer*	*0.2*	*0 - 30*	(Pai et al., 1990)
Phosphate (PO_4^{3-})	*Thermo Scientific Evolution 260 Bio UV-Visible Spectrophotometer*	*0.07*	*0 - 30*	*(Koroleff, 1983)*

Phosphate was measured by reacting an ammonium molybdate solution with ascorbic acid to produce the molybdenum blue polymer, which is proportional to phosphate concentrations present in the samples (Koroleff, 1983). Nitrite was measured using the sulfanilamide and napthal-ethylenediamine colorimetric analysis (Pai et al., 1990). Ammonium concentrations were tested via the Orthophthaldialdehyde (OPA) fluorometric method using a Turner designs *Aqua*Fluor Handheld Fluorometer (Holmes et al., 1999; Taylor et al., 2007). Nitrate(+Nitrite), NO_3+NO_2, was analyzed on an Analytical Sciences NOx 5100 Thermalox detector and a Teledyne 200 using hot vanadium sulfate to reduce nitrate to nitric oxide (Hendrix et al., 1995; Schnetger et al., 2014). Nitrate, NO_3^-, was determined by the difference of nitrite measurements on the spectrophotometer from the NO_3+NO_2, measurements.

2.2.2 Stable isotope analysis

Dissolved natural abundance stable isotopes of nitrate and ammonium were analyzed using the gas chromatography isotopic ratio mass spectrometer, GC-IRMS Thermo scientific Delta V-Conflo III coupled to a modified Isomass Precon system in the Canadian Excellence Research Chair (CERC) laboratory. NO_3^- isotopes were measured for $\delta^{15}N$ and $\delta^{18}O$ using the denitrifier method, which used *P. aureofaciens* bacteria to convert nitrate+nitrite to gaseous nitrous oxide (N_2O) (Casciotti et al., 2002). If nitrite was present in the samples, it was removed using the sulfamic acid method and was then run for NO_3^- isotopes using the denitrifier method (Granger et al., 2009; Sigman et al., 2001). Ammonium isotopes were analyzed for $\delta^{15}N$ by oxidizing ammonium to nitrite using a hypobromite solution (BrO^-) and then reducing NO_2^- further to N_2O using sodium azide (Liu et al., 2014; Zhang et al., 2007). USGS and IAEA standards were used.

Tissue samples were defrosted and dried until a constant weight at 60°C in a convection oven. The blade of the seaweed was homogenized using a Shardor stainless steel coffee grinder and stored in 15 mL centrifuge tubes. %N, %C, C:N, $\delta^{13}C$ and $\delta^{15}N$ isotopes were analyzed using an CHN elemental analyzer coupled to a Continuous Flow-Isotope Ratio Mass Spectrometer Delta^{Plus} XP – Conflo III at the University of New Brunswick, UNB, in the Stable Isotopes in Nature Laboratory (Howarth et al., 2019; Lemesle et al., 2016).

2.3 DATA ANALYSIS

2.3.1 Statistical analysis

In the first study, *survey of seaweed aquaculture farms in the Northwestern Atlantic*, an ANOVA one-way statistical analysis was conducted to examine the difference between nutrient concentrations at each site and kelp characteristics at each site. A two-way ANOVA was used to compare the effect of winter temperature and nutrients on kelp biomass characteristics.

2.3.2 Spatial analysis

In the second study, *using bioindicators to trace pollutants in Shelburne Harbour* a spatial analysis was conducted using the "kernel interpolation with barriers tool" in ArcGIS Pro. Kernel interpolation, uses the shortest distance between two points without intersecting a barrier, such as the coastline (Gribov & Krivoruchko, 2011). Maps of Shelburne Harbour were created using $[NH_4^+]$, $[NO_3^-]$, $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- and $\delta^{15}N$ of NH_4^+ data collected and analyzed in the field. Bandwidths between 2500 m and 5000 m, at 500 m increments were created. Using visual inspection, the smallest bandwidth where more random fluctuations than structural formations began to appear was selected (Wand & Jones 1995). This method was a balance between smoothing out and hyper focusing on the individual points. By finding a middle ground, broader spatial trends could be depicted.

CHAPTER 3 SURVEY OF SEAWEED AQUACULTURE FARMS IN THE NORTHWESTERN ATLANTIC FROM LONG ISLAND SOUND TO ST. ANN'S BAY

This study was conducted in collaboration with multiple organizations to determine if the seaweed aquaculture industry had potential to flourish in Nova Scotia. The Aquaculture Association of Nova Scotia (AANS) and Merinov received a 3 year Atlantic Canada Opportunities Agency (ACOA) grant to grow seaweed at 3 farms in Cape Breton. Additionally, three sites in the United States were also selected for this study to compare the results from Nova Scotia to areas that have a higher population density and have different physical characteristics. In Nova Scotia, *S. latissima* at all three sites grew, but there was a difference in the biomass growth between the sites. In the United States, only two of the sites exhibited growth. The factors that could be affecting their growth are examined in this chapter.

3.1 SEASONAL CHANGES IN NUTRIENTS AND TEMPERATURE

Nutrient concentrations were monitored at each farm to examine the seasonal changes that occur within each waterway and to determine whether the ambient nutrient concentrations present could support *S. latissima* growth in the water column. To assess the variability within each site, samples were collected at three different stations within each waterway between May and November 2019 (Figure 2.2). NO_3^- concentration were below detection at every site between May and September (Figure 3.1). In October, NO_3^- started increasing and by November was between 1 μM and 2.5 μM (Figure 3.1). During these months, there was not a significant difference in NO_3^- and PO_4^{3-} between the stations at any of the farms (Table 3.1). At St. Ann's Bay, there was a significant difference in the NH_4^+ concentrations at station 3, located at the mouth of the bay, where NH_4^+ was significantly higher, averaging 1.1 μM in November compared to 0.7 μM at the other two stations. Station 3 is located near the entrance of St. Ann's Bay approximately 3 km from any aquaculture ventures.

To further assess variability at each farm water samples were collected at 0, 2, 4 and 6 meters respectively to determine if the harbours were well mixed. Station 2 (S2) at

24

each study site is collected from the farms. The samples collected at these depths were all statistically similar to each other.

Table 3.1: P-values calculated from an ANOVA one-way analysis comparing stations and depths at each individual farms. The last row are the p values comparing the surface water at Station 2 at all three farms. Bolded values are significant.

	$[NH_4^+]$	$[NO_3^-]$	$[PO_4^{3-}]$
St. Ann's Bay	**0.01**	0.91	0.43
Arichat Harbour	0.96	0.65	0.72
Lennox Passage	0.79	0.95	0.52
All Farms (S2)	0.74	0.92	**0.01**
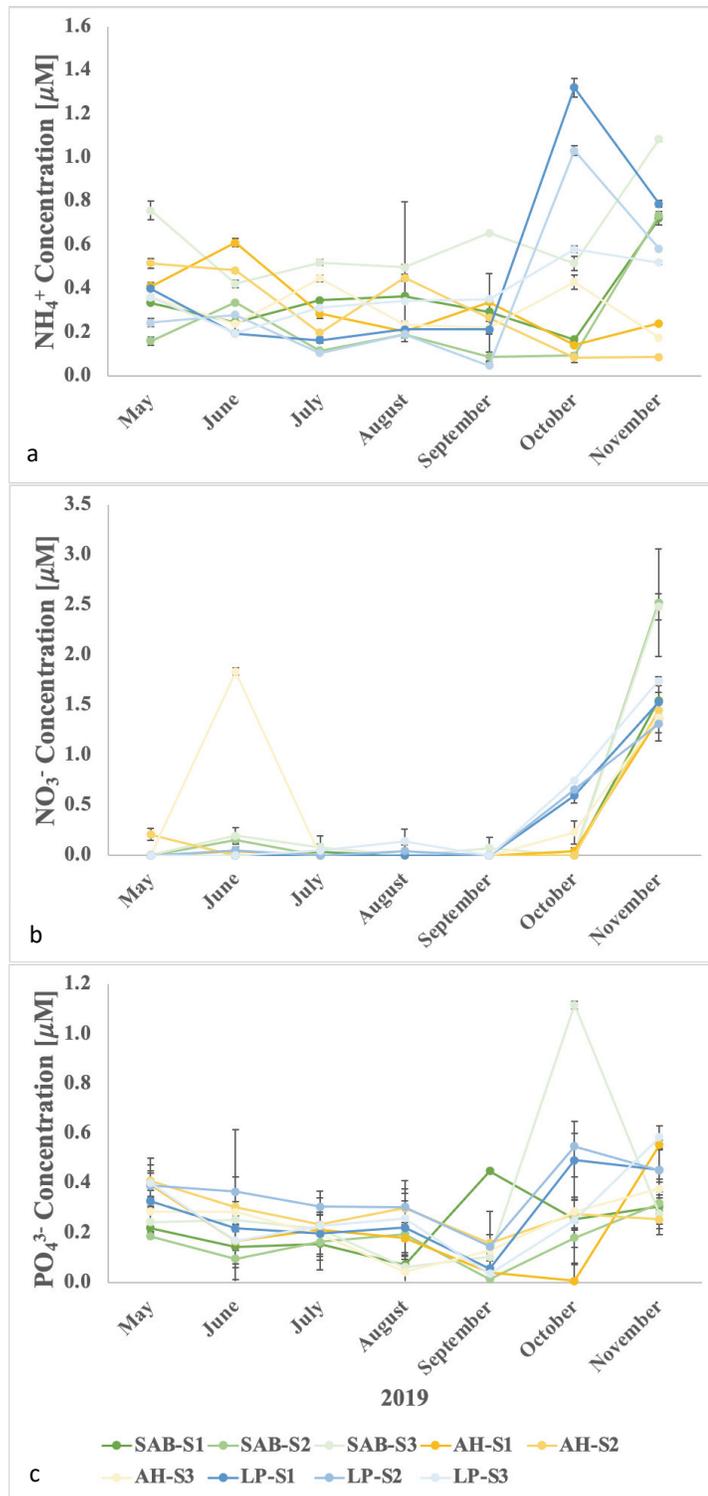
Figure 3.1: Seasonal nutrient concentrations between May and November 2019. (a) NH_4^+ concentrations, (b) NO_3^- concentrations, (c) PO_4^{3-} concentrations. Water samples were collected at 3 stations once a month and were analyzed in duplicates. SAB- St. Ann's Bay, AH- Arichat Harbour, LP-Lennox Passage

The variability seen between the stations in each bay was low due to the tidal influence and mixing capacities of each inlet. Lennox Passage and Arichat harbour, had a greater ability to mix due to their multiple large openings to the Atlantic Ocean. Along with this, the low variability in nutrients could be due to the low or below detection concentrations that were representative of the surface mixed layer. During both summer and fall months, there were detectable PO_4^{3-} and NH_4^+ concentrations, suggesting NO_3^- was the limiting nutrient at these sites (Figure 3.2). The variation in PO_4^{3-} was due to the differential amount of nutrient supply to each bay and subsequent primary production. Possibly the proximity of the towns in Lennox Passage and Arichat Harbour were increasing the amounts of PO_4^{3-} into their respective waterways. The more nitrogen available for macroalgae and phytoplankton to take up, sequentially means more phosphate would be consumed. If St. Ann's Bay had a greater nitrogen supply, the phosphorus in the bay would be taken down further leaving less behind. NO_3^- and NH_4^+ are completely consumed, as the limiting nutrient; therefore, vary less than in PO_4^{3-}.

A few other studies in Nova Scotia have conducted monthly monitoring to understand the seasonal changes in nutrients. In St. Margaret's Bay, NO_3^- peaked between December and January and again between March and April (Chapman & Craigie, 1977). Concentrations ranged from 4 μM to 6 μM during this time and then dropped to 0 μM between June and November (Chapman & Craigie, 1977). A study conducted at Indian Point and Sambro in 1996 also saw NO_3^- concentrations ranging from 1 to 6 μM (Keizer et al., 1996). In St. Margaret's Bay, PO_4^{3-} and NH_4^+ concentrations were between 0.2 μM to 0.8 μM and 0.2 to 2 μM, respectively (Chapman & Craigie, 1977). Gangé et al. (1982), also measured similar nutrient concentrations at three different bays in Nova Scotia around southern Nova Scotia. While these studies were conducted at different locations around Nova Scotia, the same nutrient patterns could be seen at our sites. Based on the similarities found in this study and the studies conducted in Nova Scotia, it can be assumed that the nutrient patterns at the Cape Breton sites follow those of relatively unpolluted regions compared to highly eutrophic regions.

Due to the difference in collection process between the three Cape Breton sites and the three United States sites, a direct comparison could not be made. From December 2019 to February 2020, Buzzards Bay had little to no NO_3^- while PO_4^{3-} was around $1\mu M$ and NH_4^+ between 0.2-0.5 μM (Figure 3.2). In contrast, the University of New Hampshire (UNH) Farm located at the mouth of the Piscataqua River and the GreenWave farm, located in the Long Island Sound had significantly higher concentrations. The mean winter nitrate concentrations at UNH were 10.8±2.0 μM and GreenWave 8.7±2.3 μM (Figure 3.2). When comparing the mean winter nutrient values between Arichat Harbor, Buzzards Bay, Long Island Sound and the Piscataqua River, there was a significant difference between the NO_3^- and PO_4^{3-} concentrations ($P<0.001$) but not for ammonium concentrations ($p=0.25$) (Figure 3.2). Based on the similarity in nutrient concentrations at the other two Cape Breton sites, it was possible that there could be a difference if sites were directly compared.
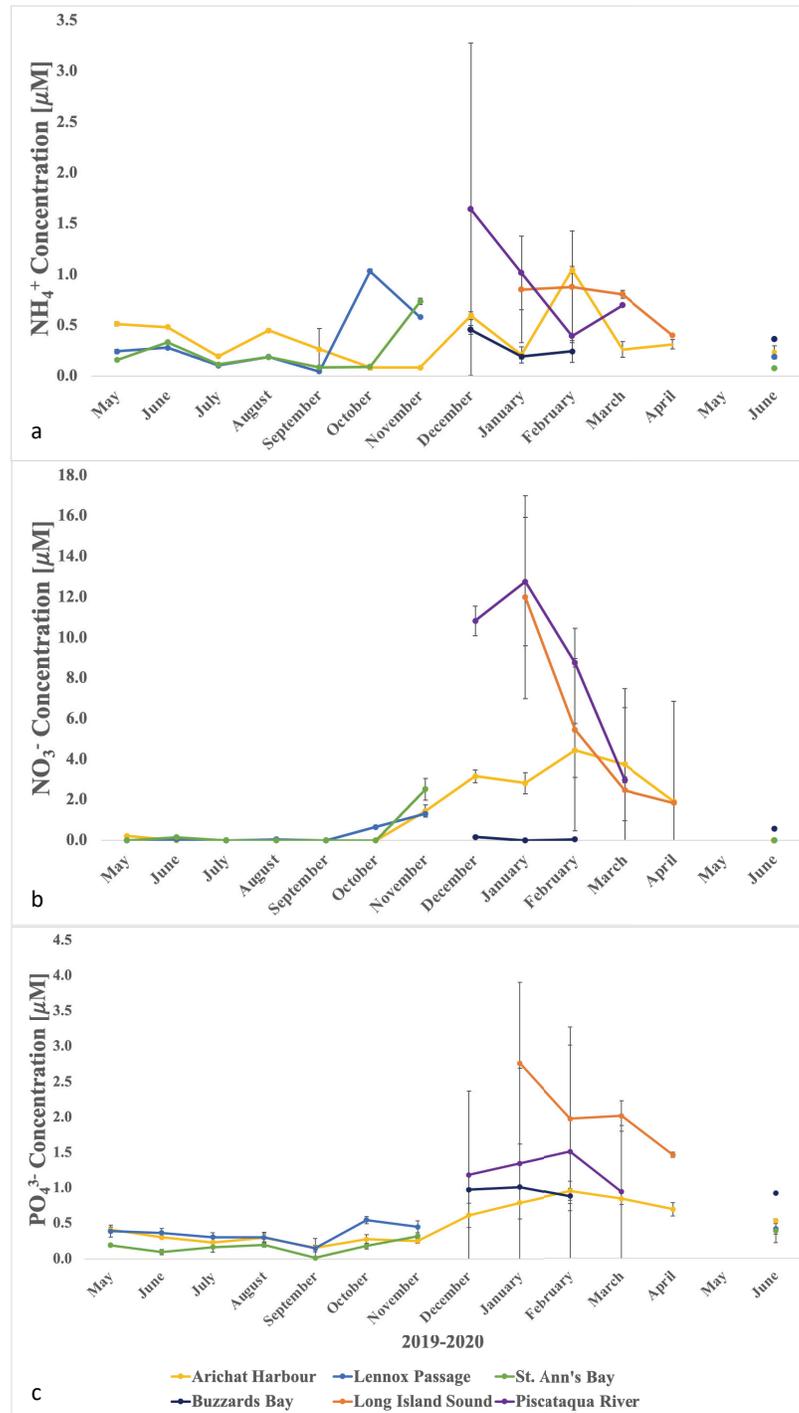
Figure 3.2: Seasonal changes in nutrient concentrations between May 2019 and June 2020. (a) NH_4^+ concentrations, (b) NO_3^- concentrations, (c) PO_4^{3-} concentrations. Arichat Harbour, Lennox Passage and St. Ann's Bay samples were taken once a month. Buzzards Bay, Long Island Sound, and Piscataqua River samples were collected biweekly when possible. Standard deviation bars from Long Island Sound, Buzzards Bay, and Piscataqua river represent the deviation of multiple samples collected over the winter from the center of the leases. All individual samples collected were run in duplicates.

Long Island Sound and Piscataqua River had higher winter concentrations for NH_4^+, NO_3^- and PO_4^{3-} compared to Arichat Harbour. This in part could be due to the location and the amount of nitrogen loading most likely occurring around the United States sites. The three sites along the eastern coast of the United States are more densely populated than the sites in Cape Breton. Changes in nutrient concentrations occurs in areas with the greatest population densities, which could be why we see higher concentrations in NO_3^-, PO_4^{3-} and NH_4^+ were seen at Long Island Sound and Piscataqua River. Point and non-point sources of anthropogenic N and P could be more prevalent in these areas than in Buzzard Bay, which had no detectable NO_3^- at the site. This would suggest that the area in which the farm was located in Buzzards Bay was nutrient deficient and would not be able to support a kelp farm industry.

Seasonal temperature changes were also examined. Lennox Passage, Arichat Harbour and St. Ann's Bay on average were colder than the sites in the United States (Figure 3.3). The data for St. Ann's Bay comes from the 2018-2019 logger deployment. Loggers from the 2019-2020 season have not been found at the time of writing this thesis. In general, the Canadian sites were colder between February and January, with temperatures reaching as low as -0.6°C. In June, Lennox Passage was the warmest site in Canada, reaching 11.6°C. Winter temperatures in the United States were closer to 4°C. The hottest temperature in June was observed in Long Island Sound at 18.3°C.
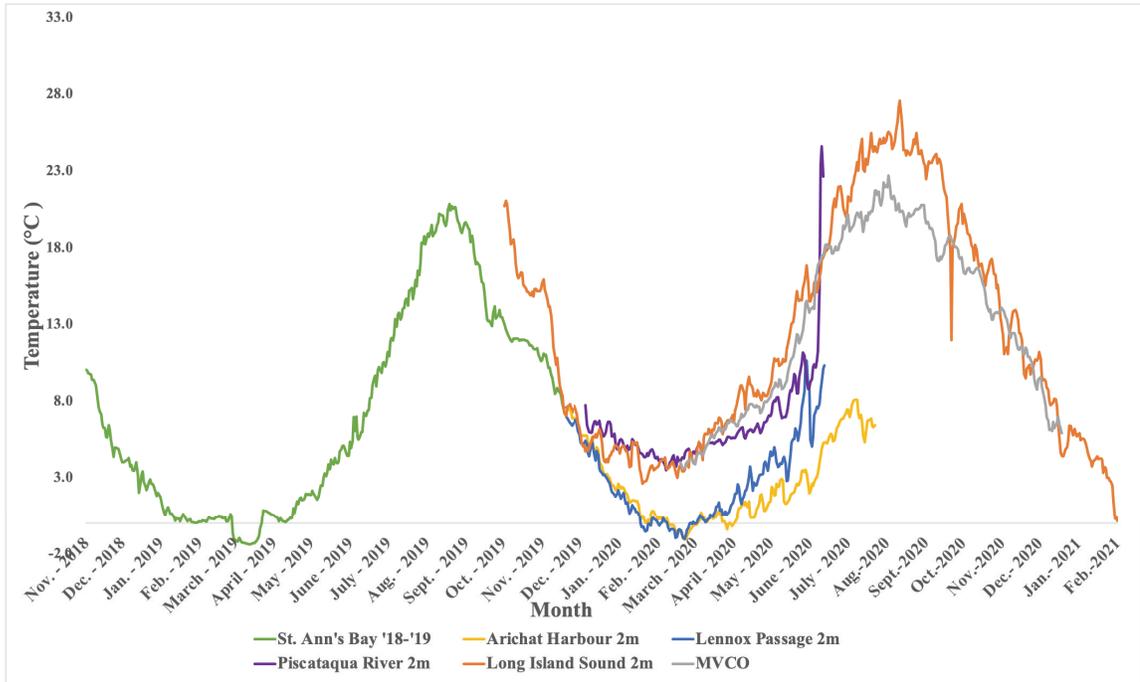
Figure 3.3: Seasonal temperature changes between November 2018 and February 2021
Data from the CTD cast at Martha's Vineyard Coastal Observatory were used for the
Buzzards Bay site. St. Ann's Bay data is from 2018-2019.

3.2 COMPARISON OF BIOMASS YIELDS FROM SUMMER HARVEST OF SACCHARINA LATISSIMA BETWEEN KELP FARMS

S. latissima was harvested from all six of the aquaculture sites in June 2020. In Cape
Breton, a plot comparison was done to check the variability along the line. Three plots
were taken each line to check this (Figure 3.4).
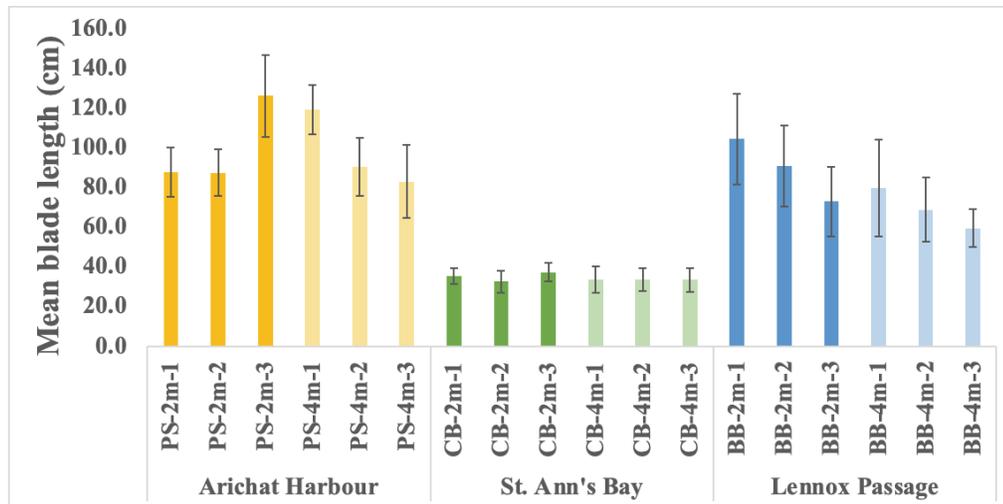
Figure 3.4: Plot comparisons across all Cape Breton sites. n=20 for each plot

At Lennox Passage and Arichat harbour there was significant difference in mean blade length between the three plots on both lines (Figure 3.4; Table 3.2). In St. Ann's Bay there was a significant difference between plots on the 2-meter lines but there was not a significant difference between blade length of the plots on the 4-meter lines (Figure 3.4; Table 3.2). These differences were likely due to where the plots were removed on the line. Where the plots were collected on the line could affect how well the seaweeds grew. As the seaweeds grew, the weight of the seaweed sunk the lines deeper than originally intended. The centers of the lines were not well supported; more buoys would have helped hold the lines in place. When the lines sink, it can reduce the amount of light reaching the seaweeds affecting growth. The variability seen in the plots collect from the 2 and 4 meter lines in Lennox Passage and Arichat Harbour could be explained by the weight of the seaweeds pulling the lines down.

Table 3.2: P-values calculated using an ANOVA one-way analysis comparing *S. latissima* plots at 2 m and 4 m depths on each farm. Bolded values were significant

	2 meters	4 meters
St. Ann's Bay	**0.009**	0.98
Arichat Harbour	**<0.001**	**<0.001**
Lennox Passage	**<0.001**	**0.003**

Biomass characteristics of all three plots were then averaged. The mean biomass characteristics measured at all locations are shown in Figure 3.5. When comparing the mean of the plots and the two depths at all three sites (n=60), there was a significant difference in Lennox Passage between both depths.
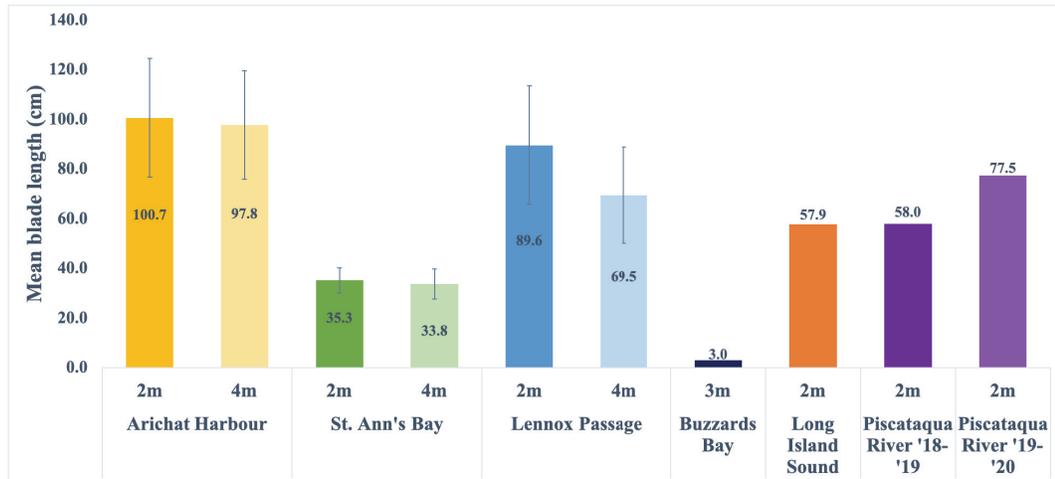
Figure 3.5: Mean blade length (cm) Arichat Harbour, St. Ann's Bay and Lennox Passage n=60. Buzzards Bay n=3. Long Island Sound and Piscataqua River.

In contrast, the lines at Arichat Harbour and St. Ann's Bay produced statistically similar results at both depths (Table 3.3). Biomass growth was found to be significantly different between all three bays (P<0.001). Blade thickness, the widest blade width and stipe diameter were also analyzed and presented similar findings. These results can be found in Appendix B: Other biomass characteristics.

Table 3.3: P-values calculated using ANOVA one way analysis comparing the growth of *S. latissima* at both 2- and 4-meter lines at each site. Bolded values were significant

	2 and 4 meters
St. Ann's Bay	0.16
Arichat Harbour	0.48
Lennox Passage	**<0.001**

Similar to the nutrient data collected, the sites in the United States could not be statistically compared to the ones in Canada due to the difference in sample numbers. However, there was a difference in the kelp growth at these sites. Buzzards Bay had no

growth. Only three seaweeds on the line were able to be measured and were 3 cm in length (Figure 3.5). Whereas the farms put in the water at Long Island Sound and Piscataqua River produced seaweeds that fell in the middle of the farms in Cape Breton. The biomass characteristics measured in Arichat Harbour, Lennox Passage, Long Island Sound, and the Piscataqua River, were similar to a study conducted by Augyte et al. (2017). In their study, they found that total length of the seaweeds ranged between 72.2 and 92.9 cm long depending on the sites (Augyte et al., 2017). However, the blade lengths in this study were shorter than the blade lengths found for *S. latissima* in Galicia Spain, which averaged 161 cm in length (Freitas et al., 2016), this was a 46% difference in length compared to Arichat Harbour, which was the site with the best growth overall. The difference seen in the sites used in our study and between the sites in the two studies mentioned could be due to hydrodynamics of the systems, as well as physical parameters such as nutrients and temperature. Johnson and Koehl (1994) found that kelp can change their blade and stipe morphologies depending on the stressors that were put upon them. While blade length, was not an indicator for measuring the growth rate of kelp, in this study the blade length was used to compare how well *S. latissima* grew between sites. This adjustment was made due to the Covid-19 pandemic.

3.3 ENVIRONMENTAL FACTORS AFFECTING KELP GROWTH IN AQUACULTURE SETTINGS

To determine the ideal location to grow *S. latissima* for aquaculture purposes, multiple coastal areas were selected, shown in Figure 2.1, that represent a gradient of water temperatures and nutrient delivery.

Temperature was one factor that affected kelp growth. Cape Breton represented an area with colder mean water temperatures throughout the year and was prone to ice coverage in the winters. Plotting the mean blade length against temperature, there was not a relationship (R^2=0.17, p=0.27) (Figure 3.6). Excluding the St. Ann's Bay and Buzzards Bay data, which were collected from the season before, the R^2 value increases to 0.72 (p=0.03), confirming that *S. latissima* prefers colder waters. This same trend can be seen when looking at the other three biomass characteristics and plotting them against

temperature (Appendix B). The temperature of the water in St. Ann's Bay was historically like the temperature at the other Cape Breton sites. In the winter, temperatures reached freezing temperatures and then warmed to roughly 15°C in the late summer. As a cold-water seaweed, *S. latissima* thrives in temperatures between 10-15°C (Bolton & Lüning, 1982; White & Marshall, 2007). When temperatures go above this for extended amounts of time, it can negatively affect seaweed growth. The maximum temperature at which *S. latissima* can still release new spores has been recorded around 21-22°C; if exposed to temperatures above 23°C for extended amounts of time, the kelp can disintegrate (Bolton & Lüning, 1982; Lüning, 1980). Between 0-10°C, the growth of kelp slowed but still grew (Bolton & Lüning, 1982).

The sites in the United States went above this threshold at the end of the growing season. Temperatures at GreenWave farms in Long Island Sound, exceed 15°C beginning at the end of May. Temperatures in Buzzards Bay and Long Island Sound did not exceed 15°C until mid-June. Seaweeds were removed after only a couple of days at that temperature. No signs of degradation to the seaweeds were noticed at the time of harvest.
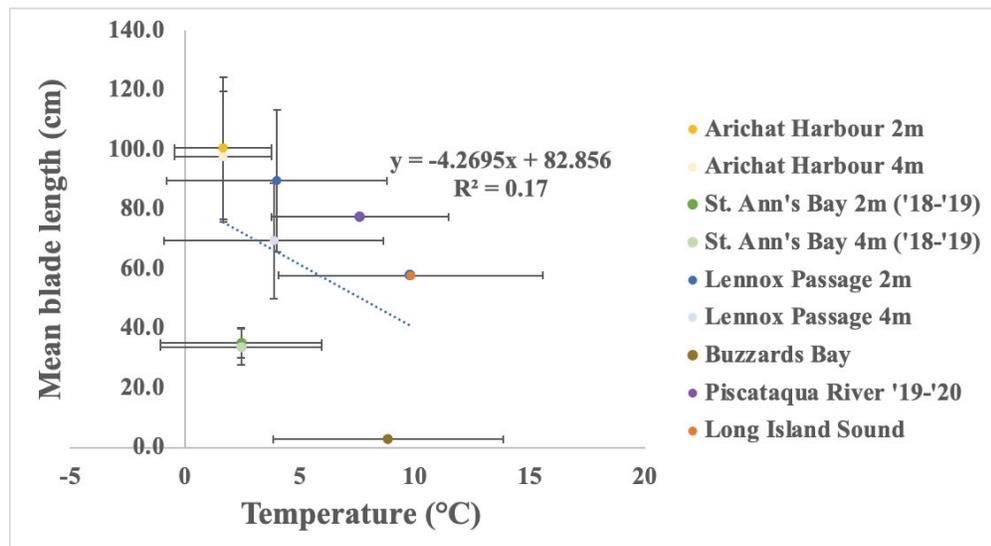
Figure 3.6: Blade length vs. mean temperature. Temperature values represent the mean monthly temperature between February and June 2020 from each site. St. Ann's Bay data come from the '18-'19 season. Loggers from this site were not recovered.

The availability of nutrients was the other factor examined in this study. Due to winter conditions and the Covid-19 pandemic, winter sampling was unable to occur, so

the November concentrations were used for the analysis. In Cape Breton, we see an inverse relationship between ammonium concentration and the biomass growth of the seaweeds based on the November concentrations collected was seen (R^2=0.86, p=0.006) (Figure 3.7). St. Ann's Bay had significantly higher NH_4^+ concentrations than the other sites. In St. Ann's Bay, there was a freshwater input coming from the North River that brought in lower salinity waters. This bay was known to have ice coverage for a portion of the winter, typically between January and March. This freshwater input could be bringing in large influxes of organic matter and NH_4^+, meaning higher turbidity that could block light from reaching the seaweeds. Compared to NH_4^+, a significant relationship between biomass growth and the other nutrients measured was not seen.

In November, St. Ann's Bay exhibited higher NH_4^+ and NO_3^- concentrations compared to Lennox Passage and Arichat Harbour. The day before sampling this site, a large rainstorm came through the area increasing the amount of fresh water in the surface water and transported more material from the North River into the bay. As such, salinity values were as low as 17 in the surface waters compared to the typical 30 at the farm. The higher NH_4^+ levels could be from the inflow of fresh water from the North River. In a study conducted in Rhode River, which flows into the Chesapeake Bay, dissolved and particulate NH_4^+ concentrations were found to be five times as high during storms but still made up lower percentages of the total N (Correll et al, 1999). The discharge, was most likely enriched with dissolved organic matter from terrigenous materials such as soil and decomposition of organic matter (Ittekkot, 1988; Opsahl & Benner, 1997) as well as anthropogenically altered sources such as fertilizers and septic tanks (Galloway et al., 2004). While this area was sparsely populated, run off from septic tanks and fertilizers could have been induced by the storm. A higher influx of organic matter could have been transported down the river during the storm creating more turbidity in the area thus blocking the light from reaching the kelp. To determine if the difference in NH_4^+ occurs only when there is more river discharge or if it is more seasonal, more sampling will need to be conducted between the farm and the river. It would also be beneficial to measure light to assess turbidity and collect samples for organic carbon as well.
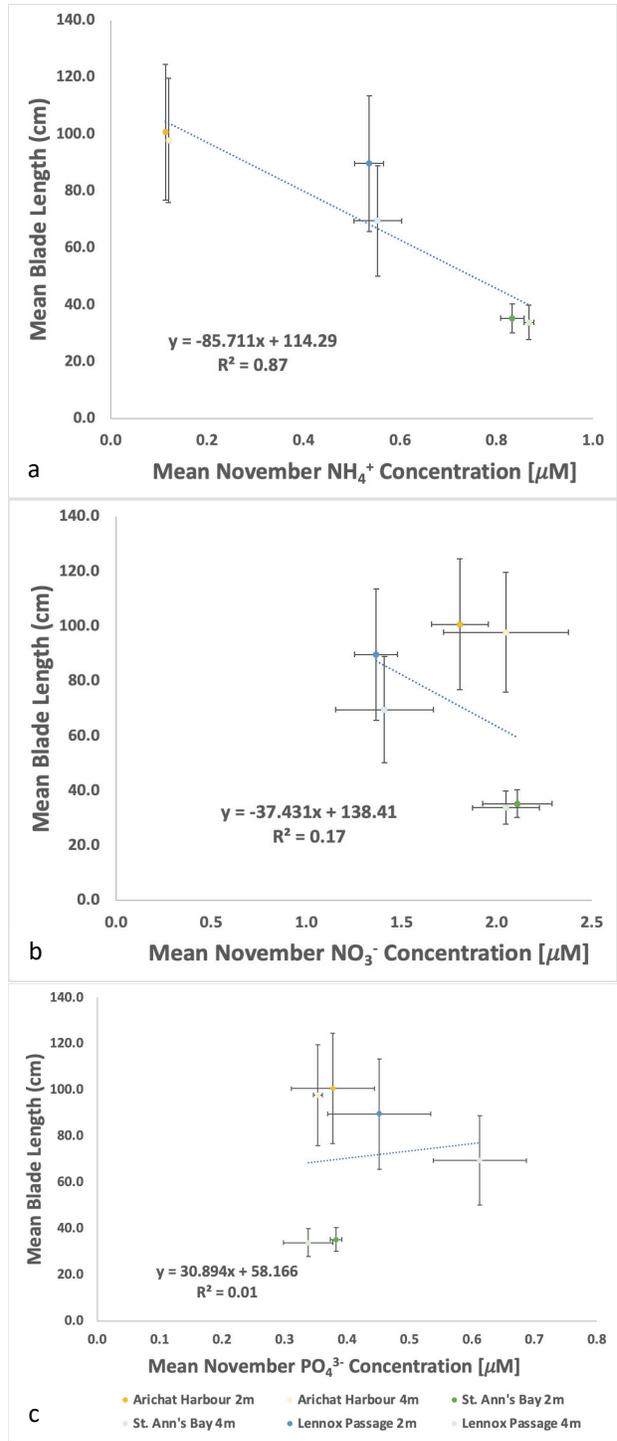
Figure 3.7 :Blade length (cm) vs. mean November nutrient concentrations. (a) NH_4^+ concentrations, (b) NO_3^- concentrations, (c) PO_4^{3-} concentrations. All samples are run in duplicate.

Figure 3.8 shows the winter nutrients collected between December and February from the sites that were able to be sampled against the mean blade length at harvest time.

There was not a significant relationship between blade length and NO_3^- and PO_4^{3-} ($R^2=$ 0.02 and 0.04, p= 0.79 and 0.69 respectively). There was a slight relationship with NH_4^+ concentrations ($R^2=0.52$, p=0.10). Overall, this inverse relationship suggests that nutrients were not driving kelp growth at most of these sites. Long Island Sound and UNH seem to have excess nutrients reaching NO_3^- concentrations as high as 18 µM in January. However, Buzzards Bay was nutrient depleted making that bay nitrogen limiting which effects the ability of seaweeds to grow. In a few studies, the growth of *S. latissima* have shown a strong correlation with the available nitrogen (Chapman & Lindley, 1980; Conolly & Drew, 1985; Gagné et al., 1982). However, when the nitrogen was present, irradiance, temperature and hydrodynamic factors played a bigger role in how the plants grew (Chapman & Craigie, 1977).

When looking at the effect that both temperature and nutrients had on the growth of *S. latissima* at the various locations, temperature had a greater effect than nutrients at the sites in the United States versus the sites in Cape Breton. However, light was likely a factor for differentiating growth between these all six sites. One can speculate that light was not the main driver in Cape Breton since most of the winter was dark and overcast and the seaweeds grew well at two of the three sites. Light and salinity are more likely the limiting factor at St. Ann's Bay due to the freshwater input from the North River. However, in a study conducted in the Arctic Ocean, *Laminaria solidungula* had sufficient light under the ice to support its growth. The larger influence was the amount of N available (Chapman & Lindley, 1980). Collecting light measurements at these sites throughout the year is still necessary as it could affect the growth at these sites. The light exposure at the US sites was greater than in Cape Breton, as the bays were further south meaning longer days in the winter and have less ice coverage and due to this irradiance should not be the driving factor at the US sites.

Overall, Cape Breton is a viable location to grow seaweeds such as *S. latissima* in an aquaculture setting. Temperatures stay cold enough throughout the year to not have a negative effect on the seaweed growth. Nutrient concentrations in the three bays, tested are high enough to support kelp growth but it is important that light, salinity and ice

coverage are taken into consideration when choosing sites. While seaweeds did grow in St. Ann's Bay, they did not grow as well likely due to fluctuations in salinity caused by the freshwater input. With more freshwater input, more ice coverage is possible in the winter as well as high turbidity and therefore less light. Buzzards Bay was nitrogen limited, which was likely why there was no growth as water temperatures during the growing season were between 3.5 and 16.8°C. The kelp grown in Long Island Sound and GreenWave grew well comparatively to the other sites. Temperature at these sites did exceed the optimal temperature threshold for these *S. latissima* but only for the last week of the season. The excess amount of nutrients available most likely contributed to good seaweed growth. When deciding between locations for seaweed aquaculture, it is important to take into consideration salinity, temperature, nutrient exchange, and light. This study looked at the effect nutrients and temperature had on the growth but was unable to examine the impacts of salinity and light. Currently, the second year of this project is being conducted. Salinity and light will be examined in more depth.
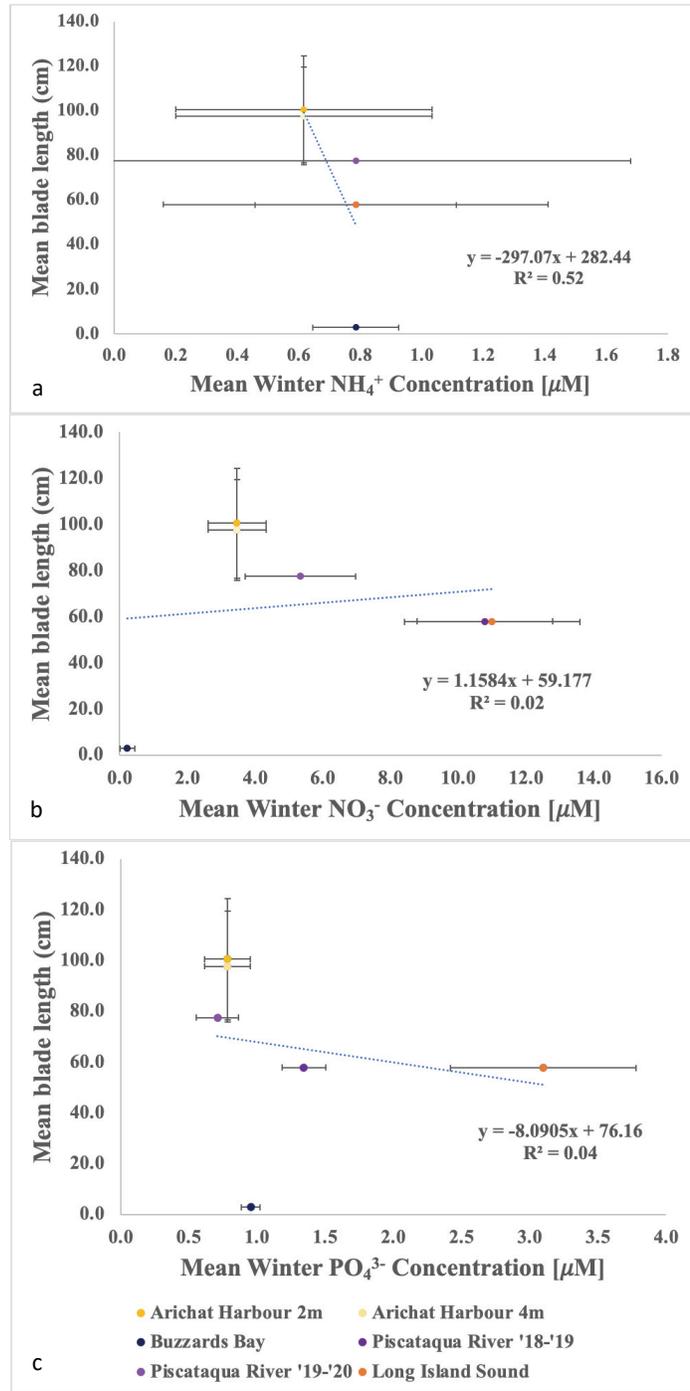
Figure 3.8: Blade length vs. winter nutrient concentrations. (a) NH_4^+ concentrations, (b) NO_3^- concentrations, (c) PO_4^{3-} concentrations. December- February for all sites except Long Island Sound which is January-February. All samples run in duplicates. Different numbers of samples were run for each month at each site. Lennox Passage and St. Ann's Bay were not included since there were no winter samples collected.

CHAPTER 4 TRACKING NUTRIENT INPUTS IN SHELBURNE HARBOUR, NOVA SCOTIA USING ISOTOPES OF DISSOLVED NITROGEN AND MACROALGAE

Increases in anthropogenic nitrogen released into the environment are affecting the health of local waterways. In this study, Shelburne Harbour, which is currently home to a large-scale finfish aquaculture industry and a wastewater treatment plant, was monitored to understand the flux of different species of nitrogen in and out of the bay. Over the sampling period, various trends can be seen when looking at dissolved nutrient concentrations (nitrate and ammonium) and dissolved natural abundances stable isotopes ($\delta^{15}N$ of nitrate, $\delta^{18}O$ of nitrate and $\delta^{15}N$ of ammonium) and the $\delta^{15}N$ and N% of *Chondrus crispus* and *Ulva lactuca.* Patterns in these seven parameters were able to indicate sources of ammonium from fish farms and the subsequent nitrification to nitrate.

Sampling for the project occurred on November 8th, 2019, and November 22nd, 2019. The week leading up to November 8th, the air temperature ranged with a low of 0°C over night to a high of 14°C during the day with winds blowing steadily from the south and southeast ("Hourly Data Report form November 8th, 2019 [HDRN8]", n.d.). On November 8th, the temperatures were warmest in the morning with a high of 7°C and cold to -2°C by the evening (HDRN8, n.d.). It rained and snowed periodically during sampling. Over the twelve-day period of the study, temperatures reached as low as -6°C at night and as high as 15°C during the day. The week leading up to November 22nd, the air temperature ranged from as low as -6°C to a high of 7°C with winds blowing from the North the entire week ("Hourly Data Report form November 22nd, 2019 [HDRN22]", n.d.). On November 22nd, the air temperature ranged from 0°C to 10°C with winds blowing from the South (HDRN22, n.d.). Overall, the day was overcast and did not rain during sampling.

4.1 DISTRIBUTION OF AMMONIUM AND NITRATE CONCENTRATION IN SHELBURNE HARBOUR

Concentrations for NH_4^+ and NO_3^- varied between sampling stations and days (Figure 4.1). NO_3^- concentrations ranged between 0.9 μM to 3.8 μM on November 8th,

2019 (Figure 4.1a). The highest concentrations were at the mouth of the harbour and decreased towards the inner bay. On November 22nd, 2019, NO_3^- concentrations more than doubled with a range between 1.3 and 12.9 μM (Figure 4.1b). The spatial patterns appeared to reverse between the two sampling days. NO_3^- increased in the inner harbour and decreased towards the entrance. The highest concentration was found northwest of the salmon farm at McNutts Island and the lowest at the entrance of Shelburne Harbour.

NH_4^+ concentrations exhibited contrasting patterns to NO_3^-. On the first day, NH_4^+ concentrations in the harbour ranged between 0.9 μM and 4.1 μM (Figure 4.1c) while on the second day NH_4^+ decreased with a range between 0.2 μM to 2.3 μM (Figure 4.1d); the trout farm near Sandy Point exhibited a concentration of 4.4 μM but was the only sample that exceeded 2.3 μM that day. On both days, the spatial trends were the reverse of those seen in the NO_3^- maps. On the first day, the highest concentrations were found southwest of the salmon farm in Shelburne Bay, and the lowest within the boundaries of the neighboring trout farms. On the second sampling day, the NH_4^+ more than halved across the harbour. The highest concentrations were again southwest of the salmon farm in Shelburne Bay but the lowest were found at the entrance to Shelburne Harbour.

Variations in both nutrients could be seen daily and between the two study periods. NO_3^- more than doubled in concentration during the two-week period with a large increase around the McNutts Salmon farm in the outer bay. Generally, these shifts were occurring due to releases of nutrients from point and non-point sources. Point sources are sources that are known to be in the area, such as four finfish aquaculture farms and a sewage plant. Non-point sources are harder to identify as their nutrient sources are unknown. It was unlikely that the spike in NO_3^- seen at the Salmon farm from McNutts Island was coming from the fish feed, as the fish predominantly excrete ammonium. Although, the nitrate could be a result of ammonium that was released in the feed and then nitrified to nitrate.
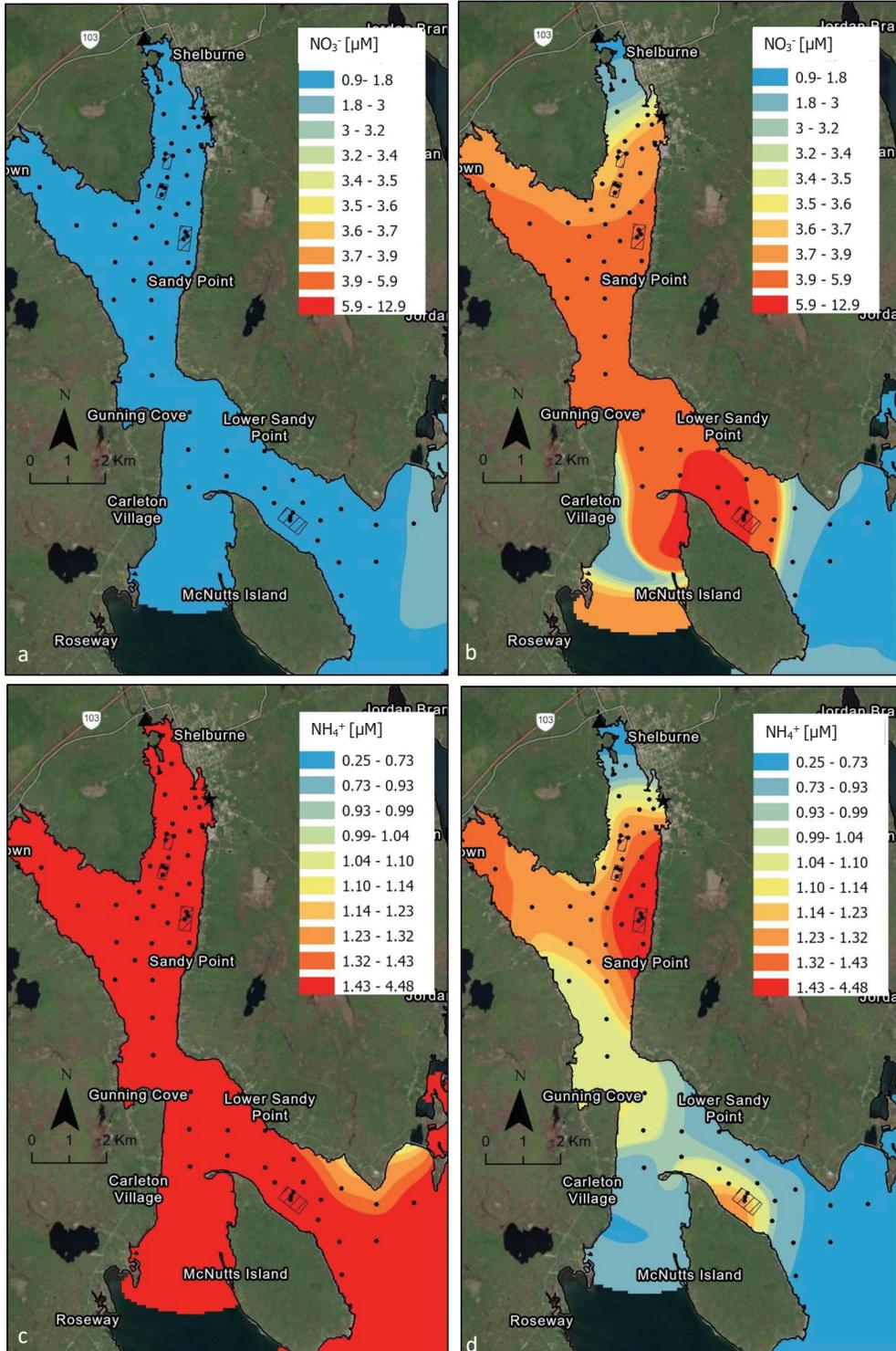
Figure 4.1: Dissolved NO_3^- and NH_4^+ concentrations from water samples collected on two different days. Points indicate sampling locations, grey boxes denote the boundaries of fish farms, the black star represents the location of the sewage treatment facility and the black triangle represent Roseway River. n=60

There are several possible causes of this increase in nitrate. First, leaking septic tanks, agricultural fertilizers or illegal dumping and runoff from non-point sources could be a cause of the increase in nitrate. Secondly, the farmers could be feeding the fish more food than was necessary, causing it to accumulate at the bottom. When this occurs, decomposition of the feed spurs microbial decay (Remen et al., 2016) which is then brought to the surface through mixing and tidal changes. These sources could explain the temporal patterns seen in the maps. With only two sampling days, it is hard to know if these patterns are continuous or episodic. It is possible that higher concentrations on any given day were due to certain points sources being released. At the time of this study, it is unknown when the wastewater was discharged or what the feeding schedule was at the farm.

Another process ongoing during the time of the experiment was nitrification, which occurs when ammonium and nitrite are available for microbes and phytoplankton to use. Nitrification is the oxidation of NH_4^+ to NO_2^- followed by the oxidation of NO_2^- to NO_3^-. On the first sampling day, NO_3^- concentrations were low throughout the harbour while NH_4^+ concentrations were high. Over the time of the experiment, there was an increase in NO_3^- occurring in the bay while generally, a decrease in NH_4^+ was seen, which was an indication that this change was from nitrification. Additionally, both NO_3^- and NH_4^+ could then be taken up and used by phytoplankton for primary production.

The increase in NO_3^- on November 22nd could be from the physical process of upwelling occurring. Upwelling is the process of deep cold nutrient rich water being brought to the surface from winds pushing surface waters in the opposite direction. In the week leading up to November 22nd, the winds switched and were coming from the north ("Past Weather in Shelburne County", n.d.), which pushed the surface waters offshore causing coastal upwelling. Over the course of the week, the flushing and tidal changes in the bay could have brought nitrate rich waters from the deep into the harbour, which could explain how the entire bay was well mixed with the outer bay having the highest concentrations.

NH_4^+ concentrations decreased by more than half between the first and second sampling period. On the first sampling day, NH_4^+ concentrations were not noticeably higher near the fish farms but instead were well-mixed. On the second excursion, a peak of high ammonium was present around the large trout farm at Sandy Point. The high ammonium concentrations were most likely from the effluents coming from the fish farms. Ammonium is excreted by fish as ammonium and urea. Finfish, such as salmon and trout, will take up a portion of the nitrogen from the feed. The nitrogen is then either stored for later use or excreted as soluble or particulate wastes (Olsen et al., 2008). The dominate forms of soluble waste was released as ammonium from the gills or urea from urine, particulate waste was released from faeces (Olsen et al., 2008). However, there were no peaks around any of the other finfish farms in Shelburne Harbour. This could be due to how quickly the bay was turned over and mixed daily.

Lower concentrations of NH_4^+ were found near the entrance to Shelburne Harbour and the mouth of Roseway River. These areas with lowered measured values of ammonium could indicate there was greater consumption of ammonium by phytoplankton and algae. Phytoplankton and algae will use these nutrients to grow and quickly die off when they are depleted. The fact that there was ammonium present means that the phytoplankton were being limited by something other than nitrogen in the bay. It is possible that PO_4^{3-} or light was the limiting factor in the phytoplankton's ability to grow. These measurements were not taken at the time of this study.

Overall, there was little evidence to suggest that water samples taken near the sewage plant had higher concentrations of dissolved nitrate or ammonium. There was some evidence to suggest that ammonium concentrations in the harbour were being produced from at least one of the finfish farms and being diluted and moved through the harbour with the tidal changes. A similar study that was conducted in Liverpool Bay saw NH_4^+ and NO_3^- concentrations that were at or below detection limit (Howarth et al., 2019). The difference between these concentration values from this study were due to sampling occurring in the late fall versus the summer. Shelburne Harbour is more exposed than Liverpool, and subject to greater levels of mixing causing the nutrients to

dissipate more quickly. In both studies, there was no evidence that sewage facilities and finfish farms overall saw notable increases in dissolved nitrogen concentrations. The lack of response may be because sampling occurred at a time when the ambient nutrient concentrations were high, increasing the nitrogen content of all samples, reducing the variation between stations, and thereby masking the detection of the fish farms and sewage facility. Collecting end member samples from the sewage facility treatment plants and particulate wastes from the fish could assist in determining the exact concentration entering the harbour from both sources.

4.2 STABLE ISOTOPE COMPOSITION OF SHELBURNE HARBOUR AS SEEN BY DISSOLVED NUTRIENT CONCENTRATIONS

Changes in the isotopic value of $\delta^{15}N$ of NH_4^+ between the sampling days are shown in Figure 4.2. On the first day, $\delta^{15}N$ of NH_4^+ ranged between 5.5‰ and 16.8‰ there were elevated signatures of $\delta^{15}N$, values greater than 11.3‰, throughout the inner bay with the lowest values between 5.5‰ and 5.9‰ near and around the trout farm at Sandy Point. The highest signatures were seen northwest of the McNutts Salmon farm. Two weeks later, $\delta^{15}N$ of NH_4^+ was seen to decrease. The isotopic signature for the harbour ranged between 7.0‰ and 17.3‰, with the values decreasing between days at each station. The highest signatures could be found northwest of the McNutts salmon farm and the stations next to the wastewater treatment plant. The lowest signatures on the second day were around the three inner finfish farms.
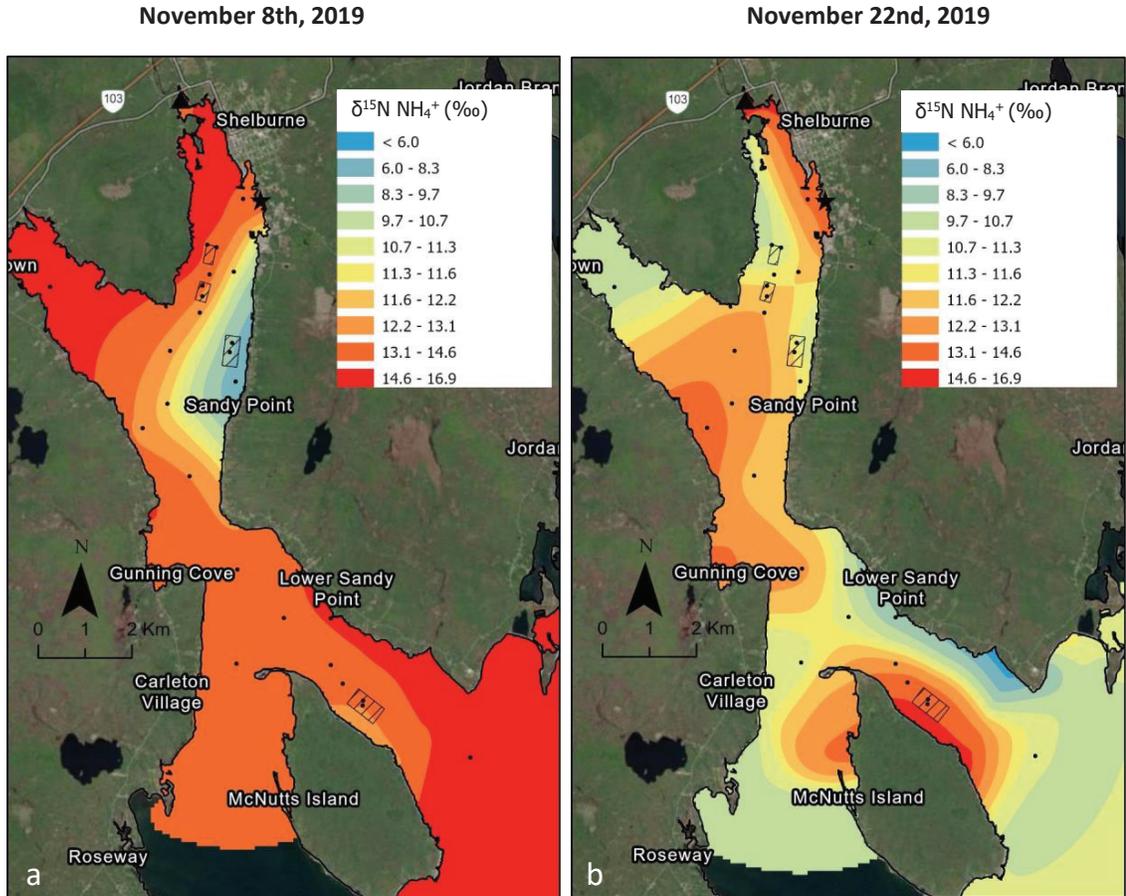
Figure 4.2: $\delta^{15}N$ of NH_4^+ from water samples collected on two different days. Points indicate sampling locations, grey boxes denote the boundaries of fish farms, the black star represents the location of the sewage treatment facility and the black triangle represent Roseway River n=25

$\delta^{15}N$ of NO_3^- shows an opposite trend compared to the $\delta^{15}N$ of NH_4^+. $\delta^{15}N$ of nitrate had moderate to low isotopic values ranging from 0.5‰ to 1.5 ‰ in the inner bay (Figure 4.3). The highest signature on the first day occurred at the trout farm closest to the sewage plant in Shelburne Harbour, 4.4‰ (Figure 4.3a). In the outer bay, there was an increase in $\delta^{15}N$ with values between 1.5‰ and 3.2‰ (Figure 4.3b). On the second sampling day, the entire harbour had an isotopic value that was at least 2.2‰, with the highest values coming from the most oceanic sample with a value of 4.8‰. The lowest values were found northwest of the McNutts salmon farm.
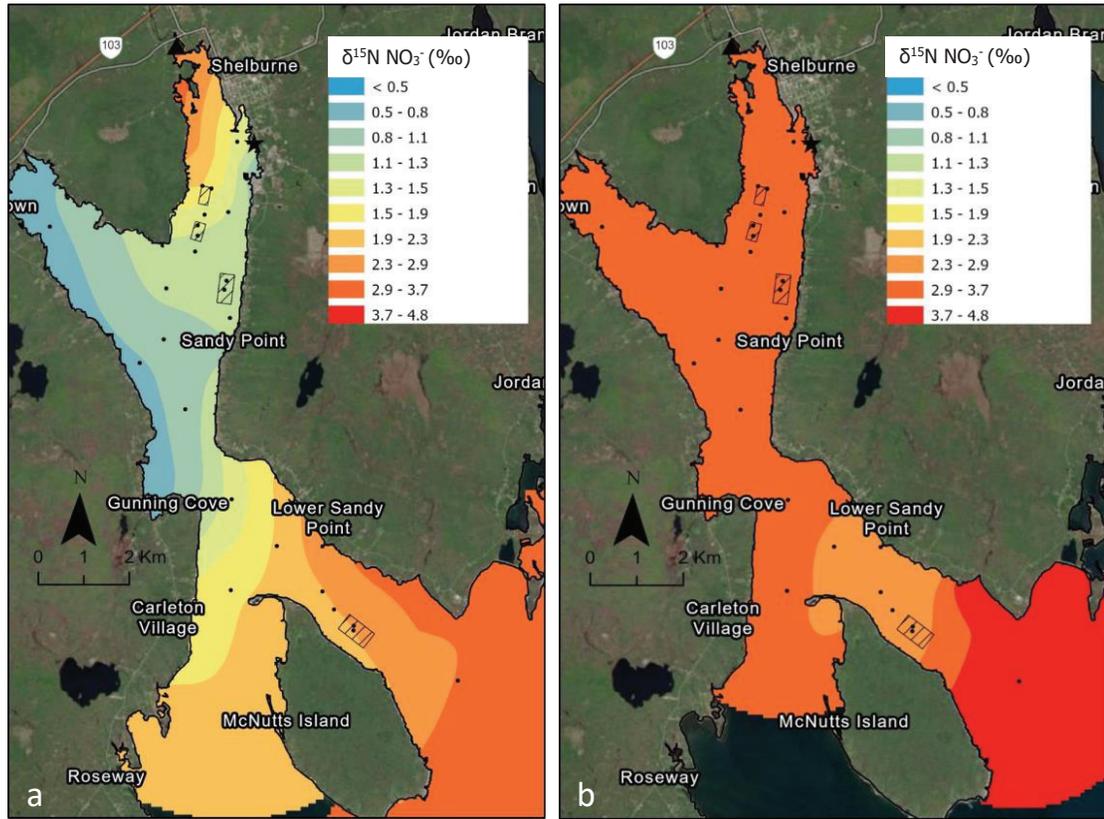
Figure 4.3: $\delta^{15}N$ of NO_3^- from water samples collected on two different days. Points indicate sampling locations, grey boxes denote the boundaries of fish farms, the black star represents the location of the sewage treatment facility and the black triangle represent Roseway River. n=25

Anthropogenic nitrogen sources have a range of $\delta^{15}N$ isotopic signatures which can be difficult to distinguish in coastal waterways. Macroalgae $\delta^{15}N$ signatures of sewage effluents can range between 7-38‰, fertilizers range between 4 and -4‰ and finfish farm effluents range between 8-11‰ (Costanzo et al., 2001; Dailer et al., 2010; García-Sanz et al., 2010; Wang et al., 2014). Coastal oceans such as Shelburne Harbour make it difficult to distinguish what these signatures could be in the bay as they were diluted via mixing.

More variability was seen in the $\delta^{15}N$ NH_4^+ than in the macroalgae data $\delta^{15}N$, discussed in greater detail in section 4.3. Ammonium was in a constant flux and the standing stock of NH_4^+ was representative of the product of the seaweeds and

phytoplankton and microbial nitrifiers consuming ammonium, while the fish were excreting it at the same time. Areas in which there was less ammonium are depicted by the lighter colors. The lighter colors could represent areas that were experiencing greater consumption or uptake of nutrients. There was an enrichment in the $\delta^{15}N$ NH_4^+ in areas where there was a high proportion of consumption versus release. Phytoplankton and seaweeds fractionate the ammonium $\delta^{15}N$ preferentially assimilating the lighter ^{14}N, leaving the dissolved NH_4^+ pool enriched in ^{15}N. Generally, since nitrate was not the preferred N source to primary producers, less signs of fractionation in nitrate were seen than in ammonium.

Nitrate overall had a much lighter $\delta^{15}N$, even lighter than what was normal for deep oceanic nitrate which was approximately 4.8-4.9‰ (Marconi et al., 2014). It was likely that this nitrate was being produced through nitrification from the fish feed. The fish feed used in this study had a lower $\delta^{15}N$ of 4.2‰ and 3.9‰ for the trout and salmon feed respectively (Table 4.1). Additionally, nitrogen fixation was another possible source of light $\delta^{15}N$ nitrate (Casciotti, 2016; Sigman et al., 2001). In the inner bay, an enrichment in $\delta^{15}N$ of NO_3^- was seen between the two sampling days. This enrichment could be from decreased release of light nitrate from the above two possible sources, fish feed or nitrogen fixation, or from some preferential assimilation of light nitrate by phytoplankton and seaweeds.

4.3 TRACING NUTRIENT SOURCES USING $\delta^{15}N$ IN MACROALGAE

Elemental and isotopic compositions of some point source nutrients in the harbour were analyzed using methods described in section 2.2.2. Sediment, direct excretion by fish and wastewater samples were unable to be collected for analysis. Table 4.1 presents the results of the Salmon and Trout feed used at the farms and the starting isotopic and nitrogen compositions of *C. crispus* and *U. lactuca*. The fish feed used in this study is the same used in Howarth et al., 2019.

Table 4.1: Elemental and isotopic composition of source nutrients collected in Shelburne Harbour

Source	$\delta^{15}N$ (‰)	N (%)
C. crispus	-4.4 ± 0.3	-2.7 ± 0.1
U. lactuca	8.8 ± 0.1	3.4 ± 0.1
Trout feed	4.18	6.54
Salmon feed	3.9 ± 0.1*	7.1 ± 0.1*

*Values taken from Howarth et al., 2019

$\delta^{15}N$ of *C. crispus* and *U. lactuca*, displayed the same general trends as $\delta^{15}N$ decreased from the entrance into the harbour into inner bay (Figure 4.4). The isotopic values of *U. lactuca* prior to incubation was 8.8‰. After the two-week incubation period in the water the isotopic values of the *U. lactuca* decreased relative to the initial values, however, 5 samples displayed higher $\delta^{15}N$ values compared to the initial value (Table 4.1). There were different $\delta^{15}N$ values within the inner and outer bay, the composition of the outer bay was close to 8‰ and the mean isotopic composition decreased to 5‰. The seaweeds collected from the outer bay had the same signature as when the seaweed was put in the water. *C. crispus* on the other hand started at -4.4‰ and increased relative to initial values (Table 4.1). In the outer bay $\delta^{15}N$ increased to 2‰. In the inner bay, *C. crispus* increased slightly to -3‰.

Unlike the $\delta^{15}N$ values, the nitrogen content of both species did not have as distinct of a trend and exhibited a smaller range in values (Figure 4.4). *C. crispus* nitrogen content ranged from 2.6% to 3.6%, the highest occurring north of McNutts. The lowest values were found between the three finfish farms in Shelburne Harbour. In general, *C. crispus* showed a trend similar to that of its $\delta^{15}N$, with nitrogen content decreasing from the entrance of Shelburne Harbour into the inner bay area. There was less of a pattern for the nitrogen content of *U. lactuca* but we were able to see high nitrogen content around the McNutts salmon farm which then decreased through the middle of the bay and increased again near the two inner most finfish farms in Shelburne Harbour. The nitrogen content of *U. lactuca* ranged between 3.2% and 4%; the highest

values occurred north of McNutts island and the lowest southwest of the Sandy Point trout farm.
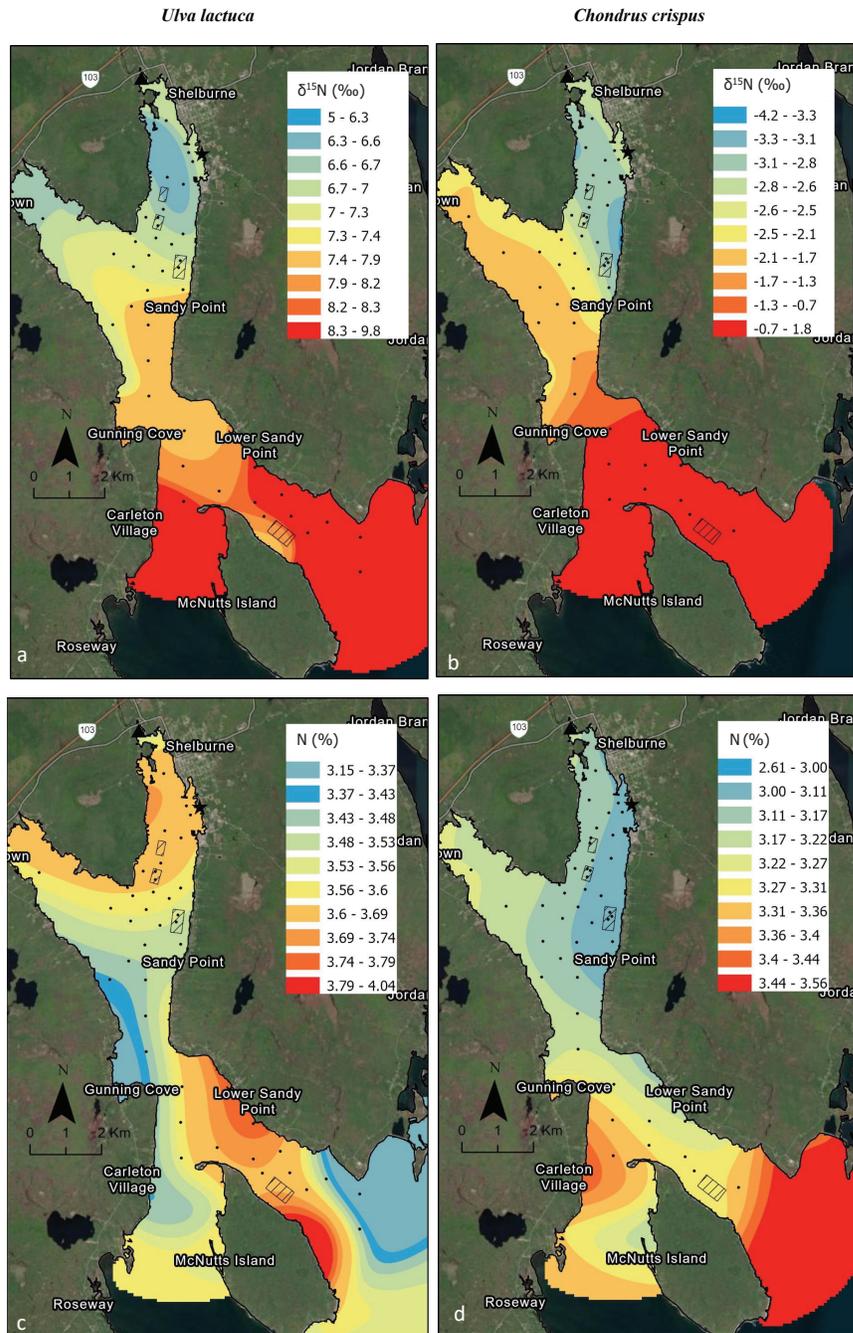
Figure 4.4: δ^{15}N and total N content of the macroalgae *U. lactuca* and *C. crispus.* Points indicate sampling locations, grey boxes denote the boundaries of fish farms, the black star represents the location of the sewage treatment facility and the black triangle represent Roseway River. n=51 for *C. crispus* and 41 for *U. lactuca*.

The ability to distinguish between nutrient sources can be extremely challenging when sampling water. However, with the use of macroalgae bioindicators, the ability to capture those signatures becomes more reliable because the macroalgae are directly able to reflect what is present in the system (Cohen & Fong, 2006). *Ulva lactuca* and *C. crispus* showed an enrichment in $\delta^{15}N$ when moving towards the entrance of Shelburne Harbour. These macroalgae took up nitrogen primarily in the bioavailable forms of nitrogen, NO_3^- and NH_4^+ (Casciotti, 2016) by absorbing the inorganic forms of nitrogen and storing them within their tissues until they were needed (Lobban & Harrison 1994; Yokoyama & Ishihi, 2010). $\delta^{15}N$ values were therefore a reflection of the mean signature present in the harbour versus a snapshot of what was happening (Mcclelland, Valiela, & Michener, 1997).

C. crispus and *U. lactuca* did not show evidence of elevated $\delta^{15}N$ coming from the fish farms and the sewage facility. $\delta^{15}N$ of both macroalgae species exhibited a general decrease from the entrance of Shelburne Harbour towards Shelburne Bay; a trend which existed independently of the fish farms and sewage facility. However, an elevated $\delta^{15}N$ signature in the Macroalgae tissue was not seen coming from the salmon farm at McNutts island which coincides with the increased amount of NO_3^- concentration. Howarth et al. (2019) conducted a similar study in Liverpool Bay that deployed *Palmaria palmata* and *C. crispus* over a ten day period and determined that the $\delta^{15}N$ of *C. crispus* showed distinctions between fin fish farms and the sewage treatment plant; *P. palamta*, however, remained unchanged during the incubation time (Howarth et al., 2019). Considering the time of year the study occurred, not seeing a distinct signature around the farms and sewage plant makes sense as ambient nutrient concentrations were likely beginning to increase clouding the $\delta^{15}N$ signature from the farms and sewage plant.

Total nitrogen content from *C. crispus* did not show any evidence of increased nitrogen coming from the three inner most farms. In general, it followed the same pattern as the $\delta^{15}N$ of both macroalgae. *U. lactuca* however, was generally higher near three of the four finfish farms and the sewage plant. According to García-Seoane et al. (2018), *U. lactuca* has fast growth rates and the ability to absorb nutrients and turn over cellular

nitrogen quickly, making it one of the most widely used bioindicators to date. It was possible that *C. crispus* did not grow as quickly during the experiment and therefore did not pick up the increased nitrogen levels around some of the farms, like the *U. lactuca* and water samples.

On the second sampling day, the highest NO_3^- concentrations were found in the outer portion of the bay with a patch of higher NO_3^- concentrations in the inner bay near the farms. NH_4^+ concentrations were greater in the bay where three of the four finfish farms were located, with a gradual decrease in concentration towards the entrance. The salmon farm at the mouth of Shelburne Harbour, did present some elevated NH_4^+ values as well. When comparing the dissolved natural abundance stable isotopes of $\delta^{15}N\ NO_3^-$ and $\delta^{15}N\ NH_4^+$, lower values of $\delta^{15}N\ NO_3^-$ were seen indicating less uptake of nitrate while $\delta^{15}N\ NH_4^+$ values were significantly higher from preferential uptake by phytoplankton and algae. $\delta^{15}N$ of *C. crispus* and *U. lactuca* were more enriched further out of the bay, following the same pattern of the NH_4^+ concentrations. Together, these findings show no evidence that the sewage facility increased the $\delta^{15}N$ of macroalgae bioindicators or water samples. However, there was evidence that at least one of the finfish farms was increasing the anthropogenic ammonium and nitrate within in the bay.

There is potential to use macroalgae as bioindicators to better monitor and improve how anthropogenic sources of nitrogen are released in coastal bays. However more research needs to be conducted to determine species and lengths of time in which samples should be left in the water. While this study was able to track increases in effluents within the harbour, it was unable to identify and distinguish the effluents from one another. Sampling in the early winter months and the sheer amount of mixing that occurs on a daily basin could both be factors as to why this study was unable to pick up distinct signatures compared to other studies.

CHAPTER 5 CONCLUSION

5.1 SUMMARY

Macroalgae is a vital resource in coastal waterways; it provides food and shelter for various organisms in the wild, is a novel tool to trace pollutants and is used in commercial products. The first objective of this thesis was to determine if the proposed sites for seaweed aquaculture implementation had enough nutrients. While winter sampling only occurred at four of the sites, the similarities between the sites suggests that nutrients were not limiting the growth of *S. latissima*. Analysis showed that *Saccharina latissima* size had no relationship with nitrate or phosphate but had an inverse relationship with ammonium, indicating another factor, such as light or salinity may be limiting the growth in St. Ann's Bay. There was also an inverse relationship with temperature which agrees with previous studies that have shown that *S. latissima* prefers colder waters. While this study was the first of its kind here in Nova Scotia, we know it may not represent all aspects of large-scale kelp growth, but it has shown the importance of picking locations that can support their growth. Some factors to take into consideration include freshwater inputs that bring in lower salinity waters and increases in organic matter that could block the light. In shallow water sites, ice coverage could also be a problem especially in some shallow bays like in Nova Scotia. A good site, would include having temperatures that stay below 15 ºC, having winter nutrient concentrations that are above detection, and having a salinity between 23-35. While production numbers might be small, this industry has the potential to flourish in many of these and others coastal harbours similar to these study sites.

The second objective was to use macroalgae and dissolved natural abundance stable isotopes in nitrate and ammonium to trace anthropogenic pollutants entering a coastal inlet home to multiple salmon and trout finfish aquaculture farms. This study shows that nitrogen is in a constant state of flux which makes monitoring difficult due to the complexity of an anthropogenically altered coastal bay. While further analysis might be necessary to fully distinguish the sources and sinks of all nitrogen species in the bay, some patterns emerged from the data. It was determined that the largest finfish farm was

a major source of ammonium. This ammonium was then fueling primary production and uptake by phytoplankton and algae, as well as nitrification to nitrate. The stable isotopic signature of the dissolved ammonium and nitrate together support the findings above. The stable isotopes of the macroalgae do not show the same patterns suggesting that daily fluctuations were not taken up. Using macroalgae as bioindicators, along with natural abundance stable isotopes in dissolved nitrogen, are novel tools that are helping scientists to better monitor and improve how anthropogenic sources of nitrogen are released in coastal bays.

5.2 FUTURE PLANS

The upcoming plans for the second year of the *Identifying nutrient controls on seaweed aquaculture* project include collecting water samples monthly during the winter months at all the Cape Breton farms and collecting tissue samples in January and March to monitor growth. This will allow us to examine the δ^{15}N and nitrogen content of *S. latissima* as it is growing. Light sensors were also installed this year to see if light levels are too low and are affecting kelp growth when ice is present. Installing nutrient sensors is another possible way to collect nutrient data during the winter months.

Future work for the industry should include trying to grow other species of seaweeds such as *Laminaria digitata* and *Agarum clathratum* which are native to Nova Scotia. While one bay may not be able to support the growth of one type of seaweed, it might have enough nutrient and light supply to support the growth of another species. Another way to help see seaweed aquaculture continue to be an industry in Nova Scotia would be to find a group or organization that could produce the seed lines for the companies. At the moment, the closest place that can create them is in Quebec. Some future work the scientists could work on include conducting experiments in the Aquatron testing how changes in temperature, light, salinity, and nutrients affect the growth of the seaweeds in the same set up as used in the field.

Some future directions proposed for studies like the macroalgae bioindicators project would be to collect more end member points as close to the sources as possible at

the time of sampling. These end member points could include sewage effluents directly from the plant on the day sampling occurred, fish waste, and sediment from various areas around the harbours including underneath the fish cages. Some of these sources may fluctuate on a daily or seasonal basis, so collecting samples at various times of the day and throughout the year would help identify what processes are occurring in the system. These end members could be used to create a model to understand how the various point and non-point sources are affecting the water quality in the harbour and to understand how anthropogenic sources are altering the harbour's natural processes. $\delta^{15}N$ signatures and nutrient concentrations would help to better decipher the point sources and sinks of nitrogen in coastal inlets. In the future, it would be important to also collect seasonal samples, to assess the temporal variability of nutrient release and uptake in this system. An additional step that could be taken is to collect samples for phosphate to further determine the limiting nutrients in bays that are anthropogenically altered.

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Appendix A: Seed line production

A.1 CAPE BRETON PRODUCTION

Seed lines were created by collecting mature wild seaweeds via a diver at a shallow water spot near each lease. Wild sporophytes were harvested to use as spawners to produce seed lines and a bank of gametophytes. The team attempted to collect mature seaweeds on July 2nd and 3rd, 2019, but sporulation of the seaweeds was not good. A second attempt to collect mature seaweeds was made on September 24th and 25th, 2019. Half of the seaweeds were lost in transportation and by the time they arrived they were in not in optimal condition and unable to spawn. The second half were sent from the Université St Anne Marine Research Center in Arichat on October 3rd and 4th, 2019 and were successfully sent to Fermes Marines du Québec (FMQ).
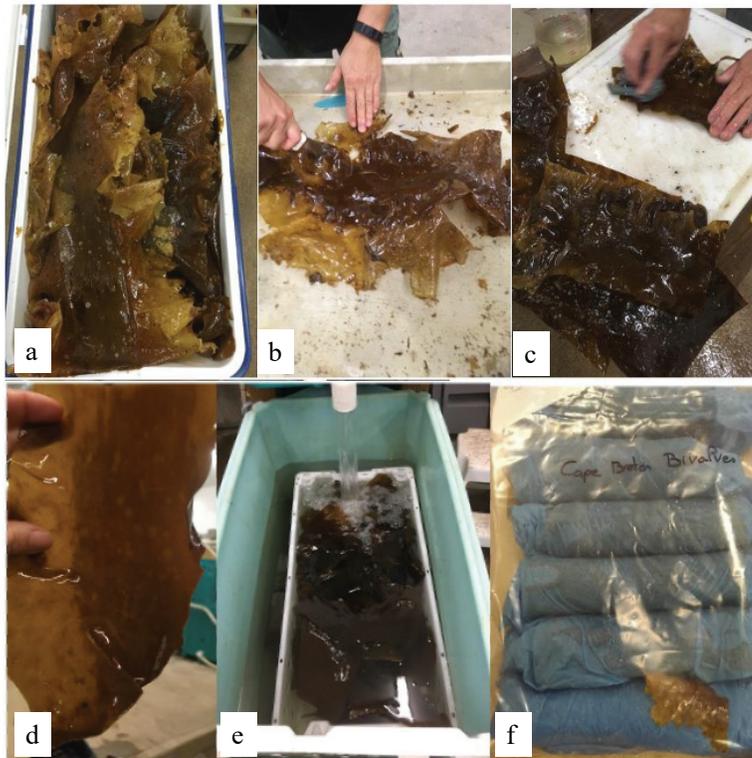
Figure A.1:Photos of cleaning and prepping the seaweeds (a)- raw seaweeds, (b)- trimming of the seaweeds, (c)- cleaning of the seaweeds, (d)- cleaned seaweed mature sore, (e)- storage of the cleaned seaweeds in tanks, and (f)- seaweeds sores packed in wet paper in a plastic bag ready to be sent to FMQ. ((Lionard & Clark, 2019); photos- Marie Lionard)

40 seaweeds were collected near Cape Breton Bivalves farm, 30 near Premium Seafoods farm, and 34 from Bounty Bay Shellfish farm. The 20 longest from each batch were cleaned to remove sediment and invasive species and then characterized for average total length, average stipe length, average blade length, average blade width and average weight. Seaweeds were then trimmed and packed in wet paper towels in plastic bags and sent to FMQ.
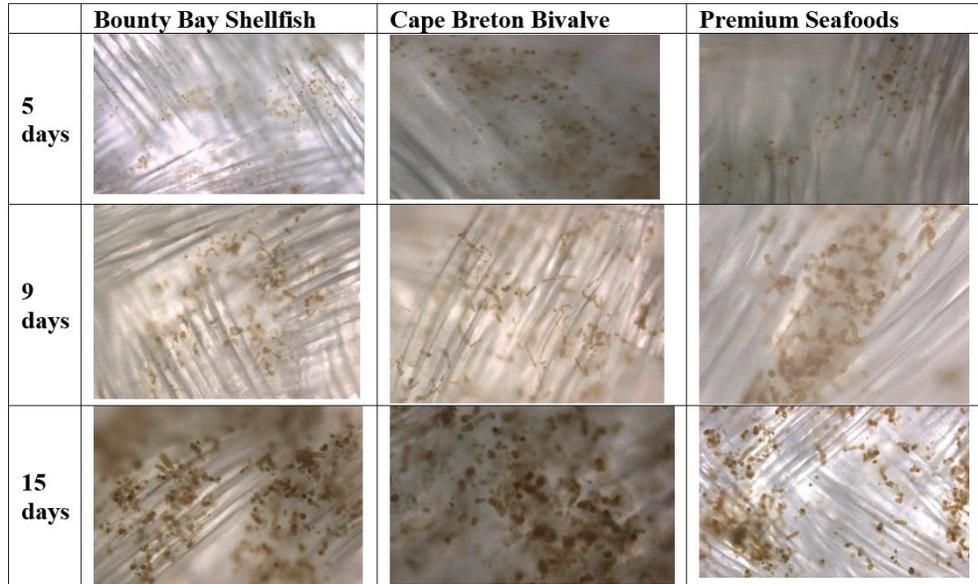
Figure A.2: Pictures (taken under a microscope - 10x) of the seeded rope after 5, 9 and 15 days for the 3 leases (Bounty Bay Shellfish, Cape Breton bivalve and Premium Seafoods). ((Lionard & Clark, 2019); photos- Jean Philippe)

Seed line production occurred at the FMQ in Québec. Upon arrival at the center, seaweeds were disinfected to prevent the spread of invasive species from Nova Scotia to Québec. The seaweed was scrubbed with absorbent paper and then rinsed twice with sterilized and filtered seawater 10°C and 1 µM. They were then further disinfected by soaking the blades in a seawater bleach solution at with 30 mg/L of sodium hypochlorite and 0.25 mL of 12% bleach per liter of seawater used, for two minutes. The seaweed then underwent two more rinses in a sterile seawater bath at 10°C containing germanium dioxide at 0.1 mL per liter of seawater (Clark & Salvo, 2020; Mooney-McAuley et al., 2016; Tamigneaux et al., 2013).

After disinfecting, seaweeds were moved into a fridge to induce sporulation of the seaweeds and were then mechanically agitated via boiling causing spores to be released

(Clark & Salvo, 2020; Mooney-McAuley et al., 2016; Tamigneaux et al., 2013). This liquid was then filtered to remove debris and colloids. A cylindrical collector was immersed in the last boiler, that contained the clean filtrate at 10°C without bubbling so the spores fixed to the seeded string. After 12 hours the collectors were moved to contain 10°C seawater, nutrient solution and biocides to prevent the growth of diatoms and *Pseudomonas alginovora* (Clark & Salvo, 2020; Mooney-McAuley et al., 2016; Tamigneaux et al., 2013). The cultures were monitored for 2-3 weeks, to verify fertilization and were transferred to a culture tank before transporting to sites (Clark & Salvo, 2020; Mooney-McAuley et al., 2016; Tamigneaux et al., 2013). After 42 days, 468 meters, 522 meters and 513 meters of seeded lines were produced for Cape Breton Bivalves, Bounty Bay Shellfish, and Premium Seafoods, respectively (Lionard & Clark, 2019). Densities on each collector were not the same, Bounty Bay and Premium lines were approximately 75-100% covered on each collector, while Cape Breton Bivalves density on the lines was 25-50% (Lionard & Clark, 2019).

A.2 UNITED STATES PRODUCTION

Part of the project at the farms in the United States was to examine how different crosses grow over the winter. On October 7, 2019, 347 different crosses were processed by mixing 10 mg of female biomass with 5 mg of male biomass (wet weight) (Lindell, 2020). The University of Connecticut had over one thousand cultures of *Saccharina latissima* or *Sacharina angustissima* which were used to create the crosses. The crosses were maintained for a week and then checked under a microscope to make sure there were signs of fertilization (Lindell, 2020). Once signs of fertilization occurred, the gametophytes and sporophytes were attached to pucks that have the string wound around them (Figure A.3). This was done using a proprietary bind from AtSea with a concentration of 0.5%. This binder was mixed with filtered sterile seawater and blended for 60 seconds (Lindell, 2020). This solution and the de-water biomass from the crossed gametophytes was mixed together in Scintillation vials and applied to the pucks by painting the material on to the strings. They were then air-dried for 10 minutes and put in polycarbonate boxes with Provasoli's solution (PES) and germanium dioxide (Lindell, 2020).
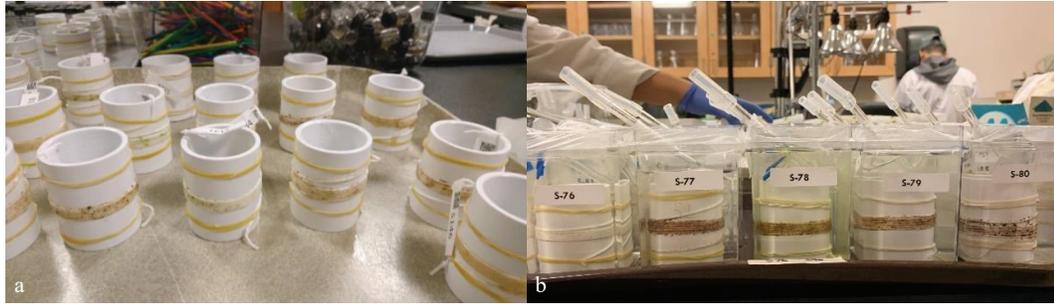
Figure A.3: (a) Pucks with gametophyte biomass from the various crosses waiting to be put into culture boxes. (b) Image of pucks settled inside the boxes with clean media prepped for incubation. Photos, courtesy of Scott Lindell and David Bailey.

Once the pucks were added to the media, they were left to acclimate for 48 hours at which time airlines were added to help avoid the detachment of algal biomass from the lines. For 3.5 weeks, the cultures were maintained at 15°C and 80 μM m^{-2} s^{-1} and then increased to 12°C and 120 μM m^{-2} s^{-1} for one week and 9°C the week leading up to putting the seeded ropes in the water (Lindell, 2020).
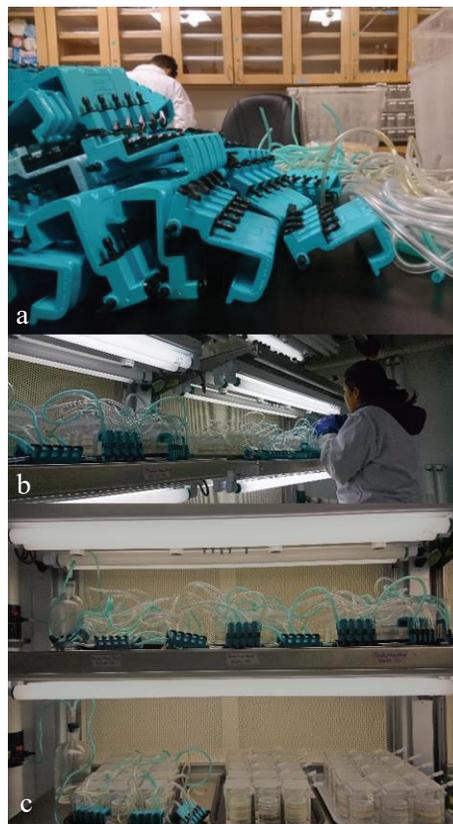
Figure A.4: (a) Prepping the airlines. (b) Connecting the airlines to the containers containing the seeded lines. (c) Seed lines sitting on the light tables light growing. Photos, courtesy of Scott Lindell and David Bailey.

Several biomass characteristics were measured during the kelp aquaculture pilot project. In the thesis, blade length is the only biomass characteristic discussed in detail. In this appendix, figures for widest blade width, blade thickness and stipe diameter are shown.

Table B.1: Mean biomass characteristics of cultivated *S. latissima* harvested in June 2020, where n is the number of *S. latissima* plants examined

Location	Line depth (m)	n	Blade length (cm)	Blade widest width (cm)	Stipe diameter (mm)	Blade thickness (mm)	Density plant/m
Arichat Harbour	2	60	100.7±23.9	19.6±5.6	4.13±1.17	1.11±0.16	769
	4	60	97.8±21.9	20.7±5.9	4.18±1.33	1.14±0.20	345
St. Ann's Bay	2	60	35.3±5.1	8.8±2.3	1.46±0.56	0.55±0.19	589
	4	60	33.8±6.0	8.4±2.2	1.32±0.55	0.51±0.30	242
Lennox Passage	2	60	89.6±23.9	14.4±3.9	N/A	N/A	522
	4	60	69.5±19.4	14.6±3.9	3.57±1.13	0.92±0.20	383
Buzzards Bay	3	3	3	1	N/A	N/A	3
GreenWave Farm	2		57.9	6.8	2.45	0.68	35
UNH Farm 2018-2019	2		58.0	7.7	2.73	0.42	74
UNH Farm 2019-2020	2		77.5	1	2.74	0.46	165
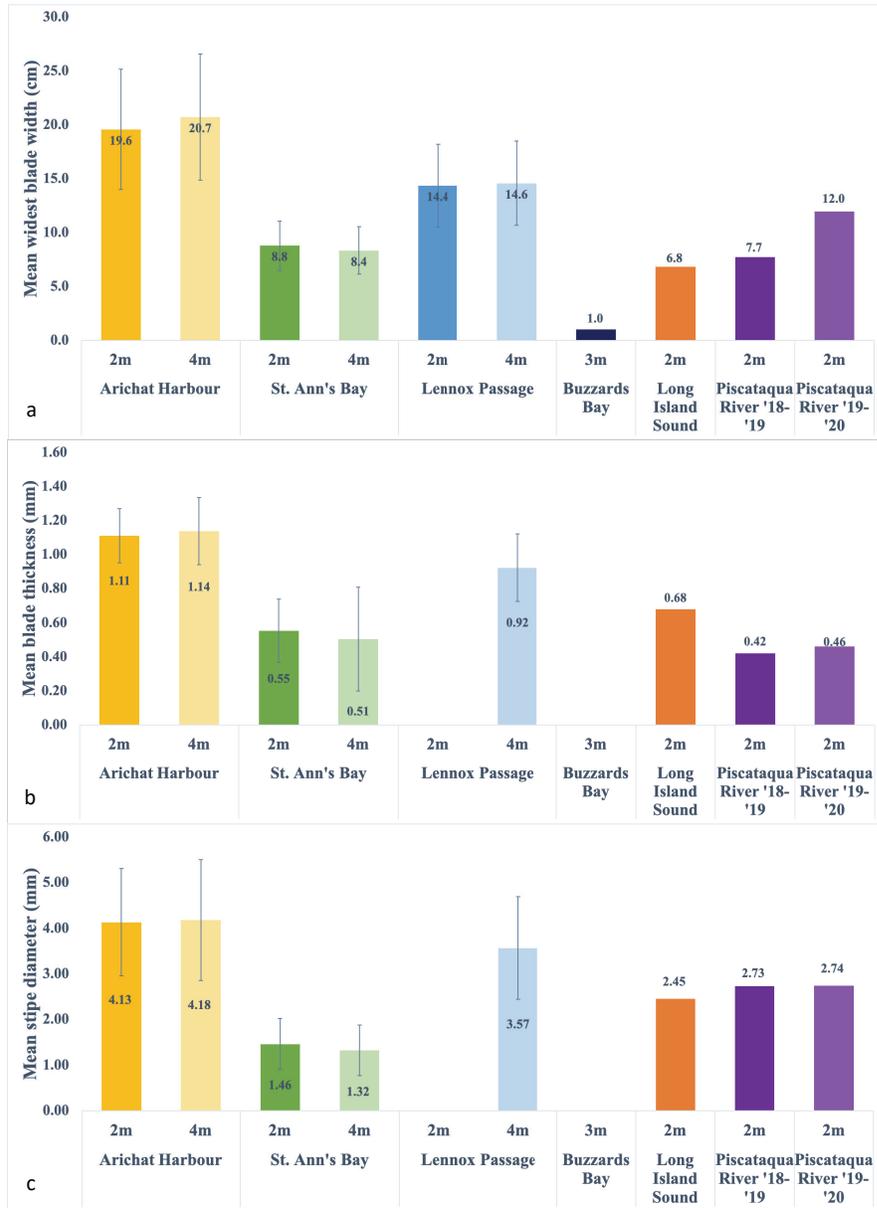
Figure B.1: Mean biomass characteristics collected in the field at all six sites. (a) mean widest blade width (cm), (b) Mean blade thickness (mm), (c) mean stipe diameter (mm) Arichat Harbour, St. Ann's Bay and Lennox Passage n=60. Buzzards Bay n=3. Long Island Sound and Pisactaqua River.
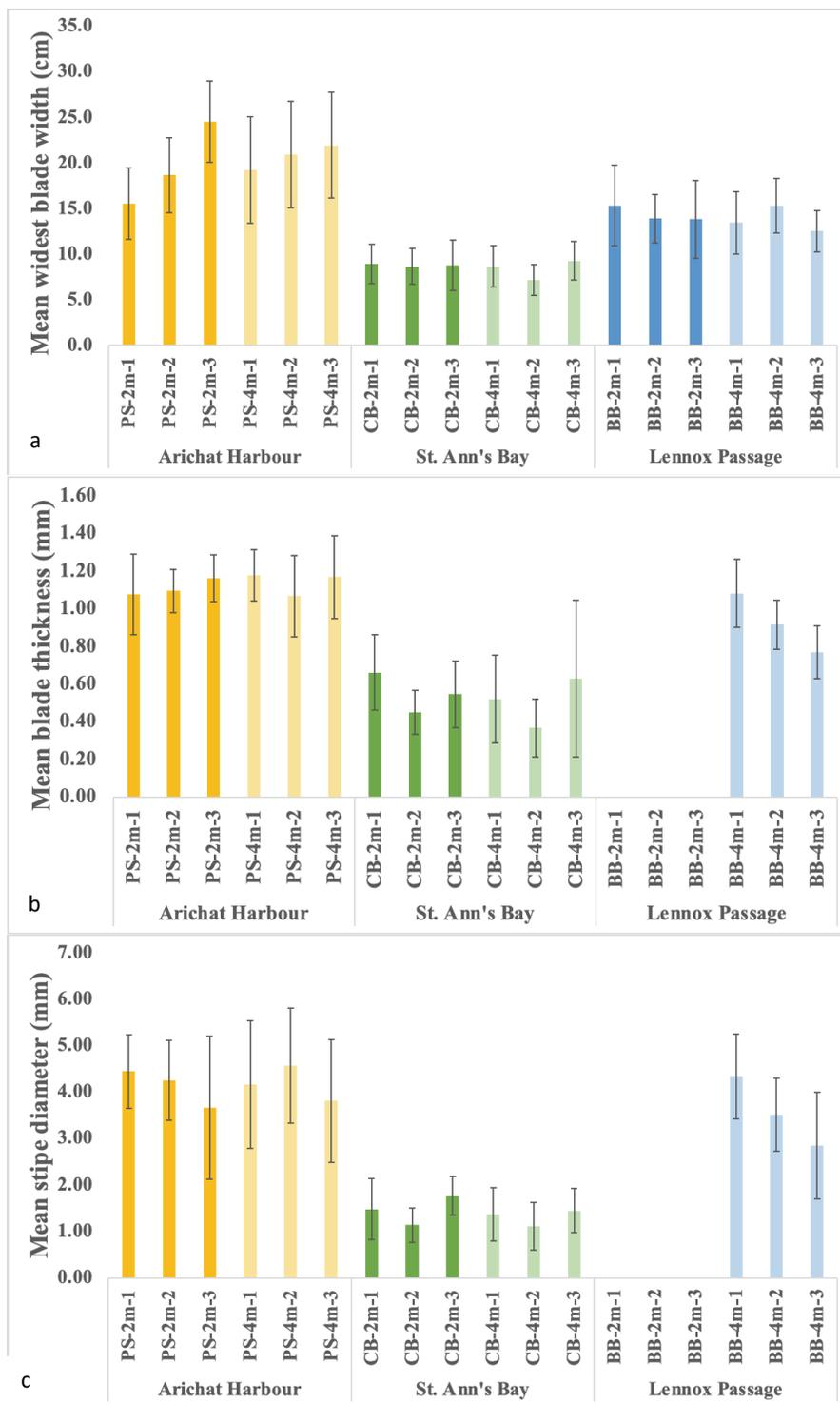
Figure B.2: Comparing plots from 2m and 4m at all three Cape Breton locations. (a) mean widest blade width (cm), (b) Mean blade thickness (mm), (c) mean stipe diameter (mm), n=20 seaweeds from every plot
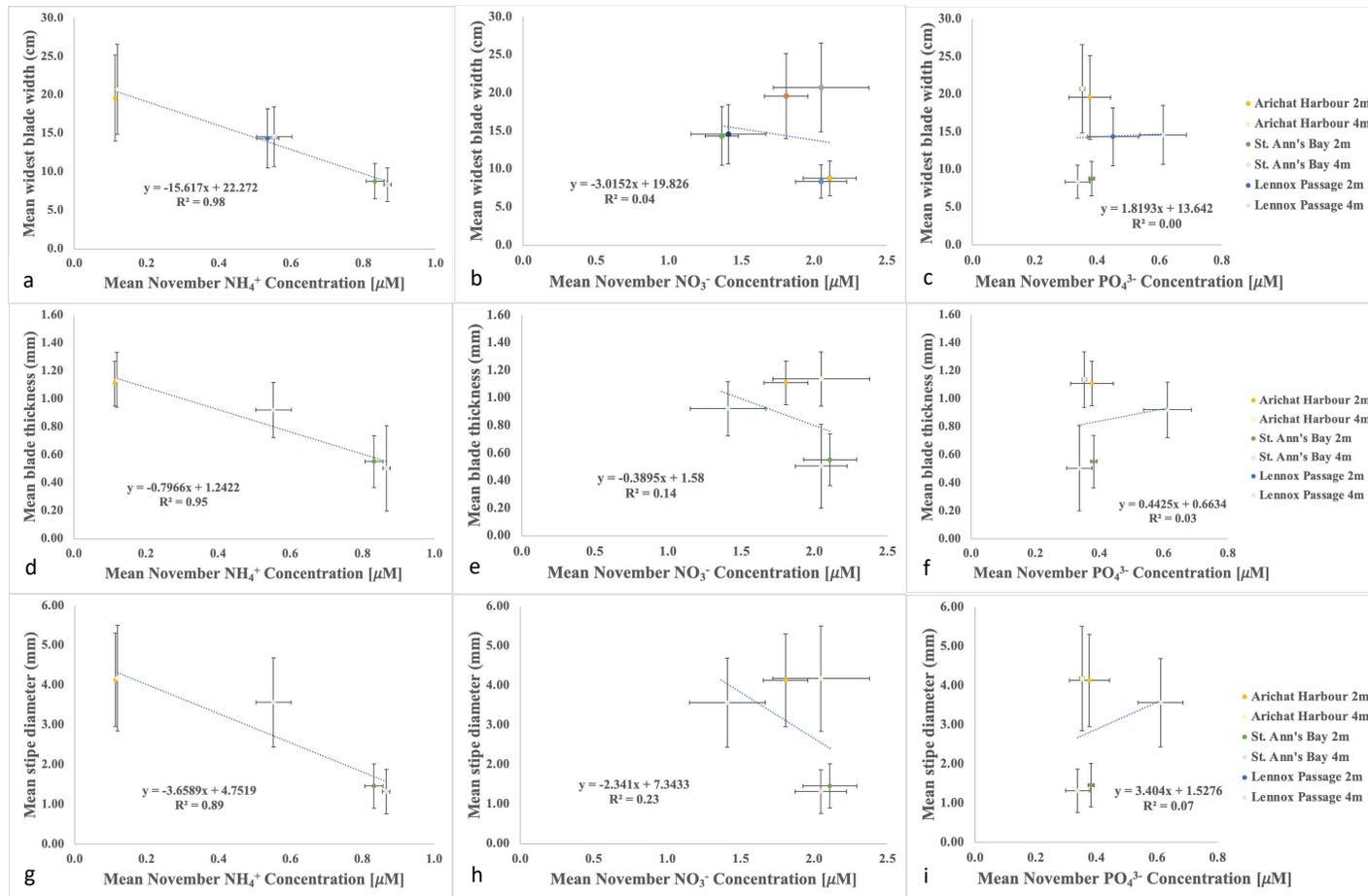
Figure B.3: Mean biomass characteristics vs. mean November nutrient concentrations. Rows: (a-c) mean widest blade width (cm), (d-f) mean blade thickness (mm), and (g-i) mean stipe diameter (mm). Columns: (a, d, g) mean November NH_4^+ concentration, (b, e, h) mean November NO_3^- concentration, (c, f, i) mean November PO_4^{3-} concentration. All samples run in duplicates. Different numbers of samples were run for each month at each site, so n is different for each site. Lennox Passage and St. Ann's Bay were not included since there was no winter samples collected.
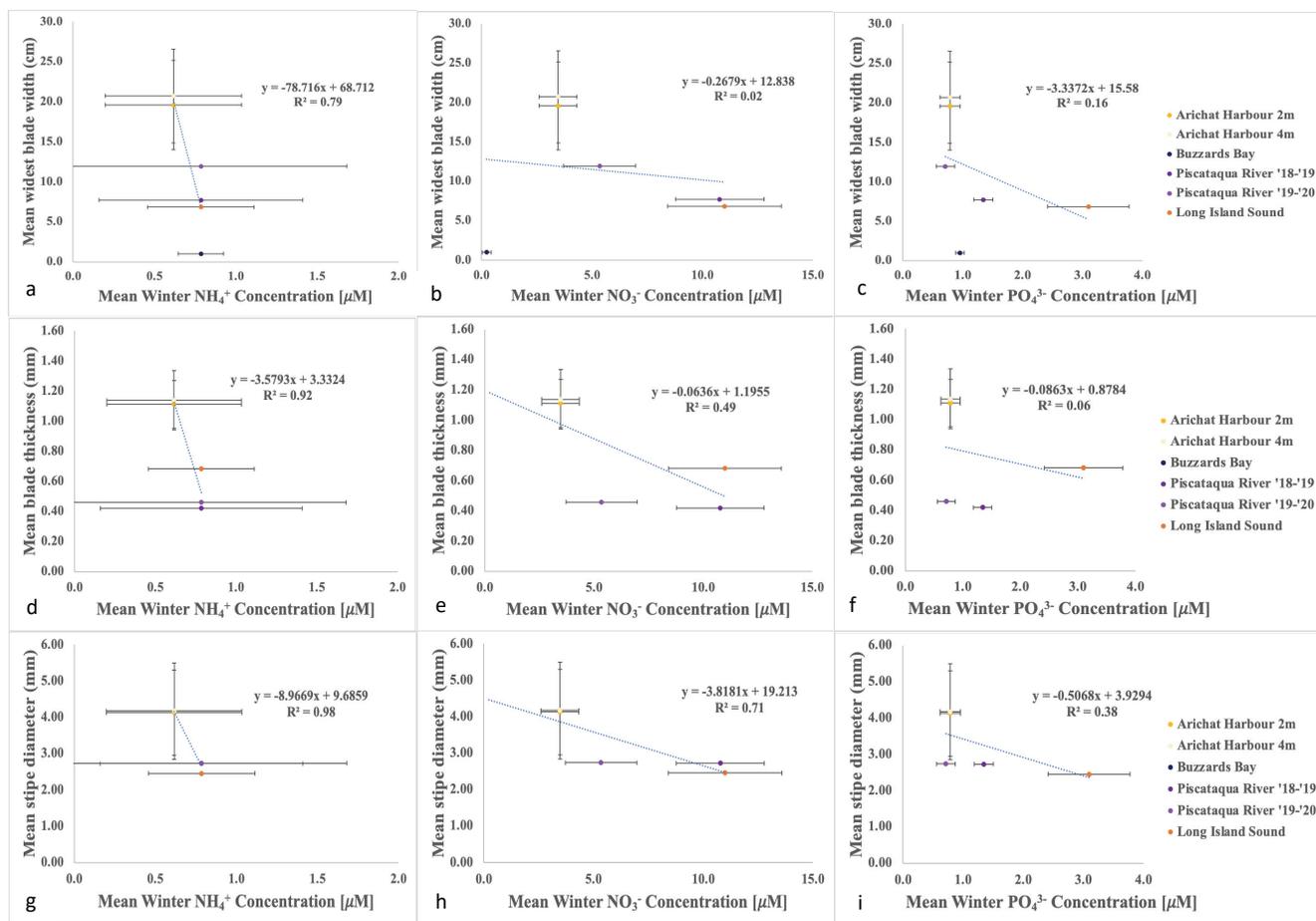
Figure B.4: Biomass characteristics vs. winter nutrient concentrations. Rows: (a-c) mean widest blade width (cm), (d-f) mean blade thickness (mm), and (g-i) mean stipe diameter (mm). Columns: (a, d, g) mean winter NH_4^+ concentration, (b, e, h) mean winter NO_3^- concentration, (c, f, i) mean winter PO_4^{3-} concentration. All samples run in duplicates. Different numbers of samples were run for each month at each site, so n is different for each site. Lennox Passage and St. Ann's Bay were not included since there was no winter samples collected.
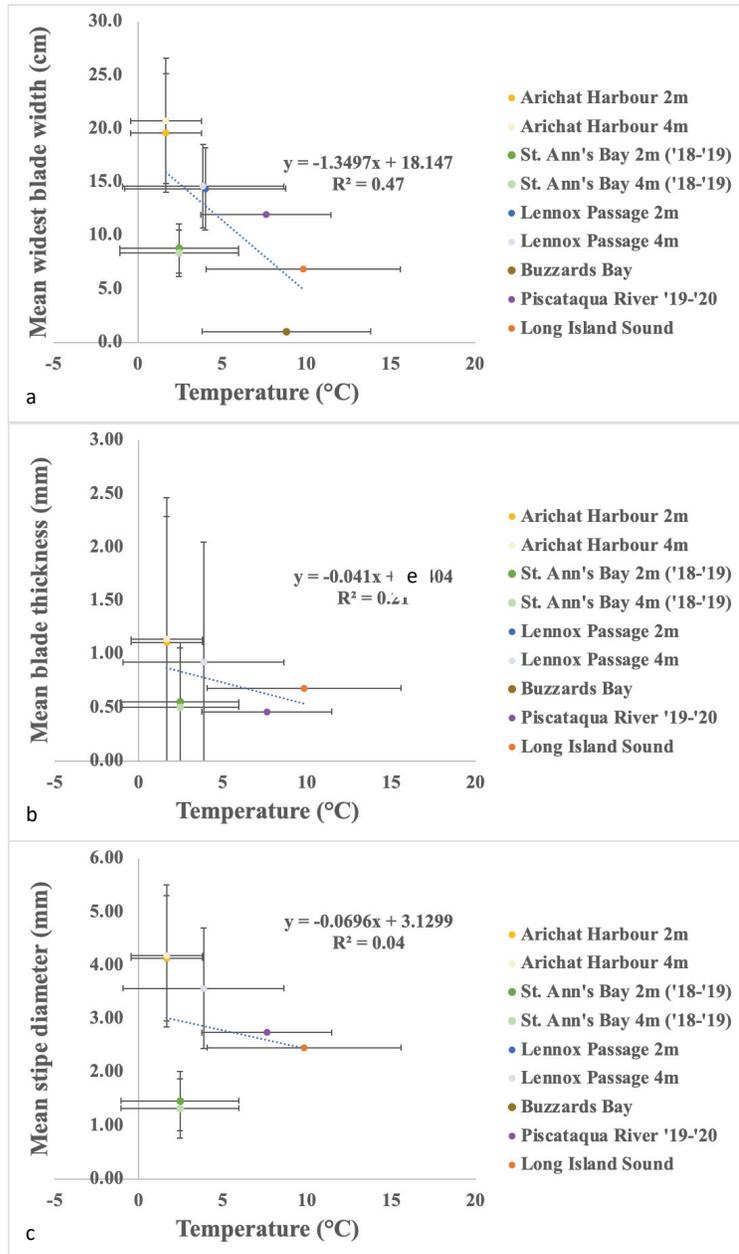
Figure B.5: Biomass Characteristics vs Mean Temperature. (a) mean widest blade width (cm), (b) Mean blade thickness (mm), (c) mean stipe diameter (mm). Temperature values represent the mean monthly temperature between February and June 2020 from each site. St. Ann's Bay data comes from the '18-'19 season. Loggers from this site were not recovered.

APPENDIX C: Shelburne nutrient and isotope data

Table C.1: Dissolved nutrient concentrations from water samples collected from Shelburne Harbour, NS on November 8th, 2019.

Station	Latitude	Longitude	Date	NO$_3^-$ +NO$_2^-$ (μM)	NH$_4^+$ (μM)	Mean NO$_2^-$ (μM)	NO$_2^-$ Standard Deviation
A	43.6565	-65.2835	11/08/2019	1.5	1.9	0.2	0.0
B	43.655528	-65.28436	11/08/2019	1.4	1.9	0.2	0.0
C	43.654611	-65.28436	11/08/2019	1.4	2.1	0.2	0.0
D	43.721472	-65.32214	11/08/2019	1.7	4.1	0.2	0.0
E	43.723278	-65.32169	11/08/2019	1.4	3.0	0.1	0.0
F	43.72225	-65.32094	11/08/2019	1.8	2.8	0.2	0.0
G	43.732083	-65.32989	11/08/2019	1.5	3.4	0.2	0.0
H	43.733667	-65.32886	11/08/2019	1.4	3.7	0.1	0.0
I	43.734083	-65.32994	11/08/2019	1.2	3.1	0.1	0.0
J	43.741528	-65.3265	11/08/2019	1.1	3.1	0.1	0.0
K	43.741972	-65.329	11/08/2019	1.0	2.6	0.1	0.0
L	43.740611	-65.32928	11/08/2019	1.3	3.3	0.2	0.0
1	43.671101	-65.2935	11/08/2019	1.8	1.3	0.3	0.0
2	43.727798	-65.3376	11/08/2019	1.2	3.0	0.1	0.0
3	43.7509	-65.3198	11/08/2019	1.3	3.1	0.2	0.0
4	43.7113	-65.3383	11/08/2019	1.3	2.7	0.2	0.0
5	43.7159	-65.3204	11/08/2019	1.5	2.8	0.2	0.1
6	43.7066	-65.3323	11/08/2019	1.0	3.1	0.2	0.0
7	43.670898	-65.3187	11/08/2019	1.3	2.3	0.2	0.1
8	43.724499	-65.3451	11/08/2019	1.2	3.0	0.2	0.0
9	43.720501	-65.3278	11/08/2019	3.7	3.1	0.2	0.0
10	43.706501	-65.3447	11/08/2019	1.2	3.0	0.2	0.0
11	43.679901	-65.3186	11/08/2019	1.3	2.8	0.3	0.0
12	43.7155	-65.3446	11/08/2019	1.1	3.0	0.3	0.0
13	43.7155	-65.3328	11/08/2019	1.2	2.8	0.2	0.0
14	43.711201	-65.3259	11/08/2019	1.1	2.6	0.2	0.0
15	43.697498	-65.3319	11/08/2019	1.0	3.1	0.2	0.0
16	43.721401	-65.338	11/08/2019	3.8	3.0	0.2	0.1
17	43.744499	-65.327	11/08/2019	1.4	3.1	0.2	0.0
18	43.748402	-65.323	11/08/2019	1.7	3.0	0.2	0.0
19	43.742802	-65.3213	11/08/2019	1.6	2.8	0.2	0.0
20	43.688499	-65.3313	11/08/2019	1.0	3.0	0.2	0.0

Station	Latitude	Longitude	Date	NO$_3^-$ +NO$_2^-$ (μM)	NH$_4^+$ (μM)	Mean NO$_2^-$ (μM)	NO$_2^-$ Standard Deviation
21	43.730099	-65.321	11/08/2019	1.4	2.6	0.2	0.0
22	43.7244	-65.3327	11/08/2019	1.1	2.6	0.2	0.0
23	43.727402	-65.3249	11/08/2019	1.3	2.8	0.1	0.0
24	43.664799	-65.3058	11/08/2019	1.2	2.1	0.2	0.0
25	43.728901	-65.3304	11/08/2019	1.1	3.3	0.1	0.0
26	43.7369	-65.3217	11/08/2019	1.1	2.8	0.2	0.0
27	43.736301	-65.3282	11/08/2019	1.0	2.9	0.1	0.0
28	43.670898	-65.3059	11/08/2019	1.2	2.1	0.2	0.0
29	43.662102	-65.2931	11/08/2019	1.4	1.8	0.2	0.0
30	43.664299	-65.2838	11/08/2019	1.4	1.5	0.1	0.0
31	43.6586	-65.2898	11/08/2019	1.3	1.4	0.1	0.0
32	43.6576	-65.2677	11/08/2019	1.3	1.0	0.1	0.0
33	43.636799	-65.2667	11/08/2019	1.3	1.6	0.1	0.0
34	43.658901	-65.2804	11/08/2019	1.2	1.7	0.2	0.0
35	43.6548	-65.2744	11/08/2019	1.2	1.3	0.2	0.0
36	43.649899	-65.2751	11/08/2019	1.2	1.4	0.1	0.0
37	43.654598	-65.2435	11/08/2019	1.9	1.7	0.2	0.0
38	43.653999	-65.2559	11/08/2019	1.3	1.0	0.1	0.0
39	43.644798	-65.2679	11/08/2019	1.3	1.5	0.2	0.0
40	43.645199	-65.2556	11/08/2019	1.6	1.8	0.2	0.0
41	43.751499	-65.3299	11/08/2019	1.1	2.8	0.2	0.0
42	43.724098	-65.3577	11/08/2019	0.9	2.8	0.1	0.0
43	43.7486	-65.3183	11/08/2019	1.4	3.0	0.2	0.0
44	43.679798	-65.3064	11/08/2019	1.4	1.7	0.2	0.0
45	43.7341	-65.3342	11/08/2019	1.0	2.8	0.2	0.0
46	43.7589	-65.328	11/08/2019	1.2	2.7	0.2	0.0
47	43.6619	-65.3181	11/08/2019	1.3	2.0	0.2	0.0
48	43.733002	-65.3703	11/08/2019	1.2	2.9	0.2	0.0

Table C.2: Dissolved nutrient concentrations from water samples collected from Shelburne Harbour, NS on November 22nd, 2019.

Station	Latitude	Longitude	Date	NO$_3^-$ +NO$_2^-$ (μM)	NH$_4^+$ (μM)	Mean NO$_2^-$ (μM)	NO$_2^-$ Standard Deviation
A	43.6565	-65.2835	11/22/2019	4.0	1.1	0.3	0.0
B	43.65553	-65.28436	11/22/2019	9.9	1.7	0.3	0.0
C	43.65461	-65.28436	11/22/2019	8.8	1.3	0.3	0.0
D	43.72147	-65.32214	11/22/2019	4.6	1.4	0.4	0.0
E	43.72328	-65.32169	11/22/2019	3.7	4.5	0.4	0.0
F	43.72225	-65.32094	11/22/2019	NA	NA	NA	NA
G	43.73208	-65.32989	11/22/2019	3.5	1.1	0.3	0.0
H	43.73367	-65.32886	11/22/2019	2.8	1.7	0.4	0.0
I	43.73408	-65.32994	11/22/2019	4.0	1.0	0.4	0.0
J	43.74153	-65.3265	11/22/2019	4.1	1.4	0.4	0.0
K	43.74197	-65.329	11/22/2019	3.8	1.0	0.4	0.0
L	43.74061	-65.32928	11/22/2019	3.9	1.1	0.4	0.0
1	43.6711	-65.2935	11/22/2019	5.9	0.7	0.2	0.0
2	43.7278	-65.3376	11/22/2019	3.8	1.0	0.3	0.0
3	43.7509	-65.3198	11/22/2019	3.4	1.0	0.3	0.0
4	43.7113	-65.3383	11/22/2019	4.4	1.2	0.4	0.0
5	43.7159	-65.3204	11/22/2019	3.8	1.3	0.4	0.0
6	43.7066	-65.3323	11/22/2019	4.5	1.1	0.4	0.0
7	43.6709	-65.3187	11/22/2019	5.2	1.2	0.4	0.0
8	43.7245	-65.3451	11/22/2019	3.7	1.4	0.3	0.0
9	43.7205	-65.3278	11/22/2019	3.5	1.1	0.3	0.0
10	43.7065	-65.3447	11/22/2019	4.0	1.1	0.3	0.0
11	43.6799	-65.3186	11/22/2019	4.0	1.2	0.4	0.0
12	43.7155	-65.3446	11/22/2019	4.1	1.4	0.4	0.0
13	43.7155	-65.3328	11/22/2019	4.1	1.1	0.4	0.0
14	43.7112	-65.3259	11/22/2019	4.3	1.3	0.4	0.0
15	43.6975	-65.3319	11/22/2019	4.4	1.0	0.4	0.0
16	43.7214	-65.338	11/22/2019	3.9	1.1	0.4	0.0
17	43.7445	-65.327	11/22/2019	3.9	2.3	0.4	0.0
18	43.7484	-65.323	11/22/2019	4.1	1.2	0.4	0.0
19	43.7428	-65.3213	11/22/2019	4.1	1.2	0.4	0.0
20	43.6885	-65.3313	11/22/2019	4.1	1.0	0.4	0.0
21	43.7301	-65.321	11/22/2019	4.0	1.0	0.4	0.0

Station	Latitude	Longitude	Date	NO$_3^-$ +NO$_2^-$ (μM)	NH$_4^+$ (μM)	Mean NO$_2^-$ (μM)	NO$_2^-$ Standard Deviation
22	43.7244	-65.3327	11/22/2019	4.3	1.4	0.4	0.0
23	43.7274	-65.3249	11/22/2019	4.2	1.1	0.4	0.0
24	43.6648	-65.3058	11/22/2019	4.2	1.0	0.4	0.1
25	43.7289	-65.3304	11/22/2019	3.6	1.1	0.4	0.0
26	43.7369	-65.3217	11/22/2019	3.5	1.2	0.3	0.0
27	43.7363	-65.3282	11/22/2019	3.8	1.3	0.4	0.0
28	43.6709	-65.3059	11/22/2019	6.6	0.8	0.3	0.0
29	43.6621	-65.2931	11/22/2019	13.3	1.0	0.3	0.0
30	43.6643	-65.2838	11/22/2019	7.7	0.8	0.3	0.0
31	43.6586	-65.2898	11/22/2019	11.5	1.1	0.3	0.0
32	43.6576	-65.2677	11/22/2019	1.9	1.0	0.2	0.0
33	43.6368	-65.2667	11/22/2019	2.1	0.7	0.3	0.0
34	43.6589	-65.2804	11/22/2019	2.9	1.0	0.3	0.0
35	43.6548	-65.2744	11/22/2019	2.4	1.0	0.3	0.0
36	43.6499	-65.2751	11/22/2019	2.4	1.0	0.3	0.0
37	43.6546	-65.2435	11/22/2019	1.6	0.3	0.2	0.0
38	43.654	-65.2559	11/22/2019	1.4	0.2	0.1	0.0
39	43.6448	-65.2679	11/22/2019	2.1	0.7	0.2	0.0
40	43.6452	-65.2556	11/22/2019	1.6	0.6	0.2	0.0
41	43.7515	-65.3299	11/22/2019	3.4	1.0	0.3	0.0
42	43.7241	-65.3577	11/22/2019	4.1	1.2	0.4	0.1
43	43.7486	-65.3183	11/22/2019	3.4	0.9	0.3	0.0
44	43.6798	-65.3064	11/22/2019	3.9	0.9	0.3	0.0
45	43.7341	-65.3342	11/22/2019	3.2	0.9	0.3	0.0
46	43.7589	-65.328	11/22/2019	1.9	0.7	0.3	0.0
47	43.6619	-65.3181	11/22/2019	3.7	0.8	0.3	0.0
48	43.733	-65.3703	11/22/2019	3.9	1.3	0.4	0.0

Table C.3: Dissolved isotopic values from water samples collected from Shelburne Harbour, NS on November 8th, 2019.

Station	Date	Latitude	Longitude	Mean δ^{15}N of NO$_3^-$ (‰)	δ^{15}N of NO$_3^-$ (‰) Standard Deviation	Mean δ^{18}O of NO$_3^-$ (‰)	δ^{18}O of NO$_3^-$ (‰) Standard Deviation	Mean δ^{15}N of NH$_4^+$ (‰)	δ^{15}N of NH$_4^+$ Standard Deviation (‰)
1	11/08/2019	43.671101	-65.293503	3.23	0.13	1.17	0.48	15.01	0.08
10	11/08/2019	43.706501	-65.344704	0.79	0.47	7.74	0.35	12.66	0.59
11	11/08/2019	43.679901	-65.318604	1.07	0.19	2.10	1.54	14.14	0.00
15	11/08/2019	43.697498	-65.331902	0.74	0.43	3.53	0.77	13.62	0.03
16	11/08/2019	43.721401	-65.337997	1.53	0.09	16.78	0.11	12.21	0.43
25	11/08/2019	43.728901	-65.330399	1.19	0.26	6.04	0.33	12.88	0.38
26	11/08/2019	43.7369	-65.321701	1.02	0.47	5.95	0.41	11.18	0.13
27	11/08/2019	43.736301	-65.328201	0.64	0.53	5.52	0.30	15.36	0.51
28	11/08/2019	43.670898	-65.305901	1.93	0.50	2.22	0.13	13.75	0.20
29	11/08/2019	43.662102	-65.293098	2.27	0.24	2.37	1.21	14.96	0.09
3	11/08/2019	43.7509	-65.319801	1.21	0.78	4.25	0.36	14.27	0.13
31	11/08/2019	43.6586	-65.289803	2.53	0.19	2.45	1.21	14.58	0.34
4	11/08/2019	43.7113	-65.338303	1.07	0.69	6.93	0.50	10.67	0.09
40	11/08/2019	43.645199	-65.2556	3.06	0.06	4.27	0.67	15.54	0.24
47	11/08/2019	43.6619	-65.3181	1.97	0.56	2.73	0.22	13.75	0.41
48	11/08/2019	43.733002	-65.3703	0.78	0.36	3.90	0.74	16.82	0.34
5	11/08/2019	43.7159	-65.320396	1.29	0.10	7.70	0.81	5.92	0.37
B	11/08/2019	43.655528	-65.284361	2.33	0.14	1.73	1.20	13.03	0.04
C	11/08/2019	43.654611	-65.284361	2.23	0.44	2.46	1.32	12.15	0.15

Station	Date	Latitude	Longitude	Mean $\delta^{15}N$ of NO_3^- (‰)	$\delta^{15}N$ of NO_3^- (‰) Standard Deviation	Mean $\delta^{18}O$ of NO_3^- (‰)	$\delta^{18}O$ of NO_3^- (‰) Standard Deviation	Mean $\delta^{15}N$ of NH_4^+ (‰)	$\delta^{15}N$ of NH_4^+ Standard Deviation (‰)
D	11/08/2019	43.721472	-65.322139	1.43	0.12	7.61	0.53	11.70	0.16
E	11/08/2019	43.723278	-65.321694	1.00	0.07	7.78	0.96	5.51	0.53
G	11/08/2019	43.732083	-65.329889	1.13	0.10	5.99	0.98	13.27	0.08
I	11/08/2019	43.734083	-65.329944	1.04	0.48	5.99	0.84	14.17	0.55
J	11/08/2019	43.741528	-65.3265	0.83	0.08	5.10	1.19	14.31	0.03
K	11/08/2019	43.741972	-65.329	4.38	0.57	6.88	0.29	12.97	0.45

Table C.4: Dissolved isotopic values from water samples collected from Shelburne Harbour, NS on November 22nd, 2019.

Station	Date	Latitude	Longitude	Mean $\delta^{15}N$ of NO_3^- (‰)	$\delta^{15}N$ of NO_3^- (‰) Standard Deviation	Mean $\delta^{18}O$ of NO_3^- (‰)	$\delta^{18}O$ of NO_3^- (‰) Standard Deviation	Mean $\delta^{15}N$ of NH_4^+ (‰)	$\delta^{15}N$ of NH_4^+ Standard Deviation (‰)
1	11/22/2019	43.671101	-65.293503	2.62	0.07	13.89	0.31	6.95	NA
10	11/22/2019	43.706501	-65.344704	3.82	0.16	3.14	0.15	14.81	0.51
11	11/22/2019	43.679901	-65.318604	3.55	0.14	3.04	0.99	14.04	0.43
15	11/22/2019	43.697498	-65.331902	3.52	0.01	3.10	0.31	10.34	0.21
16	11/22/2019	43.721401	-65.337997	3.62	0.02	1.69	0.55	14.13	0.14
25	11/22/2019	43.728901	-65.330399	3.63	0.06	1.61	0.67	14.42	0.31
26	11/22/2019	43.7369	-65.321701	3.63	0.09	1.92	0.52	13.23	0.15
27	11/22/2019	43.736301	-65.328201	3.60	0.03	1.41	0.51	11.50	0.08
28	11/22/2019	43.670898	-65.305901	2.69	0.25	13.39	0.25	10.19	1.09

Station	Date	Latitude	Longitude	Mean $\delta^{15}N$ of NO_3^- (‰)	$\delta^{15}N$ of NO_3^- (‰) Standard Deviation	Mean $\delta^{18}O$ of NO_3^- (‰)	$\delta^{18}O$ of NO_3^- (‰) Standard Deviation	Mean $\delta^{15}N$ of NH_4^+ (‰)	$\delta^{15}N$ of NH_4^+ Standard Deviation (‰)
29	11/22/2019	43.662102	-65.293098	2.18	0.04	16.37	0.03	11.53	0.40
3	11/22/2019	43.7509	-65.319801	3.62	0.18	2.00	0.36	14.93	0.05
31	11/22/2019	43.6586	-65.289803	2.29	0.04	15.84	0.26	17.29	0.21
4	11/22/2019	43.7113	-65.338303	3.40	0.09	3.56	0.53	12.59	0.27
40	11/22/2019	43.645199	-65.2556	4.90	0.11	2.00	0.63	10.89	0.28
47	11/22/2019	43.6619	-65.3181	3.22	0.15	6.47	0.49	10.55	0.32
48	11/22/2019	43.733002	-65.3703	3.21	0.06	2.89	0.15	10.23	0.14
5	11/22/2019	43.7159	-65.320396	3.59	0.04	2.29	0.50	12.17	0.32
B	11/22/2019	43.655528	-65.284361	2.66	0.06	15.20	0.10	13.38	0.05
C	11/22/2019	43.654611	-65.284361	2.62	0.03	13.45	0.19	14.90	0.15
D	11/22/2019	43.721472	-65.322139	3.48	0.05	4.58	0.03	14.00	0.28
E	11/22/2019	43.723278	-65.321694	3.68	0.23	1.76	0.25	7.68	0.03
G	11/22/2019	43.732083	-65.329889	3.56	0.14	2.44	0.29	14.12	0.14
I	11/22/2019	43.734083	-65.329944	3.65	0.06	1.60	0.30	9.54	0.95
J	11/22/2019	43.741528	-65.3265	3.70	0.14	1.77	0.21	7.55	0.13
K	11/22/2019	43.741972	-65.329	3.75	0.03	1.85	0.31	10.04	0.83

Table C.5: δ^{15}N and N% in macroalgae tissue of *Chondrus crispus* from Shelburne Harbour, NS between November 8-22, 2019.

Station	Species	Latitude	Longitude	δ^{15}N	% N
1	*Chondrus crispus*	43.671101	-65.293503	0.58	3.271
2	*Chondrus crispus*	43.727798	-65.337601	-2.34	3.041
3	*Chondrus crispus*	43.7509	-65.319801	-3.01	2.99
4	*Chondrus crispus*	43.7113	-65.338303	-1.35	3.22
5	*Chondrus crispus*	43.7159	-65.320396	-1.72	3.155
6	*Chondrus crispus*	43.7066	-65.332298	-2.23	2.919
7	*Chondrus crispus*	43.670898	-65.318703	-0.22	3.387
8	*Chondrus crispus*	43.724499	-65.3451	-2.52	2.699
9	*Chondrus crispus*	43.720501	-65.327797	-1.97	3.334
10	*Chondrus crispus*	43.706501	-65.344704	-2.72	2.826
11	*Chondrus crispus*	43.679901	-65.318604	0.01	3.303
12	*Chondrus crispus*	43.7155	-65.344597	-1.65	3.425
13	*Chondrus crispus*	43.7155	-65.332802	-1.6	3.164
15	*Chondrus crispus*	43.697498	-65.331902	-1.3	3.347
16	*Chondrus crispus*	43.721401	-65.337997	-2.07	3.303
17	*Chondrus crispus*	43.744499	-65.327003	-2.61	3.242
18	*Chondrus crispus*	43.748402	-65.322998	-3.42	2.809
19	*Chondrus crispus*	43.742802	-65.321297	-2.59	3.413
20	*Chondrus crispus*	43.688499	-65.331299	-2.12	3.148
22	*Chondrus crispus*	43.7244	-65.332703	-0.87	3.197
23	*Chondrus crispus*	43.727402	-65.324898	-2.49	3.174
24	*Chondrus crispus*	43.664799	-65.305801	-0.71	3.208
25	*Chondrus crispus*	43.728901	-65.330399	-2.77	3.215
26	*Chondrus crispus*	43.7369	-65.321701	-3.1	3.348
27	*Chondrus crispus*	43.736301	-65.328201	-2.75	3.239
28	*Chondrus crispus*	43.670898	-65.305901	0.6	3.556
29	*Chondrus crispus*	43.662102	-65.293098	0.59	3.251
31	*Chondrus crispus*	43.6586	-65.289803	-1.01	3.098
35	*Chondrus crispus*	43.6548	-65.274399	1.77	3.442
41	*Chondrus crispus*	43.751499	-65.329903	-2.77	3.236
42	*Chondrus crispus*	43.724098	-65.357697	-1.73	3.348
43	*Chondrus crispus*	43.7486	-65.318298	-2.37	3.02
44	*Chondrus crispus*	43.679798	-65.306396	-0.76	2.976
45	*Chondrus crispus*	43.7341	-65.334198	-3.04	3.169
46	*Chondrus crispus*	43.7589	-65.328003	-2.81	3.18

Station	Species	Latitude	Longitude	δ¹⁵N	% N
47	*Chondrus crispus*	43.6619	-65.3181	0.83	3.335
48	*Chondrus crispus*	43.733002	-65.3703	-2.1	3.204
D	*Chondrus crispus*	43.721472	-65.322139	-4.21	2.937
E	*Chondrus crispus*	43.723278	-65.321694	-3.47	2.728
F	*Chondrus crispus*	43.72225	-65.320944	-3.84	2.609
G	*Chondrus crispus*	43.732083	-65.329889	-3.04	3.268
H	*Chondrus crispus*	43.733667	-65.328861	-3.52	2.853
I	*Chondrus crispus*	43.734083	-65.329944	-2.49	3.11
K	*Chondrus crispus*	43.741972	-65.329	-3.92	3.044
L	*Chondrus crispus*	43.740611	-65.329278	-3.82	3.097

Table C.6: δ¹⁵N and N% in macroalgae tissue of *Ulva lactuca* from Shelburne Harbour, NS between November 8-22, 2019.

Station	Species	Latitude	Longitude	δ¹⁵N	% N
1	*Ulva lactuca*	43.671101	-65.293503	8.26	3.745
2	*Ulva lactuca*	43.727798	-65.337601	7.08	3.649
3	*Ulva lactuca*	43.7509	-65.319801	6.32	3.742
4	*Ulva lactuca*	43.7113	-65.338303	8.29	3.586
5	*Ulva lactuca*	43.7159	-65.320396	8.23	3.562
6	*Ulva lactuca*	43.7066	-65.332298	8.24	3.405
7	*Ulva lactuca*	43.670898	-65.318703	7.69	3.706
8	*Ulva lactuca*	43.724499	-65.3451	7.9	3.484
9	*Ulva lactuca*	43.720501	-65.327797	6.58	3.627
10	*Ulva lactuca*	43.706501	-65.344704	6.31	3.366
11	*Ulva lactuca*	43.679901	-65.318604	7.06	3.721
13	*Ulva lactuca*	43.7155	-65.332802	7.41	3.381
15	*Ulva lactuca*	43.697498	-65.331902	7.33	3.475
16	*Ulva lactuca*	43.721401	-65.337997	7.21	3.528
17	*Ulva lactuca*	43.744499	-65.327003	5.65	3.755
19	*Ulva lactuca*	43.742802	-65.321297	6.52	3.428
20	*Ulva lactuca*	43.688499	-65.331299	8.04	3.19
21	*Ulva lactuca*	43.730099	-65.320999	6.7	3.502
22	*Ulva lactuca*	43.7244	-65.332703	6.77	3.756
23	*Ulva lactuca*	43.727402	-65.324898	7.39	3.443
24	*Ulva lactuca*	43.664799	-65.305801	8.16	3.49
25	*Ulva lactuca*	43.728901	-65.330399	6.49	3.481
27	*Ulva lactuca*	43.736301	-65.328201	4.98	3.909

Station	Species	Latitude	Longitude	$\delta^{15}N$	% N
29	*Ulva lactuca*	43.662102	-65.293098	8.13	3.791
30	*Ulva lactuca*	43.664299	-65.283798	9.84	3.823
31	*Ulva lactuca*	43.6586	-65.289803	6.99	3.983
32	*Ulva lactuca*	43.6576	-65.2677	7.5	3.148
34	*Ulva lactuca*	43.658901	-65.280403	8.98	3.793
35	*Ulva lactuca*	43.6548	-65.274399	9.34	3.826
38	*Ulva lactuca*	43.653999	-65.255898	9.85	3.237
40	*Ulva lactuca*	43.645199	-65.2556	9.48	3.54
43	*Ulva lactuca*	43.7486	-65.318298	7.38	3.728
44	*Ulva lactuca*	43.679798	-65.306396	7.96	4.043
45	*Ulva lactuca*	43.7341	-65.334198	7.28	3.66
46	*Ulva lactuca*	43.7589	-65.328003	6.79	3.554
47	*Ulva lactuca*	43.6619	-65.3181	8.74	3.583
48	*Ulva lactuca*	43.733002	-65.3703	6.61	3.597
D	*Ulva lactuca*	43.721472	-65.322139	6.59	3.589
E	*Ulva lactuca*	43.723278	-65.321694	6.65	3.432
G	*Ulva lactuca*	43.732083	-65.329889	5.63	3.555
I	*Ulva lactuca*	43.734083	-65.329944	8.19	3.835