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## Evaluating and Advancing Opportunities for Ecosystem Services, Increased Production, and Sustainability of Kelp Aquaculture in the Gulf of Maine

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**EVALUATING AND ADVANCING OPPORTUNITIES FOR ECOSYSTEM SERVICES,  
INCREASED PRODUCTION, AND SUSTAINABILITY OF KELP AQUACULTURE IN  
THE GULF OF MAINE**

By

Gretchen Schott Grebe

B.A. Bates College, 2009

M.S. University of California Santa Barbara, 2013

A DISSERTATION

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Doctor of Philosophy

(in Aquaculture and Aquatic Resources)

The Graduate School

The University of Maine

May 2021

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By Gretchen Schott Grebe

Dissertation Advisors: Dr. Carrie J. Byron and Dr. Damian Brady

An Abstract of the Dissertation Presented  
in Partial Fulfillment of the Requirements for the  
Degree of Doctor of Philosophy  
(in Aquaculture and Aquatic Resources)  
May 2021

Commercial seaweed farming efforts are expanding worldwide. The use of new species, ecosystems, and practices present many opportunities for novel research and resource management. The purpose of this project was to evaluate and advance seaweed aquaculture practices in the Western Gulf of Maine. *Saccharina latissima* (sugar kelp) is the species of focus because it is currently the most farmed macroalga in the Northeastern United States. Fieldwork supporting the empirical studies was conducted January 2016 - May 2019 in Casco and Saco Bays, ME. Growth, yield, morphology, elemental and isotopic composition, and enzymatic activity of *S. latissima* were quantified across four sites. These observations were used to evaluate the nitrogen bioextraction efficiency of *S. latissima* farms in this region and the effect of distal-end trimming on the morphology and yield of *S. latissima*. Additionally, Maine kelp aquaculture was used as a case study to determine if the Ecosystem Approach to Aquaculture was suitable for seaweed farming and to explore the application of a multi-criteria screening model to guide the market-specific siting of seaweed aquaculture.

Findings from these studies depict a high variability in ambient environmental conditions between sites and in morphological and compositional variability between individual

sporophytes. On a hectare-to-hectare basis, the nitrogen bioextraction by kelp farming in the region far exceeded the nitrogen loading from riverine or atmospheric sources. Distal-end trimming had a significant effect on *S. latissima* stipe and blade morphology and increased late-season production yields. Furthermore, the Ecosystem Approach to Aquaculture was found to be applicable to the development of kelp aquaculture beyond Asia. Specific attention should be given to maintaining genetic diversity, developing best management practices, and integrating wild and farmed kelp management. Lastly, the screening analysis showed that the application of criteria specific to end-market uses of seaweed biomass results in uniquely optimal areas for cultivation. These findings are novel contributions to the fields of aquaculture research, coastal management, and phycology, and they provide a platform for continued research and development of seaweed aquaculture in the region.

## **DEDICATION**

For Tutu Web, who doggedly believed in the value of a graduate education and women in STEM, who sent me more issues of *Science* than I could ever read, and who always talked me into continuing.

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*“Find a group of people who challenge and inspire you, spend a lot of time with them, and it will change your life”. – Amy Poehler*

I am fortunate to have been challenged, inspired, and supported by many individuals during my doctoral work. Firstly, I would like to thank my advisors, Carrie Byron and Damian Brady, for diving into the world of seaweed aquaculture with me, providing unwavering support for broad approaches to academic and professional development, and aiding new connections across institutions, geography, and discipline. You have been incredible mentors. I am also especially grateful to the faculty and staff at the University of New England’s Marine Science Center for sharing their time, lab spaces, materials, and insight with me. Special thanks are due to Adam St. Gelais, Addie Waters, Tim Arenti, and Liz Johndrow for their field support and creative and practical approaches to winter fieldwork on the water in Maine. Thank you to all the undergraduate and graduate members of the Byron lab who provided countless hours of laboratory assistance and inspired me with their endless enthusiasm for marine biology. I would like to specifically acknowledge the contributions of Mary Hollandbeck, Kyle Brennan, Alex Geisser, Emma Jones, Everett Pierce, Olivia Barberi, and Connor Jones.

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extraordinarily steady and pragmatic example to follow, and Emma Tacardi, Charlotte Quigley, and Julek Chawarski for helping me to feel connected to the UMaine community despite being a remote student.

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## LIST OF ABBREVIATIONS

ANOVA – Analysis of variance

C – Carbon

$\delta^{13}\text{C}$  – Delta C-13, the ratio of stable isotopes  $^{13}\text{C}$ : $^{12}\text{C}$  in parts per thousand (‰)

$\delta^{15}\text{N}$  – Delta-N-15, the ratio of stable isotopes  $^{15}\text{N}$ : $^{14}\text{N}$  in parts per thousand (‰)

DW – Dry weight

EAA – Ecosystem Approach to Aquaculture

m – meter

MANOVA – Multivariate analysis of variance

MT – Metric tonne

N – Nitrogen

NERACOOS – Northeast regional association of coastal ocean observing systems

NO<sub>3</sub> – Nitrate

NRA – Nitrate reductase activity

P – Phosphorus

PAR – Photosynthetically active radiation

PY – Percent yield

SIA – Stable isotope analysis

WW – Wet weight

WWTF- Wastewater treatment facility

# CHAPTER 1

## INTRODUCTION

Approximately 29.4 million tonnes of farmed marine seaweeds, worth approximately \$6 billion per annum, were produced in 2018 (FAO 2018, 2020). This yield of cultivated macroalgae was more than double the global production of 13.5 million tonnes recorded in 2015 (FAO 2018), and growth projections for both domestic and international potential algal production and market expansion are favorable. This growth is predicted in part because seaweeds and their derivatives are used in so many of our current manufacturing processes for processed foods, animal feed, pharmaceuticals, biofuels, and agricultural enhancers like fertilizers (Graham *et al.* 2016; Wells *et al.* 2016). Additionally, numerous emerging applications like seaweed-based textiles and bioplastics are currently in development (van den Burg *et al.* 2020).

Seaweed farms are found in over fifty countries, but the practice of cultivating seaweed originates from the temperate coastlines of China, Japan, and Korea (FAO 2016). It has only been recently, within the last 20 years or so, that commercial cultivation of seaweeds has gained interest in Europe (Sweden, Norway, Iceland, Ireland, Scotland, Spain, and the Faroe Islands), North America (Canada and USA), and South America (Chile). Much of this recent attention to seaweed aquaculture has been supported by contributions from peer-reviewed and extension literature, which provided invaluable instructions regarding the husbandry and out-planting of the popular kelp species *Saccharina latissima* (Linnaeus) C.E.Lane, C.Mayes, Druehl & G.W.Saunders 2006. These contributions include but are not limited to: Bartsch *et al.* 2008; Forbord *et al.* 2012; Sanderson *et al.* 2012; Redmond *et al.* 2014; Kim *et al.* 2015, 2017, 2019; Freitas *et al.* 2016; Augyte *et al.* 2017; Bak *et al.* 2018; and Goecke 2020. This support has

rendered *S. latissima*, commonly referred to as sugar kelp, to currently be the most widely farmed seaweed in the United States. *Saccharina latissima* farms are currently found in Rhode Island, Connecticut, Massachusetts, New Hampshire, Alaska, Oregon, and Maine. *Saccharina latissima* has also gained popularity as a cultivated seaweed because it grows quickly, and it has a life cycle that can be easily manipulated in the laboratory.

The life cycle of *S. latissima* includes a heteromorphic alternation of generations between a gametophyte and sporophyte (Schreiber 1930) and cultivation of *S. latissima* is based around these phases. *Saccharina latissima* is a member of the class Phaeophyceae (Guiry & Guiry 2017) and was formerly taxonomically classified as *Laminaria saccharina* (Linnaeus) J.V.Lamouroux 1813. Phaeophytes are commonly referred to as brown algae, because they contain an accessory pigment called fucoxanthin that gives them a distinctive greenish-brown color (Graham *et al.* 2016). Brown algae are abundant along many temperate coasts (Young *et al.* 2007) and in the Gulf of Maine; many commonly observed genera like *Fucus*, *Laminaria*, and *Alaria* belong to the Phaeophyceae (Graham *et al.* 2016).

The studies comprising this dissertation have been designed with a focus on *S. latissima* with the hope that the research findings can be directly applicable to the Maine, U.S., and European aquaculture industries. Nonetheless, many of the topics and research needs presented here could be extended to other species and genera in the family *Laminariaceae*. Species in the *Laminariaceae* family grow from the intercalary meristem, or the basal end of the blade between the stipe and the blade (Parke 1948). They typically exhibit a distinct seasonal cycle of growth in late winter/early spring and degeneration during summer (Egan and Yarish 1990; Henley & Dunton 1995; Nielsen *et al.* 2014; Peteiro 2006). For more details and discussion about the seasonal growth and composition of *S. latissima*, I refer the reader to Chapters 3 and Chapter 4.

Despite the diverse existing and emerging applications of seaweed biomass, almost all seaweed cultivated in the U.S. and Europe is currently sold as raw material for value-added food products (*i.e.*, kelp noodles, kelp puree, kelp spice mix). Thus, the discussions in Chapter 2 and Chapter 4 include heavy consideration of the dynamics and needs of food markets. However, I note here that daily consumption of *S. latissima* has been found to result in excessive iodine intakes (Stevant *et al.* 2018), and these high levels of iodine are also observed in food products using *S. latissima* as a raw ingredient (Dawczynski *et al.* 2007b; Desideri *et al.* 2016). Stevant *et al.* (2018) have shown that soaking seaweed biomass in warm fresh water (32 °C) can reduce the iodine in *S. latissima*, but this treatment also reduces the nutrient content of the biomass. This issue of high iodine content must be addressed if *S. latissima* tissue continues to be the macroalga of choice for human food applications. Alternatively, *S. latissima* can be used as raw feedstock for a plethora of other purposes, some of which may require a large shift in cultivation strategies towards larger scale, automated cultivation arrays. I refer the reader to Chapter 5 for more discussion of the divergence in cultivation systems as a factor of target market for the raw seaweed biomass.

### **Scope and motivation**

In this dissertation I take an in-depth look at the Western world's recent interest in kelp aquaculture and generate new knowledge about the opportunities and limitations of *S. latissima* aquaculture to provide ecosystem services and a source of organic biomass. Motivation for my work originated in large part from conversations with aquaculture industry members and researchers engaged in the Sustainable Ecological Aquaculture Network (SEANET). This project brought together aquatic farmers and researchers throughout the state of Maine under the common goal of advancing economically viable, and socially and ecologically responsible,

aquaculture. SEANET's overall research approach was founded on principles of socio-ecological systems, and thus, too, were many of the supporting research projects, including this dissertation. In Chapter 1, I explore the ecological, social, and management implications of increasing kelp aquaculture along the Maine Coast and applies guidance from the Ecosystem Approach to Aquaculture (FAO 2010) to generate recommendations for research, business, and management priorities. In Chapter 2, I evaluate the nitrogen bioextraction potential of *S. latissima* aquaculture in the Western Gulf of Maine and provides context for the magnitude of nitrogen removed compared to nitrogen inputs within the larger ecosystem. In Chapter 3, I assess the potential of a crop treatment, distal-end trimming, to alter *S. latissima* morphology and increase harvest yields and nutrient assimilation from kelp farms. Lastly, in Chapter 4, I explore the possibilities for aquaculture site screening that incorporates end-market considerations and supports the further development of ocean area prioritized for aquaculture activities.

## CHAPTER 2

### AN ECOSYSTEM APPROACH TO KELP AQUACULTURE IN THE AMERICAS AND EUROPE

This chapter is an updated version of the following publication:

Grebe, G.S., Byron, C.J., Gelais, A.S., Kotowicz, D.M., Olson, T.K. (2019). An ecosystem approach to kelp aquaculture in the Americas and Europe. *Aquaculture Reports*, 15:100215. <https://doi.org/10.1016/j.aqrep.2019.100215>

#### 2.1 Chapter abstract

Kelp farming is increasing along the temperate coastlines of the Americas and Europe. The economic, ecological, and social frameworks surrounding kelp farming in these new areas are in contrast with the conditions of progenitor kelp farming regions in China, Japan, and Korea. Thus, identifying and addressing the environmental and social impacts of kelp farming in these regions is vital to ensuring the industry's long-term sustainability. Here, a conceptual model of the human and natural systems supporting this nascent kelp aquaculture sector was developed using Maine, USA as a focal region. Potential negative impacts of kelp aquaculture were identified to be habitat degradation, overfishing of wild "seeds" (*i.e.*, parent material), predation and competition with wild fish and genes, and transmission of diseases. Increased food security, improved restoration efforts, greater fisheries productivity, and alternative livelihoods development were determined to be potential positive impacts of kelp aquaculture. The interconnectedness of kelp aquaculture activities means that biodiversity and productivity resulting from either negative or positive impacts of kelp aquaculture could have downstream effects on local fisheries and coastal communities.

Our recommendations to improve or protect the ecosystem services tangential to kelp farming include: define ecosystem and management boundaries, assess ecosystem services and environmental carrying capacity, pursue ecologically and socially considerate engineering, and protect the health and genetic diversity of wild kelp beds. Our recommendations to ensure that kelp farming improves the well-being of all stakeholders include: increase horizontal expansion, expand and teach Best Management Practices, and develop resiliency against climate change. Additionally, we recommend that an integrated management strategy should be developed for wild and farmed kelp to ensure that kelp aquaculture is developed in the context of other sectors and goals.

## **2.2 Introduction**

Marine seaweed farming is a rapidly expanding practice. In 2016, the global production of farmed seaweed reached an estimated 30 million tonnes (FAO 2018). Approximately 27% of this production was kelp; a group of ca. 30 genera of large brown seaweeds in the order Laminariales (Guiry & Guiry 2017). The temperate coastlines of China, Japan, and Korea have historically been the epicenters of kelp farming (FAO 2016). Recently, the practice has expanded to regions in Europe (Sweden, Norway, Iceland, Ireland, Scotland, Spain, and the Faroe Islands), North America (Canada and USA), and South America (Chile). In the USA, kelp have been farmed in Maine, New Hampshire, Massachusetts, Connecticut, Rhode Island, Washington, and Alaska. Total production in the Americas and Europe in 2014 was approximately 54,000 tonnes valued at US \$51 million (FAO 2016).

American and European production of cultivated kelp was equivalent to 1.5% of global gross production in 2014 (FAO 2016). However, it accounted for 4% of the value (FAO 2016), because European and American economic, ecological, and social frameworks surrounding kelp

farming contrast with the conditions of progenitor kelp farming regions in Asia. Much of the kelp from Asia is grown and traded at commodity scales (FAO 2017), although there are exceptions to this (e.g., Japanese wakame industry). Regardless, kelp consumption in Asia has been mostly contingent on price and taste (Chapman *et al.* 2015). In contrast, kelp of European and American origin is considered a specialty product. It is typically selected for its nutritional value and ecological and ethical farming practices (Chapman *et al.* 2015). Consequently, kelp produced in the Americas and Europe for food sells for an average of US\$ 944 tonne<sup>-1</sup> wet weight (WW) (FAO 2016), whereas in Korea kelp sold for hydrocolloids sells for ca. US\$ 177 tonne<sup>-1</sup> (FAO 2017). As such, the sustainability of American and European kelp farming is crucial to its viability. Established aquaculture industries (e.g., tilapia, carp, and shrimp) have undergone similar evaluations which resulted in Best Management Practices (BMPs) and guidelines to increase the industry sustainability (Lebel *et al.* 2002; Azad *et al.* 2009; Fletcher 2012; Mungkung *et al.* 2013).

The Ecosystem Approach to Aquaculture (EAA) is a framework for evaluating aquaculture practices (FAO 2010). It was developed by aquaculture experts at the FAO using observations of well-established industries farming aquatic animals. Three strategic principles define the EAA guidance (FAO 2010):

- 1) “Aquaculture development and management should take account of the full range of ecosystem functions and services, and should not threaten the sustained delivery of these to society.
- 2) Aquaculture should improve human well-being and equity for all relevant stakeholders.
- 3) Aquaculture should be developed in the context of other sectors, policies, and goals.”

In addition to functioning as a stand-alone strategy, the EAA principles contributed to many of the Sustainable Development Goals and Targets set by the United Nations in 2015 (Hambrey 2017) and have helped to steer the aquaculture sector to more sustainable and holistic practices (Brugère *et al.* 2018). However, acceptance of the approach has been nonuniform across user groups (Brugère *et al.* 2018). Thus, the present study sought to explore the appropriateness and the value of the EAA for the incipient kelp aquaculture subsector outside Asia. The FAO literature on the EAA was assessed for its relevance to small-scale kelp aquaculture. Then, the EAA strategy and principles were used to recommend practices that can be adopted to promote the long-term sustainability of the kelp industry.

## **2.3 Methods**

### **2.3.1 Site description**

Maine, USA, was used as a case study to explore the pertinence of the EAA to the new kelp aquaculture industry. Aquaculture has generally been supported by Maine's economy and culture historically centered around fishing, shipbuilding, forestry, agriculture, extractive industries, manufacturing, and tourism (MSOP 2003). The region's protected coastline and water temperature ranging from 0.5 to 17.5 °C (NOAA 2018a, 2018b) are particularly well-suited for kelp aquaculture. In 2010, the first kelp farm in the United States was started in Casco Bay, Maine. The farmers used techniques originating from Europe and Asia, which were adapted and further developed with Dr. Charles Yarish and Dr. Jang Kim at the University of Connecticut (Flavin *et al.* 2013). In the decade since, many small kelp farms have been established along Maine's 5,500 km of rocky coastline. State-wide harvest data depict a 3-fold increase in the production of farmed marine seaweeds from 2015 to 2018 (Maine DMR 2018). In 2018, sixteen

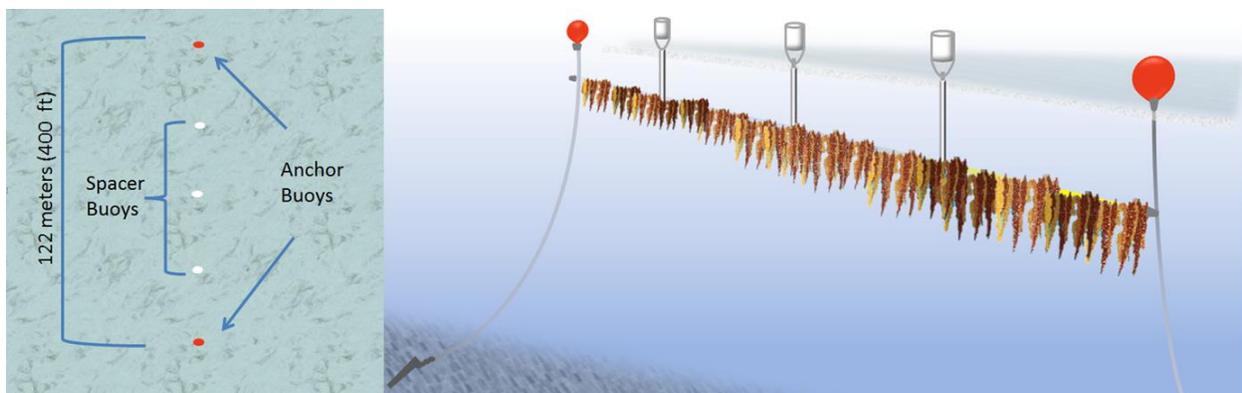
entities collectively reported harvest of 24.2 tonnes (WW) of farmed marine algae from Maine, the majority of which was *Saccharina spp.* (Maine DMR 2018).

The sugar kelp *Saccharina latissima* and the winged kelp *Alaria esculenta* are the most extensively farmed kelp species in Maine and in the United States (Kim *et al.* 2015, 2017; Rose *et al.* 2015; Augyte *et al.* 2017). Both species are members of the Phaeophyceae, commonly referred to as brown algae. *Saccharina latissima* and *Alaria esculenta* are abundant throughout much of the Arctic and along temperate coasts between the 16°C summer isotherm and the 19 - 20°C isotherms, respectively (Breeman 1988; Lüning 1990). *Saccharina latissima* and *Alaria esculenta* exhibit rapid growth from early winter to late spring, reaching 2 to 5 meters (m) within approximately six months (Handå *et al.* 2013; Redmond *et al.* 2014; Azevedo *et al.* 2016).

Cultivation of *S. latissima* and *A. esculenta* is based around the species' life cycle, which includes a heteromorphic alternation of generations between a microscopic gametophyte and a “frond-like” sporophyte (Schreiber 1930). The latter is targeted for grow-out. There are multiple ways of seeding sporophytes for grow-out, but they all begin at least once by collecting sorus, or reproductive tissue, from mature, diploid sporophytes. Spore release from the sorus is achieved using desiccation and warming (Flavin *et al.* 2013). The released zoospores then mature as microscopic, filamentous gametophytes (Graham *et al.* 2016). In more advanced nurseries, these gametophytes are sorted by sex, and then either held indefinitely, crossed to produce specific strains, or cloned before being blended to produce juvenile sporophytes (Flavin *et al.* 2013; Redmond *et al.* 2014). Otherwise, the gametophytes can be applied to a thin seed line with spray-seeding or settling techniques (Flavin *et al.* 2013; Redmond *et al.* 2014). Following application to a substrate, the gametophytes become fertile. Mature eggs release a pheromone that causes the antheridium to break apart and directs sperm to an egg for fertilization (Graham *et al.* 2016).

Then zygotes grow in place of the female gametophyte to form juvenile sporophytes (Flavin *et al.* 2013; Graham *et al.* 2016). The young sporophytes are raised on land in aquaria with artificial nutrients and light until the sporophytes are 2 to 10 mm in length (Flavin *et al.* 2013; Redmond *et al.* 2014).

In the grow-out phase, the juvenile sporophytes are transferred from the aquaria to longlines in the ocean. There they will continue to grow using natural light and available nutrients in the water column. The most common deployments in Maine consist of 1 to 1.25 cm sinking rope, called a *longline*, anchored with moorings and chain (Fig. 2.1). Longlines are typically 122 m, but some variation occurs. Intermediate floats and spacers with counter-weights are used to maintain the longline 2 to 2.5 m below the water surface. Suspending the longline at this depth ensures that the kelp receives adequate, but not excessive, light to grow while also protecting it from wave action and boat travel.



**Figure 2.1** Longline kelp aquaculture as commonly practiced in Maine: 122 m longline as seen from above (A) and the side (B).

In the Northwest Atlantic, the grow-out cycle for *S. latissima* and *A. esculenta* spans roughly late September through early May, although this varies somewhat according to location along the coast (Bricknell *et al.* 2020). Sometimes, the late availability of reproductive tissue

from wild kelp beds has delayed the seeding of individual farms into October or November.

Laboratory techniques to produce and maintain gametophyte cultures could prevent delayed farm deployment, but this is not yet commercial practice in Maine. In this region, most kelp farms are harvested once in late April or May to maximize total farm biomass and minimize fouling (*e.g.*, snails, tunicates, hydroids, bryozoans, and amphipods). Harvesting practices vary according to the end-use of the kelp.

All kelp aquaculture sites in the State must be approved by the Maine Department of Marine Resources (DMR). Under guidelines set by the Maine Legislative Branch, the DMR has the authority to issue three types of aquaculture agreements: Limited Purpose Aquaculture Licenses (LPAs), Experimental Leases, and Standard Leases (Maine Legislature 2017). The Maine DMR (2019a) provides the following guidance regarding each agreement:

- LPAs are typically 122 m<sup>2</sup>. They are the easiest to acquire and can be issued by permit. LPAs are licenses, not leases, which are valid for one year. They can be renewed but are not transferrable. An individual can apply for a maximum of 4 LPAs per year but can supervise up to 12. As with any license, the State reserves the right to revoke issuance or decline renewal of the license should the holder fail to comply with all requirements.
- Experimental leases can encompass up to 1.6 hectares. They are valid for three years and the lease cannot be renewed unless they are used for scientific research. A site visit by the Maine DMR's environmental scientists is necessary to approve the lease. An adjudicated hearing is required if the DMR receives 3 or more letters from interveners.
- Standard leases can be up to 40 hectares. The application process is stringent and includes an adjudicated hearing. Standard leases are valid for 20 years, renewable, and transferable if they are active and in compliance with all existing regulations. Applicants

for standard leases must attend a pre-application meeting and share a draft application with the DMR. A public scoping session must also be held with the host municipality before submitting a final lease application for review. Applicants for a standard lease are required to obtain a permit from the U.S. Army Corps of Engineers (USACE). They must also alert the United States Coast Guard to ensure that the site is included in the agency's navigational updates.

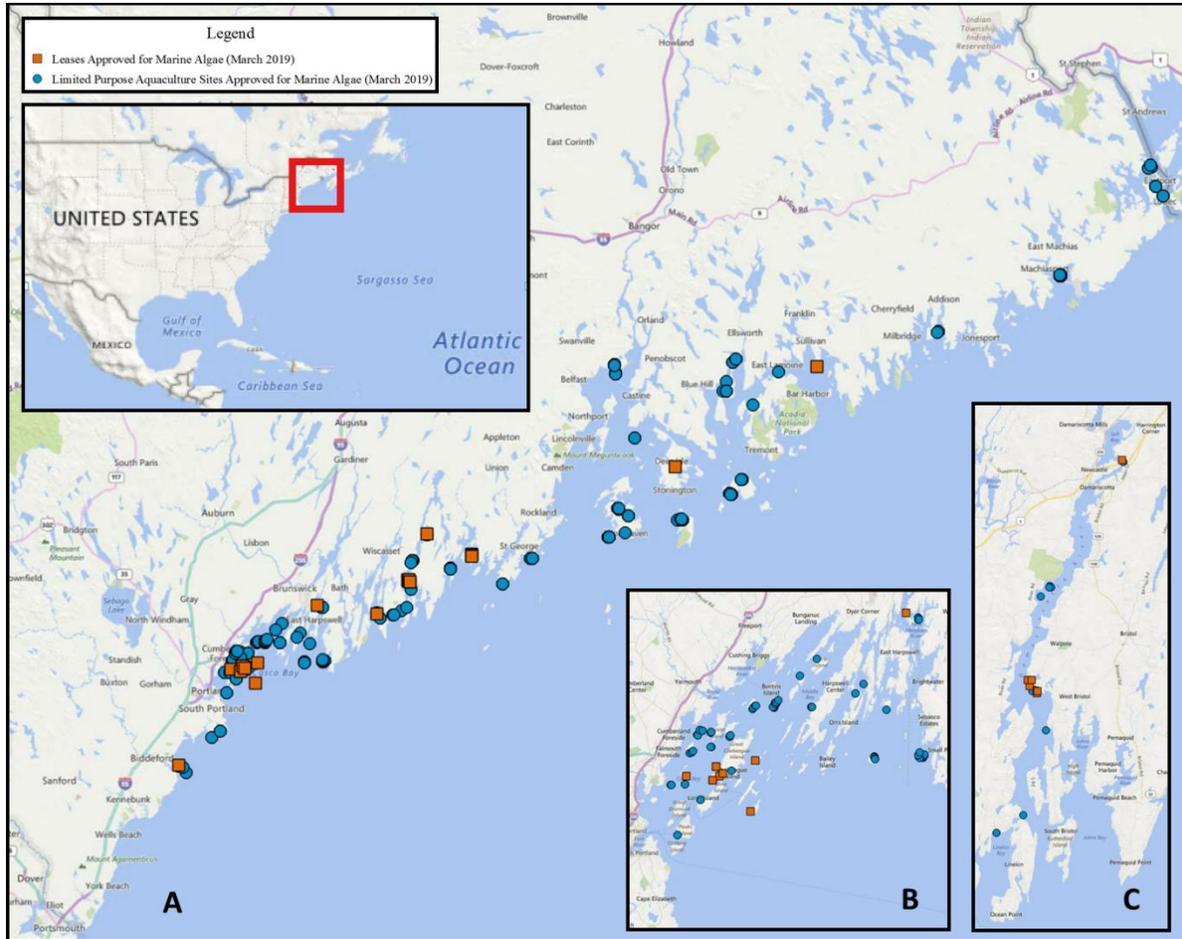
The DMR review criteria for both aquaculture LPAs and leases include consideration of existing fisheries and licensed sites, navigation, essential wildlife habitat, recreational use, riparian landowners, and ecologically sensitive flora and fauna (Maine DMR 2019a). Thus, success in the lease application process requires working knowledge of the social and ecological systems connected to the proposed site. Careful site selection and evaluation are critical to ensuring a smooth application process.

Maine's tiered system for aquaculture agreements has facilitated the expansion of seaweed aquaculture in the region. In the spring of 2019, there were 189 LPAs and 23 standard or experimental leases approved for marine seaweeds within Maine state waters (Maine DMR 2019b, 2019c). However, many of the LPAs may be purely speculative at this time. LPAs and leases approved for marine seaweed cultivation are widely distributed along the State's coast and in two areas of higher concentration: Casco Bay and the Damariscotta River (Fig. 2.2).

### **2.3.2 Analytical approach**

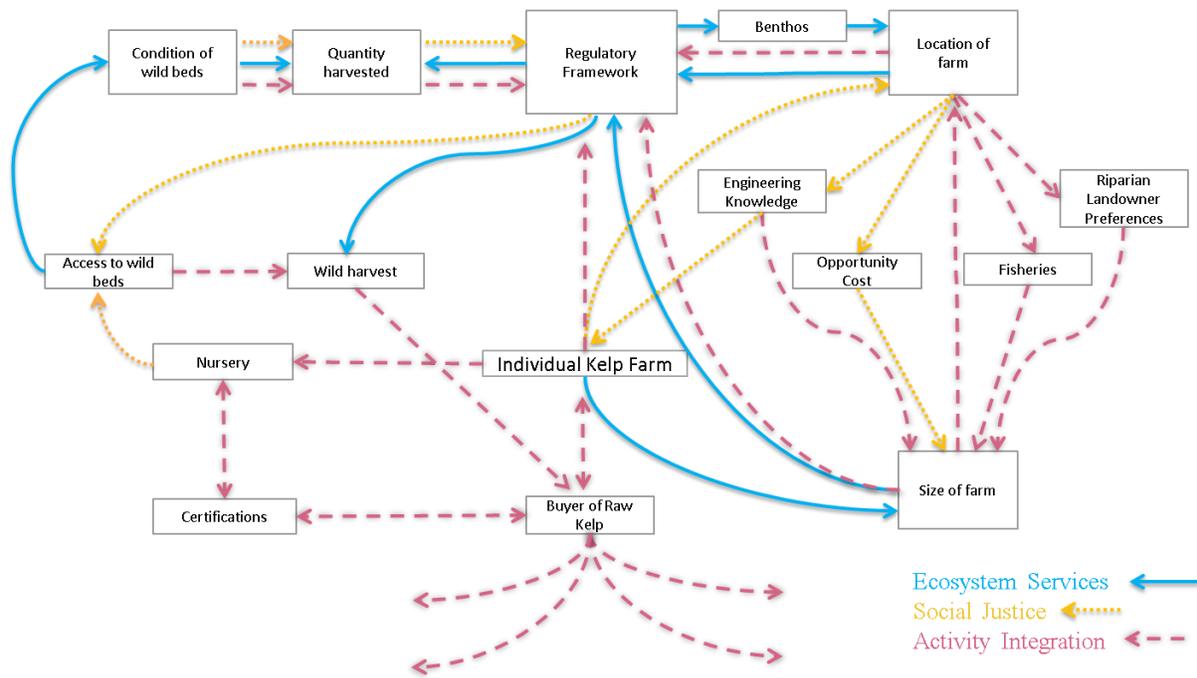
Kelp aquaculture is a practice that leverages biology and ecology within a social, economic, and political context. Thus, identifying the human and natural components of the broader kelp aquaculture system is required to evaluate its sustainability (Liu *et al.* 2007; Whitney *et al.* 2017). Organizational, temporal, and spatial interactions occurring between the

components are equally important (Liu *et al.* 2007b; Pulver *et al.* 2018). Industry observation, along with data collected through four focus groups and 24 semi-structured interviews with industry participants, regulators and extension staff, provided the data used to determine the physical and social components of kelp aquaculture.



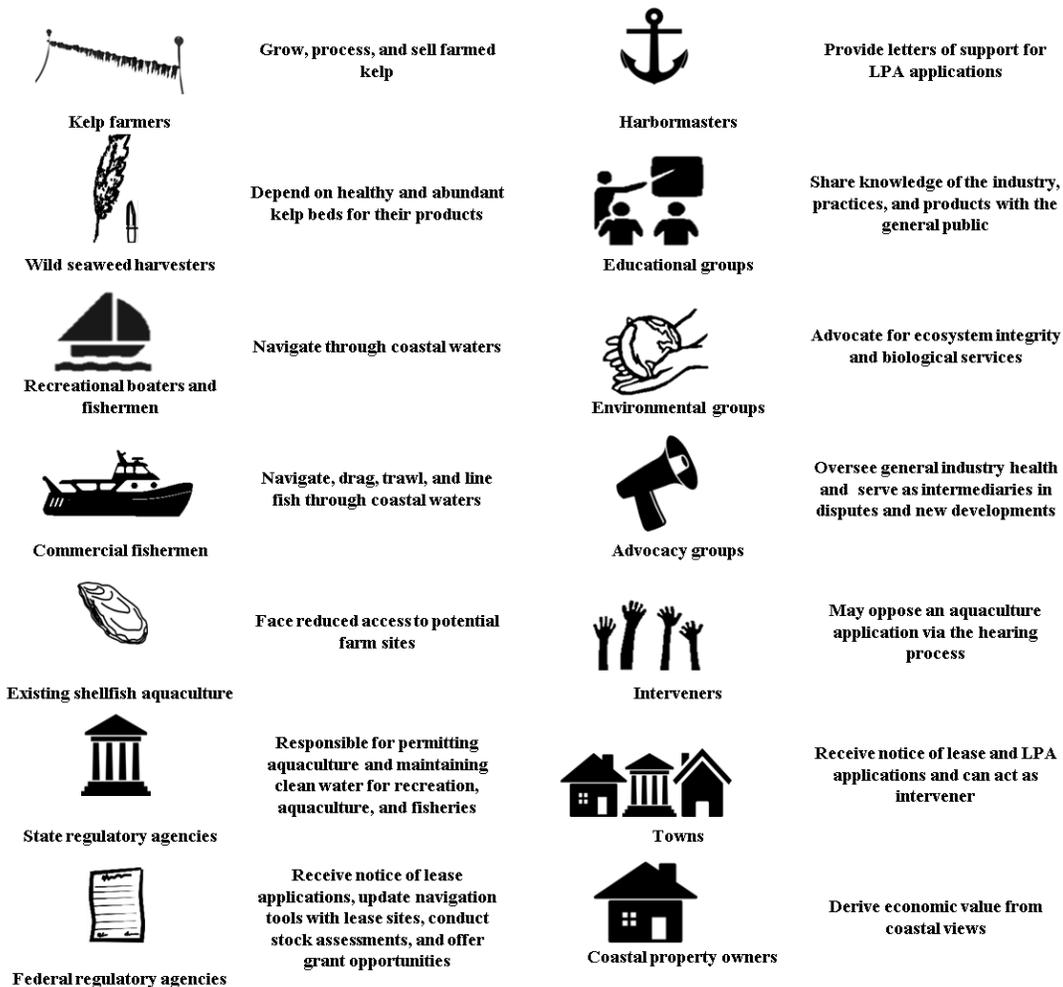
**Figure 2.2** Aquaculture Lease Sites (orange squares) and Limited Purpose Aquaculture Sites (blue circles) approved to grow marine seaweeds along the coastline of Maine (A), in Casco Bay (B) and on the Damariscotta River (C). Data source: Maine Department of Marine Resources (2019b, 2019c).

The scope of this study was limited to activities and relationships directly tied to the farming of raw kelp. Buyers of raw kelp, primary and secondary kelp processing facilities, buyers and retailers of kelp products, consumers of kelp products, and vertically-integrated business models rest outside the scope of this evaluation. We inserted the human and ecological relationships connecting each physical or social component to generate a conceptual model of kelp aquaculture in Maine (Fig. 2.3). These causal relationships were classified according to the EAA principle that best defines the relationship. The principles have been abbreviated as 1) *Ecosystem Services*, 2) *Social Justice*, and 3) *Activity Integration*.

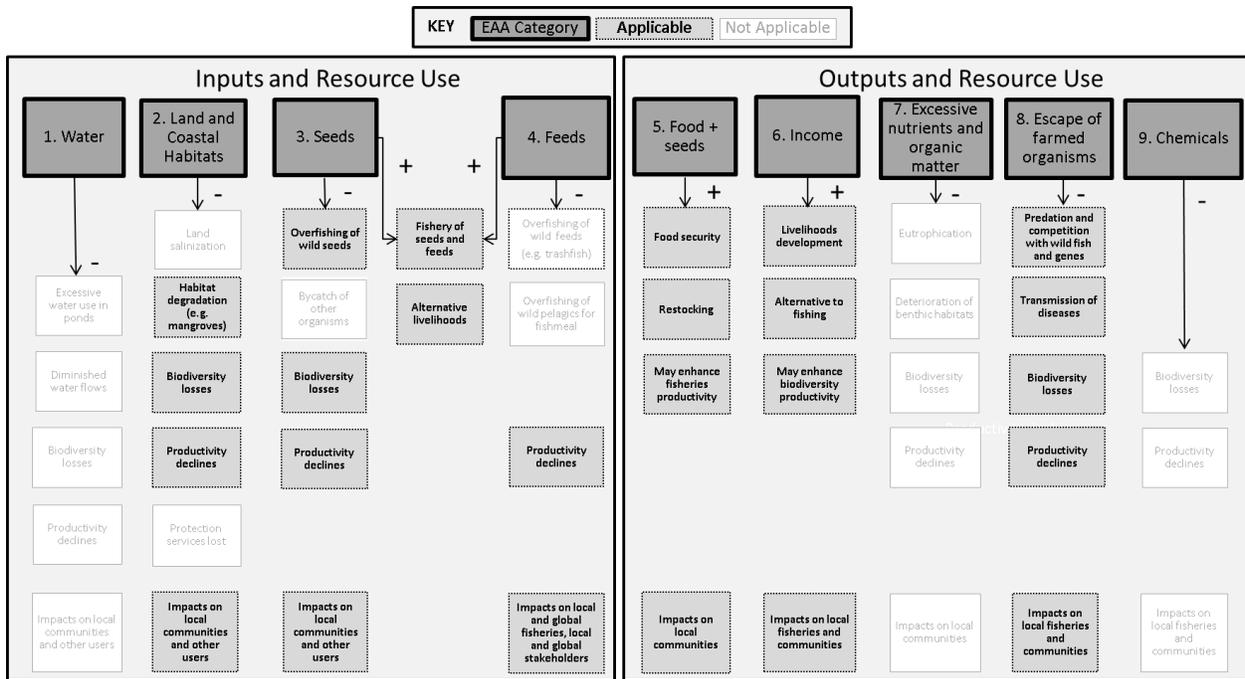


**Figure 2.3** The human and environmental relationships supporting kelp aquaculture. Relevance to Ecological Approach to Aquaculture guiding principles (FAO 2010) is indicated as 1) Ecosystem Services (green lines), 2) Social Justice (blue lines), and 3) Activity Integration (yellow lines). Directional arrows depict a chain of events or decisions associated with each factor.

Then, the conceptual model representing the kelp aquaculture system was used to identify and describe stakeholders in the production of farmed kelp (Fig. 2.4). Together the list of stakeholders and conceptual model were used to evaluate the relevance of the FAO’s identified common issues and impacts of aquaculture for kelp farming (Fig. 2.5). If an FAO-listed issue or impact was determined applicable to kelp aquaculture in Maine, we used the associated EAA guiding principle in combination with peer-reviewed literature and information from the industry observation, focus groups, and interviews to propose actions addressing the potential concern.



**Figure 2.4** Stakeholders in the kelp aquaculture industry. All post-harvest activities were excluded.



**Figure 2.5** Potential issues and impacts related to aquaculture inputs (left) and outputs (right) identified by FAO (2010). The potential issues and impacts are organized by category (dark grey boxes). Light grey boxes denote issues and impacts that are applicable to kelp aquaculture. White boxes signify issues and impacts that do not apply to kelp aquaculture. Plus signs indicate positive impacts and minus signs represent negative impacts.

The FAO technical report on the Ecosystem Approach to Aquaculture (2010) lists the most common ecological and social impacts associated with aquaculture systems (Fig. 2.5). Both positive and negative impacts are considered, and the impacts are sorted according to whether they are inputs or outputs in the aquaculture system. The FAO’s list was developed primarily considering fed aquaculture (e.g., fed finfish and shrimp culture) and not seaweed aquaculture. Thus, there is a need for careful assessment of the appropriateness and applicability of these

stated impacts for kelp aquaculture. The conceptual map and stakeholder list were both used in this evaluation. If an FAO potential impact was identified as not applicable to kelp aquaculture in Maine (white boxes in Fig. 2.5), then a justification for this decision is provided in the subsections of this article. Conversely, if an FAO potential impact is relevant to kelp aquaculture in the Americas and Europe (light grey boxes in Fig. 2.5), the nature of the concern is described in the appropriate subsection. A precautionary approach is especially warranted when evaluating an emerging industry. The EAA guidance also emphasizes precautionary measures (FAO 2010). As such, each potential impact is considered at a coastline-scale (*i.e.*, multiple kelp farms) and with the expectation that the industry will continue to grow rapidly. The common issues and impacts are presented and discussed in order of appearance (left to right).

## **2.4 Results**

### **2.4.1 Potential input impacts of kelp aquaculture**

Possible input impacts are grouped under the FAO's previously defined categories of water, land and coastal habitats, seeds (*i.e.*, sporelings), and feeds (Fig. 2.5).

#### **Water**

The production of farmed kelp has little consumptive freshwater use. In the nursery phase, minimal freshwater is used to rinse tanks during water changes. Inland nurseries using artificial seawater require additional freshwater as the solvent in the seawater preparation. This water need is equivalent to the size of the aquarium, typically 100 to 500 L, but it can be sterilized and recirculated. Inland nurseries using pumped and filtered seawater have similar rates of saltwater consumption. During grow-out, all water use is, by definition, non-consumptive.



## **Land and coastal habitats**

The FAO concerns regarding negative impacts to land and coastal habitats vary in their applicability to kelp aquaculture. Land salinization, the first concern listed, is associated with inland aquaculture of marine and estuarine organisms and does not apply to marine kelp aquaculture. The potential for physical habitat degradation and associated biodiversity losses, productivity declines, and protection services lost are relevant to kelp aquaculture. These potential impacts are associated with the possibility of marine mammal entanglement in the longlines, the mooring system, and seafloor shading at shallow farm sites. The FAO does not list potential positive impacts to habitat resulting from aquaculture. However, preliminary work suggests that some seaweed farms can have higher marine species richness and abundance than wild kelp beds or nearby areas without aquaculture.

The possibility of marine mammal entanglement in kelp longlines is an emerging concern among stakeholders in kelp aquaculture. For instance, habitat for the endangered North Atlantic right whale *Eubalaena glacialis*, extends along the Maine coastline (Kraus *et al.* 2005; NOAA 2016). Entanglement in non-mobile fishing gear has historically been one of the primary causes of individual mortalities (Kraus *et al.* 2005; NOAA 2016). No case of entanglement in kelp longlines has been reported, but the concern for possible marine mammal entanglement will be amplified as a growing number of kelp farms are deployed. Risks of right whale entanglement are also expected to increase as kelp farms expand in size or move further offshore.

Localized impacts to the benthos could potentially result from moorings used to secure the longline or bottom-shading by kelp grown in shallow waters. The permitting process in the State of Maine, by way of USACE, requires eel-grass delineation and consideration of the potential loss of any benthic vegetation (USACE 2015; Maine DMR 2019). As a result, most

farms are sited above sand or mud substrate where marine life is less abundant or diverse. Mooring chain scour can cause a small loss of physical habitat, but the tension through the longline system keeps the mooring chain and line from rotating. The impact on the benthos is less than the disturbance caused by a small boat mooring. Another concern is that shading from large-scale seaweed farms could affect primary production or other ecosystem dynamics (Stévant *et al.* 2017). Seafloor shading has been associated with decreased heterogeneity in subtidal communities in estuaries and the nearshore environment (Glasby 1999; Miller & Etter 2008), where kelp aquaculture is predominately sited. Impacts of shading are likely negligible for kelp farms installed at sites where the seafloor is deeper than the euphotic zone. Similar to concerns with marine mammal entanglement, the potential impacts to the benthic habitat are primarily related to the size of an individual farm and the density of farms along the coast.

A few studies have investigated the positive habitat contributions from seaweed farming. A study on the coast of Ireland found different species assemblages and higher species richness in the holdfasts of suspended kelp farms when compared to wild kelp beds (Walls *et al.* 2016). On the Pacific and Caribbean coasts of Costa Rica, the waters around cultivated *Codium* sp., *Gracilaria* sp., *Sargassum* sp., and *Ulva* sp. plots had a significantly higher number of fish species and individuals than areas without aquaculture (Radulovich *et al.* 2015). These initial studies are promising, yet more research is needed to fully understand the extent to which seaweed installations can serve as robust marine habitat. For example, little is known about how harvesting at the end of the season, effectively complete removal of the cultivated kelp canopy, influences the fauna shown to congregate around the farms (Wood *et al.* 2017). More studies are also needed to understand how much variation occurs between regions and seaweed species.

### **“Seeds” (i.e., sporeling)**

Efforts towards gametophytes cultures or laboratory-based sorus management and induction are underway, but the Maine kelp aquaculture industry is presently reliant on wild kelp beds as the source of reproductive tissue for sporelings (Kim *et al.* 2017). Consequently, concerns related to seed (sporelings) production for kelp aquaculture include potential over-harvesting of wild sorus tissue and the spread of parasites or non-indigenous species. These ecological concerns are further accentuated by the lack of studies examining existing or prospective biodiversity losses, productivity declines, and protection services lost or gained as the result of kelp farming.

Wild kelp is a perennial primary producer and foundation species providing habitat and food that affects community composition in the rocky subtidal zone (Lüning 1990; Steneck *et al.* 2002; Christie *et al.* 2009). Epiphytic algae, gastropods, amphipods, sea urchins, sea stars, and fish inhabit kelp beds (Steneck *et al.* 2002). These, in turn, become food for large crabs, lobsters, carnivorous fish, and other predators (Steneck *et al.* 2002) which are often consumed by humans. Therefore, seemingly small changes to the structure or genetic makeup of the wild population could cause reverberations throughout the ecosystem. Decreased abundance in another subtidal foundation species (*Mytilus edulis*) has led to community composition shifts in the Gulf of Maine (Sorte *et al.* 2017). These impacts could ultimately affect the marine food web structure and the coastal ecosystem’s ability to provide supporting services for marine organisms and humans. A change in wild kelp populations would also directly affect wild kelp harvesters. Indirect impacts could reach wild coastal fisheries which provide an essential source of protein for human consumption and a source of income for marine fishermen.

Currently, harvesters access and trim reproductive kelp from natural beds at low tide. Bycatch is not a concern because they can selectively trim their target species. Some harvesters remove only half of the blade and leave the rest to grow back. The ecological risk associated with wild sorus harvesting lies in the potential for over-harvesting quantities of sorus tissue that might impact the natural life cycle of the organism or the longevity of the kelp community. For example, historically targeting exclusively reproductive individuals has had drastic consequences for continued success in reproduction and recruitment of other marine species (Sala *et al.* 2001; Sadovy & Domeier 2005; Johnson *et al.* 2012). However, we emphasize that the amount of sorus tissue currently collected for kelp farming is minimal compared to kelp biomass removed by wild harvesting and winter storms.

The EAA recommends applying the precautionary approach when ecosystem resilience or thresholds are unknown (FAO 2010). Sorus tissue harvesting in Maine falls into this category. As more individuals enter the industry, it is plausible that sorus tissue could be harvested at a rate impacting natural replenishment or juvenile sporophyte recruitment. If replenishment or recruitment is substantially reduced, it will negatively affect the biodiversity and productivity of wild kelp beds. The marine organisms that inhabit the kelp beds and people that rely on them will also be impacted.

Seaweed aquaculture also has the potential to spread parasites or introduce non-indigenous species to new regions (Skjermo *et al.* 2014; Cottier-Cook *et al.* 2016). The current recommended methodology for producing kelp sporelings in Maine encourages cleaning of sorus tissue with a razor blade, Betadine-R solution at 5 mL/L, and a series of rinses with sterilized seawater (Flavin *et al.* 2013; Redmond *et al.* 2014). This methodology is designed to remove epiphytic algae and attached organisms like ciliates and bryozoans (Flavin *et al.* 2013; Redmond

*et al.* 2014). No standardized sanitary guidance is provided for kelp gametophyte or sporeling production in Maine.

## **Feeds**

No added “feeds” are used in kelp farming. Kelps are autotrophic and able to use energy from the sun, carbon dioxide, oxygen, and nutrients to grow. Most of the FAO concerns with aquaculture feeds are not applicable to kelp farming, except for the potential impact on marine ecosystem productivity. This impact could be either positive or negative.

It has been proposed that kelp farms installed in nutrient-poor areas may have a negative impact on marine ecosystem productivity (Wood *et al.* 2017). The farmed kelp can compete with other marine algae and plants for dissolved nutrients and minerals (Wood *et al.* 2017). No detrimental effects on marine water conditions have been reported around small and dispersed farms currently established in Maine. Nevertheless, this potential impact should be considered as kelp farming intensity increases along the coastline. For instance, severe nutrient limitation has been documented in areas with intensive seaweed cultivation, such as Korea and Japan (Park *et al.* 2018; Shim *et al.* 2014; Zhang *et al.* 2004).

Contrariwise, kelp farming activities may positively influence marine ecosystem productivity when used as a *bioextraction*, or *bioremediation* strategy. This approach exploits the metabolic needs of kelp to intentionally remove excess nutrients or carbon dioxide in nearshore waters experiencing nutrient pollution, ocean acidification, and carbonate limitation (Chung *et al.* 2011; Duarte *et al.* 2017; Krause-Jensen & Duarte 2016; Rose *et al.* 2014, 2015). Studies regarding the nitrogen bioremediation potential and the degree of photosynthetically-driven carbon dioxide assimilation of kelp aquaculture in Maine are in progress. Meanwhile, findings from other species and regions help to characterize the potential benefits. Studies from nearby

Connecticut, USA, show that *S. latissima* farms can address eutrophication by removing 38 to 180 kg of nitrogen hectare<sup>-1</sup> at the time of harvest (Kim *et al.* 2015). In China, harmful algal blooms along the coast have been effectively mitigated by large-scale cultivation of the red algae *G. lemaneiformis* and *P. yezoensis* (Wu *et al.* 2015, 2017; Yang *et al.* 2015a, 2015b). At a farm-level scale, the localized alkalization offered by seaweed is thought to be beneficial for both corals and shellfish using calcification to make shells (Branch *et al.* 2013; Bricknell *et al.* 2020; Pfister *et al.* 2019). With regards to carbon sequestration, it has been estimated that the world's seaweeds could potentially sequester a 61 – 268 Tg C yr<sup>-1</sup> through export to the deep sea or burial in coastal sediments (Krause-Jensen & Duarte 2016). The high end of this range is more carbon burial than salt marshes, mangroves, and seagrasses combined (Duarte *et al.* 2013).

#### **2.4.2 Potential output impacts of kelp aquaculture**

Output impacts are grouped by the FAO's previously defined categories of food and “seeds” (sporelings), income, excessive nutrients and organic matter, escape of farmed organisms, and chemicals (Fig. 2.5).

##### **Food and “seeds” (sporelings)**

Kelp aquaculture can have a positive impact on food security. The practice offers direct benefits to food security when kelp is used as food for humans. It indirectly benefits food security when used as a livestock feed, fertilizer, an input in aquaculture systems, or for fisheries enhancement. These contributions, combined with negligible needs for freshwater or arable land, make kelp aquaculture an increasingly attractive method for providing food for a growing global population.

Farmed kelp can contribute to the protein and energy requirements of both humans and livestock (Morrissey *et al.* 2001; Makkar *et al.* 2016). Kelps are a source of carbohydrates, fiber, vitamins (A, B, and B-12), minerals (iron, iodine, potassium, calcium), and omega-3 long-chain fatty acids (Morrissey *et al.* 2001; Wells *et al.* 2016; FAO 2018). Beyond basic nutritional requirements, there is also evidence that alginates derived from brown seaweeds can have ample benefits for human gut health (Brownlee *et al.* 2005). Some brown seaweeds have bioactive compounds that could be used in small doses as prebiotics for ruminants and other livestock (Makkar *et al.* 2016).

Research into appropriate serving sizes and bioavailability of these nutrients for humans and other organisms is imperative. In a similar manner to plants, seaweeds assimilate inorganic elements like arsenic, iodine, and other minerals from their surrounding environment (Graham *et al.* 2016). Thus, more guidelines for serving size are needed. Biorefinery studies (*i.e.*, application of enzymes) to increase the bioavailability of nutrients or remove unwanted minerals from kelps (Schiener *et al.* 2017) would also be helpful. Advancements in both arenas will further validate the potential for farmed kelp to contribute to food security.

Seaweed installations can make indirect contributions to food security by enhancing fisheries productivity and output efficiencies of other aquaculture operations. The longlines may provide habitat and food for wild organisms (see subsection 3.1.2) or cultured organisms. For example, in Chile and on the West Coast of the United States, wild *Macrocystis pyrifera* is harvested to feed cultured abalone (Camus *et al.* 2019; The Cultured Abalone LLC 2015). In the future, this biomass could come from aquaculture (Camus *et al.* 2019). Additionally, kelp farms may offer localized alkalization of coastal water benefitting wild and cultured shellfish growth (subsection 3.1.5). The extractive properties of seaweeds have also been shown to mitigate the

potential impacts of animal excrement when used in integrated multi-trophic aquaculture (IMTA) systems (Neori *et al.* 1996; Chopin *et al.* 1999; Troell *et al.* 1999a, 1999b).

## **Income**

Kelp aquaculture is an accessible marine livelihood that can supplement or replace income from existing ocean foods production. Small-scale kelp farming requires little capital investment, which makes it more realizable to newcomers than other forms of aquaculture. In Maine, the equipment cost for a 122 m longline is less than US \$1,000 (T. Olson, pers. comm., 2016). Collaborative relationships between industry, researchers, and extension agents have also played an instrumental role in supporting new entrants to the industry. Of particular note are the numerous, free or low-cost, educational resources available to prospective kelp farmers. The Kelp Farming Manual (Flavin *et al.* 2013) is a digital document providing detailed guidance for site selection, farm equipment, and nursery techniques. The New England Seaweed Culture Handbook, Nursery Systems (Redmond *et al.* 2014) focuses on the biology, cultivation methods, and cultivation systems for kelp and three other seaweeds. It is also available online. In addition to these print resources, many nonprofit organizations and academic institutions have provided workshops in culturing techniques and business management for prospective kelp farmers. In Maine, Coastal Enterprises Inc. (CEI), Island Institute, Maine Sea Grant, Maine Seaweed Exchange, and University of Maine Cooperative Extension have all offered classes or workshops on topics related to seaweed aquaculture.

Thus, market issues, perhaps more than grow-out technology, most threaten the economic viability of kelp farming. Substantial market development is still necessary for American and European growers (Bjerregaard *et al.* 2016; Skjermo *et al.* 2014). On a global scale, seaweeds and their derivatives are used in food products, animal feed, pharmaceuticals, beauty products,

biofuels, and agricultural products (FAO 2018; Graham *et al.* 2016). However, almost all kelp farmed in Maine is used in food products (*i.e.*, kelp noodles, kelp puree, kelp spice mix) because individual farmers struggle to access larger purchasers or have chosen to integrate vertically (Griffin & Warner 2017). Better access and competitiveness within existing markets, and the creation of new markets, will help to solidify kelp aquaculture as an alternative or supplemental livelihood.

### **Excessive nutrients and organic matter**

Kelp is an autotroph so there are no water-quality impacts of excess feed or feces associated with its cultivation. Kelp does produce a large amount of water and oxygen as the byproducts of photosynthesis. However, both water and oxygen are readily incorporated by saltwater, so the direct byproducts of seaweed cultivation are not of ecological concern. On the contrary, the byproduct oxygen from seaweed farms has been understood to provide the ecosystem service of oxygenation (Vásquez *et al.* 2014) and the dissolved organic carbon contributions are likely positive as well.

There is some concern that organic matter sloughed or dislodged from kelp farms could have a negative environmental impact. There can be a loss of organic matter from the farm during winter storms or due to natural blade erosion. Sloughing of material from wild kelp beds is generally understood to be a positive contribution to secondary production (Krumhansl & Scheibling 2012). Nonetheless, it has been suggested that sloughed cultivated kelp could contribute to nutrient over-enrichment or the de-oxygenation of sediments if a sizeable amount were to settle on the seafloor (Skjermo *et al.* 2014). This risk applies mostly to areas with low water exchange rates or naturally abundant algae. Ultimately, further investigation into the fate

and quantity of biomass leaving kelp farms is needed to fully evaluate the potential impacts of this organic matter (Skjermo *et al.* 2014).

### **Escape of farmed organisms**

The FAO presents concerns that farm escapees could prey on, or compete with, wild organisms in the farm vicinity. The concern of predation does not apply because kelp is an autotroph, but the threat of competition with wild organisms is still valid. If cultivated kelp enters its reproductive phase, the sorus tissue or released zoospores can be carried by ocean currents to areas where they might compete for habitat or interbreed with wild kelp. Uncontrolled, this potential crop-to-wild gene flow could lead to loss of genetic diversity, the transmission of diseases to wild kelp populations, and an overall decline in ecosystem resilience (Hutchings & Fraser 2008; Cottier-Cook *et al.* 2016; Buschmann *et al.* 2017).

The risk of decreased genetic diversity resulting from crop-to-wild gene flow is highly related to industry sporeling production strategies. Currently, a small amount of reproductive tissue, generally from 1 – 3 mature individuals, is used to produce billions of spores (Flavin *et al.* 2014; Redmond *et al.* 2014). This renders enough sporelings for multiple small kelp farms. As a result, the organisms on an individual farm have a similar genetic composition. If these individuals reach maturity, they will release gametes into the surrounding ecosystem that could outcompete or replace wild gametes. Then, over time, the local kelp populations could experience genetic erosion trending towards a genetic makeup similar to that of the farmed species.

Genetic diversity in algal populations is correlated with disease resistance because it helps to maintain the adaptive capacity of individuals to persist in stressful environments (Coleman *et al.* 2013; Wernberg *et al.* 2018). Therefore, the current sporeling production

methods used in Maine may leave kelp more susceptible to disease. Industry-wide disease outbreaks in cultured Pacific white shrimp (*Litopenaeus vannamei*) and Atlantic salmon (*Salmo salar*) demonstrate the potential impacts of limited breeders and inbreeding practices (Luvesuto *et al.* 2007; Cottier-Cook *et al.* 2016; Doyle 2016). Intensive culture of the red seaweeds *Kappaphycus alvarezii* and *Eucheuma denticulatum* in Asia and Africa have also been significantly affected by ice-ice and other diseases (Ward *et al.* 2020). While environmental factors likely play a role, these disease outbreaks are also believed to be more prevalent due to the monoculture approach of cultivation and low genetic variation in cultured stocks (Halling *et al.* 2013; Hafting *et al.* 2015). Several diseases have been observed in cultivated *Saccharina japonica*, a close relative to *S. latissima*, which is intensively cultivated in Asia. These include rot disease, twisting disease, and blister disease, which are believed to be environmentally induced (Getchis *et al.* 2014; Tseng 1986). Stipe blotch and dark spot disease have also been observed in *S. japonica* and believed to result from interactions with marine bacteria or fungi (Getchis *et al.* 2014; Tseng 1986).

It remains uncertain whether the aforementioned diseases will appear in Maine or other parts of the Americas and Europe (Getchis *et al.* 2014). As with many of the potential output impacts, disease risks will become more relevant as the scale of commercial cultivation increases (Buschmann *et al.* 2014). In the face of uncertainty, the precautionary principle should be applied. A kelp-disease outbreak could be devastating to the Maine kelp industry and associated human communities. It also poses considerable risk to wild kelp populations.

## **Chemicals**

Current kelp aquaculture practices exclude the application of chemicals to the farmed area or surrounding marine environment. Therefore, additional concerns listed under this category are not applicable.

## **2.5 Discussion**

Long-term ecological and social sustainability is vital to the continued growth and success of kelp farming. The strategy and principles of the Ecosystem Approach to Aquaculture can be used, in combination with lessons learned from other industries, to proactively address the relevant concerns presented above. Recommendations for practices, research, and resource management to address the potential impacts of kelp aquaculture are presented below. The recommendations are grouped by EAA principle providing the most considerable guidance (Table 2.1). Stakeholders connected with each recommendation are also listed.

### **2.5.1 Recommendations using EAA principle of ecosystem services**

The first principle of the EAA advises that aquaculture planning and development should not threaten ecosystem functions or services (FAO 2010). This principle rests on the assumptions that ecosystems provide services benefiting living beings and humans are an integrated part of ecosystems. Multiple high-priority actions can be undertaken to bring kelp aquaculture in Maine into greater alignment with the principle of Ecosystem Services. These recommended actions are: 1) define ecosystem and management boundaries, 2) assess ecosystem services and environmental carrying capacity, 3) pursue ecologically and socially considerate engineering and siting, and 4) protect health and genetic diversity of wild kelp beds.

**Table 2.1** Recommendations for new actions, research, and resource management to further ensure the long-term sustainability of kelp aquaculture in the Americas and Europe.

Recommendations were developed using the FAO’s Ecosystem Approach to Aquaculture strategy and principles (2010).

 <b>Ecosystem Services</b>	 <b>Social Justice</b>	 <b>Activity Integration</b>
<ul style="list-style-type: none"> <li>• Define Ecosystem and Management Boundaries</li> <li>• Assess Ecosystem Services and Environmental Carrying Capacity</li> <li>• Pursue Ecologically and Socially Considerate Engineering</li> <li>• Protect Health and Genetic Diversity of Wild Kelp Beds</li> </ul>	<ul style="list-style-type: none"> <li>• Increase Horizontal Expansion</li> <li>• Expand and Teach Best Management Practices (BMPs)</li> <li>• Develop Climate Change Resiliency</li> </ul>	<ul style="list-style-type: none"> <li>• Integrate Kelp Aquaculture and Kelp Harvesting into a Seaweed Management Plan</li> </ul>

### Define ecosystem and management boundaries

Defining the ecosystem and management boundaries for kelp aquaculture will work to prevent habitat degradation and associated biodiversity losses, productivity declines, and impacts on local communities and other users. This effort will facilitate monitoring and more targeted use-designations according to the biophysical conditions of the region. The marine commons frequently experiences mismatches between ecosystem and management scales, but socio-ecological systems that share the same ecosystem and management boundaries have higher chances at sustainability (Berkes *et al.* 2006). Defined management boundaries, or zoning, can also help to limit potential competition with wild kelp, crop-to-wild gene flow, and the transmission of diseases. Additionally, this action addresses the risk of overfishing for wild “seeds” (parent material) by providing a framework for regional oversight of sorus tissue

collection. Stakeholders connected with this recommendation include kelp farmers, wild seaweed harvesters, recreational boaters and fishermen, commercial fishermen, existing shellfish aquaculture, state regulatory agencies, federal regulatory agencies, harbormasters, educational groups, environmental groups, advocacy groups, interveners, towns, and coastal property owners.

Specific zones for sorus tissue harvesting could be defined using a variety of methods ranging from low to high levels of required effort and expense. The presence or absence of *S. latissima* or *A. esculenta* could be used to determine the bioregions. Either existing observations, historical records, or some combination of both could be employed. Using existing datasets would produce a relatively inexpensive assessment if done at a bay scale. A more sophisticated approach would be to model and analyze the direction and velocity of currents, which facilitates the movement of spores, using studies of wild kelp spore dispersal as a baseline. At least one spatial predictive probability model of potential spore distribution has been developed by combining field-measured geophysical attributes with modeled variables (Bekkby & Moy 2011). A study in support of this approach found that the connectivity of kelp beds (*Ecklonia radiata*) in Australia varies according to the strength of boundary currents (Coleman *et al.* 2009). The most comprehensive method for defining the bioregions, although quite costly, would be to conduct and use a detailed analysis of the wild kelp population structure. For example, along the relatively linear coast of California, a genetic distance-based model showed that habitat continuity and geographic distance played critical roles in population structure and gene flow (Alberto *et al.* 2010). This effect may be amplified along Maine's highly rugose coastline.

Urgent explication of management boundaries will also inform guidelines for the collection of production of sporelings and localization of strain selection. Genetic and population

structure studies on macrophytes in the Northwest Atlantic have been sparse. However, a fine-scale structure assessment of *S. latissima* in eastern portions of the state was recently completed (Brenton *et al.* 2018). This study found overall low genetic diversity but did note significant fine-scale structuring of populations along portions of Maine's somewhat continuous eastern coastline (Brenton *et al.* 2018). Moreover, the most considerable genetic difference was observed between two populations separated by a small geographic distance. These findings suggest that the driving factors influencing the interconnectivity of Maine's sugar kelp populations are dynamic and not entirely explained by location. As a first step towards bioregional sporeling/seedstock guidelines, seaweed nurseries could commit to only using genetic strains and reproductive material collected from the same bioregion as the farm site (Yarish *et al.* 2017).

#### **Assess ecosystem services and environmental carrying capacity**

Further quantifying the ecosystem services and environmental carrying capacity associated with kelp aquaculture will lessen the potential for habitat degradation and associated biodiversity losses and productivity declines. It will aid in the establishment of an evidence-based limit for aquaculture expansion. Such efforts will also further understanding of the interactions between kelp farms and productive fisheries. Increased knowledge of the ecosystem services offered by kelp farms will allow for more strategic placement of farms to maintain and enhance biodiversity, ecosystem productivity, and income. Stakeholders connected with this recommendation include kelp farmers, wild seaweed harvesters, recreational boaters and fishermen, commercial fishermen, existing shellfish aquaculture, state regulatory agencies, federal regulatory agencies, harbormasters, educational groups, environmental groups, advocacy groups, interveners, towns, and coastal property owners.

The term *environmental carrying capacity* refers to the ability of ecosystem services to tolerate a particular activity without unacceptable impact (Group of Experts on the Scientific Aspects of Marine Environmental Protection 1986). Environmental carrying capacity assessment is a core tenet of the EAA principle of Ecosystem Services. The scale at which environmental carrying capacity evaluation occurs should be a function of the features or resource services targeted for protection (*i.e.*, estuary, bay, or basin-wide). Therefore, careful delineation of ecosystem and management boundaries (subsection 4.1.1.) is the first step to assessing environmental carrying capacity. Resource managers and policymakers should use best available science to delineate these ecosystem and management boundaries.

Once the boundaries are established, the ability of each designated region to support kelp farming activities should be assessed. Kelp farming occupies physical space in the ecosystem and also requires dissolved carbon, nitrogen, phosphorus, and trace metals. There is a need for regional studies exploring the origin and availability of these elements and nutrients. A greater understanding of regional water circulation and exchange is also important (Park *et al.* 2018). More insight into the ecological interactions between the farms and other associated organisms is warranted. For example, little is known about the microbial communities associated with kelp farms, the degree of fish and invertebrate aggregation around these installations (Walls *et al.* 2017), or the final destination of algal material sloughing from the degrading kelp tips. Information regarding the changes in planktonic ecosystems near kelp farms and the impacts of respiration by kelp farms during the night is also scarce.

An environmental carrying capacity assessment for the Gulf of Maine would include an evidence-based estimate of the maximum hectares of kelp farms that could be supported by each region without affecting any ecosystem services. One such effort concluded that a two-hectare

seaweed farm in Sweden had either a positive effect, or no effect, on the supporting (e.g., biogeochemical cycling, habitat), regulating (e.g., mitigating eutrophication), and provisioning ecosystem services (e.g., food) in the region (Hasselström *et al.* 2018). This Swedish study serves as a useful starting point. Similar evaluations should be repeated in each region where kelp is cultivated. Regional repetition of studies will help to ensure that variations in geophysical and ecological processes are adequately captured.

A greater understanding of the ecosystem services provided by kelp aquaculture installations will foster social acceptance of the industry (Alleway *et al.* 2019; Rose *et al.* 2014). Wild seaweed communities provide numerous ecosystem services, and many of these functions are also accredited to seaweed aquaculture installations (Chung *et al.* 2017; MEA 2005). These include food provision, raw materials, biodiversity enrichment, increased habitat volume, provision of food and shelter, nutrient mitigation, wave attenuation, and carbon-dioxide removal (Chung *et al.* 2011; Duarte *et al.* 2013; FAO 2003; Kim *et al.* 2015; MacArtain *et al.* 2007; Mork 1996; Radulovich *et al.* 2015; Rose *et al.* 2010, 2014, 2015; Sondak *et al.* 2017). New knowledge regarding the magnitude of these ecosystem services will further inform estimates for the equilibrium between increased kelp aquaculture and sustained health of the surrounding marine ecosystem.

### **Pursue ecologically and socially considerate engineering**

The ecologically considerate engineering of aquaculture installations will ensure that kelp farms do not have unacceptable impact on other marine organisms. Avoiding impact on marine fauna will become increasingly important as the kelp industry grows and moves further offshore. Socially considerate engineering will minimize potential impacts to the viewsheds of local communities. Stakeholders connected with this recommendation include kelp farmers,

recreational boaters and fishermen, commercial fishermen, existing shellfish aquaculture, state regulatory agencies, federal regulatory agencies, harbor masters, environmental groups, interveners, towns, and coastal property owners.

Minimizing opportunities for marine mammal entanglement is the most pressing issue not currently addressed by the regulatory process or BMPs. Gear modification has been proposed for various fisheries to reduce North Atlantic right whale entanglements in fishing gear. These measures have not been successful (Knowlton *et al.* 2012; Rolland *et al.* 2016), so there are few proven examples of gear modifications for the kelp industry to follow. Until effective modification for non-mobile gear is determined, kelp farmers can demonstrate effort towards preventing entanglement by ensuring that their farms are sited outside of critical habitat for the North Atlantic right whale (NOAA 2016). Due to the current LPA limits set by the Maine DMR, the most common longline length in Maine is 122 m. Farmers applying for a larger, full lease could maintain short longlines and provide passageways between longlines to facilitate marine mammal movement through the farm. Dispersed longlines may also reduce the possible impacts from seafloor shading at shallow sites and minimize benthic disturbance from the mooring system. Effects of seaweed farms on the benthos should be better researched and systematically documented (Stévant *et al.* 2017) so that siting criteria can be re-evaluated if substantial changes to farm size or density occur. Each of farm management strategies to reduce marine mammal entanglement, benthic shading, and mooring scour would also likely reduce the density of surface buoys. Consequently, the visual impact of kelp farming would be lessened for coastal landowners and other water users.

## **Protect health and genetic diversity of wild kelp beds**

Best practices and continued scientific efforts to protect the health and genetic diversity of wild kelp beds will lessen the risks associated with the dislodgement of farmed kelp. Genetic impacts and the loss of genetic diversity have been pinpointed as critical challenges for aquaculture (CBD 2011; FAO 2010; United Nations 2015). Specifically, Aichi Strategic Goal B, Target 6 challenges that, by 2020, ecosystem-based approaches should be used for sustainable management and harvest of aquatic plants to reduce pressure on biodiversity (CBD 2011). Defining bioregions for reproductive strain production, developing specific and disease-resistant strains, and building diverse seed banks can reduce the likelihood of disease outbreak and prevent related biodiversity and productivity losses. Reducing harvest of sorus tissue, developing regional sorus harvesting guidelines, and gravitating away from the use of wild reproductive tissue also preemptively address the threat of overharvesting wild sorus tissue. Stakeholders connected with this recommendation include kelp farmers, wild kelp harvesters, and environmental groups.

Establishing laboratory-based seedbanks will provide a reliable seedstock for the industry, expedite the outplanting of new material via direct seeding, and improve reduce the impact of kelp aquaculture on wild kelp beds (Forbord *et al.* 2012; Kim *et al.* 2017; Redmond *et al.* 2014). The maintenance of kelp gametophyte cultures for aquaculture purposes is routine practice in Japan, Korea, and China. In the U.S., research groups are using laboratory-based kelp germplasm (S. Lindell, pers. comm., 2019; Martins *et al.* 2017; Peteiro *et al.* 2016, Quigley 2018), but these practices have yet to be widely adopted by the U.S. kelp industry. This lack of adoption is mostly due to inexperience, lack of instruments, and limited nursery facilities.

Although the establishment and maintenance of gametophyte cultures is very important to the long-term success of kelp aquaculture in the region, there may be a hybrid approach that could be utilized while the industry is building resources and expertise. More specifically, methods have been developed to induce sorus tissue production in a laboratory setting by manipulating the photoperiod and mechanically preventing the transport of the sporulation inhibitors (Forbord *et al.* 2012; Pang & Lüning 2004). This technique can also be used to maintain year-round production of zoospores and sporophytes in nurseries (Forbord *et al.* 2012).

Ultimately, creating an industry independent of wild sorus tissue sources will ensure the scalability and sustainability of kelp aquaculture (Kim *et al.* 2017). Developing specific kelp strains will allow farmers to have a reliable source of seed throughout the year while targeting specific crop characteristics. It may also provide more reliability regarding the morphometric attributes of the farmed product. Strain development offers the opportunity for novel product and intellectual property development (Loureiro *et al.* 2015). However, there remains a concern that cultivated strains originating from native genotypes could cross-hybridize with wild individuals. This effect has been studied in *S. japonica* by collecting wild kelp from an area with no seaweed cultivation and two cultivars from intensive seaweed culture in China and Japan (Liu *et al.* 2012). Higher genetic diversity was observed in the wild kelp, and this was interpreted as an indication that domestication might be accompanied by decreased genetic diversity and a narrower germplasm base of cultivars (Liu *et al.* 2012). In due time, sterile kelp strains could be developed to prevent crop-to-wild gene flow (Loureiro *et al.* 2015). Techniques for sterile-strain production of *S. latissima* are of interest to multiple research teams (Sjøtun 2017, S. Lindell, pers. comm., 2019).

Continued prospecting of nursery and grow-out strategies for other disease-resistant strains and previously uncultivated species is also essential. Intensive seaweed monoculture, or the widespread cultivation of a single species or strain, has been linked with disease (Hafting *et al.* 2015). Just as in agriculture, diversified cultivation and crop rotation can interrupt disease cycles and help producers reduce and manage the risk of disease (Krupinsky *et al.* 2002). Parallel work on both fronts is needed. Diversified cultivation, supported by the development of cultivation strategies for previously uncultivated species, may be within shorter reach than the establishment of disease-resistant strains. In Chile, for example, seeding and grow-out of two previously uncultivated Laminariales, *Lessonia trabeculata* and *Macrocystis pyrifera*, has been successful (Camus *et al.* 2018, 2019). Voluntary dissemination of these methods, similar to the widespread sharing of seeding and grow-out techniques for *S. latissima*, will increase the resilience of the budding industry. As seen in other cultivated species, the establishment of disease-resistant strains and disease-free nurseries can also help to prevent crop damage (Hafting *et al.* 2015). Disease-resistant strains will be vital to restocking efforts if crops are lost to disease (Cottier-Cook *et al.* 2016).

Small changes to existing sorus tissue harvesting can help to protect the health of wild kelp beds until wild sorus tissue harvesting is no longer needed. For example, harvesters could commit to removing only half the thallus of an individual kelp sporophyte and leaving the rest to regrow. In Maine, a minimum cutting height requirement is already in place for rockweed, or *Ascophyllum nodosum* (Maine DMR 2014). *Ascophyllum* spp. physiology and harvesting practices are dissimilar from *S. latissima* and *A. esculenta*; namely, rockweeds have apical meristems and grow from the tips, whereas kelps have intercalary meristems and grow from the

basal-blade region. However, the existing legislation sets a precedent that may result in more readily available social acceptance for a minimum cutting height BMP.

### **2.5.2 Recommendations using EAA principle of social justice**

The second EAA principle counsels that aquaculture activities should be equitable and improve human well-being (FAO 2010). This principle assumes that educated stakeholders participating in a transparent process will make decisions that support maximum well-being (FAO 2010). An additional perspective from a finer-resolution assessment of successful socio-ecological systems identified governance, decision-making, livelihoods, well-being, and adaption to current and future climate change as critical components for successful interactions in a marine-based socio-ecological system (Charles *et al.* 2012). Maine's robust state aquaculture legislation means that governance and decision-making in the kelp industry are already highly transparent and aimed at providing maximum well-being. Therefore, the areas of most considerable improvement under the EAA principle of Social Justice include: 1) increase horizontal expansion, 2) share education in Best Management Practices (BMPs), and 3) develop climate change resiliency.

#### **Increase horizontal expansion**

Increasing horizontal expansion within the kelp industry will create more jobs and maximize the potential income generated by kelp aquaculture. Diversification of labor across multiple organizations at each step of the supply chain will also result in more stability around kelp production activities and provide opportunities for specialization. Independent kelp seed providers, or nurseries, are an example of a specialization that could occur within the supply chain. Improving the reliability of seed production and access will help to ensure that kelp farming is an accessible alternative to fishing. Stakeholders connected with this recommendation

include kelp farmers, commercial fishermen, existing shellfish aquaculture, educational groups, and advocacy groups.

Diffusion and Innovation Theory (Rogers 1962) explains how new ideas, practices, or products are adopted over time. Innovations are not readily accepted by the entirety of society, but rather, they "diffuse" through it gradually because individuals sit along a spectrum of risk-seeking to risk-adverse (Rogers 1962). This theory can be used to anticipate new entrants to, and continued development of, the kelp industry. Kelp aquaculture has been promoted as an alternative or supplement to other ocean-based livelihoods (*i.e.*, commercial fishing, shellfish aquaculture, tourism) (Lem 2016; Redmond *et al.* 2014). In Maine, kelp farming has already captured the *innovators* and *early adopters*. They comprise a small segment of the total population that sees the need for change, is willing to take the risk, and can serve as leaders (Rogers 1962). The limited, but successful, and vertically-integrated companies in the state are a testament to the work of innovators and early adopters (Engle *et al.* 2018).

The *early* and late *majorities* are the much larger sectors of the population that need evidence of success before adopting an innovation (Rogers 1962). Adoption of kelp aquaculture by the early and late majorities will require more investment in seaweed production and processing systems (Bjerregaard *et al.* 2016), post-harvest storage, distribution, and value-added product development. Creation of a robust primary market will also increase the attractiveness and sustainability of kelp farming as an alternative livelihood. Similarly, kelp seed production needs to become more predictable. Nurseries must be able to reliably supply large quantities of high-quality seed (Skjermo *et al.* 2014). New entrants in the industry may have more specialized, targeted experience in automation and distribution that could be applied to kelp seed production. Alternatively, the formation of a nursery cooperative would help to improve the reliability of

kelp seed in the region. Equipment, knowledge, and seeded line could be collectively shared and produced by the cooperative.

### **Expand and teach Best Management Practices (BMPs)**

Industry-wide BMPs for seaweed harvesting, management, cultivation, and processing need to be developed quickly (Rebours *et al.* 2014) and in parallel with the expansion of American and European kelp aquaculture. The entrepreneurs, foodies, fishermen, and biotech companies entering the industry have varying levels of education in aquaculture, husbandry, crop management, and marine ecosystems. In the absence of unified industry standards, there is a risk that uninformed individuals could act in a manner that subjects an entire region or industry to economic or ecological risk. Dissemination and development of additional BMPs support new entrants to the industry and thereby promotes livelihood development. More specifically, widespread awareness and application of on-farm BMPs will address the potential transmission of diseases from cultivated to wild kelp. Educating growers on these same practices can also reduce potential crop loss from fouling or disease which would otherwise affect local businesses and communities developing around kelp aquaculture. Stakeholders connected with this recommendation include kelp farmers, wild kelp harvesters, educational groups, and state regulatory agencies.

An independent, neutral entity should develop a unifying list of BMPs for the nascent kelp industry. This entity could be a council, a non-profit organization, an industry alliance, or a growers' guild. This group is advised to confer an advisory board comprised of members from each stakeholder group (Fig. 4). It will be beneficial to consult terrestrial farmers and land managers as experts on transferrable crop and ecosystem management strategies. Maine has a history of collaborative decision-making via stakeholder advisory boards regarding the

management of marine resources. For example, the salmon farming companies in Maine, recognizing impending threats to the ecological and social sustainability, penned the Finfish Bay Management Agreement through a neutral third-party entity, the Maine Aquaculture Association (MAA 2002). More recently, the Maine Legislature passed legislation requiring the development of a Fishery Management Plan (FMP) for rockweed (Maine DMR 2014). A diverse stakeholder group comprised of industry, academic, and environmental organizations was convened by the Maine Department of Marine Resources who oversaw the FMP's development and Maine Sea Grant facilitated the meetings.

The advisory board would document existing BMPs and develop new ones. The board could also establish a centralized repository for this information. The Manual for the Identification and Management of Aquaculture Production Hazards (Getchis *et al.* 2014) provides a list of some BMPs that can help to reduce risk in seaweed aquaculture. Examples include selecting sites with sufficient current flow and nutrient levels, only out-planting during optimal growing conditions, and maintaining optimal densities to reduce fouling from epiphytes. Additional BMPs could be developed around this existing guidance.

Farmed seaweeds are at risk for diseases and severe fouling from epiphytes (FAO 2017). In these two technical problems lie immediate opportunities for the industry to raise awareness and develop BMPs. Study of intensive seaweed cultivation in other parts of the world suggests that Maine will see an increased prevalence of disease and fouling in the future. Adopting BMPs from these established industries could help to prevent future crop loss or, in the case of a very severe outbreak, industry collapse. Some examples of BMPs specifically designed to prevent disease outbreak include preventing culture lines from touching the seafloor at low tide, planting and harvesting around settlement windows of planktonic herbivores, harvesting early, and

optimizing culture conditions to prevent physiological stress (Cottier-Cook *et al.* 2016; Getchis *et al.* 2014; Walls *et al.* 2017). In the event of a specific disease outbreak, necessary quarantine procedures will include keeping a log of environmental parameters, removing all visibly infected or unhealthy kelp, and preventing cross-contamination before sanitation (Cottier-Cook *et al.* 2016; Getchis *et al.* 2014; Walls *et al.* 2017).

Once they are developed, it is imperative that the BMPs be effectively shared with all relevant stakeholders. Over the last decade in Maine, public-sector entities have provided education for prospective kelp growers through general aquaculture training programs (Island Institute 2017; Maine Sea Grant 2018). However, these programs are not seaweed-specific, and the growth of the industry has outpaced them. More recently, a few fee-for-service and contract farmer-training options have been offered (see: Ocean Approved, Sea Greens Farms, and Springtide Seaweed). The benefit of new entrants paying for training is that they can learn about BMPs. However, the second principle of EAA mandates equal access for all stakeholders (FAO 2010). Paid-training programs may exclude some potential entrants due to cost. Thus, they may not be the optimal pathway for educating stakeholders and industry members when other institutional resources are available. In Maine for example, the Maine Seaweed Council (MSC) is well-poised to draft and provide training on Maine-specific kelp aquaculture BMPs.

### **Develop climate change resiliency**

The FAO does mention climate change as a potential concern for aquaculture in the 2010 technical guidelines. Almost ten years later, the imminent ecological and social impacts of climate change cannot be overlooked. The forecasted shifts in ranges and distributions of algae resulting from rising water temperatures and changes in ice cover, salinity, dissolved oxygen, and circulation are particularly relevant to aquaculture (IPCC 2007; Bricknell *et al.* 2020). More

broadly, coastal development and pollution, combined with climate change impacts, will also create increased stress on coastal communities and habitats (IPPC 2007). Consideration of climate change impacts in integrated planning and development stages will increase the capacity for stakeholders to adapt to them (IPCC 2007, 2014; Whitney *et al.* 2017). Developing climate change resiliency within the budding kelp aquaculture industry will help to ensure that farmed kelp can contribute to food security despite a changing climate. Protecting strong genetic diversity in wild kelp populations should help to maintain the population's performance during heatwaves (Wernberg *et al.* 2018), and similarly, building up the genetic diversity in gametophyte cultures and cultivated kelp strains will support the resilience of cultivated kelp stocks. Further efforts towards temperature-tolerant strain development can uphold kelp aquaculture as a marine-based livelihood in warmer water. Stakeholders connected with this recommendation include kelp farmers, wild seaweed harvesters, recreational boaters and fishermen, commercial fishermen, existing shellfish aquaculture, state regulatory agencies, federal regulatory agencies, harbor masters, educational groups, environmental groups, advocacy groups, interveners, towns, and coastal property owners. Each stakeholder in kelp aquaculture is likely to experience impacts of climate change, but the degree and timing of the impact remain unknown.

The forecasted changes in water temperatures pose a threat to the cultivation of *S. latissima* and *A. esculenta* that rely on cool water (Park *et al.* 2017). Ambient water temperature affects recruitment, photosynthesis, growth, and reproduction of seaweeds (Lüning 1988, 1990; Wiencke *et al.* 1994). Studies of *S. latissima* and *A. esculenta* gametophyte survival under high temperatures show a switch from reproduction to vegetative growth with increasing water temperature (Park *et al.* 2017). These findings suggest that more southern kelp populations may

be negatively impacted by the forecasted warming (Park *et al.* 2017). Increased water temperatures could also affect the beneficial microbiome associated with the organisms. For instance, a study of the red alga *Delisea pulchra* showed that increased water temperatures could negatively affect the holobiont, or microbes living on the alga, that provide chemical defenses against disease (Harder *et al.* 2012).

Recent observations show that the Gulf of Maine is warming faster than 99% of the global ocean (Pershing *et al.* 2015). Research into culture and grow-out techniques for temperature-tolerant strains of kelp has been prompted by the observed and projected warming in the Gulf of Maine. Recently, laboratory protocols for producing temperature tolerant strains of *A. esculenta* were developed (Quigley 2018; Bricknell *et al.* 2020) and Burdett *et al.* (2019) further demonstrated the resilience of photosynthesis in *L. digitata* and *L. hyperborea* exposed to a three-day heat spike of +2 or +4 °C. These developments are excellent first steps in climate change resiliency for the industry because *A. esculenta* appears to be more temperature constrained than *S. latissima* (Park *et al.* 2017). High-temperature tolerant strains for *S. latissima* are a high priority for research due to the prolific cultivation of this species (Kim *et al.* 2017) and they are likely to be available soon. In Korea, they have employed selective breeding technologies to develop two temperature-tolerant strains of *Saccharina japonica* (Hwang *et al.* 2018). In addition to tolerating higher seawater temperatures, these strains also performed well in strong wave action and yielded more biomass than the control algae (Hwang *et al.* 2018).

More basic physiology experiments, culturing-method development, and grow-out assays will also help to improve the industry's climate change resiliency. Insufficient knowledge of seaweed biology, physiology, and reproduction is a significant hurdle for large-scale commercialization of seaweed aquaculture in Chile (Bushman *et al.* 2017). This paucity is also

highly evident in Maine. Efforts in each of these research tracks will support crop diversification and increase the adaptive capacity of the industry to respond to the potential consequences and opportunities resulting from climate change.

### **2.5.3 Recommendations using EAA principle of activity integration**

The third principle of the EAA instructs that aquaculture development should be integrated with other sectors and management efforts (FAO 2010). The FAO further conveys that this can be achieved through multi-sectoral, or integrated planning and management. Indeed, case studies and conceptual modeling from across the world demonstrate that conservation is more successful if the users of shared environmental resources are also linked together socially (Bodin *et al.* 2014). With the development of kelp aquaculture in Maine, there are now multiple users of wild kelp beds. Therefore, one of the most straightforward actions to reconcile kelp aquaculture within the existing use of the resource is to integrate the management and planning of kelp harvesting.

#### **Integrate kelp aquaculture and kelp harvesting into a seaweed management plan**

This recommendation addresses the potential overharvesting of wild sorus tissue by consolidating requests for, and records of, all kelp harvesting. More comprehensive management of wild kelp beds ensures the viability of wild kelp harvesting as an economic livelihood. Harvesting BMPs and zonation of sorus harvesting areas will also protect the seed source for future research and industry development. Stakeholders connected with this recommendation include kelp farmers, wild kelp harvesters, and state regulatory agencies.

With the growth of kelp aquaculture, the need for more comprehensive monitoring and management of natural kelp beds is increasingly important (Buschmann *et al.* 2013; Frangoudes 2011). Similar to many other kelp farming regions, Maine has an existing fishery in which

harvesters collect mature *S. latissima*, *A. esculenta*, and *L. digitata* sporophytes by hand. Harvesters in the wild kelp fishery are required to keep and report detailed effort and landings records, including area harvested, seaweed species, and biomass landed (Maine DMR 2015). However, recreational harvest rules in Maine allow harvesting of  $\leq 22.6$  kg of seaweed per day without a license. Sorus tissue harvest can go unreported because the amount of tissue required for kelp nursery operations is usually much lower (see subsection 2.1) than the reporting threshold. Under reporting of wild tissue harvest renders effective monitoring and sustainable management of the fishery more challenging.

An integrated kelp management plan can support the development of the cultivated kelp industry while providing more protection for the natural kelp beds. In such a plan, individuals or companies harvesting wild sorus tissue for seed stock production would be held to the broader management regulations for the seaweed fishery. Integrated management for all interactions with wild kelp beds will, at a minimum, allow regulators to track effort, quantity, and spatial distribution of sorus harvest. This data can be integrated into the broader fisheries management plan for seaweeds. Ecological indicators like density, biomass, recruitment, and population structure could be used to link regions with different harvesting regimes under a co-management effort (Vega *et al.* 2014). Informed and integrated management is needed to ensure the sustainability of wild kelp beds and the livelihood of both kelp farmers and wild kelp harvesters.

## **2.6 Conclusion**

Approximately 58% (25) of the 43 potential issues and impacts originally described by the FAO working group in the EAA document are relevant to kelp aquaculture. Thus, most of the strategy and principles of the EAA can be used to establish protocols and actions to promote the

ecological and social sustainability of the nascent kelp industry. The concerns and recommendations described in the present study address ecological, social, and management aspects of kelp production. The major ecological concerns are the alignment of management and ecosystem boundaries and the potential impact to the wild kelp beds from seed sourcing and transfer of species beyond natural limits. Best Management Practices applied at key leverage points within the system would help the kelp industry to address many of the relevant ecological concerns. Low barriers to entry and rapid growth of the industry are the leading factors accentuating potential social conflicts. Recommendations to address the social sustainability of the industry are focused on the development of BMPs and the education of stakeholders to accept them, increasing horizontal expansion, and the development of climate-change resiliency. It is also recommended that kelp aquaculture and sorus harvesting activities be integrated into a broader fishery management plan for seaweeds.

The assessment and recommendations developed with the focus on the Maine kelp industry are believed to be applicable to other kelp industries in the Americas and Europe. Some adaptations will be necessary to fit the practices, ecosystems, and attitudes of the different kelp-producing countries and latitudes. Further studies in other regions where kelp farming is starting are necessary to establish a general and predictive model for development of this nascent industry.

## CHAPTER 3

### THE NITROGEN BIOEXTRACTION POTENTIAL OF NEARSHORE SACCHARINA LATISSIMA CULTIVATION AND HARVEST IN THE WESTERN GULF OF MAINE

Grebe, G.S., Byron, C.J., Brady, D.C., Geisser, A., Brennan, K. (2021) The nitrogen bioextraction potential of nearshore *Saccharina latissima* cultivation and harvest in the Western Gulf of Maine. *Journal of Applied Phycology*, <https://doi.org/10.1007/s10811-021-02367-6>

#### 3.1 Chapter abstract

In-water remediation strategies, implemented in conjunction with traditional watershed management, could help minimize the impact of excess nitrogen (N) on marine ecosystems. Seaweed farming and harvesting may have potential as in-water N remediation tools in the Western Gulf of Maine (WGoM), but more understanding of the associated spatial and temporal variability is needed. In this study, *Saccharina latissima* was grown and collected from four WGoM sites in 2016 – 2019 and analyzed for tissue N content and stable isotopes. The source of N taken by the kelp was not obvious from monthly nor interannual mean  $\delta^{15}\text{N}$  measured in the kelp tissue, and the interannual means were significantly different between sites in the same bay. Mean kelp biomass across all sites and years was 9.84 ( $\pm 2.53$ ) – 14.84 kg (wet weight) per meter of longline at time of harvest (late May – early June). Nitrogen content of the *S. latissima* tissue was 1.04% – 3.82% ( $\pm 0.22$ ) (dry weight) throughout the growing season and generally decreased through the spring. Using these results, we estimated that harvesting a hypothetical hectare of *S. latissima* after 6 – 7 months of cultivation in the WGoM would have the potential to remove 19.2 ( $\pm 4.8$ ) – 176.0 ( $\pm 7.7$ ) kg N ha<sup>-1</sup>, depending on the density of longlines. The wide ranges of both biomass at time of harvest, and  $\delta^{15}\text{N}$  and percent N content in the kelp tissue,

highlight the need for site-specific pilot studies, even within a specific bay, prior to implementing kelp aquaculture as an in-water tool for N bioextraction.

### **3.2 Introduction**

Nutrient pollution is one of the principal causes of poor coastal water quality and habitat degradation (Nixon 1995, 1998; Diaz and Rosenberg 2008; Paerl *et al.* 2014). Globally, an estimated 245,000 km of coastline are considered “dead zones” triggered by excessive input of reactive nitrogen (N) and phosphorus (P) (Diaz and Rosenberg 2008). In the United States of America (USA), a nationwide excess of reactive N from anthropogenic sources has caused impairment to an estimated two-thirds of the country’s coastal waters (Bricker *et al.* 1999; Howarth *et al.* 2002). Moreover, the degree of coastal nutrient loading to the Northeastern USA coastline is considered one of the highest on Earth (Boesch 2002; Howarth 2008). Nutrient pollution, in combination with other trace elements supporting primary production, results in areas of hypoxia and anoxia, habitat degradation, altered food webs, loss of biodiversity, increased instances of green or harmful algal blooms, and greater susceptibility to localized ocean acidification (Nixon 1987; 1995; Paerl 1997; Paerl and Whitall 1999; Breitburg *et al.* 2009, 2018; Wallace *et al.* 2014).

In this study, we focus on nutrient concerns in the Western Gulf of Maine (WGoM) bordering Massachusetts (MA), New Hampshire (NH), and Maine (ME), USA. Bays and estuaries adjacent to these states are waterbodies of emerging concern due to both point and nonpoint sources of reactive N (Castro *et al.* 2003; Liebman *et al.* 2012). Effluent from wastewater treatment facilities (WWTFs) is the most common point source of N to the WGoM, however substantial N contributions from nonpoint N sources like stormwater runoff, agricultural runoff, and atmospheric deposition also occur in the region (Castro *et al.* 2003;

Liebman *et al.* 2012; Trowbridge *et al.* 2014). Atmospheric N deposition is estimated to be 30 – 40% of the total N load in many locations and stormwater runoff has been estimated to contribute another 30 – 35% of the nonpoint source N loading (Castro *et al.* 2003; Liebman *et al.* 2012; Trowbridge *et al.* 2014). New Hampshire and Massachusetts have implemented N discharge limits and strategies targeting both point and nonpoint source N to address and minimize the deleterious effects of excess nutrients on the WGoM (Reitsma *et al.* 2017). Maine, the state with the most coastline bordering the WGoM, has yet to establish nutrient criteria.

In addition to improving point-source discharges, resource managers in Maine are interested in nutrient bioextraction as part of a system-wide approach integrating watershed load reductions and enhanced nutrient assimilation (Liebman *et al.* 2012). Nutrient bioextraction strategies, also referred to as *bioremediation*, aim to remove nutrients that exceed the flushing and assimilation capacity of the system, regardless of their source (Krom 1986; Chopin *et al.* 2001; Neori *et al.* 2004). Bioextraction efforts in coastal water bodies typically target dissolved inorganic nitrogen (DIN) because it often limits primary production in temperate marine ecosystems (Ryther and Dunstan 1971; Lobban and Harrison 1994). Excess dissolved inorganic P and dissolved carbon (C), and small amounts of dissolved organic N and P when inorganic nutrient levels are low (Li *et al.* 2016), are also removed from the environment during bioextraction (Bianchi 2007).

Many primary producers are suitable for use in bioextraction, but recently more attention has been given to the use of macroalgae in this role. Macroalgae naturally extract N from the marine environment because N is one of the key macronutrients required for protein and nucleic acid synthesis; and kelps are highly productive (Gao and McKinley 1994; Valiela *et al.* 1997; Neori *et al.* 2004). Previous studies have evaluated a range of macroalgal species and cultivation

systems, including temperate and tropical macroalgae, land-based systems, integrated multi-trophic aquaculture (IMTA) systems, and nearshore marine installations. Many of these studies strategically cultivated a desirable alga to remove DIN from the surrounding water (Goldman *et al.* 1974; Ryther *et al.* 1975; Neori *et al.* 1996, 2004; Chopin *et al.* 1999, 2001, 2012; Buschmann *et al.* 2001; Troell *et al.* 2003; Abreu *et al.* 2011; Sanderson *et al.* 2012; Wang *et al.* 2012, 2014; Broch *et al.* 2013; Handå *et al.* 2013; Kim *et al.* 2014, 2015; Yarish *et al.* 2017; Fossberg *et al.* 2018). To determine the origin of the removed DIN, the N isotope ratio ( $\delta^{15}\text{N}$ ) in the algal tissue can be compared to the isotopically distinct  $\delta^{15}\text{N}$  of nitrogen originating from oceanic, atmospheric, treated wastewater, or fertilizer (Heaton 1986; Owens 1987; Peterson and Fry 1987).

Seaweed aquaculture and harvesting activities are expanding in the WGoM (Grebe *et al.* 2019; Maine Department of Marine Resources (MEDMR) 2019c), which raises the question: can this growing industry potentially contribute to the maintenance or enhancement of the WGoM's assimilative capacity for nutrients? Aquaculture leaseholders in Maine reported harvesting approximately 127 mt wet weight (WW) of cultivated macroalgae in 2019 (MEDMR 2019c), the majority of which was processed or sold as edible (Piconi *et al.* 2020). Maine's seaweed production is projected to grow at 12 – 15% annually to reach a total annual yield of 1360 – 2720 mt (WW) by 2035 and new market opportunities in livestock feed, fertilizer, pharmaceuticals, and carbon or nutrient offsets are expected (Piconi *et al.* 2020). Most of the current seaweed aquaculture expansion is focused on kelp (order Laminariales). The most commonly grown species in Maine are: *Saccharina latissima* (Linnaeus) C.E.Lane, C.Mayes, Druehl & G.W.Saunders 2006 (sugar kelp), *Saccharina angustissima* (Collins) Augyte, Yarish & Neefus 2018 (*skinny kelp*), and *Alaria esculenta* (Linnaeus) Greville 1830 (winged kelp or horsetail

kelp) (Grebe *et al.* 2019; Bricknell *et al.* 2020). Of the three, *S. latissima* is also the most frequently grown species in the USA (Kim *et al.* 2015; Yarish *et al.* 2017). In this study, we focus only on the bioextraction potential of *S. latissima*.

Previous studies have estimated N bioextraction by *S. latissima* grown in other regions by multiplying the percent N content in the kelp tissue by biomass harvested and extrapolating to a larger area (Neori *et al.* 2004; He *et al.* 2008; Chopin *et al.* 2012; Kim *et al.* 2015; Wu *et al.* 2015; Xiao *et al.* 2017; Yarish *et al.* 2017). Findings from these studies suggest that *S. latissima* aquaculture can be a useful nutrient extraction strategy in specific regions or seasons, but there is a need for more long-term estimates from a wide range of locations. Along the Eastern USA coastline, the need for improved understanding of the temporal and spatial variability of N dynamics and the related bioextraction efficiencies of specific macroalgal species is especially strong (Kim *et al.* 2007; Liebman *et al.* 2012; Kim *et al.* 2015). Kim *et al.* (2015) and Yarish *et al.* (2017) provided N bioextraction estimates by *S. latissima* grown in New York, Connecticut, and southern Massachusetts, but the temperature gradient along the Eastern USA coastline is one of the steepest in the world, and temperature has a strong influence on *S. latissima* growth (Fortes and Lüning 1980; Bolton and Lüning 1982). A better understanding of the expected macroalgal N bioremediation ranges is essential from a management perspective because bioextraction can be expensive (Neori *et al.* 2004). Some commonly used nutrient management practices are not adequately assessed and later found to be moderately ineffective (Boesch *et al.* 2001). Overestimating efficiencies of management measures is costly from a financial perspective, but it also damages social capital that had to be built by resource managers prior to initiating the treatment strategy. Thus, identifying local-regional patterns or commonalities

across local studies can help build a better understanding of the range of results expected from bioextraction efforts.

In this study, we aimed to expand on previous work evaluating bioextraction by macroalgae along the Eastern USA coast to gain a more comprehensive understanding of the N extraction potential in the region. First, we estimated the N-extraction of kelp harvested from the WGoM in late spring and throughout the growing season to determine the effect of harvest timing, biomass, and percent tissue N content on the total N removed from the surrounding water. Then, we sought to characterize the source of DIN taken up by the kelp by measuring the  $\delta^{15}\text{N}$  in the collected tissue. Lastly, we provided regional context for the potential N removed through harvesting cultivated *S. latissima* from the WGoM by estimating the amount of harvested kelp needed to extract N equivalent to the N-loading from atmospheric deposition, activities in the watershed upland of the coast, and treated wastewater effluent.

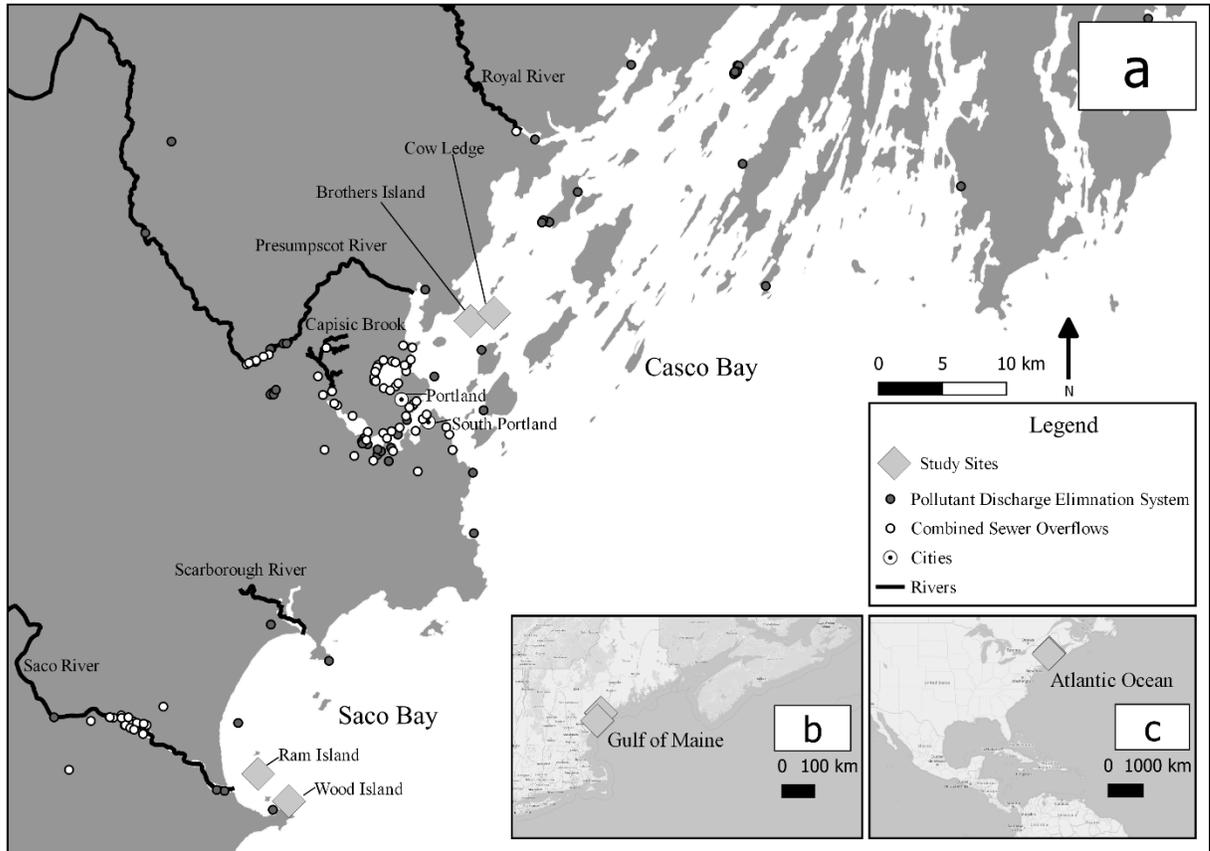
### **3.3 Materials & methods**

#### **3.3.1 Study site descriptions**

The Gulf of Maine (GoM) is a temperate, biologically productive, waterbody extending from Nova Scotia, Canada, to Cape Cod, Massachusetts, USA (Fig. 3.1). Offshore, much of the GoM's productivity is from the upwelling of nutrient-rich water from deep on the continental slope (Townsend 1998; Bricknell *et al.* 2020). In the coastal zone, nutrient delivery and cycling are influenced by vertical mixing by tides, wind-driven transport, small and large-scale buoyancy forcing, large freshwater sources, atmospheric deposition, wastewater treatment facilities (WWTFs), and stormwater runoff (Garret *et al.* 1978; Townsend 1991; Pettigrew *et al.* 1998, 2005; Castro *et al.* 2003; Trowbridge *et al.* 2014).

Cultivation and sampling occurred at four sites in the Western Gulf of Maine. Two of the sites were in Casco Bay, Maine. We refer to these sites as *Brothers (Bros.) Island* and *Cow Ledge* because they were near these geographical features. The Brothers Island and Cow Ledge sites were < 3 km apart and the longlines were oriented in a similar cardinal direction (North-South) which was parallel to the prevailing current. The other two cultivation and sampling sites, *Ram Island* and *Wood Island*, were in Saco Bay, Maine (Fig. 1). The Ram Island and Wood Island sites were < 4 km apart and the longlines were oriented in a similar cardinal direction (East-West) and parallel to the prevailing current.

Casco Bay has a relatively complex, indented shoreline, whereas Saco Bay is a relatively uniform, crescent bay (Tanner *et al.* 2006). Previous studies have concluded that land-based N sources dominate nearshore N concentrations in Casco Bay (Castro *et al.* 2003; Gray 2019). Less information is available for Saco Bay, but it is presumably also heavily impacted by land-based N. Both bays receive substantial freshwater and nutrient contributions from rivers draining upland watersheds (Wade *et al.* 2008; Tilburg *et al.* 2011, 2015; Gray 2019), WWTFs employing secondary treatment, and combined sewer overflows (Maine Department of Environmental Protection [MEDEP] 2019). Combined sewer overflows (CSOs) contribute land-based nutrients to the bays after heavy rainstorms when stormwater runoff is channeled into the combined sewer collection system at a volume that exceeds the capacity of the treatment facility (MEDEP 2019). In 2019, these CSOs collectively discharged 768,000 m<sup>3</sup> of untreated stormwater runoff and wastewater into Casco Bay and 273 m<sup>3</sup> into Saco Bay (Riley 2020).



**Figure 3.1** Map of the four study sites: Wood Island, Ram Island, Brothers Island, and Cow Ledge (gray diamonds). Panels: a) Casco and Saco Bay, Maine, b) the Western Gulf of Maine (WGoM), c) the Northwestern Atlantic Ocean. Freshwater sources of interest are the Saco River, Scarborough River, Capisic Brook, Presumpscot River, and Royal River (black lines). White-circled black dots are the cities of Portland and South Portland, white dots are combined sewer overflows, and gray dots designate pollutant discharge elimination system outfalls administered by the Maine Department of Environmental Protection (MEDEP 2018). Basemaps: QGIS Open Street Map and GADM (<https://gadm.org/>)

### 3.3.2 Field measurements and laboratory procedures

#### Kelp cultivation and collection

*Saccharina latissima* sporelings were produced using the methodology described in Redmond *et al.* (2014). Briefly, we collected wild *S. latissima* reproductive tissue from nearby bays and stressed it in the laboratory to release spores. Thin line was inoculated in water containing the released spores (6,000 – 8,000 spores mL<sup>-1</sup>) over night and then transferred to aquaria. The sporelings grew in light and temperature-controlled aquaria for approximately 6 – 8 weeks. Outplanting occurred between October and December each year. Kelp installations at each site consisted of 1 (Wood Island and Ram Island), 2 (Brothers Island and Cow Ledge in 2018), and 5 (Cow Ledge in 2019) longlines suspended 2 m below the water's surface. Each longline was 60 – 120 m long, and the spacing between each line was  $\geq 6$  m. Each site was less than 1 km from shore. Water depths on site were 7 – 17 m mean lower low water (MLLW).

Kelp cultivation occurred during four growing seasons: October to June 2016 – 2019. Sample collection typically began in January or February when the individual sporophytes were 30 – 50 cm long and 7 – 8 g (WW). The sporophytes were too small to obtain density estimates at that time. However, in mid-March, mean sporophyte density was typically 200 – 500 sporophytes m<sup>-1</sup>. At maturity in late May, mean sporophyte density was approximately 200 sporophytes m<sup>-1</sup>.

During sampling events, we maintained the sample integrity by removing the entire organism (holdfast, stipe, and blade) using nitrile gloves. Access to the sampling sites was weather-dependent, and thus, sampling frequency varied throughout the season and from year to year. During the most rigorous sampling season (2019), we completed approximately 10 sampling events at each site: roughly once per month, December through February, and 2 – 4

times per month from March to June. The timing of sampling was also variable across tides and time of day. At Cow Ledge in 2019, where there were 5 longlines, we collected kelp from the outermost line. All collected kelp was stored in plastic bags, transported in a covered cooler, and refrigerated at 8 °C until further processing. Transportation between the field and the laboratory was 1 – 2 h.

### **Biomass analysis**

We removed and weighed all sporophytes from three, 10 cm sections of the longline to generate a mean biomass estimate for each sampling date. The location of the sections along the longline were haphazardly determined. (During a few sampling events and seasons only one biomass measurement was possible. We do not report standard deviations for these cases). Then, we multiplied the mean biomass (WW) per 10cm by 10 to obtain an estimate of kelp biomass (WW) per longline-meter. We also established a wet to dry ratio for the samples by weighing the collected kelp upon removal from the plastic bag in the laboratory and again immediately after it had been lyophilized. The difference between the two weights was attributed to water loss and used to establish a wet to dry ratio.

### **Elemental and stable isotope analysis**

On each sampling event, we haphazardly collected five individual sporophytes for elemental and stable isotope analysis. Within 12 h of collection, we excised a 4 cm<sup>2</sup> cutout from the basal tissue near the meristem, where metabolic activity is concentrated (Nielsen *et al.* 2014; Boderskov *et al.* 2016). The tissue was rinsed with deionized water and lightly rubbed between gloved hands for 30 seconds. No epiphytic algae were visibly present on the sporophytes. A small percentage (< 5%) of the sporophytes had snails (*Lacuna vincta*) or egg rings attached to them, which we manually removed. The tissue samples were stored in a -40 °C freezer. The

frozen tissue was lyophilized at -50 °C using a Labconco FreeZone Legacy 2.5 Liter Benchtop Freeze Dryer, (115V, 60Hz, Model #:7670520). After 24 h of drying, the lyophilized samples were homogenized into a fine powder using a mortar and pestle. The powder (2.5 – 5 mg) was encapsulated in tin capsules and shipped to the University of California Davis Stable Isotope Facility [UC Davis SIF] (<https://stableisotopefacility.ucdavis.edu>). The SIF analyzed each sample for total N, total C, <sup>15</sup>N, and <sup>14</sup>C using a PDZ Europa ANCA-GSL elemental analyzer interfaced with a PDZ Europa 20-20 isotope ratio mass spectrometer. The instruments have an analytical precision of 0.3‰ for <sup>15</sup>N, and the instruments were calibrated before analysis with certified standards (UC Davis SIF 2017). Overall, 364 samples were analyzed for elemental content and stable isotope ratios. We calculated elemental ratios for samples using the measurements obtained from the UC Davis SIF. We obtained percent tissue N or C content by dividing the total weight of N or C measured in each sample by the encapsulated dry sample weight. Then, we calculated the C:N ratio (*M:M*) for each sample from these percentages.

A common approach for estimating N removed from the marine ecosystem is to multiply the percent tissue N content in the kelp at harvest by the biomass harvested (Neori *et al.* 2004; He *et al.* 2008; Kim *et al.* 2013, 2014, 2015). This methodology stems from the understanding that some N is immediately used to fuel macroalgal growth, and the surplus is stored as pigments, amino acids, and proteins (Martínez *et al.* 2012). Therefore, we also opted for this approach and estimated total N removed by *S. latissima* at the time of harvest using the mean percent tissue N calculated for the dry weight (DW) of the kelp during each sampling event (Eqn.1):

$$\text{Equation 1: } N \text{ removed} = \frac{g N}{g DW} * \frac{g DW}{g WW} * \frac{g WW}{m}$$

We adjusted the percent tissue N content by the WW:DW ratio and then multiplied by the estimated mean of kelp biomass (WW g m<sup>-1</sup>) for that site on the same sampling date. No assumptions regarding forms of N were included in these calculations.

The UC Davis SIF calculated the stable isotope ratios for each sample by comparing the difference in the <sup>15</sup>N measured in the sample against the <sup>15</sup>N in at least four different laboratory reference materials (Eqn. 2) (Peterson and Fry 1987):

$$\text{Equation 2: } \delta^{15}\text{N} = ((R \text{ sample}/R \text{ reference}) - 1) \times 1000 (\text{‰}),$$

where R equals <sup>15</sup>N/<sup>14</sup>N. The  $\delta^{15}\text{N}$  of primary producers reflects the inorganic N sources used, plus a variable amount of fractionation (differential use of <sup>15</sup>N vs. <sup>14</sup>N during N uptake) (Fogel and Cifuentes 1993; Fry 2006). Thus, we compared the calculated ratios to known  $\delta^{15}\text{N}$  ranges for N from specific sources. The  $\delta^{15}\text{N}$  ranges most attributed to each N source are: -2 – 0‰ for atmospheric N, -3 – 3‰ for N from commercial fertilizers, 4 – 8‰ marine N from natural sources, and >10‰ for N discharged from wastewater treatment processes (Heaton 1986; Macko and Ostrom 1994; McClelland *et al.* 1997; McClelland and Valiela 1998; Costanzo *et al.* 2001; Gartner *et al.* 2002; Cole *et al.* 2004; Kendall *et al.* 2007).

### **3.3.3 Environmental measurements**

Water temperature was continuously measured using Hobo Pendant Temperature/Light 8K Data Loggers (Part #: UA-002-08). If a temperature logger was lost or compromised, we used temperature readings from the nearby University of Maine Land Ocean Biogeochemical Observatory buoys (2016 only) and a buoy maintained by the U.S. National Oceanic and Atmospheric Administration (NOAA) (Station CASM 1). A Tilt Current Meter (TCM-1; Lowell Instruments LLC) hanging inverted from the middle spacer-buoy on each longline continuously measured current velocity and direction. Photosynthetically active radiation (PAR) was

measured at 2 m underwater using an LI-193 Spherical Quantum Sensor (LI-COR) during each sampling event May 2018 – June 2019. Before these dates, we estimated photosynthetically active radiation (PAR) for each bay by transforming daily Global Horizontal Irradiance obtained from the National Solar Radiation Database (<https://maps.nrel.gov/nsrdb-viewer>) Physical Solar Model V.3 for the Portland International Jetport station (location ID 1364086). We considered all rainfall up to 60 h before sampling as potential runoff affecting the collected kelp but excluded snowfall. Rainfall data for Casco Bay sites came from the Portland International Airport weather station maintained by NOAA. The University of New England Marine Science Center Weather Station in Biddeford, ME provided rainfall data for the Saco Bay sites.

We collected triplicate water samples from 2 m underwater during the 2018 and 2019 growing seasons using a horizontal Niskin bottle. All water samples were stored in sealed Whirl-pak bags, transported in a covered cooler to the laboratory, refrigerated at 8 °C, and processed within 4 h of collection. Each water sample was analyzed to estimate salinity, pH, and  $\text{NO}_3^-$ -N concentration at the time of sampling. We measured salinity using a Cole-Parmer RSA-BR90A Refractometer (0 - 90%) and a HACH benchtop meter (model #: PW172KB0703F01) calibrated to certified standards to measure pH. We determined the concentrations of  $\text{NO}_3^-$ -N in each sample spectrophotometrically using HACH Nitrate TNTplus Low Range Vial Tests and a HACH DR3900 Laboratory VIS Spectrophotometer calibrated before analysis with certified standards. We chose to enumerate  $\text{NO}_3^-$ -N because it is a common form of problematic reactive N in waterways impacted by anthropogenic activities (Galloway *et al.* 2004) and more easily measured through grab sampling than nitrite or ammonium.

### 3.3.4 Statistical analysis

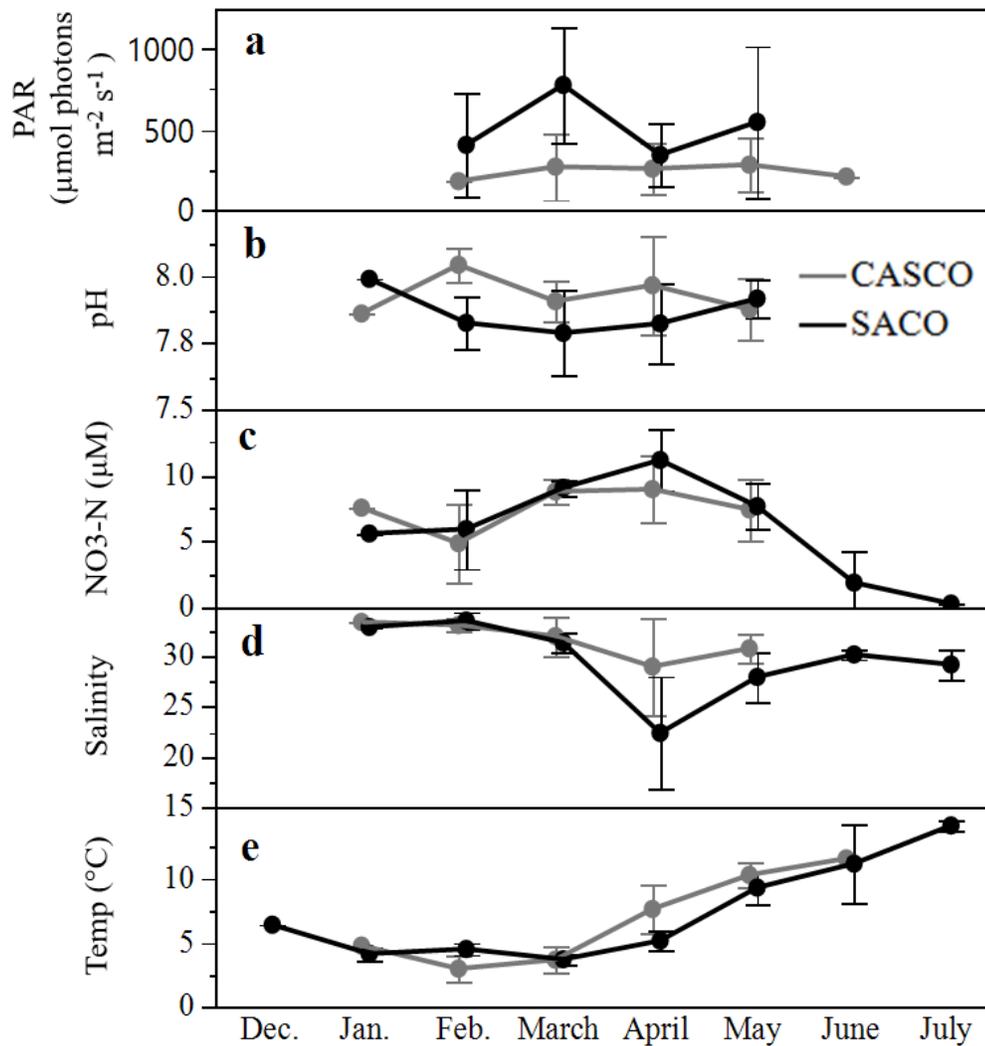
We examined all data for assumptions related to normality and homogeneity of variance. We identified and removed outliers using quantile ranges, robust fit, a k-nearest neighbor analysis. Then, we examined data from each study area separately (*i.e.*, individual sites) and collectively (all sites). We used multivariate analyses of variance (MANOVA) to compare the effect of 13 environmental factors on percent tissue N, percent tissue C,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  measured in the kelp tissue. The 13 environmental factors were: site, bay, temperature, current, light, pH, salinity, ambient  $\text{NO}_3^-$ -N at the surface, ambient  $\text{NO}_3^-$ -N at 2 m deep, total rainfall received 60 h before sampling, growout week, distance from shore, and distance from nearest WWTF. When significant effects ( $p \leq 0.05$ ) were detected, each dependent variable was analyzed separately using one-way analyses of variance (ANOVA). We performed post-hoc comparisons using Tukey's Honest Significant Difference HSD tests and measured Pearson R correlations between  $\delta^{15}\text{N}$ , percent tissue N, and the environmental factors. We used JMP Pro 14 for all statistical analyses.

## 3.4 Results

### 3.4.1 Environmental conditions

Monthly mean PAR, pH, salinity,  $\text{NO}_3^-$ -N, and water temperatures measured during the study varied by bay (Fig. 3.2). The ambient water conditions at the Saco Bay sites were generally colder, higher in nutrients, and lower in pH and salinity than the Casco Bay sites. The highest salinity (S) measured was in February (S = 35) and the lowest in early May (S = 8). The pH of water collected from the sites was 7.5 – 8.2. The highest pH values occurred in November, and the lowest values occurred in May. Mean monthly nitrate in the water samples was 0.29 – 11.8  $\mu\text{M}$   $\text{NO}_3^-$ -N. Peak nitrate values occurred in early March to mid-April and then declined mid-

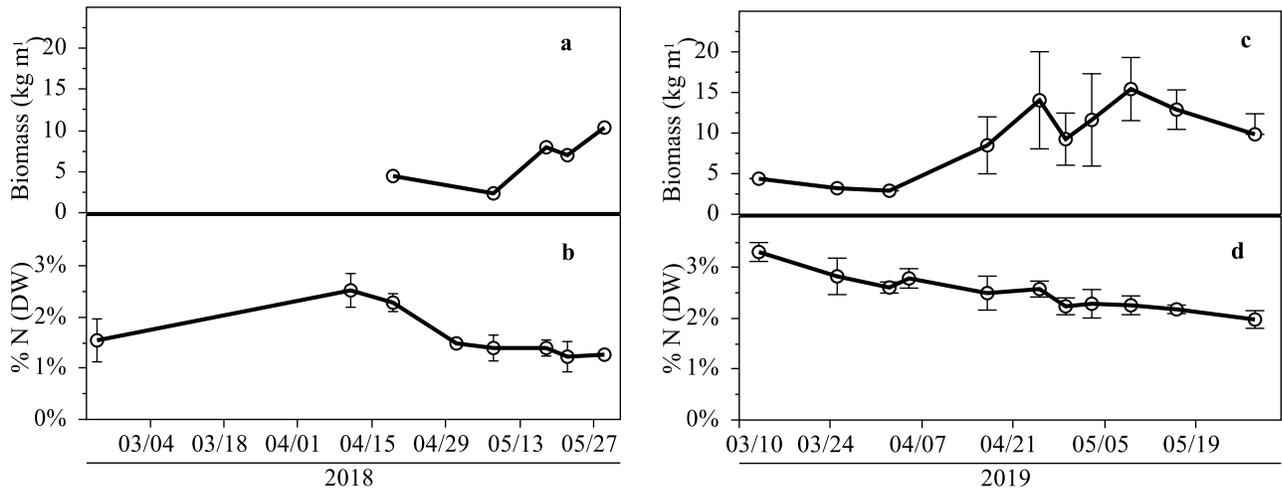
April through May. The water temperature at the kelp farms was 1 – 12 °C from November to June. We observed three distinct temperature intervals. The water temperature steadily declined from 10 °C in November to approximately 2 – 4 °C in mid-February. Then it oscillated between 2 – 4 °C from mid-February until mid-March. Then, water temperature began to increase before reaching 10 – 12 °C in late May. The current velocities measured at the Cow Ledge and Wood Island in 2018 and 2019 were 3 – 54 cm s<sup>-1</sup>. Specific current velocities are not available for Ram Island or Brothers Island, but they are probably like those at Cow Ledge and Wood Island, because tidal cycles drive most of the variability in currents within the nearshore WGoM.



**Figure 3.2** Mean monthly ambient conditions measured in Saco Bay (black lines) and Casco Bay (gray lines) from 2016 – 2019. Panels: a) PAR (photons  $m^{-2} s^{-1}$ ) measured at 2 m below water surface, b) ambient water pH, c) nitrate ( $\mu M$ ), d) salinity, and e) temperature ( $^{\circ}C$ ). Error bars are standard deviation

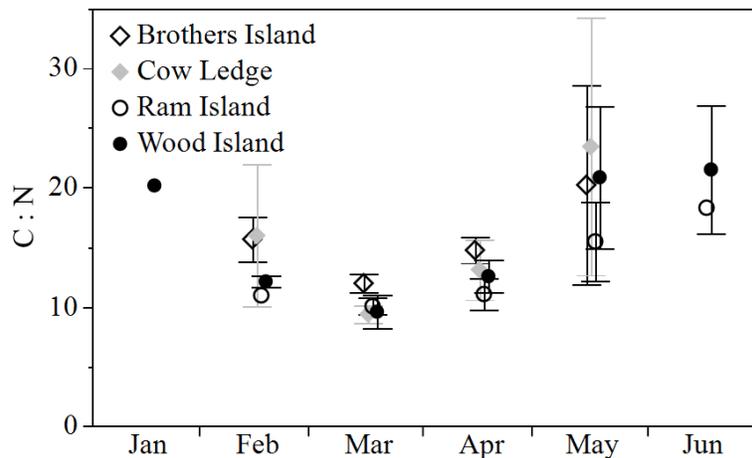
### 3.4.2 Biomass and elemental analysis

Mean kelp biomass across all sites and years was  $9.84 (\pm 2.53)$  to  $14.84 \text{ kg WW m}^{-1}$  longline at the time of harvest (Table 3.1). Wet to dry ratios of the kelp were 7.4:1 in March to 8.7:1 in May. The highest sampling frequency, and thus insight into biomass increase, occurred in Spring 2018 and Spring 2019 at the Wood Island site (Fig. 3.3). In 2018, biomass measurements at this site show that peak growth occurred in late May. Interestingly, measurements from 2019 at this site show peak growth from April until mid-May, followed by a decline in biomass in late May.



**Figure 3.3** Kelp characteristics from the Wood Island site in 2018 and 2019. Panels: a) mean biomass ( $\text{kg m}^{-1}$ ) in 2018, and b) percent tissue nitrogen (DW) in 2018, c) mean biomass ( $\text{kg m}^{-1}$ ) in 2019, d) percent tissue nitrogen (DW) in 2019. Error bars are standard deviation of  $\geq 3$  samples, replicate biomass measurements were not collected in 2018.

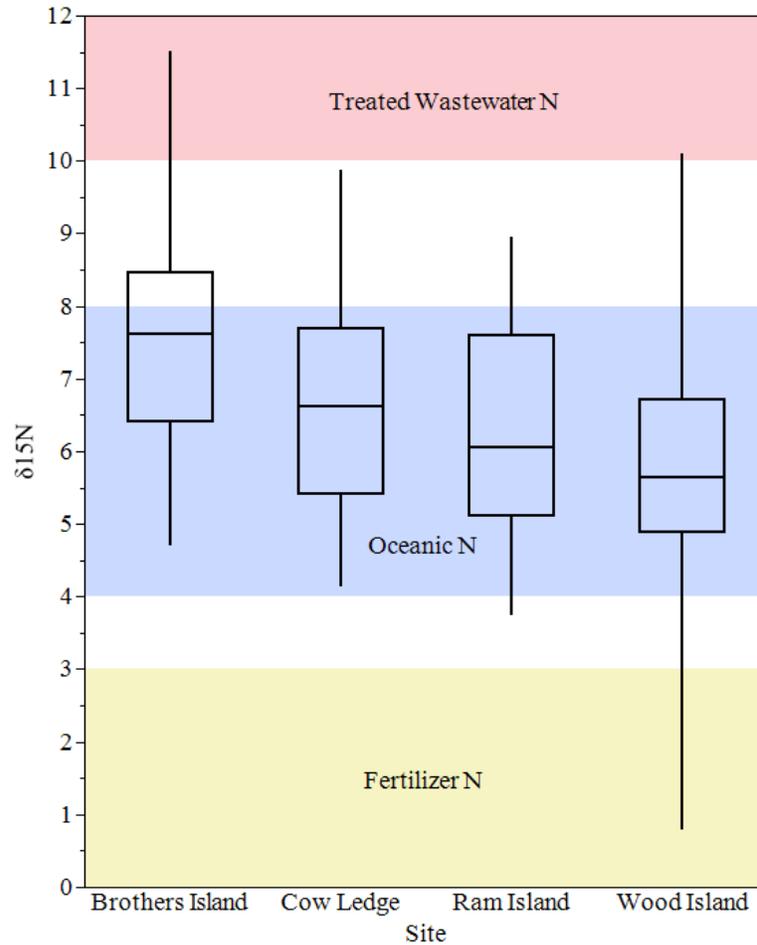
Mean N content of the *S. latissima* tissue, calculated for each sampling event, was 1.04 – 3.82% ( $\pm 0.22$ ) DW throughout the growing season and generally decreased through the spring. Tissue N content at Wood Island in 2018 and 2019 illustrated the general trend: at this site, percent tissue N decreased 0.08 – 0.17% week<sup>-1</sup> from mid-April to late-May (Fig. 3). In late May, percent tissue N contents were 1.04 – 2.29% ( $\pm 0.09$ ) DW. Increasing water temperature was negatively associated with percent tissue N (MANOVA;  $F(9, 155) = 37.49, p < 0.0001$ ). Photosynthetically active radiation (PAR) was positively associated with percent tissue N (MANOVA;  $F(9, 155) = 4.65, p = 0.0325$ ). Site also had significant effects on percent tissue N (MANOVA;  $F(9, 155) = 4.10, p = 0.0078$ ). There were no significant direct correlations (Pearson's  $r$ ) between the percent tissue N and environmental data. Across sites and all years, the mean C:N ratio ( $M:M$ ) measured in the *S. latissima* tissue was 9.4 ( $\pm 0.7$ ) to 23.4 ( $\pm 10.8$ ). The lowest C:N ratios were in March; the highest ratios were in May and June (Fig. 3.4).



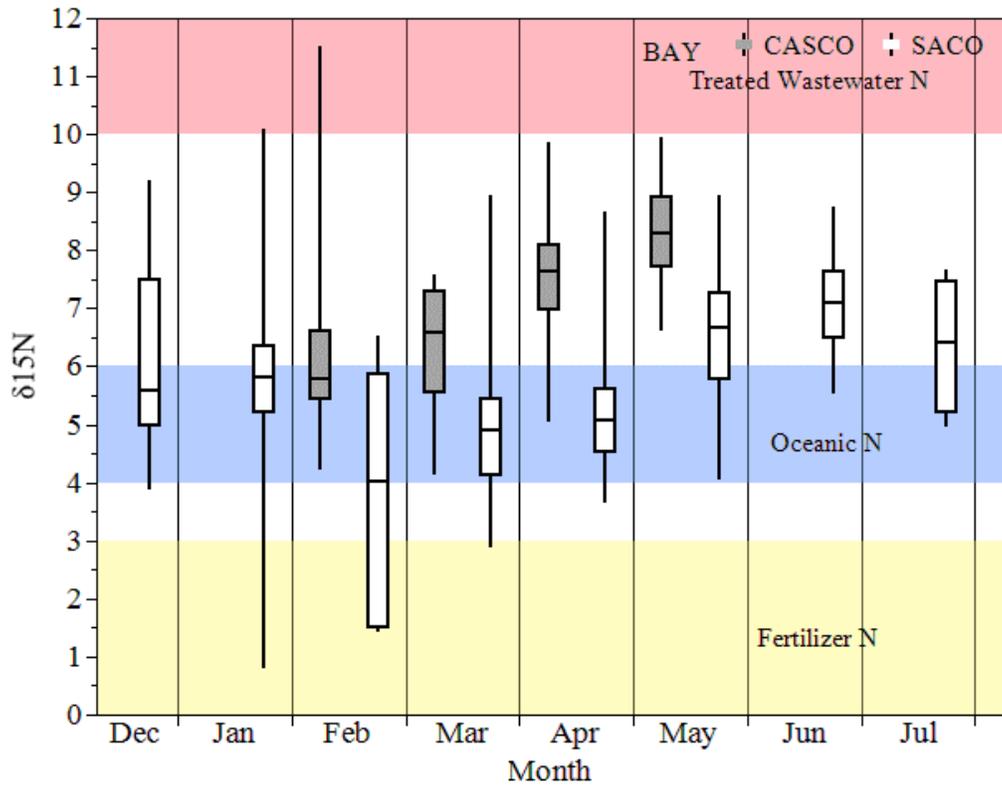
**Figure 3.4** Mean monthly C:N ratio ( $M:M$ ) in *Saccharina latissima* tissue cultivated near Brothers Island (open diamond), Cow Ledge (gray-filled diamond), Ram Island (open circle), and Wood Island (black-filled circle). Error bars are standard deviation.

### 3.4.3 Stable isotopes

The interannual mean  $\delta^{15}\text{N}$  measured in the kelp tissue grown at the two Casco Bay sites was significantly different from the interannual mean  $\delta^{15}\text{N}$  measured in the kelp tissue grown at the two Saco Bay sites (Tukey's HSD,  $df = 258$ ,  $p < 0.0001$ ). The widest range in distribution of  $\delta^{15}\text{N}$  was at the Wood Island site (0.79 – 10.09‰), and the narrowest range in distribution was at the Ram Island site (3.95 – 8.96‰) (Fig. 3.5). Cultivation site significantly affected  $\delta^{15}\text{N}$  values (MANOVA;  $F(9, 155) = 19.33$ ,  $p < 0.0001$ ). Grow-out week had a positive effect on  $\delta^{15}\text{N}$  values (MANOVA;  $F(9, 155) = 5.88$ ,  $p < 0.0160$ ), and ambient  $\text{NO}_3\text{-N}$  had a negative effect on  $\delta^{15}\text{N}$  values (MANOVA;  $F(9, 155) = 5.12$ ,  $p < 0.0240$ ). There were no significant correlations (Pearson's  $r$ ) between  $\delta^{15}\text{N}$ , % tissue N, or environmental conditions at the sites. When combined by bay, the  $\delta^{15}\text{N}$  values mirror each other with the lowest values observed in February and then rising throughout the spring (Fig. 3.6).



**Figure 3.5** Nitrogen isotope ratios ( $\delta^{15}\text{N}$ ) in *Saccharina latissima* cultivated at Brothers Island, Cow Ledge, Ram Island, and Wood Island from 2016 - 2019. Shaded areas represent the range of  $\delta^{15}\text{N}$  commonly associated with nitrogen from treated wastewater ( $\delta^{15}\text{N} = > 10\text{‰}$ ), oceanic nitrogen ( $\delta^{15}\text{N} = 4 - 8\text{‰}$ ), and fertilizers ( $\delta^{15}\text{N} = -3 - 3\text{‰}$ ). Unshaded ranges represent overlap between nitrogen sources. Ranges for  $\delta^{15}\text{N}$  from different sources were obtained from: Heaton 1986; Macko and Ostrom 1994; McClelland *et al.* 1997; McClelland and Valiela 1998; Costanzo *et al.* 2001; Gartner *et al.* 2002; Cole *et al.* 2004; Kendall *et al.* 2007.



**Figure 3.6** Monthly nitrogen isotope ratios (‰) measured in *Saccharina latissima* from Casco (gray fill) and Saco Bay (white fill) from 2016 – 2019. The range of  $\delta^{15}\text{N}$  commonly associated with nitrogen (N) from treated wastewater is ( $\delta^{15}\text{N} = > 10\text{‰}$ ), oceanic N ( $\delta^{15}\text{N} = 4 - 8\text{‰}$ ), and fertilizers ( $\delta^{15}\text{N} = -3 - 3\text{‰}$ ). Unshaded ranges represent overlap between N sources. Ranges for  $\delta^{15}\text{N}$  from different sources were obtained from: Heaton 1986; Macko and Ostrom 1994; McClelland *et al.* 1997; McClelland and Valiela 1998; Costanzo *et al.* 2001; Gartner *et al.* 2002; Cole *et al.* 2004; Kendall *et al.* 2007.

### 3.5 Discussion

#### 3.5.1 Biomass and bioextraction estimates

*Saccharina latissima* grew well in both Casco and Saco Bay. Biomass per longline-meter at harvest ( $10 - 15 \text{ kg m}^{-1}$ ) (WW) was on the higher end of the ranges previously reported in the

literature (Table 3.1). The mean wet weight to dry weight ratio (WW:DW) ratio of the sporophytes at harvest was also slightly higher than the 7:1 reported by Sanderson *et al.* (2012) for cultivated *S. latissima* from an IMTA system in Scotland. In our data, the highest and lowest biomass measurements have almost 1% difference in the tissue N content (Cow 2018 vs. Wood 2019). The observed 1 – 4% N content is comparable to the range reported by other studies where *S. latissima* was grown in water with high DIN from anthropogenic or fish waste (Handå *et al.* 2013; Kim *et al.* 2015; Marinho *et al.* 2015; Yarish *et al.* 2017), but the higher 4% N tissue content also exceeded the upper value reported by several studies where *S. latissima* was grown in IMTA or relatively unimpacted water (Sanderson *et al.* 2012; Bruhn *et al.* 2016; Freitas *et al.* 2016; Fossberg *et al.* 2018). Maximum potential N removal did not coincide with peak percent tissue N and percent tissue N observed in the kelp at the time of May harvest was lower than previously observed for *S. latissima* in the Northwest Atlantic (Kim *et al.* 2015; Yarish *et al.* 2017). Almost all percent N and C:N ratios measured in the kelp tissue at harvest indicate that nitrogen was limiting. Like Kim *et al.* (2015) and Yarish *et al.* (2017), we observed a high degree of temporal and spatial variability of tissue N content in *S. latissima*.

**Table 3.1** Comparison of environmental conditions in the Western Gulf of Maine with previous studies. Metrics include water temperature, salinity, current) and bioextraction parameters (harvest time, biomass, tissue N, total WW biomass ha<sup>-1</sup> and total N ha<sup>-1</sup> removed). All decimals rounded to nearest whole number.

Location	Cond. Notes	Temp (°C)	Salinity	Current (cm s <sup>-1</sup> )	Harvest Time	Biomass (kg WW m <sup>-1</sup> )	% Tissue N (DW)	Longline Spacing (m)	Total Biomass (WW mt ha <sup>-1</sup> )	Total N removed (kg ha <sup>-1</sup> )	Reference
Gulf of Maine, USA	-	2 – 12	8 – 35	1 – 3	May	10 – 15	1 – 4	6	16 – 24	19 – 46	Current study
Long Island Sound, USA	-	0 – 17	21 – 33	-	May/June	1 – 19	1 – 4	6	9 – 10	10 – 35	Kim <i>et al.</i> (2015); Yarish <i>et al.</i> (2017)
Badcall and Calbha, Scotland	IMTA	7 - 15	-	-	June	-*	1 – 3	-*	-*	-*	Sanderson <i>et al.</i> (2012)
Horsen's Fjord, Denmark	-	0 – 19	12 – 27	0 – 34	May	0 – 1	1 – 3	8 – 10	2 – 7	3 – 26	Marinho <i>et al.</i> (2015); Bruhn <i>et al.</i> (2016)
	IMTA	0 – 19	12 – 29	0 – 36	May	1	1 – 4	8 – 10	7	31	Marinho <i>et al.</i> (2015)
Galicia and Canabria, Spain	-	13 – 17	-	12 – 92	May	4 – 16	-	4	30 – 46	-	Peteiro <i>et al.</i> (2006, 2016); Peteiro and Freire (2013)
Tristeinrasa, Norway	IMTA	4 – 14	27 – 34	0 - 20	June	-*	2 – 5	-*	-*	-*	Handå <i>et al.</i> (2013); Wang <i>et al.</i> (2014)
Sogn and Fjordane, Norway	-	4 – 15	-	-	-	-	1 – 2	-	-	-	Fossberg <i>et al.</i> (2018)
Galicia, Spain	IMTA	11 – 16	-	-	April	-*	2 – 3	-*	-*	-*	Freitas <i>et al.</i> (2016)
New Brunswick, Canada	-	-	-	-	-	8 – 16	-	-	-	-	Druehl <i>et al.</i> (1988); Chopin <i>et al.</i> (2004)
Bocabec Bay, Canada	IMTA	-	-	-	-	8 – 21	-	-	-	-	Chopin <i>et al.</i> (2004)

- Information was not provided

-\* Information provided was for a cultivation array that does not allow for cross-comparisons to horizontal longlines

As previously demonstrated, our results can be extrapolated to generate rough, hectare-scale estimates of potential bioextraction by kelp harvesting in the region. With a moderate, 6 m of spacing between longlines, a total of 1,767 m of longline fit in one hectare of ocean surface. Multiplying this by the calculated kg kelp m<sup>-1</sup> would result in a kelp harvest of 17.3 (±4.4) – 26.1 mt WW ha<sup>-1</sup>. The intensive cultivation scenario, with 1.5 m spacing between longlines, has 6,768 longline-meters per hectare, which would produce 70.3 – 100.1 mt WW ha<sup>-1</sup>. Converting by the WW:DW ratio (8.7:1), and then multiplying by the mean % tissue N measured in kelp from each site at harvest, results in an estimated 19.2 (±4.8) – 46.0 (± 2.0) kg N ha<sup>-1</sup> that could be removed by harvesting a hectare of *S. latissima* with 6 m spacing between longlines and 73.5 (±18.4) – 176.0 (±7.7) kg N ha<sup>-1</sup> with 1.5 m spacing between longlines (Table 3.2).

Previously published estimates of N loading to Casco Bay (*i.e.*, atmospheric N deposition, N loading from upland activities in the watersheds, and effluents from large WWTFs), help to put the potential bioextraction from kelp aquaculture in context (Table 3.3). We can calculate the approximate area of *S. latissima* harvest needed to remove a quantity of N equivalent to the N that is delivered to Casco Bay from these sources. In all examples considered, the quantity of N removed from Casco Bay by harvesting one hectare of *S. latissima* would be greater than the amount of N contributed to the Bay from one hectare of any loading sources. For example, even with 6 m spacing between longlines, the N extraction by 1 ha of *S. latissima* harvest is equivalent to the annual atmospheric deposition of N across 2.7 (±0.7) – 10.7 (±0.5) ha of Casco Bay or 5.1 (±1.3) – 12.1 (±0.5) ha of activities in a nearby urban subwatershed. Insufficient data on N inputs prevents a direct comparison for Saco Bay, but we expect that the pattern would be similar.

**Table 3.2** Mean *Saccharina latissima* biomass ( $\text{kg m}^{-1}$ ), % N content, and tissue C:N (*M*) measured at harvest from Spring 2016 – 2019. These observations were used to estimate the potential biomass produced from 1 ha of farming activity at two densities (6 m and 1.5 m spacing between longlines) and the potential N ( $\text{kg ha}^{-1}$ ) removed when harvesting this biomass. Values are rounded to the nearest tenth. Standard deviations are reported in the parentheses when possible, but replicates were not collected for all years.

Year	Biomass ( $\text{kg m}^{-1}$ WW)				% N (DW)				C: N				Total N (kg) removed $\text{ha}^{-1}$ 6 m spacing				Total N (kg) removed $\text{ha}^{-1}$ 1.5 m spacing			
	Bros. Island	Cow Ledge	Ram Island	Wood Island	Bros. Island	Cow Ledge	Ram Island	Wood Island	Bros. Island	Cow Ledge	Ram Island	Wood Island	Bros. Island	Cow Ledge	Ram Island	Wood Island	Bros. Island	Cow Ledge	Ram Island	Wood Island
<b>2016</b>	-	-	-	<b>13.0</b>	-	-	-	<b>2.3</b> ( $\pm 0.1$ )	-	-	-	-	-	-	-	<b>46.0</b> ( $\pm 2.0$ )	-	-	-	<b>176.0</b> ( $\pm 7.7$ )
<b>2017</b>	-	-	-	<b>12.5</b>	<b>1.0</b> ( $\pm 0$ )	<b>1.9</b> ( $\pm 0$ )	<b>1.8</b> ( $\pm 0.6$ )	<b>2.0</b> ( $\pm 1$ )	<b>34.6</b>	<b>15.3</b> ( $\pm 0$ )	<b>18.3</b> ( $\pm 3.5$ )	<b>17.7</b> ( $\pm 4.8$ )	-	-	-	<b>39.0</b> ( $\pm 19.5$ )	-	-	-	<b>149.5</b> ( $\pm 74.8$ )
<b>2018</b>	-	<b>14.8</b>	-	<b>10.4</b>	-	<b>1.1</b> ( $\pm 0.5$ )	-	<b>1.2</b> ( $\pm 0.3$ )	-	<b>35.7</b> ( $\pm 4.9$ )	-	<b>28.3</b>	-	<b>25.0</b> ( $\pm 11.4$ )	-	<b>19.2</b> ( $\pm 4.8$ )	-	<b>95.8</b> ( $\pm 43.6$ )	-	<b>73.5</b> ( $\pm 18.4$ )
<b>2019</b>	<b>13.4</b>	<b>10.4</b>	<b>12.7</b> ( $\pm 0.4$ )	<b>9.8</b> ( $\pm 2.5$ )	<b>1.8</b> ( $\pm 0.1$ )	<b>1.7</b> ( $\pm 0.1$ )	<b>1.8</b> ( $\pm 0.2$ )	<b>2.0</b> ( $\pm 0.2$ )	<b>18.7</b> ( $\pm 1.7$ )	<b>19.4</b> ( $\pm 1.4$ )	<b>20.1</b> ( $\pm 1.4$ )	<b>17.8</b> ( $\pm 1.7$ )	<b>37.1</b> ( $\pm 2.1$ )	<b>27.2</b> ( $\pm 1.6$ )	<b>35.1</b> ( $\pm 3.9$ )	<b>30.1</b> ( $\pm 8.2$ )	<b>142.0</b> ( $\pm 7.9$ )	<b>104.1</b> ( $\pm 6.1$ )	<b>134.6</b> ( $\pm 15.0$ )	<b>115.4</b> ( $\pm 31.6$ )

**Table 3.3** Amount of estimated atmospheric, riverine, and treated wastewater N loading into Casco Bay potentially offset by the harvest of 1 ha of *Saccharina latissima*.

	Annual N (kg ha <sup>-1</sup> )	Hectares offset by <i>S. latissima</i> bioextraction	
		6 m spacing	1.5 m spacing
Atmospheric Deposition (Dry + Wet) Low Estimate	4.3 <sup>a</sup>	<b>4.5</b> (±1.1) – <b>10.7</b> (±0.5)	<b>17.1</b> (±4.3) – <b>40.9</b> (±1.8)
Atmospheric Deposition (Dry + Wet) High Estimate	7.2 <sup>a</sup>	<b>2.7</b> (±0.7) – <b>6.4</b> (±0.3)	<b>10.2</b> (±2.5) – <b>24.4</b> (±1.1)
Presumpscot River Watershed (Forested)	1.5 <sup>b</sup>	<b>12.8</b> (±3.2) – <b>30.7</b> (±1.3)	<b>49.0</b> (±12.3) – <b>117.3</b> (±5.1)
Royal River Watershed (Forested)	5.3 <sup>b</sup>	<b>3.6</b> (±0.9) – <b>8.7</b> (±0.4)	<b>13.8</b> (±3.5) – <b>33.1</b> (±1.5)
Capisic Brook Watershed (Urban)	3.8 <sup>b</sup>	<b>5.1</b> (±1.3) – <b>12.1</b> (±0.5)	<b>19.4</b> (±4.9) – <b>46.4</b> (±2.0)
Effluent from large WWTFs	3.5 <sup>c</sup>	<b>5.5</b> (±1.4) – <b>13.2</b> (±0.6)	<b>21.1</b> (±0.9) – <b>8.7</b> (±0.4)

<sup>a</sup> Sonoma Technology Inc.(2003) estimated that atmospheric N deposition (wet + dry) to Casco Bay is 4.3 – 7.22 kg ha<sup>-1</sup> yr<sup>-1</sup> inorganic N

<sup>b</sup> Recent work by Gray (2019) suggested that the large and predominantly forested Presumpscot and Royal River watersheds respectively export 1.5 kg to 3.79 kg N ha<sup>-1</sup> yr<sup>-1</sup> into Casco Bay. She estimated nitrogen loading from the smaller, but urbanized, Capisic Brook watershed to be 5.31 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Gray 2019)

<sup>c</sup> Annually, the six largest WWTFs near Casco Bay discharge an estimated 914 mt of N into the bay (MEDEP 2008), which is approximately 3.5 kg N ha<sup>-1</sup> yr<sup>-1</sup> across the area of Casco Bay.

These estimates of total N removed per hectare of kelp harvested from the WGoM (19.2 – 46 kg ha<sup>-1</sup> with 6-m longline spacing) are higher than many of the ranges reported by other studies evaluating *S. latissima* for bioextraction at nearshore and IMTA sites (Table 3.3). Of particular interest, again, is the comparison between this study and those in closest proximity. In the Long Island Sound, CT and the Bronx River Estuary, NY, Kim *et al.* (2014, 2015) and Yarish *et al.* (2017) calculated 10 – 35 kg N ha<sup>-1</sup> removed with 6-m longline spacing and 29 – 139 kg N ha<sup>-1</sup> with 1.5-m longline spacing (Kim *et al.* 2014, 2015; Yarish *et al.* 2017). Additionally, Augyte *et al.* (2017) estimated 88.7 kg ha<sup>-1</sup> N removal by closely related species, *Saccharina angustissima* (formerly *Saccharina latissima* forma *angustissima*), cultivated near Bristol, ME and Sorrento, ME using a 2.5 m spacing between longlines. We recalculated this to be 124 kg ha<sup>-1</sup> N removal by *S. angustissima* with 1.5 m spacing between longlines and note that this estimate lies in the middle of the range reported by this study for *S. latissima* grown with the same longline spacing in Saco and Casco Bay.

This emphasizes the importance of considering cultivation density and harvest timing when evaluating bioextraction applications. Unsurprisingly, increasing the *density* of longlines on a hectare of ocean surface produced a much higher estimate of N extraction per hectare. However, we must consider these estimates with caution. The risk of overestimating bioextraction increases when extrapolating from dispersed longlines to higher densities because intensive cultivation reduces the water flow delivering nutrients, and thus the tissue N content, but values from low-density field studies do not reflect this (Kerrison *et al.* 2015; Marinho *et al.* 2015). Additionally, higher density cultivation could exceed the environmental or social carrying

capacity<sup>1</sup> for kelp aquaculture in the region, which is why we have both evaluated a range of longline densities and underline the need for integrated management of N pollution.

The *timing* of kelp farm deployment and harvesting also influences bioextraction services of kelp grown in the WGoM. For example, from February through early May 2019, even as percent tissue N decreased throughout the spring, the biomass increased, and thus, so did the potential N removed through the harvest of all cultivated kelp. However, biomass did not increase in the same way during the last couple weeks of May 2019 due to reduced growth rates and sloughing, possibly associated with ambient water temperatures exceeding 10°C. Therefore, to maximize the N extracted in 2019, the sugar kelp should have been harvested in early May rather than late May. The most dramatic example is from the Wood Island site in 2019, where harvesting one month earlier would have doubled N removal (27.4 kg ha<sup>-1</sup> vs. 51 kg ha<sup>-1</sup>). However, these gains also appear to vary by site. At the other sites that same spring, harvesting 3 – 4 weeks earlier would have resulted in 3 – 22 kg ha<sup>-1</sup> more N removed. Additionally, in 2018 the highest estimates of N removal were obtained in late May, possibly because the ambient water temperature did not reach 10°C until that time. This highlights an opportunity for active monitoring of the ambient DIN and dissolved inorganic carbon at kelp aquaculture installations and N and C content in the kelp tissue. Using real-time estimates of N removal and ambient environmental conditions to schedule harvesting could maximize bioextraction effects.

Even with optimizations to harvest timing and density of longline arrays, kelp bioextraction must be part of a comprehensive N management strategy. Human activity has added reactive N to the landscape and changed nearshore habitats in ways that enhance N

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<sup>1</sup> Carrying capacity is a system's ability to tolerate activity without unacceptable impact to ecosystem characteristics like wildlife movement and habitat, recreational and commercial uses, water circulation, viewsheds, and other cultural uses (Group of Experts on the Scientific Aspects of Marine Environmental Protection 1986).

delivery to coastal ecosystems (Cleveland *et al.* 1999; Galloway *et al.* 2004). Comparing the maximum N potentially removed by harvesting a hectare of *S. latissima* to sources of nitrogen loading in Casco Bay reinforces the magnitude of anthropogenic disturbance in the N cycle. Encouragingly, the hectare-level comparisons generated for Casco Bay suggest that kelp bioextraction may be an efficient in-water tool to intercept nonpoint source pollution like atmospheric N deposition which, again, can be 30 – 40% of total N load to Casco Bay (Castro *et al.* 2003; Sonoma Technology 2003). However, the application of N bioextraction technologies must only be an additional measure for mitigating anthropogenic impacts on the environment. It should not be an alternative to improved management of point source and nonpoint source N by reducing combustion of fossil fuels, decreasing the application of N-based fertilizers, and tertiary treatment of wastewater. Using kelp aquaculture to remediate any substantial quantities of N will require a considerable shift in social acceptance of marine development and would have to be carefully evaluated against other commercial and ecological needs for this bay.

### **3.5.2 Environmental conditions**

Careful consideration of environmental variables' potential effect is important when anticipating how potential yields and nutrient concentrations reported by this study might vary. Many of the measured environmental conditions exhibited patterns like those reported by Kim *et al.* (2014, 2015) and Yarish *et al.* (2017), however ambient salinity at our sites exhibited more dramatic swings than those observed in Long Island Sound. Mean ambient salinity measured at each sampling event declined from 30 to 23 in Saco Bay and 32 to 29 in Casco Bay in March and April, and salinity dropped as low as 16 – 17 at Cow Ledge and Wood Island in mid-April. This decline in salinity is earlier and steeper than the lowest salinities of 22 – 26 that Kim *et al.* observed

at their sites in May. This discrepancy is notable regarding the timing of stress on the kelp crop. *Saccharina latissima* is semi-euryhaline; it can withstand 23 – 35 with no reduction in growth (Druehl 1967; Bartsch *et al.* 2008), but stress responses often develop at salinities below this range. A sharp decline in growth occurred in *S. latissima* in salinities consistently below 16 (Bartsch *et al.* 2008; Nielsen *et al.* 2014), and Gordillo *et al.* (2002) found that the closely related, *Laminaria digitata*, exhibited reduced nitrate uptake rates in low salinity conditions. Thus, the spring flush timing leading to freshening events in the WGoM could have affected the growth and tissue composition of the sampled kelp and may ultimately impact the potential N removed by kelp in this region.

The potential impact of combined stressors should also be considered when interpreting results from this study. No statistically significant relationships were observed between percent tissue N,  $\delta^{15}\text{N}$ , and the measured environmental conditions. One explanation for this may be that an alga's tolerance range for one environmental factor may be influenced by other environmental factors (Hurd *et al.* 2014). For example, when Mortensen (2017) grew *S. latissima* and *L. digitata* in water enriched with nitrate and phosphate, the algae survived almost two weeks in brackish water (salinity = 18). In our study, one or several of the environmental conditions measured were less than optimal for *S. latissima* growth at some point during the growing seasons. For instance, temperatures at the sampling sites did not reach the 5 – 15 °C optimal growth range for *S. latissima* (Fortes and Lüning 1980; Bolton and Lüning 1982; Kim *et al.* 2015; Yarish *et al.* 2017) until mid-March. Photosynthetically active radiation measured during some sampling events was lower than the light-saturating level of 150 – 215  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  reported for adult *S. latissima* sporophytes (Lüning 1979; Bartsch *et al.* 2008). Similarly, the range of current speeds (3 – 54  $\text{cm s}^{-1}$ ) during periods of 2018 and 2019 seasons is broader than

the optimal 10 – 25 cm s<sup>-1</sup> flow rate for *S. latissima* (Kerrison *et al.* 2015). Lastly, initial sporophyte density (200 – 500 m<sup>-1</sup>) may have resulted in clumping and shading preventing adequate light and nutrients from reaching all sporophytes. The statistically significant effect of site on both the  $\delta^{15}\text{N}$  and percent tissue N observed in our results may be the result a combined stressor effect involving any of these ambient conditions and perhaps even other stressors that were not detected. Or inversely, the absence of a clear relationship between percent tissue N,  $\delta^{15}\text{N}$ , and may be because the algae were able to tolerate passing colder temperatures, low light, lower or higher current, or higher cultivation densities because the other environmental conditions were more than adequate.

### 3.5.3 Sources of nitrogen – stable isotopes

Mean  $\delta^{15}\text{N}$  measured in the kelp tissue did not show a clear indication that kelp grown and collected from Saco and Casco Bay took up N from anthropogenic sources. This finding contrasts with the general picture of coastal WGoM dynamics presented by Castro *et al.* (2003), Liebman *et al.* (2012), and Trowbridge *et al.* (2014). The absence of a clear N source relationship is also dissimilar to conclusions presented by Kim *et al.* (2015), who described clear indications that anthropogenic N-sources were taken up by *S. latissima* in the Bronx River Estuary (-2 – 6‰) and Long Island Sounds (9 – 19‰). The interannual, site-specific means  $\delta^{15}\text{N}$  for samples from Brothers Island (7.6 ‰), Cow Ledge (6.6‰), Ram Island (6.3‰), and Wood Island (5.7‰) sites fell within the  $\delta^{15}\text{N}$  ranges commonly attributed to N of marine origin (4 – 8‰) (Fig. 5). However, the high-end of the  $\delta^{15}\text{N}$  range measured in kelp grown at Brothers Island (4.7 – 11.51‰) spans into the  $\delta^{15}\text{N}$  values commonly attributed to N from treated wastewater (10 – 12‰). In Saco Bay, the low end of the  $\delta^{15}\text{N}$  range measured in kelp grown at Wood Island reached into the  $\delta^{15}\text{N}$  values commonly attributed to N from fertilizers (-3 – 3‰).

Looking at  $\delta^{15}\text{N}$  by bay (Fig. 6), the range of tissue  $\delta^{15}\text{N}$  in Saco Bay *S. latissima* reached its lowest values, indicative of N originating from fertilizer, in February. Also, during February, some measurements of tissue  $\delta^{15}\text{N}$  in Casco Bay *S. latissima* had values indicative of N originating from treated wastewater (> 10‰) but the sample mean was much lower (5.78‰). The monthly mean tissue  $\delta^{15}\text{N}$  in Casco Bay *S. latissima* continued to rise through May. Nutrient bioavailability, *S. latissima* ecophysiology, or unmeasured environmental changes may have influenced these results obtained in the present study.

*Nutrient bioavailability* at the study sites, affected by flushing rates and uptake by wild species, may have also limited exposure of the sampled kelp to anthropogenic N. Slow N supply rates and low amounts of N substrate are key considerations for N isotope distributions in primary producers because they limit reactions important for growth (Peterson and Fry 1987). In N-limited systems, macroalgae do little fractionation of their source material during N uptake (Peterson and Fry 1987; Savage and Elmgren 2004; Thornber *et al.* 2008); all available N will be consumed regardless of isotope content so long as redox conditions remain relatively stable. Given the stable redox conditions in this well-mixed, highly oxygenated environment, we assume the observed  $\delta^{15}\text{N}$  values in the tissue were representative of the N source (Wada and Hattori 1978; Mariotti *et al.* 1982; Pennock *et al.* 1996). However, fractionation by some macroalgae has occurred in water with high DIN concentrations, which resulted in tissue- $\delta^{15}\text{N}$  values lower than that of the  $\delta^{15}\text{N}$  measured in the source N (Wada and Hattori 1978; Mariotti *et al.* 1982; Peterson and Fry 1987; Pennock *et al.* 1996; Wang *et al.* 2014). Examining the mean percent tissue N and the tissue C:N ratio in the *S. latissima* each month and at harvest indicates that there were periods during many of the growing seasons when the kelp was N-limited. In *S. latissima*, >3% DW tissue N content suggests N sufficiency, 1.9% is the minimum required for

maximal growth, and <1.3% DW tissue N indicates N limitation (Chapman *et al.* 1978; Wheeler and Weidner 1983; Kim *et al.* 2015). Therefore,  $\delta^{15}\text{N}$  values measured during or after a period of N-limitation may not be comparable to when N was replete in the kelp tissue (Aberle and Malzahn 2007). The natural assimilatory capacity and high flushing rates of the WGoM may also explain the absence of a clear anthropogenic isotopic signature in the cultivated *S. latissima* despite the known contributions of anthropogenic N. Additionally, the WGoM has large, naturally occurring, *Fucus* spp. and *Ascophyllum nodosum* beds in the intertidal and subtidal zones. These wild algae may have also intercepted some anthropogenic N before it reached the study sites.

The *nutrient ecophysiology* of the sampled kelp (*i.e.*, starving or N-saturated) may have affected fractionation rates that are crucial assumptions for the application of stable isotope ratio assessments of primary producers. Fernandes *et al.* (2012) found that large N reserves in algal tissue can mask the isotopic signal of newly acquired N, and kelp cells have large vacuoles enabling N storage. When ambient N is abundant, kelp cells can store N as nitrate in cellular vacuoles and cytoplasm (Fong *et al.* 1994). Then, they draw on these reserves when ambient N is low (Chapman and Craigie 1977; Egan and Yarish 1990). It is plausible that this nutrient ecophysiology resulted in a muddled  $\delta^{15}\text{N}$  that is not representative of recent N use. For example, if *S. latissima* took up and stored N from the marine environment in December – February, the stored N would have a  $\delta^{15}\text{N}$  reflecting that source. When this stored N was assimilated into algal tissue later in the spring, because ambient N was insufficient for the sporophytes' accelerated growth rates, the tissue sampled at that time would still exhibit a  $\delta^{15}\text{N}$  that was influenced by a marine N source despite the possibility that the algae could be using N from another source.

Cellular N reserves in the *S. latissima* could also explain why there were no statistically significant relationships between percent tissue N, tissue  $\delta^{15}\text{N}$ , and ambient nitrate at each site.

Lastly, undetected *environmental changes* in the N sources or at the study sites may have affected the  $\delta^{15}\text{N}$  results. The isotopic composition of N species within aquatic environments is affected by many environmental processes including assimilation, denitrification, nitrification, mineralization (Wada *et al.* 1975; Wada and Hattori 1978; McCready *et al.* 1983). Substantial changes in ambient environmental conditions can result in a shifted  $\delta^{15}\text{N}$  ratio for N sources, making it challenging to use stable isotope techniques to identify nutrient sources in field studies (Fry 2006; Wayland & Hobson 2001). For example, the presence, or pulses of, ammonium at the sites may help to explain why there was no correlation between ambient nitrate concentrations and  $\delta^{15}\text{N}$ . *Saccharina latissima* exhibits a preference for ammonium. Harrison *et al.* (1986) found that nitrate uptake in *S. latissima* was completely suppressed for 30 minutes following a pulse of ammonium. We assumed that any ammonium delivered to the sites would be immediately taken up, so we did not attempt to quantify ammonium in this study. However, frequent ammonium supplies or an ammonium pulse shortly prior to a sampling event may have also influenced N uptake rates or provided a contrasting  $\delta^{15}\text{N}$  signal.

Undocumented phytoplankton blooms are another example of an undetected environmental event that may be a source of variability influencing our dataset. Yarish *et al.* (2017) attributed low tissue N in kelp to a prolonged spring phytoplankton bloom, which may have been supported by mild winter conditions (*i.e.*, harsh winter and spring results in more DIN available for the macroalgae). Anderson *et al.* (2005) also found correlations between spring snowmelt and spring phytoplankton blooms in southern New England. Releases of N from  $^{15}\text{N}$ -depleted sediments would have also affected the  $\delta^{15}\text{N}$  measured in the kelp tissue (Altabet 2006;

Bianchi 2007; Sigman *et al.* 2009). Without knowledge or measurement of a release, it would be hard to correct for it when interpreting the data presented here. It is also possible that the natural variation between sites, or between published  $\delta^{15}\text{N}$  values for N sources and those in the WGoM, is so considerable that it exceeds the capacity of stable isotope analysis to differentiate between the N sources (Ostrom *et al.* 1997; Fry 2006). Due to logistical constraints, characterization of the  $\delta^{15}\text{N}$  in  $\text{NO}_3^-$  from specific N sources in Casco and Saco Bay was not possible. However, if future work can do this, it will reduce uncertainty regarding unmeasured environmental conditions and support the development of a stable-isotope specific mixing model for these locations.

Importantly, the isotope values reported in this study can help us to understand the current WGoM biogeochemistry and the existing degree of human perturbation in Casco and Saco Bay. If used in future studies, they will also help to better describe the direction and magnitude of nutrient cycling in the WGoM (Peterson & Fry 1987; Ostrom *et al.* 1997; Dethier *et al.* 2013). Establishing baseline stable isotope values for *S. latissima* in this region will help with the detection of potentially incipient eutrophication, which is preferable to restoration (McClelland *et al.* 1997). Additionally, if future studies can demonstrate a closer relationship between anthropogenic N pollution and bioextraction provided by kelp in the WGoM, it will garner stronger public support for cap and trade programs to include bioextraction as an eligible activity.

### **3.6 Conclusion**

Identifying and implementing effective nutrient management technologies is critical to mitigating the impact of human activities on coastal ecosystems. This study measured biomass,  $\delta^{15}\text{N}$ , and tissue N content of *Saccharina latissima* grown from 2016 – 2019 at four sites in

Casco and Saco Bay, Maine to better understand how the N bioextraction achieved by harvesting cultivated kelp varies across space and time. Although the patterns in elemental content of the *S. latissima* tissue from the WGoM are like those reported from further south, total biomass at time of harvest was higher. Significant variation in biomass and tissue N content was observed between sites between the two bays, potentially due to combined environmental stressors, or the timing of seasonal temperature and salinity changes between the bays. High variation in  $\delta^{15}\text{N}$  also occurred between sites, and the monthly and interannual mean  $\delta^{15}\text{N}$  did not show explicit use of anthropogenic N sources like wastewater or fertilizer. The absence of clear source-N relationships may be the result of physiological traits of *S. latissima*, biogeochemical characteristics of the WGoM, or unmeasured environmental changes. Our results further highlight the need for site-level pilot studies, even within the same bay, to characterize the seasonal and spatial variation of N assimilation before any kelp aquaculture is developed solely for bioextraction purposes in the WGoM. Finally, we extrapolated our results to estimate that harvesting cultivated kelp from the WGoM has the potential to extract  $19.2 (\pm 4.8) - 176.0 (\pm 7.7)$  kg N ha<sup>-1</sup> depending on the cultivation density used, which emphasizes the importance of cultivation density and harvest time on theoretical kelp aquaculture bioextraction efficiencies. We conclude that kelp farming and harvesting could be a component within a broader, integrated approach to N mitigation in the region, but a substantial increase in kelp production and social acceptance of aquaculture will be required.

## CHAPTER 4

### THE EFFECT OF DISTAL-END TRIMMING ON SACCHARINA LATISSIMA

#### MORPHOLOGY, COMPOSITION, AND PRODUCTIVITY

Grebe, G.S., Byron, C.J., Brady, D.C., St. Gelais, A.S, Costa-Pierce, B.A. The effect of distal-end trimming on *Saccharina latissima* morphology, composition, and productivity. *Journal of World Aquaculture Society*, In Press.

#### 4.1 Chapter abstract

As kelp cultivation increases around the world, so does the need for farm management strategies that produce specific crop characteristics, optimize yield, widen harvesting windows, and prevent biomass loss. Distal-end trimming of macroalgae has been recommended as a farm management method addressing these needs. In this study, we trimmed cultivated *Saccharina latissima* sporophytes grown in the Western Gulf of Maine (WGoM) to 60 cm above the stipe-blade interface. We characterized the effect of trimming on the morphology, tissue nutrient content, stable isotope ratio, and nitrate reductase activity of the kelp. We also evaluated the economic trade-offs of trimming using a simple production model. The results suggest that trimming the blade to 60 cm may have minimal biological consequences. Additionally, the trimming appears to benefit “short” kelp blades in proximity to the trimmed blades. Daily yield (% increase in weight day<sup>-1</sup>) after trimming was initially lower than the control, but late-season daily yields and crop-retention following storms were markedly improved. Ultimately, we conclude that growers could use trimming to acquire kelp biomass earlier in the season, retain late-season biomass, and potentially increase the total revenue gained from kelp farming if price premiums can be exacted for this biomass.

## 4.2 Introduction

Kelp farming in the Americas and Europe is on the rise (Augyte *et al.* 2017; Kim *et al.* 2019; Grebe *et al.* 2019; FAO 2020). Seaweed farmers in the United States produced an estimated 249 - 272 wet MT of farmed edible macroalgae in 2019; the majority of which was kelp (Piconi *et al.* 2020 and references therein). This growth is a ~ 20-fold increase from the estimated 11 – 14 wet MT harvested in 2015 (MEDMR 2019c; Piconi *et al.* 2020 and references therein). In Europe, kelp is now permitted as a species for cultivation on at least thirty aquaculture leases across Spain, France, The Netherlands, Denmark, Ireland, The United Kingdom, Sweden, and Norway (Camia *et al.* 2018; EMODnet 2020). Now, for the budding European and American kelp farming industry to grow beyond initial proof-of-concept, there is a need for additional optimization of farming and engineering practices that maximize algal yields, extend the harvest season to provide a consistent supply of kelp for buyers, and increase revenue for kelp farmers (Handå *et al.* 2013; Boderskov *et al.* 2016; Rolin *et al.* 2017; Fredriksson *et al.* 2020).

Distal-end trimming, a crop management strategy in which a portion of the older blade is removed using a transverse cut, may be a practice that can help bring the industry closer to these necessary yields, consistency, and revenue gains. In northern China, distal-end trimming has been widely practiced on *Saccharina japonica* farms for decades, and it has been shown to improve the health of *S. japonica* blades and minimize the occurrence of diseases by increasing the light availability, water flow, and nutrients around the blades (Tseng 1962, 1981, 1986; Wu & Zheng 1981; Scoggan *et al.* 1989). Furthermore, the economic benefits of the trimming are understood to be the capture of biomass that would otherwise be lost to natural blade erosion or storms, an extended growing season, and reduced production costs (Wu 1962, Wu & Zheng

1981; Scoggan *et al.* 1989). More specifically, natural shedding can result in the loss of up to 30% of total seasonal biomass production on these *S. japonica* farms, but distal-end trimming captures this material before shedding occurs in late spring (Tseng 1962, 1986; Wu 1962, Wu & Zheng 1981; Scoggan *et al.* 1989). In more recent studies, Gao *et al.* (2013b) found that distal-end trimming extended the growth phase of *Undaria pinnatifida* in Japanese waters by 1 month and Bak *et al.* (2018) calculated that trimming could reduce that total cost per kg of wet cultivated kelp by more than  $\frac{2}{3}$  because it increased the number of harvests possible without reseeded (Bak *et al.* 2018).

Conversely, several studies examining the effect of trimming on other algal species suggest that distal-end trimming can result in biological disadvantages. In Laminariales, translocation brings photosynthates and nutrients from their point of production or uptake in the mature, distal blade to the growing tissue in the basal region (Parker 1963, 1965; Tseng 1986; Schmitz & Lobban 1976; Wu & Zheng 1981; Davison & Stewart 1984). So, one potential disadvantage of distal-end trimming is the decline of translocated photosynthates and nutrients which may potentially result in periods of decreased growth. Wu *et al.* (1981) observed a close relationship between trimming and growth in the intercalary region of *S. japonica* blades; the length and weight of *S. japonica* were not diminished if only  $\frac{1}{3}$  of the distal end was removed but removing any more material than this resulted in reduced growth in length. Several experiments with wild *Nereocystis luetkeana* reported reduced growth rates up to two weeks following blade trimming; presumably, again, because the amount of photosynthate translocated back to the growth region was reduced (Nicholson 1970; Schmitz & Lobban 1976; Nicholson & Briggs 1972; Roland 1985). In some instances, it appears that the deficiencies resulting from the removed blade area are too great for the organism to overcome. For example, trimming

*Saccharina diabolica* < 30 cm from the base of the blade had a strong negative impact on its growth and total yield (Sanbonsuba *et al.* 1987). Rolin *et al.* (2017) trimmed *Laminaria digitata* 10 cm from the base of the blade and it did not regrow. In contrast, trimming wild *Ecklonia maxima* 10 – 30 cm above the base of the primary blade every 4 months has been shown to result in a 4 to 5-fold increase in total yields (Levitt *et al.* 2002).

Only a few studies have investigated the effect of distal-end trimming on *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders; despite its current status as one of the most widely cultivated macroalgal species in Europe and North America (Yarish *et al.* 2017; Grebe *et al.* 2019, 2021; EMODnet 2020). In one study, Rolin *et al.* (2017) grew *S. latissima* (and *L. digitata*) off the coast of the Shetland Islands, and then trimmed the kelp thalli 10 cm above the stipe-blade transition zone. The trimmed *S. latissima* regrew and did not show the same summer erosion or fouling as the control blades (Rolin *et al.* 2017). A second study, Bak *et al.* (2018), grew and trimmed *S. latissima* (and *Alaria esculenta*) near the Faroe Islands down to 5 – 15 cm from the stipe-blade transition zone to ensure preservation of the meristematic region. In the end, Bak *et al.* (2018) trimmed the same *S. latissima* thalli four times in a season and calculated that trimming could reduce that total cost per kg of wet cultivated *S. latissima* by more than two-thirds because it increased the number of harvests possible without reseeded (Bak *et al.* 2018). The results of these initial studies suggest that distal-end trimming has promise as an improved farming practice for *S. latissima*, but more understanding of the species-specific physiological response to this technique is needed (Rolin *et al.* 2017; Bak *et al.* 2018; Grandorf Bak 2019).

Before incorporating distal-end trimming into existing *S. latissima* farm management, it is important to know if, and when, the practice stimulates growth or other physiological changes

in the remaining sporophyte. Several studies have used changes in morphology or metabolic processes as clues. For instance, Gao *et al.* (2013b) removed most of the thallus (trim location 30 cm above the stipe-blade interface) of *U. pinnatifida* and observed increased gross photosynthetic rates in the remaining thallus for several months afterward. Although they were not directly studying distal-end trimming, Burnett & Koehl (2019) punched a 1 cm x 4 mm hole through the rachis of wild *Egregia menziesii* to mimic herbivore damage, and they observed that the rachis grew wider near the location of the injury. We propose that changes in thallus morphology and blade composition, and more specifically differences in blade %N, %C,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , C:N, or NRA may serve as additional indicators of trimming-induced stress. A reduced %N or %C could indicate an impact on the organism's ability to sustain itself with the remaining blade. If this N or C stress was severe, then the trimmed blades would have a lower  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$  in their basal tissue when compared to control blades of the same length because when N or C is abundant, algae prefer to use lighter  $^{14}\text{N}$  and  $^{12}\text{C}$  isotopes for their metabolic processes (Peterson & Fry 1987; Savage & Elmgren 2004; Thornber *et al.* 2008). If combined with other morphological or compositional observations, a shift in C:N ratio in the blade post-trim might also serve as an indicator of increased production of defense compounds (Royer *et al.* 2013) and elevated nitrate reductase activity in the remaining blade might signal a shift in nitrogen metabolism of the thalli (Hurd *et al.* 1995).

In this study, we explored the effect of distal-end trimming on the morphology, composition, and yields of cultivated *S. latissima* to help kelp farmers weigh the costs and benefits of incorporating this practice into their operations. Eager to build on the recent studies by Rolin *et al.* (2017) and Bak *et al.* (2018) and the best practices reported by Wu *et al.* (1981), we increased the length between the stipe-blade interface and the trim to 60 cm. We

hypothesized that kelp trimmed to this length would exhibit an injury response to the trimming by growing wider near the trim. We also hypothesized that, even with an increased trimming length, the trimmed kelp tissue would have a lower %C, %N,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  resulting from the reduction of blade area for photosynthesis and nutrient uptake. Our third hypothesis was that nitrate reductase, one of the key enzymes for N assimilation in algae, would increase in the remaining (basal) regions of the trimmed blades as the kelp attempted to meet the nutrient demands of the growth region despite a reduced blade area. Our fourth hypothesis was that daily yield from the trimmed sections of *S. latissima* would be lower than the control sections due to our previous hypotheses about injury response and reduction of blade area. Lastly, we sought to evaluate the economic tradeoffs of an expanded *S. latissima* harvest season with smaller, more frequent harvests by developing a model of biomass production from kelp aquaculture in the state of Maine, USA with varying growth rates, total production, and sale prices.

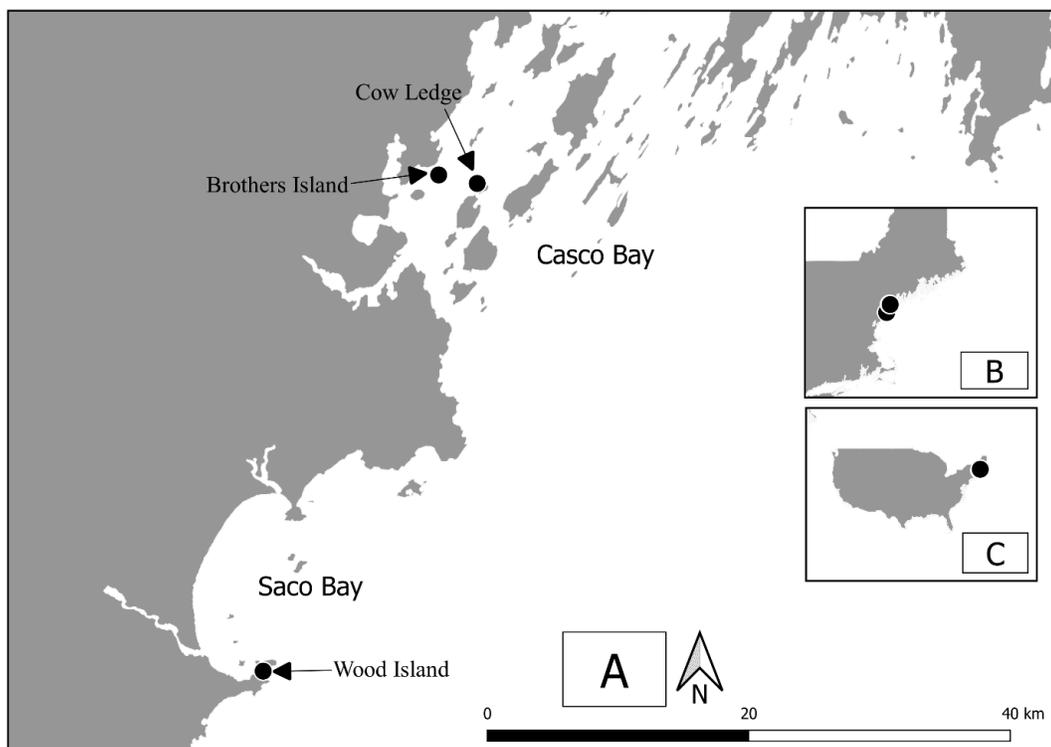
## **4.3 Materials and methods**

### **4.3.1 Site characteristics**

*Saccharina latissima* was cultivated at three sites in the Gulf of Maine, USA (Fig. 4.1). One site, Wood Island (43.4553, -70.3367), was in Saco Bay, and two sites, Cow Ledge (43.7025, -70.1877,) and Brothers Island (43.6968, -70.2095), were in Casco Bay. Site depth ranged from 8 to 17 m Mean Lower Low Water. Water temperature at each site was continuously recorded using suspended loggers (Hobo Pendant Temperature/Light Loggers; UA-002-08). Precipitation data for Saco Bay were obtained from the weather station located at the Arthur P. Girard Marine Science Center on the University of New England's Biddeford, ME campus. Regional wind and precipitation data for Casco Bay were retrieved from the National Oceanic and Atmospheric Administration's (NOAA) Climate Data Online (Station

USW00014764; Portland Jetport). Surface solar radiation downwards (SSRD) was obtained from the National Aeronautics and Space Administration (NASA) Earth Observing System data collection. Current velocities at the sites were estimated using an inverted Tilt Current Meter (Lowell Instruments LLC) suspended from the longline spacer buoys.

Thirty sampling trips were completed in total: 9 during Spring 2018 and 21 during Spring 2019. Salinity, pH, and nitrate-N concentrations at each cultivation site were characterized using grab samples collected from 2 m depth during each sampling trip. Salinity was measured with a refractometer (Cole-Parmer RSA-BR90A; 0 – 90%). Water pH was assessed using a benchtop meter (Hach model #: PW172KB0703F01) calibrated to certified standards. Nitrate-N at the site was quantified spectrophotometrically (Hach Nitrate TNTplus Low Range Vial Tests; Hach DR3900 VIS spectrophotometer). Photosynthetically active radiation was also measured during these sampling trips using a spherical quantum sensor (LI-COR LI-193). Readings were taken above the water surface and at 2 m deep. The difference between the readings was used to determine light attenuation.



**Figure 4.1** *Saccharina latissima* cultivation and sampling sites in relation to: A) Saco Bay and Casco Bay, B) the Gulf of Maine, C) Northeastern USA. A single, 60 m longline was deployed at Wood Island, two 120 m longlines were deployed at Brothers Island, and six 120 m longlines were deployed at Cow Ledge. Brothers Island and Cow Ledge are < 3 km apart. Samples were collected from Wood Island and Cow Ledge in 2018, and from all three sites in 2019.

### 4.3.2 Kelp cultivation

Kelp sporelings were produced using the techniques described in Redmond *et al.* (2014). In brief, reproductive *Saccharina latissima* tissue was collected from wild beds in Casco Bay via boat using a hand-rake. In the lab, the sorus tissue was wiped dry, treated with betadine, placed between layers of paper towels, and kept cool overnight to induce spore release. Inoculation water was prepared to approximately 6,000 – 8,000 spores mL<sup>-1</sup>. The spores settled on thin nylon

button twine (Ludlow/A&E brand). Temperature and light-controlled aquaria were used to grow the sporophytes until they were deployed at sea in November 2018 and December 2019. At deployment, the sporophyte-covered string was wound around a thicker (1.25 cm) diameter longline. A single, 60 m longline was deployed at Wood Island in 2018 and 2019. This longline was oriented parallel to the prevailing current (east-west). Fredriksson *et al.* (2020) provide an additional description of the hydrodynamics of this site. Two 120 m longlines were deployed at Brothers Island in 2019 and Cow Ledge in 2018. In 2019, five 120 m longlines were deployed at Cow Ledge. The longlines at Brothers Island and Cow Ledge were oriented parallel to the prevailing current (north-south) and spacing between the longlines at the same site was  $\geq 6$  m. All longlines were maintained at a depth of 2 m using buoys attached to rigid PVC spacers. After outplanting, the sporophytes were left untouched until trimming was initiated.

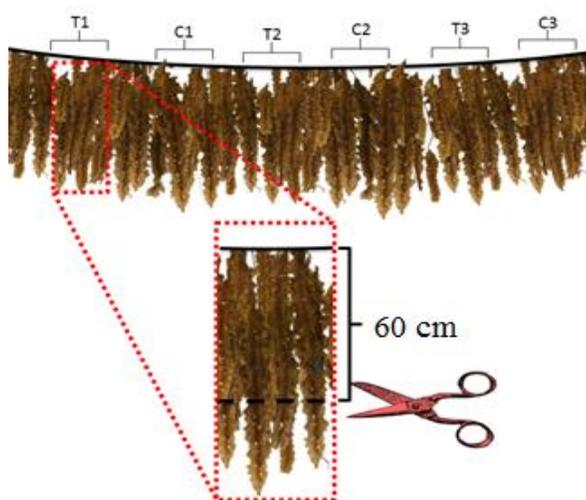
#### **4.3.3 Trimming technique**

Trimming treatments were initiated in late March of 2018 and 2019 and continued through mid-May 2018 and 2019. During each trimming event, three 1-m sections of the line were haphazardly designated for trimming (Fig. 4.2). We marked the start and end of these sections by attaching different-colored zip ties to the longline. In these sections, the kelp thalli were trimmed to approximately 60 cm in length using scissors. The basal end, stipe, and holdfast were left attached to the longline. The longline outside the trimming sections was left uncut to serve as a control. Overall, we conducted 12 trimming events.

Approximately 8 - 20 days after trimming, depending on weather, 10 cm of longline was randomly subsampled from within the trimmed section. All thalli from this subsection were harvested for analysis. A nearby section of longline was used for harvesting a subsample of the control (untrimmed) thalli. This section was at least 2 meters from any previous harvesting to

ensure that there was no spill-over effect of prior trimming or sample harvesting on the control. The harvested thalli were transported in plastic bags in a covered cooler at 8 - 10°C to the laboratory where they were stored in the refrigerator at 8 - 10°C and processed within 24 hours of collection.

We returned to the same trimmed sections throughout the growing season to see how the characteristics of the trimmed kelp changed. We used the different-colored zip ties to quickly identify the same trimmed sections from one sampling event to the next. At each visit, we collected new, 10 cm wide subsamples. We were able to sample the earliest trimmed sections up to nine times before the longlines were removed from the water in June. The later-trimmed sections were sampled a minimum of three times.

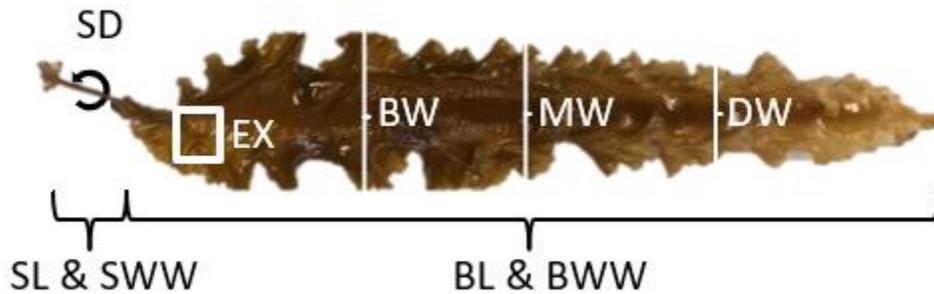


**Figure 4.2** Schematic of *Saccharina latissima* longline depicting relative positioning of trimmed (T) and control (C) sections (figure not to scale). Trimming treatments were administered to three 1-m sections of the line by trimming all thalli in the section to approximately 60 cm in length (measured from the stipe-blade transition zone). Three other sections of the longline were left untrimmed as controls. Then, 8 – 20 days after each trimming event, all thalli from a 10 cm section of each trimmed or control section were harvested for analysis.

#### 4.3.4 Processing and analysis

During each sampling event, five thalli from the trimmed subsection and the control subsection were preserved for nitrate reductase analysis immediately after removing them from the water. A 5 x 5 cm section of tissue was excised from the basal region of individual kelp blades, frozen in liquid N, and stored at -80°C until processed. The individuals were haphazardly selected with one exception; the basal portion of the thalli had to be  $\geq 7$  cm wide so that the 5 x 5 cm section could be removed. The nitrate reductase activity (NRA) of each tissue sample was measured using the methodology described by Young *et al.* (2005) and nitrite produced during the process was measured using a Hach 3900 spectrophotometer and TNT 880 nitrite vials.

Twenty individuals from each treatment group were analyzed for changes in morphology: the five thalli selected for NRA and an additional fifteen individuals haphazardly selected. Morphology measurements (Fig. 4.3) were made by caliper, a fish measuring board, and a precision balance. Total blade length (BL), width at  $\frac{1}{4}$  of the blade (basal-region) (BW), width at  $\frac{1}{2}$  of the blade (mid-region) (MW), width at  $\frac{3}{4}$  of the blade (distal-region) (DW), stipe length (SL), stipe diameter (SD), stipe wet weight (SWW), and blade wet weight (BWW). A representative surface area to weight ratio was used to calculate the mass removed by the basal tissue sample, and this weight was added to the measured total weight to correct for the removed tissue.



**Figure 4.3** Location of morphological measurements for *Saccharina latissima*. The recorded characteristics included: total blade length (BL), width at  $\frac{1}{4}$  of the blade (basal region) (BW), width at  $\frac{1}{2}$  of the blade (mid region) (MW), width at  $\frac{3}{4}$  of the blade (distal region) (DW), stipe length (SL), stipe diameter (SD) stipe wet weight (SWW), and blade wet weight (BWW). The location of tissue excised for carbon and nitrogen stable isotope analysis and nitrate reductase analysis is also indicated (EX). The location of the BW measurement (0.25L) was intended to capture maximum blade width (Vettori and Nikora 2017).

Next, the samples that were analyzed for NRA were also analyzed for C and N content and stable isotope ratios. A second tissue sample was excised from the basal region of each blade, as close as possible to the original excision. This tissue was rinsed with deionized water, frozen, and then lyophilized at  $-50^{\circ}\text{C}$  for 24 hours. Each sample was ground with a mortar and pestle and encapsulated in tin (2.5 – 5 mg). These samples were analyzed by the UC Davis Stable Isotope Facility (SIF) using an elemental analyzer (PDZ Europa ANCA-GSL) interfaced with an isotope ratio mass spectrometer (PDZ Europa 20-20). Sample precision was 0.2‰ for  $^{13}\text{C}$  and 0.3‰ for  $^{15}\text{N}$  (UC Davis SIF 2020). The total C and N content measured in the samples was divided by the dry sample weight, to obtain %C and %N of the dry excised tissue sample.

The UC Davis SIF calculated the stable isotope ratios for each sample by comparing the difference in the  $^{15}\text{N}$  or  $^{13}\text{C}$  measured in the sample against the  $^{15}\text{N}$  or  $^{13}\text{C}$  measured in at least four different laboratory reference materials (Eqn. 1) (Peterson & Fry 1987):

$$\text{Equation 1: } \delta^{15}\text{N or } \delta^{13}\text{C} = ((R \text{ sample}/R \text{ reference}) - 1) \times 1000 (\text{‰}),$$

where R equals  $^{15}\text{N}/^{14}\text{N}$  or  $^{13}\text{C}/^{12}\text{C}$ .

#### 4.3.5 Statistical analysis

One-way analysis of variance (ANOVA) was used to determine the effect of sampling site on environmental characteristics, and whether the data from different sites could be combined for analysis. Determining whether samples across sites could be pooled was important because site-specific environmental conditions (*i.e.*, temperature, water motion, light, and nutrients) and their interactions affect seasonal growth patterns, morphology, and productivity of kelp (Gerard 1987; Egan & Yarish 1990; Hymanson *et al.* 1990; Hurd 2000). The morphological and compositional data were sorted into blades harvested from a trimming section or a control section. Distributions for each group were plotted and tested for normality (Shapiro-Wilk) and equal variance (Levene's test) (Levene 1960; Shapiro & Wilk 1965). When the distributions and variance were normal, Tukey's HSD post-hoc tests were used to test the difference in the means between the trimmed treatment and the control. Non-parametric tests (Wilcoxon signed rank) were used when assumptions of normality and equal variance were not met. Significance levels of 0.05 were used for all tests unless otherwise indicated. Bivariate regression analyses were used to explore the relationship between the environmental and morphological data. Statistical analyses were conducted using JMP Pro V. 14.2 (SAS Institute) and RStudio V.1.2.1335 (RStudio, PBC).

#### 4.3.6 Daily yield and the production and harvesting model

We calculated biomass (wet kg m<sup>-1</sup>) and daily yield (%) for each sampling date. Biomass was calculated as wet weight (WW) per longline meter by weighing the complete 10 cm sample and linearly extrapolating to 1 m of longline. Daily yield (DY) was calculated using a Ricker relationship (Eqn. 2) (Ricker 1979):

$$\text{Equation 2: } DY (\% \text{ day}^{-1}) = (\ln(X_t) - \ln(X_0))/t \times 100$$

where  $\ln(X_t)$  is the natural logarithm of the wet biomass weight per longline meter at time  $t$  and  $\ln(X_0)$  is the natural logarithm of the initial weight. Daily yield integrates new production, erosion, and sloughing and so is not solely an indicator of growth rate. Bivariate regression analyses were used to explore the relationship between the environmental observations and daily yield.

The daily yield was used as an input in the production and harvesting model. To generate production and harvest estimates, we assumed that the observations of daily yield for trimmed and control *S. latissima* were applicable across the WGoM. Additional economic inputs were determined using a recent market analysis for U.S. seaweed production (Piconi *et al.* 2020). They included: 1) Maine's farmed edible seaweed production in 2019 was 147 t (WW), 2) presently harvesters can expect \$880 - \$1,540 MT<sup>-1</sup> for bulk unprocessed seaweed, and 3) high-end projections suggest Maine's farmed edible seaweed production could reach up to 2,722 MT (WW) by 2035 (Piconi *et al.* 2020). We set April 1st as the model start date to reflect the approximate initiation of trimming treatments in the field experiment and May 15th as the end date because the large majority of farmed *S. latissima* harvesting in the WGoM currently occurs in mid to late May. To determine the starting biomass for current scenarios we used the highest

daily yield consistently measured ( $6\% \text{ day}^{-1}$ ) in the control sections from April 1 to May 15, and back-calculated from the total production reported in 2019 (147 MT) to obtain an estimate of 133 MT on April 1<sup>st</sup>. This same methodology was used for the future scenarios; back-calculating from the forecasted 2,722 MT of kelp that could be produced by 2035 using a 6% daily yield to set a starting biomass of 2,708 MT on April 1<sup>st</sup>.

We examined the sensitivity of model output as a function of price per MT of *S. latissima*. In ten scenarios, the price per MT was constant at  $\$880 \text{ MT}^{-1}$ ,  $\$1,210 \text{ MT}^{-1}$ , or  $\$1,540 \text{ MT}^{-1}$ . In two additional scenarios, we allowed the price of harvested kelp to vary as a function of harvest timing (from  $\$880 \text{ MT}^{-1}$  to  $\$1,540 \text{ MT}^{-1}$ ). These latter scenarios were developed to incorporate the possibility that the regional sale price of raw kelp may be inversely related to supply or directly related to its morphological or nutritional characteristics. Incorporating the observed daily yields, economic analysis, and price variability resulted in the consideration of twelve scenarios (Table 4.1).

**Table 4.1** Description of each scenario used in *Saccharina latissima* biomass production and harvesting model. Business as usual (BAU) is one final harvest with no trimming or intermediate harvests of kelp. Daily yield (DY) is % increase in weight per day.

Scenario
<b>1A:</b> BAU with 6% DY, low sale price (\$880 WW t <sup>-1</sup> ) on May 15 harvest
<b>1B:</b> BAU with 6% DY, high sale price (\$1540 WW t <sup>-1</sup> ) on May 15 harvest
<b>2A:</b> BAU, 6% DY, full early harvest (April 1) at high sale price (\$1540 WW t <sup>-1</sup> )
<b>2B:</b> BAU, 6% DY, full mid-window harvest (May 1) at middle sale price (\$1210 WW t <sup>-1</sup> )
<b>2C:</b> BAU, 6% DY, full late-window harvest (May 15) at low sale price (\$880 WW t <sup>-1</sup> )
<b>3A:</b> BAU, 6% DY April 1- May 1, -3% DY for May 1 – 15, full late-window harvest (May 15) at low sale price (\$880 WW t <sup>-1</sup> )
<b>4A:</b> Trim 30% April 1 with 3% DY after trim, low sale price (\$880 WW t <sup>-1</sup> ) for both April 1 and May 15 harvest
<b>4B:</b> Trim 30% April 1st, 3% DY after trim, high sale price for early harvest (\$1540 WW t <sup>-1</sup> ) and low sale price (\$880 WW t <sup>-1</sup> ) for late harvest
<b>4C:</b> Trim 30% April 1 with 3% DY after trim, higher price (\$1540 WW t <sup>-1</sup> ) for both April 1 and May 15 harvest
<b>5A:</b> Increased production in 2035, harvest May 15 with - 3% DY May 1 -15
<b>5B:</b> Increased production in 2035, remove 30% April 1 with 3% DY after trim, high sale price (\$1540 WW t <sup>-1</sup> ) for April 1 harvest and low sale price (\$880 WW t <sup>-1</sup> ) for May 15 harvest
<b>5C:</b> Increased production in 2035, remove 30% April 1 with 3% DY after trim, high sale price (\$1540 WW t <sup>-1</sup> ) for April 1 and May 15 harvest

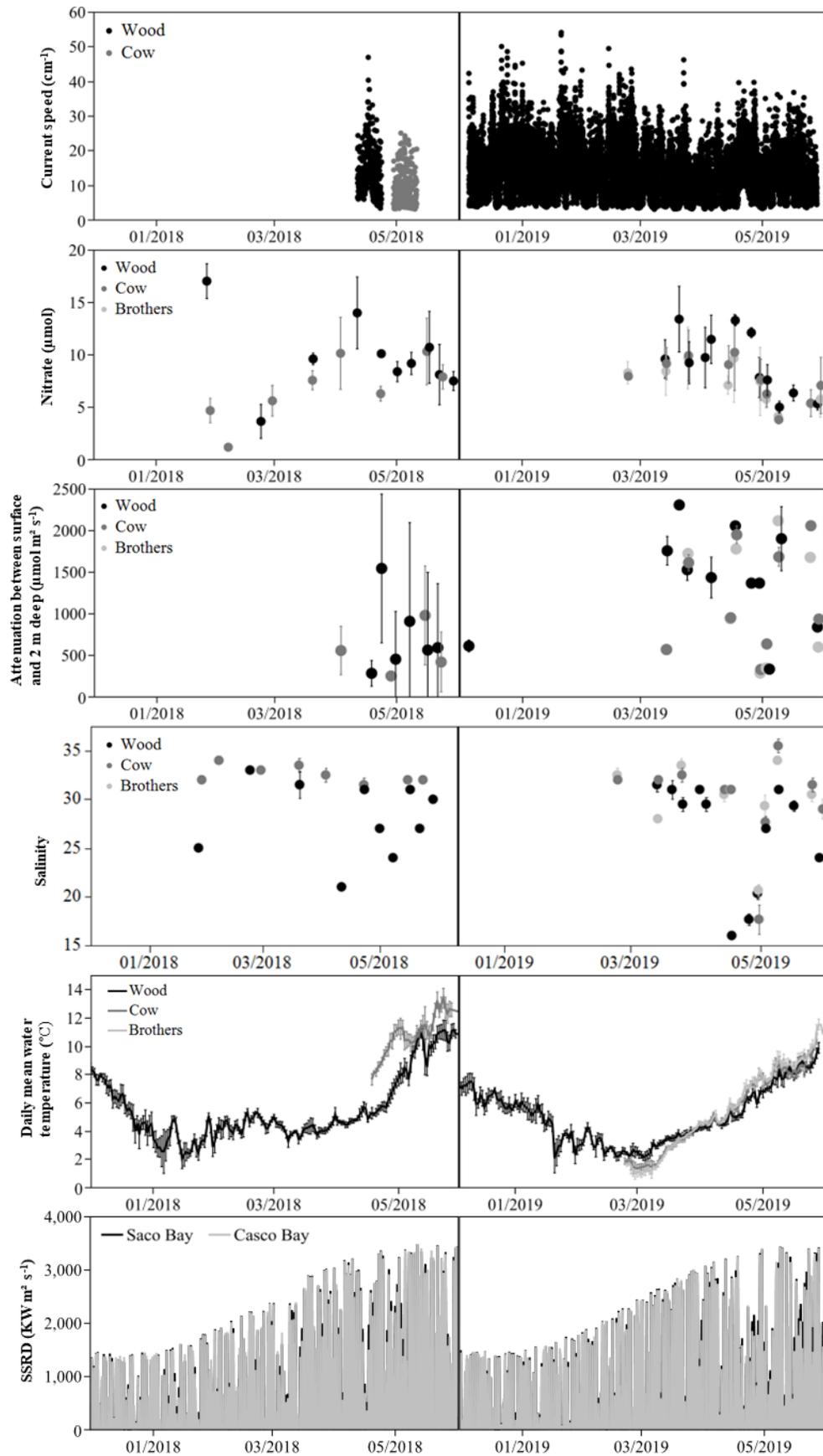
## 4.4. Results

### 4.4.1 Environmental conditions

Environmental conditions measured during the 2018 and 2019 cultivation periods were significantly different between the Cow Ledge, Brothers Island, and Wood Island sites. There was a significant effect of “site” on ambient temperature [ANOVA;  $F(2,584) = 12.0, p < 0.001$ ], salinity [ANOVA;  $F(2,709) = 40.8, p < 0.001$ ], pH [ANOVA;  $F(2,710) = 15.7, p < 0.001$ ], and nitrate [ANOVA;  $F(2,704) = 13.9, p < 0.001$ ]. Due to this high amount of environmental variability, the response variables (*i.e.*, morphology, tissue composition, NRA, biomass, daily yield) were not pooled and observations from Spring 2018 and Spring 2019 were also treated as separate. Overall, Saco Bay (Wood Island) had higher ambient nitrate and minimum water temperatures, but lower salinity and maximum water temperatures than Casco Bay (Cow Ledge and Brothers). More specifically, during the growing season mean daily water temperature ranged from 1 – 14 °C across sites (Fig. 4.5). Salinity ranged from 16 to 35. The lowest salinities were observed in early May 2018 and late April 2019. Salinity levels at Wood Island were generally lower than those at Cow Ledge and Brothers Island. Sea surface radiation (SSRD) was similar between Casco and Saco Bays, although in many instances Saco Bay had slightly more SSRD than Casco Bay on the same given day. Nitrate levels in the water column ranged from 1.2 – 17  $\mu\text{M}$  and they varied by season, with the highest levels of nitrate recorded at Wood Island and the lowest levels observed at Brothers Island. A decline in ambient nitrate was observed in both May 2018 and 2019, with an earlier and more severe decline in May 2019. The range of current velocities recorded at Wood Island and Cow Ledge were similar (5 – 54  $\text{cm s}^{-1}$  and 3 – 50  $\text{cm s}^{-1}$ , respectively). Current data is not available for Brothers Island due to logger battery

failure, but the current velocities are likely similar to those at Cow Ledge because the sites are close and most of the variability in current speed within the region is driven by tidal cycles.

**Figure 4.4 (next page)** Environmental conditions measured at Wood Island (black), Cow Ledge (dark gray), and Brothers Island (light gray) sites during the 2018 and 2019 *Saccharina latissima* growing seasons. The panels presented are: A) daily mean current velocity, B) mean nitrate measured at each sampling event, C) mean PAR attenuation measured at each sampling event, D) mean salinity measured at each sampling event, E) mean daily water temperature, and F) maximum daily surface solar radiation downwards (SSRD). Error bars are standard deviation.



#### 4.4.2 Morphology

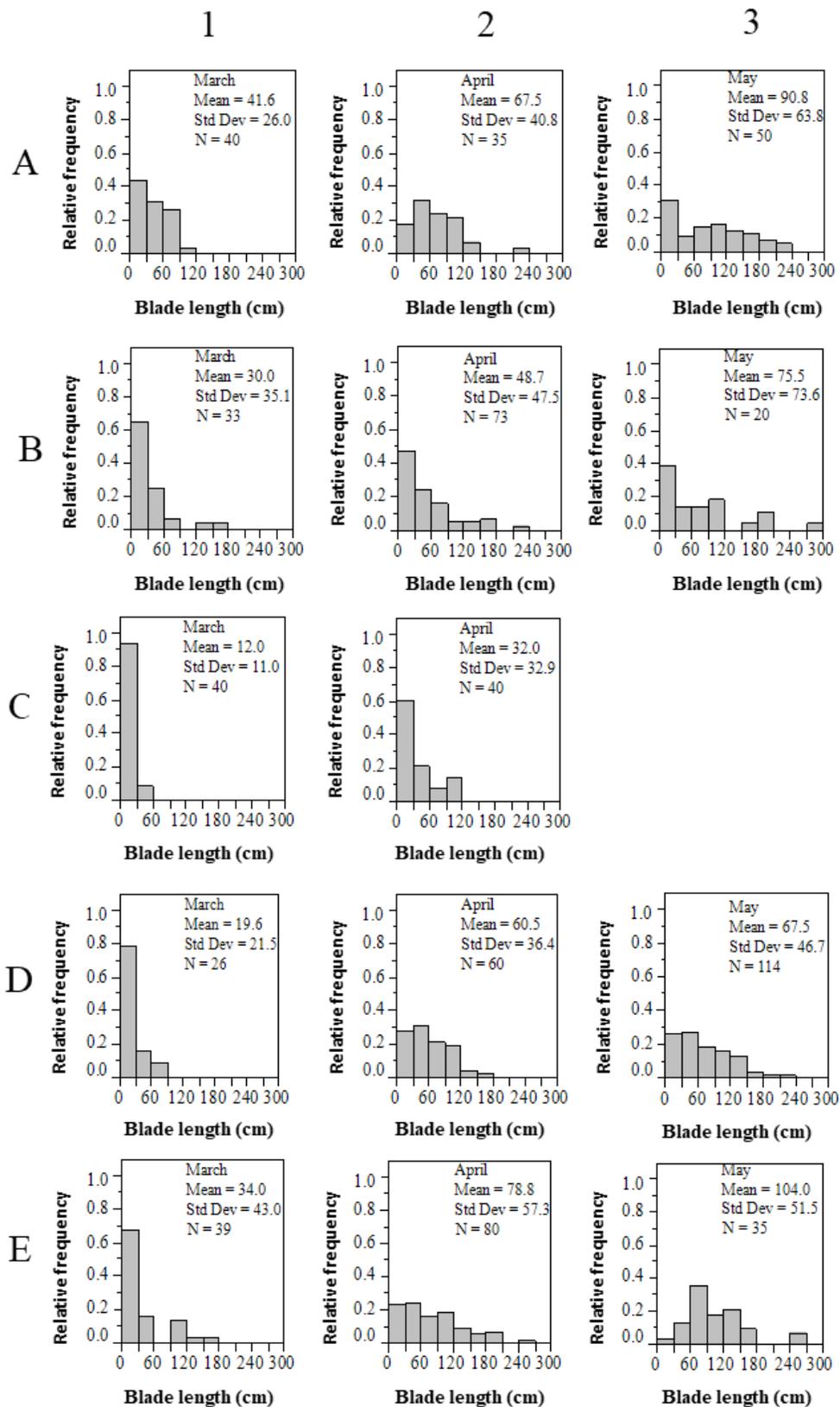
Distributions of the untrimmed (control) blades had high variation and they were right-skewed (Fig. 4.5). To facilitate comparison of the trimmed and control blades, we addressed the high variation among blade lengths by dividing the morphological variation into two groups based on size classes: hereafter called (1) Long Blade Class (LBC) and (2) Short Blade Class (SBC). The trimming and control LBC had blades that were  $\geq 60$  cm. The SBC had blades that were  $< 60$  cm, and these are the thalli that were interspersed between the trimmed thalli (or long control thalli) but were too short to be trimmed themselves.

The Wood Island LBC ( $\geq 60$  cm) exhibited significant differences between mean stipe length, stipe diameter, and stipe weight between the trimmed and control group (Table 4.2). When compared to the control LBC, we observed a significantly higher mean stipe length (12.5%), stipe diameter (16%), and stipe weight (25%) in the trimmed LBC. There were no significant differences for the other traits. When comparing the trimmed Wood Island SBC ( $< 60$  cm) to the control SBC, we observed a significantly higher mean blade length (37%), blade basal width (75%), blade mid width (52%), blade distal width (16%), blade weight (144%), stipe length (57%), stipe diameter (63%) and stipe weight (28%) (Table 4.2). There were no significant differences for the other traits.

No significant morphological differences were observed between trimmed and control blades in the LBC from Brothers Island. However, most of the morphological characteristics of trimmed SBC from Brothers Island were higher than those from the control sections: mean blade length (42%), basal width (113%), mid width (145%), blade weight (300%), stipe length (151%),

stipe diameter (67%), and stipe weight (233%). The Cow Ledge site was not analyzed for specific morphological differences due to a low sample size ( $n < 20$ ) in each size class.

**Figure 4.5 (next page)** Un-trimmed *Saccharina latissima* blade lengths (cm) at the study sites in 2018 and 2019. Rows are ordered by year and site: A) Brothers Island in 2019, B) Cow Ledge in 2018, C) Cow Ledge in 2019, D) Wood Island in 2018, and E) Wood Island in 2019. Columns are in ordered by month: 1) March, 2) April, and 3) May. Means and standard deviations are rounded to the nearest tenth.



**Table 4.2** Results of Tukey’s HSD (T) or Wilcoxon signed rank tests (W) that tested significance of difference between trimmed and control individuals. The Long Blade Class (LBC) consists of blades  $\geq 60$  cm and the Short Blade Class (SBC) is comprised of blades  $< 60$  cm. Reported means, standard deviations, and t-values rounded to the nearest tenth, p-values rounded to the nearest thousandth. Traits with no significance are not shown.

Site	Class	Variable	Test	Trimmed N	Control N	Treatment mean	Control mean	df	t-value	p-value
<b>Wood Island 2019</b>	LBC	stipe diameter (mm)	W	78	73	3.6 (0.9)	3.2 (0.9)	72	-748.5	<0.0001
		stipe length (cm)	W	69	70	5.8 (4.1)	5.0 (2.7)	69	-648.5	<0.0001
		stipe weight (g)	W	69	70	1.0 (1.3)	0.8 (0.7)	69	-804.5	<0.0001
	SBC	blade length (cm)	W	61	74	34.2 (16.9)	24.9 (17.4)	73	-767.5	<0.0001
		basal width (cm)	W	61	74	5.6 (4.2)	3.2 (2.2)	73	-1234.5	<0.0001
		mid width (cm)	W	59	74	5.0 (4.6)	3.3 (2.3)	73	-1171.5	<0.0001
		distal width (cm)	W	46	74	2.9 (1.4)	2.5 (1.9)	73	-554.5	0.0011
		blade weight (g)	W	61	69	10.5 (13.8)	4.3 (10.1)	68	-1112.5	<0.0001
		stipe length (cm)	W	56	67	4.4 (4.4)	2.8 (1.6)	66	-959.0	<0.0001
		stipe weight (g)	W	54	36	1.0 (1.3)	0.8 (0.7)	69	-804.5	<0.0001
stipe diameter (mm)	W	56	67	2.6 (1.0)	1.6 (0.7)	66	-1068.0	<0.0001		

<b>Bros. Island 2019</b>		% C	W	17	7	27.1 (2.2)	24.8 (2.7)	6	-11.0	0.0391
		$\delta^{15}\text{N}$	W	17	7	6.2 (1.6)	4.6 (1.9)	6	-11.0	0.0391
		C:N	W	17	7	11.7 (2.0)	10.1 (1.3)	6	-13.0	0.0156
	LBC	$\delta^{13}\text{C}$	W	18	33	-16.6 (1.6)	-17.3 (1.5)	32	-129.5	0.0091
		% C	T	18	33	26.0 (0.0)	27.0 (0.0)	32	3.8	0.0003
	SBC	blade length (cm)	W	28	83	41.2 (13.5)	29.0 (14.7)	82	-1290.0	<0.0001
		basal width (cm)	W	28	83	11.5 (67.2)	5.4 (2.8)	82	-1709.0	<0.0001
		mid width (cm)	W	28	83	12.0 (7.6)	4.9 (2.3)	82	-1740.0	<0.0001
		blade weight (g)	W	27	75	20.6 (17.5)	5.2 (6.0)	74	-1397.0	<0.0001
		stipe length (cm)	W	26	77	6.8 (39.7)	2.7 (20.1)	76	-1415.5	<0.0001
		stipe diameter (mm)	W	24	75	3.0 (1.2)	1.8 (0.9)	75	-1368.0	<0.0001
		stipe weight (g)	W	25	48	1.0 (1.2)	0.3 (0.4)	47	-555.6	<0.0001
		% C	T	15	3	24.5 (1.4)	23.0 (0.0)	2	-32.6	0.0005

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#### 4.4.3 Composition

No significant differences were observed in the %C, %N,  $\delta^{13}\text{C}$ , or  $\delta^{15}\text{N}$  of the trimmed LBC from Wood Island (Table 4.2). Trimmed thalli in the LBC at Brothers Island had significantly lower %C and  $\delta^{13}\text{C}$  in their blades. The SBC in the trimmed sections at Wood Island had a 9% higher %C, a 19% higher C:N (16%), and 35% higher  $\delta^{15}\text{N}$  in their blades than the SBC blades from the control sections. The %C of the trimmed SBC at Brothers Island was also 9% higher than the control. No significant differences in NRA were observed between individuals in the treatment or control group, regardless of length class or site.

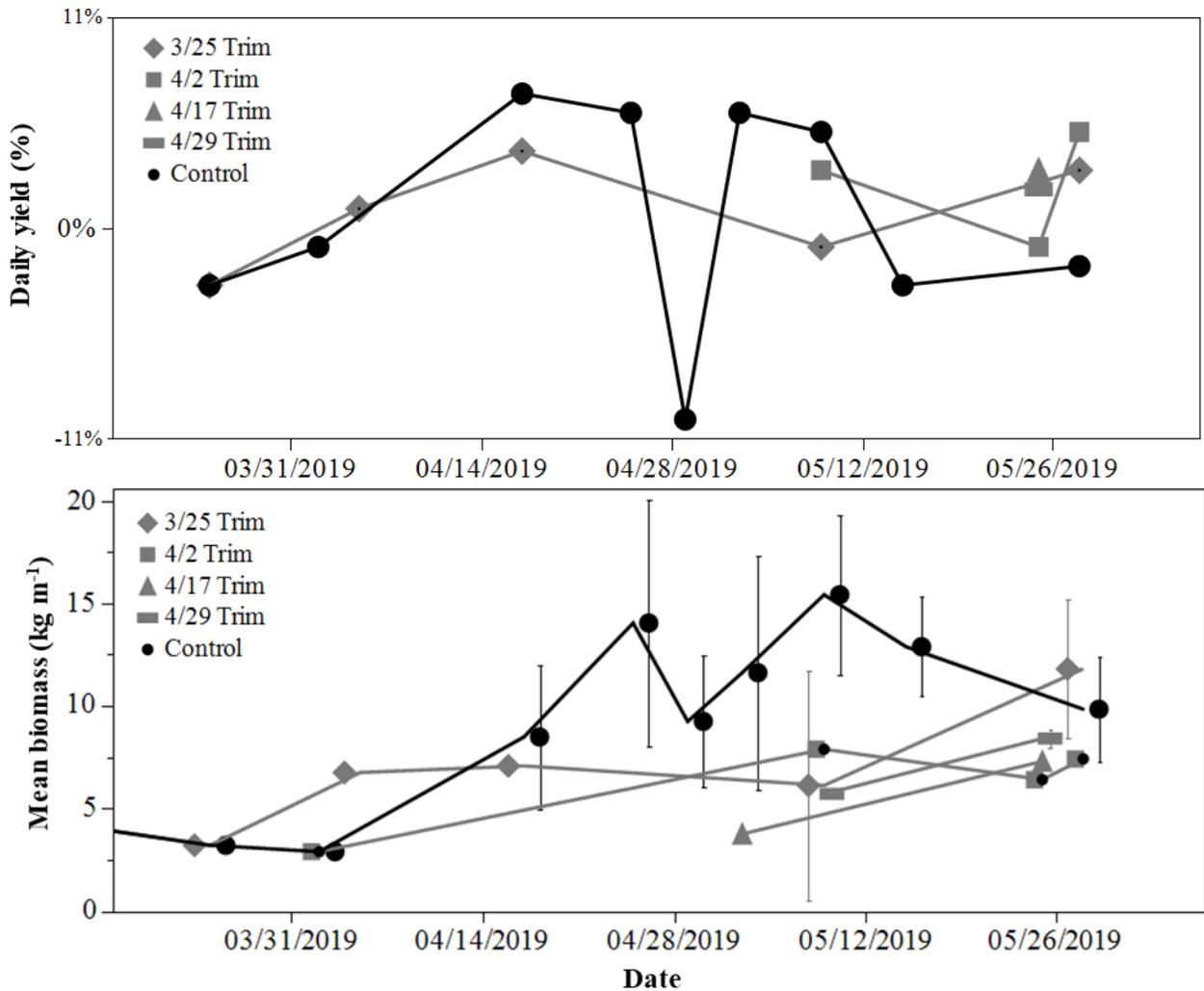
#### 4.4.4 Biomass and daily yield

We analyzed mean biomass and daily yield for the control and trimmed sections at Wood Island in 2019 because this site-year combination had the most consistent biomass measurements (winter-early spring conditions in Maine precluded consistent visits to other sites Fig. 4.6; Table 4.3). We were able to measure biomass 12 times at this site in 2019: sampling approximately every 4 – 15 days from March to June 2019. The highest observed daily yield of the control group (6 – 7%  $\text{day}^{-1}$ ) was observed between mid-April and early-May. After this point, daily yield of the control group was negative (-2 to -3%  $\text{day}^{-1}$ ). The trimmed sections, in contrast, exhibited a mostly positive daily yield through the end of the season (-1 – 5%  $\text{day}^{-1}$ ). The mean daily yield of all the trimmed sections throughout the season was 2.25%  $\text{day}^{-1}$ , and the mean daily yield resulting from a trim administered on April 17<sup>th</sup> was 3% (Table 4.3).

**Table 4.3** Calculated daily yield (% increase in weight day<sup>-1</sup>) of *Saccharina latissima* cultivated at Wood Island, Spring 2019. Mean and standard deviations (SD) rounded to the nearest tenth.

Date	Trim Date				
	Control	3/25	4/2	4/17	4/29
3/13	-	-	-	-	-
3/25	-3%	-	-	-	-
4/2	-1%	-	-	-	-
4/17	7%	4%	-	-	-
4/25	6%	-	-	-	-
4/29	-10%	-	-	-	-
5/3	6%	-	-	-	-
5/9	5%	-1%	3%	-	-
5/15	-3%	-	-	-	-
5/25	-	-	-1%	3%	2%
5/28	-2%	3%	5%	-	-
Mean PY during treatment period	0%	2%	2%	3%	2%

**Figure 4.6** Measured *Saccharina latissima* biomass ( $\text{kg m}^{-1}$ ) and daily yield for control and trimmed groups at Wood Island, Spring 2019. The lines represent different trimming treatments, and the points indicate sampling events. The steep drop in daily yield of the control group in late April 2019 was a storm that dislodged kelp off the longline. Error bars are standard deviation.

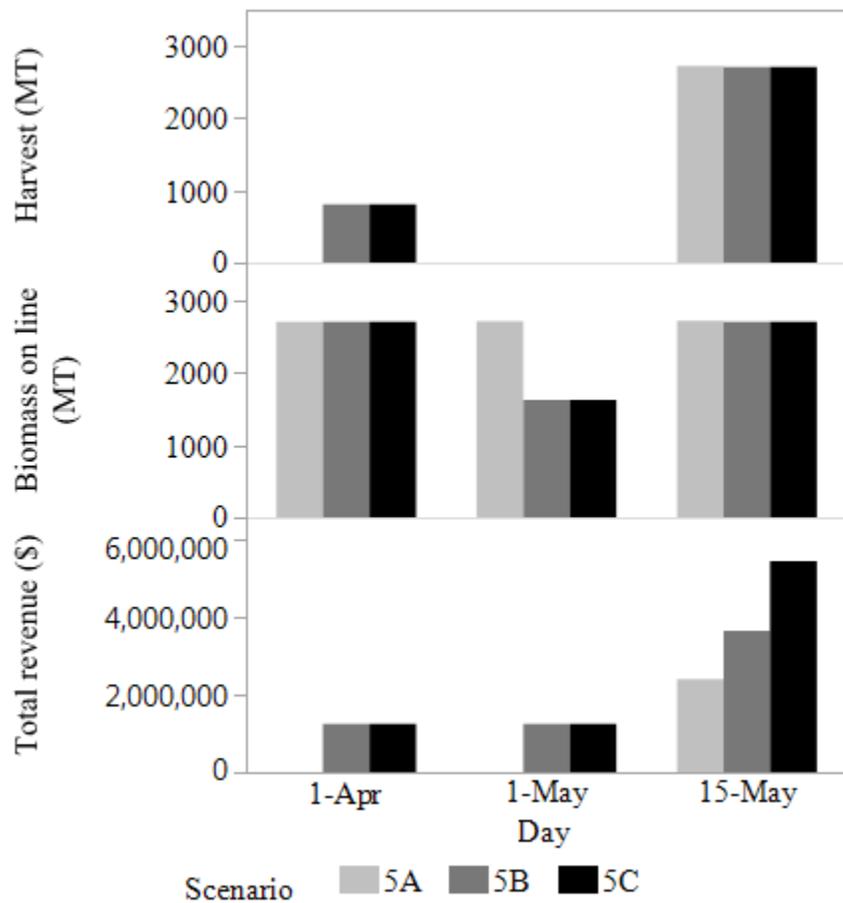


#### 4.4.5 Biomass production and harvesting model

Using Maine's forecasted 2035 production of 2,722 MT of seaweed (Piconi *et al.* 2020), the model predicts that trimming could result in an additional \$3 million in revenue for the

industry if the maximum price per tonne (\$1540) is assigned to the early season and late season harvests (Scenario 5C; Fig. 4.7). Using the 2019 production reported for Maine (147 WW MT year<sup>-1</sup>), the model estimates that late-season erosion and sloughing results in approximately \$4,700 of lost revenue when kelp is sold at the lowest reported price (\$880; Table 4.4, Scenario 3A). If 30% of the kelp is harvested via trimming on April 1<sup>st</sup>, and no price premium is associated with the sale of early-season kelp, then the subsequently reduced growth rate from trimming (3%) results in approximately \$4,300 of lost revenue (Scenario 4A). When the highest price per MT (\$1540) is assigned to the early season kelp, then the model predicts that early-season trimming would result in an additional \$17,293 of revenue, even if the final harvest is still assigned the lowest price per MT (Scenario 4B). If all the kelp produced were assigned the highest price, the model predicts that an additional \$40,000 in revenue would be captured by trimming that maintained a 3% daily yield until May 15<sup>th</sup>.

**Figure 4.7** Forecasted harvest, biomass, and total revenue for Scenario 5A – 5C of *Saccharina latissima* biomass production model. Scenario 5A shows increased production in 2035 (2722 MT total) and a harvest on May 15 with - 3% daily yield (DY) May 1 -15. Scenario 5B shows increased production in 2035, removal of 30% biomass on April 1 with 3% DY after trim, a high sale price (\$1540 WW t<sup>-1</sup>) for April 1 harvest and low sale price (\$880 WW t<sup>-1</sup>) for May 15 harvest. Scenario 5C shows increased production in 2035, removal of 30% biomass on April 1 with 3% DY after trim, and a high sale price (\$1540 WW t<sup>-1</sup>) for April 1 and May 15 harvest.



**Table 4.4** Results from biomass production and harvesting model.

Scenario	Date (m/d)	Biomass (MT)	Harvest (MT)	USD \$ MT <sup>-1</sup>	Revenue (\$)	Total revenue (\$)	Losses/gains (\$)
1A	4/1	133					
	5/1	141					
	5/15	147	147	880	129,360	129,360	
1B	4/1	133					
	5/1	141					
	5/15	147	147	1540	226,380	226,380	
2A	4/1	133	133	1540	204,799	204,799	
	5/1	141					
	5/15	147					
2B	4/1	133					
	5/1	141	141	1210	170,549	170,549	
	5/15	147					
2C	4/1	133					
	5/1	141					
	5/15	147	147	880	129,360	129,360	
3A	4/1	133					
	5/1	141					
	5/15	142	142	880	124,641	124,641	(4,718)
4A	4/1	133	40	880	35,108	120,321	(4,319)
	5/1	96	0				
	5/15	97	97	880	85,213		
4B	4/1	133	40	1540	61,439	146,653	17,293
	5/1	96	0				
	5/15	97	97	880	85,213		
4C	4/1	133	40	1540	61,439	210,563	40,013
	5/1	96	0				
	5/15	97	97	1540	149,123		
5A	4/1	2708					
	5/1	2720					
	5/15	2722	2722	880	2,395,360	2,395,360	
5B	4/1	2708	812	1540	1,251,096		
	5/1	1627	0				
	5/15	2712	2712	880	2,386,296	3,637,392	1,242,032
5C	4/1	2708	812	1540	1,251,096		
	5/1	1627	0				
	5/15	2712	2712	1540	4,176,018	5,427,114	3,031,754

## 4.5 Discussion

### 4.5.1 Morphology

Our hypothesis that trimmed kelp blades would grow wider than the control kelp blades in response to the injury from trimming was not supported. No significant differences in basal or mid-region blade width were observed between the trimmed and control blades in the LBC. We did, however, observe a significant difference in the stipe morphology between the trimmed and control thalli in the LBC at Wood Island. The stipes of the trimmed blades were significantly longer, thicker, and heavier than the stipes of the control blades.

There are several possible explanations for the longer, thicker, and heavier stipes. One reason might be that the removal of the distal end of the blade altered the hydrodynamic forces on the blade and the stipe. Johnson & Koehl (1994) observed that drag coefficients on wild *N. luetkeana* varied with blade shape and that kelp compensated for higher drag coefficients with stipe elongation and thickening to maintain elastic strain similarity. Wernberg & Vanderklift (2010) showed that elongation and thickening of stipes can even occur in response to short-term fluctuations in wave exposure. It is also possible that trimming triggered a shift in the allocation of photosynthate and nutrients between blade and stipe, in which a greater quantity than normal was sent to the stipe. Under normal circumstances, *Nereocystis* sp. stipes receive a minimal amount of photosynthate and are not dependent on translocated materials (Nicholson 1970; Nicholson & Briggs 1972). However, two-way translocation of organic material has been observed between mature *Macrocystis* blades and stipes (Sargent & Lantrip 1952; Parker 1963, 1965). Increased delivery of photosynthate or nutrients to the stipe following distal-end trimming cannot be determined with the results of this study, but it could be further explored by quantifying the %C, %N in the stipes before and after trimming, or with the use of <sup>14</sup>C-labeled

products as demonstrated by Parker (1965). Genetics are an additional factor influencing kelp blade and stipe morphology, so incorporating genotype investigations into future studies may also shed additional light on this observed response to trimming. From the perspective of the kelp farmer, substantially longer, thicker, and heavier stipes can lead to issues of entanglement and floating stipes on the farm, so additional characterization of the potential relationship between distal-end trimming and stipe growth is important.

Several studies have promoted staggered harvesting to mitigate light or nutrient shading (Scoggan *et al.* 1989; Sanderson *et al.* 2012; Gao *et al.* 2013b; van den Burg *et al.* 2016; Bak *et al.* 2018, Grandorf Bak 2019), which is also common practice in parts of Asia. We observed evidence that distal-end trimming affected adjacent blades in the SBC. The SBC individuals in the trimmed sections had longer, wider, and heavier blades than those in the control sections. Their stipes were also longer, thicker, and heavier. The starting density of sporophytes on the lines was relatively high, so before trimming, so we suspect that the SBC may have been receiving insufficient light or nutrients. When trimming removed biomass, this might have allowed increased light and nutrients to reach the shorter sporophytes, enabling them to grow longer and wider than the SBC sporophytes in the control sections. We did not directly measure the change in light or nutrient availability after trimming but this effect has been previously documented by Tseng (1962, 1981, 1986), Wu & Zheng (1981), and Scoggan *et al.* (1989).

#### 4.5.2 Composition

We hypothesized that trimmed blades would have a lower %C and  $\delta^{13}\text{C}$  resulting from the loss in blade photosynthetic area. The trimmed LBC from Brothers Island did indeed have a significantly lower mean %C and  $\delta^{13}\text{C}$  than the control LBC in the control sections. In contrast, the mean %C and  $\delta^{13}\text{C}$  of trimmed blades was not significantly different than the control at Woods Island. We also observed evidence that biomass removal via trimming affected the composition of the adjacent SBC. At both sites, the SBC in the trimmed sections had a significantly higher %C than the control sections which would support the notion that more light and nutrients were available to these sporophytes after trimming the LBC. Interestingly, the site variability in carbon content of the trimmed LBC matched the difference in stipe morphology between the two sites. However, linear regression analyses showed little relationship between LBC stipe morphology, %C, and  $\delta^{13}\text{C}$  at either site. Another possibility is that trimming changed the floating angle of the kelp and its access to light. Wu & Zheng (1981) observed that distal-end trimming changed the floating angle of the *S. japonica* blades (from  $8^\circ$  to  $27^\circ$  in  $5 \text{ cm s}^{-1}$  current), which resulted in better lighting of the blade and higher photosynthetic activity. We did not attempt to measure a potential change in floating angle, but future studies could build on the results presented here by incorporating this measurement. A final explanation for the incongruency of %C and  $\delta^{13}\text{C}$  in the trimmed LBC between sites could be disparate environmental conditions between the sites.

Our hypothesis that blades in the trimmed LBC would have lower %N and  $\delta^{15}\text{N}$  than the control resulting from the loss of available tissue for nutrient uptake was not supported. Additionally, we hypothesized that NRA in the remaining thallus would be elevated as the kelp compensated for the loss of nutrient-acquiring blade area, but this was not supported either. The

lack of different %N,  $\delta^{15}\text{N}$ , and NRA in the trimmed LBC suggests that trimming the blade down to 60 cm may not result in physiologically induced nutrient stress on the remaining *S. latissima* thallus. However, we recommend further assessment of this trimming length in regions with dissimilar environmental conditions because NRA, %N, and  $\delta^{15}\text{N}$  in algae are highly influenced by ambient nutrient levels, irradiance, and temperature (Chapman 1978; Davison & Stewart 1984; Peterson & Fry 1987).

### **4.5.3 Daily yield**

Our hypothesis that distal-end trimming would result in reduced daily yields from the trimmed sections was not supported. Rather, the late-season negative daily yield we observed in the control group (-2 to -3% day<sup>-1</sup>) and mostly positive daily yield observed in the trimmed groups (-1 to 5% day<sup>-1</sup>) suggest that trimming facilitates the capture kelp distal biomass that would otherwise be fouled or lost to the environment. Routine and substantial loss of cultivated seaweed biomass has been shown to occur through dislodgement, thalli breakage, and seasonal erosion of distal tissue (Buck & Buchholz 2005; Peteiro *et al.* 2006; Zhang *et al.* 2012). At our sites, it appears that the distal-end trimming helped to prevent biomass loss from both dislodgement and erosion of the LBC.

The case for retaining thalli that would otherwise be dislodged or broken during a storm is most compelling at Wood Island in April 2019. On April 25, 2019, a mean biomass of 14.0 ( $\pm$  6.0) WW kg m<sup>-1</sup> was calculated for the Wood Island site. Then on the night of April 26, a powerful Nor'easter moved through the region which produced wind gusts up to 17.4 m s<sup>-1</sup> (62.6 km hr<sup>-1</sup>) within a 5-minute period at the closest weather station (Portland Jetport). Approximately 53 mm of precipitation in 24 hours were recorded at the Portland Jetport. At the UNE weather station near Wood Island, approximately 33 mm of precipitation was recorded in a 12-hour

period and 44 mm in 24 hours. These conditions, likely in combination with large waves, resulted in the considerable loss of kelp from the Wood Island site. On April 29, just four days after the previous biomass measurement, the mean biomass was only two-thirds ( $9.3 (\pm 3.2)$  WW  $\text{kg m}^{-1}$ ) of the prior measurement. The sampling interval of the trimmed section biomass for Wood Island is, unfortunately, not conducive to direct comparison for that short window. However, the mean biomass for kelp trimmed earlier in the spring during a three-week window including the storm only declined by 13% ( $7.08$  to  $6.13 (\pm 5.6)$  WW  $\text{kg m}^{-1}$ ) and the daily yield ranged from  $-1$  to  $4\% \text{ day}^{-1}$ . This is an indication that the distal-end trimming can significantly reduce risk for kelp farmers as it allows them to reap biomass that would otherwise be lost during extreme weather events.

In addition to severe weather that results in biomass loss, blade erosion can also lead to reduced daily yields in the late spring. Wild Laminariales commonly shed up to 25% of their mass from the distal blade in the late spring or summer and this, combined with a decline in new growth, results in a “seasonally-determined negative length growth” for many kelp species ((Tseng 1986; Boderskov *et al.* 2016). We posit that this erosion contributes to the decline in daily yield observed in the control sections at Wood Island in mid and late-May 2019, although some additional dislodgement or breakage of the LBC may have also occurred during this time. Encouragingly, the daily yield of the trimmed sections during this period was higher than the control and the rates are generally positive. Based on the increased SBC blade and stipe weight in the trimmed sections, we venture that these positive daily yields may be associated with reduced erosion of the LBC and growth of the SBC without erosion. Additional parsing of the relative contributions of each of these factors was beyond the scope of this study, but our results concur with previous studies promoting the use of distal-end trimming to extend the kelp grow-

out season (Scoggan *et al.* 1989; Sanderson *et al.* 2012; Gao *et al.* 2013b; van den Burg *et al.* 2016; Bak *et al.* 2018).

Furthermore, the comparison of the daily yield associated with specific trimming time suggests that there may be an optimal window for distal-end trimming based on the ambient water temperature at the farm. Scoggan *et al.* (1989), who examined trimming practiced on *S. japonica* farms in China, also concluded that timing the trimming with water temperature may be important. They observed that the yields of *S. japonica* were highest if blade tip cutting occurred when seawater temperatures were 5 - 6°C; ostensibly, because nutrients had accumulated in the distal end, and the remaining blades received more light, which supported growth (Scoggan *et al.* 1989). Trimming blade tips when the water was < 5°C was ineffective because there was insufficient nutrient content in the tips and trimming once the water temperature surpassed 6°C was ineffective because thalli overcrowding on the line resulted in insufficient light, photosynthesis, and accumulation of photosynthates (Scoggan *et al.* 1989). We suspect that the observation from Scoggan *et al.* (1989) may hold for *S. latissima* farms in the WGoM. For instance, at Wood Island, when trimming was conducted in late March, the calculated daily yield 4 weeks later in mid-April was 4% day<sup>-1</sup> for the trimmed group and 7% day<sup>-1</sup> for the control group. However, when trimming occurred in early to late-April, the calculated daily yield was -1 to 3% day<sup>-1</sup> for the trimmed groups and -2 to -3% day<sup>-1</sup> for the control group. The highest daily yields observed from the trimmed kelp in the present study were measured following trims made before the end of April 2018 and 2019, when the water temperature was between 5 and 6°C. More observations linking water temperature, daily yield, and the timing of distal-end trimming can help to confirm this relationship for the WGoM.

#### 4.5.4 Production and harvesting model

We sought to evaluate the tradeoffs of an expanded *S. latissima* harvest season with smaller, more frequent harvests with a relatively simple biomass and harvesting model. The results of this modeling effort indicate that the potential economic gains from distal-end trimming depend on a price premium for the trimmed kelp. Scenario 4A in our model showed that at current US production rates, distal-end trimming in early April results in a loss of approximately \$4,300 if all the kelp is sold at the lowest price per tonne (\$880 WW MT<sup>-1</sup>). In this scenario, the combination of the short-term reduction in daily yield following trimming and a flat price for the *S. latissima* biomass does not support the incorporation of distal-end trimming into current farming practices if the purpose of trimming is simply to reduce the risk of losing product during storms. Moreover, the real financial loss is likely greater because the biomass and production model does not consider the additional, and potentially considerable (Scoggan *et al.* 1989), labor costs of trimming.

Conversely, if a price premium can be obtained for trimmed kelp, then the model predicts financial gains from the trimming practice. We considered two obvious ways that the price premium could emerge. In a supply-limited or seasonally-limited market, kelp biomass harvested in April could demand a higher price per tonne before the mid-May harvest significantly increases market supply. Scenario 4B demonstrates that an early-season price premium (\$1540 MT<sup>-1</sup> WW) could result in an additional \$17,000 in revenue at current US production rates and \$1.2 million at forecasted production for 2035. Although this forecast is encouraging, the current US market for raw seaweed has relatively high price elasticity (*i.e.*, demand for seaweed, measured in \$ kg<sup>-1</sup>, does not change much with supply) so a large shift in this market would be required to obtain these financial gains from distal-end trimming. A second possibility is that

price premiums emerge for *S. latissima* that is more tender and flavorful (Fossberg *et al.* 2018). Tenderness and blade morphology is already a central factor driving price in the more established Asian seaweed markets (Kawashima 1984, 1993; Mairh *et al.* 1991; Peteiro & Freire 2011; Gao *et al.* 2013a). In Japan, *U. pinnatifida* thalli harvested during their growth phase typically sell for five times more than the regular-priced adult thalli (Gao *et al.* 2013b); and thalli tenderness presumably factors into this consumer preference. The results of our field experiments with *S. latissima* illustrate the potential of distal-end trimming to promote the growth of the SBC, and because the SBC is likely more tender than the LBC at final harvest, it could also exact a price premium in a market that favors tender thalli. Ultimately, if all kelp biomass harvested from US farms employing distal-end trimming were sold at the highest market price considered in the biomass and production model (\$1540 MT<sup>-1</sup> WW), then this would result in an additional \$40,000 - \$3 million in revenue.

#### **4.6 Conclusion**

Given the recent boom in the cultivation of *Saccharina latissima*, additional knowledge about the costs and benefits of distal-end trimming on this species is especially timely and important. Previous studies have shown that removing the distal-end of long blades can offer biological benefits like increased sunlight and nutrients for the remaining thalli. Our study confirms these biological benefits for *S. latissima*. When compared to the control group, the thalli in proximity to the trimmed blades had wider, longer, and heavier blades and stipes, and greater %C in their blade tissue. The potentially negative impacts of distal-end trimming on kelps can vary with species, season, the location of the trim relative to the growth region, and the ambient environmental conditions at the cultivation site. However, at *S. latissima* farms in the

WGoM, trimming blades to 60 cm in late March or April does not appear to cause stress to the remaining blade. We did not observe differences in the %N, NRA, C:N ratio,  $\delta^{15}\text{N}$ , or width of the trimmed blades that would suggest trimming-induced nutrient limitation or an injury-related growth response. Furthermore, trimming the distal-ends of *S. latissima* shows promise as a tool to help farmers in the WGoM maintain positive daily yields into late spring and capture biomass that would otherwise be naturally lost or dislodged during storms. Our biomass and production model illustrates that the potential economic benefits of this practice are highly dependent on production scale and price premiums for early season kelp; both of which are largely speculative for the present-day European & North American cultivated kelp markets.

## CHAPTER 5

### SUPPORTING VALUE-ADDED SITING OF KELP FARMS IN CASCO BAY, ME

#### 5.1 Chapter Abstract

Macroalgal biomass can be incorporated into a variety of value chains. As such, the optimal cultivation sites for macroalgal biomass destined for food and livestock feed likely differ from optimal sites for macroalgal biomass destined for biofuels or bioextraction. We explored this question using Casco Bay, ME as a model system, because it has one of the highest concentrations of seaweed farms in the US. We first applied biological, social, and ecological constraints on seaweed suitability across the Bay. Then we evaluated how the distributions of these areas shift when applying additional criteria for high-end food and feed markets or the contrasting biofuel and bioextraction markets. The results predict that Casco Bay has optimal ocean area for macroalgal production to support either market and identified a portion of the Bay that would be well-suited for an aquaculture opportunity area or prioritized zone. Further exploration showed that increasing the depth criterion for arrays targeting biofuel and bioextraction does not result in expanded optimal area. However, predicted optimal area does increase when arrays producing macroalgae for high-end food and feed markets are accepted near the shoreline. This screening analysis is the first spatial assessment for seaweed in the region, and it indicates general spatial patterns and limitations associated with seaweed industry growth and development.

#### 5.2 Introduction

The call for aquaculture planning in the coastal zone has become increasingly strong within the fields of ocean sciences (Aguilar-Manjarrez *et al.* 2010). Minimizing the impact of aquaculture on other ocean-based activities and the environment is now understood to be

important to aquaculture sustainability throughout the world, and it has been identified as an especially critical imperative in the United States and Europe where the potential for profitable expansion of marine aquaculture is high, but social license for aquaculture is generally low (Kapetsky *et al.* 2013; Gentry *et al.* 2017; Lester *et al.* 2018; Johnson *et al.* 2019; Costello *et al.* 2020). For aquaculture to expand responsibly in these regions, the tradeoffs between aquaculture activities, other water uses, and ecosystem services must be balanced to the greatest extent possible (McKindsey *et al.* 2006; Liu *et al.* 2007; Aguilar-Manjarrez *et al.* 2010). However, this balance between tradeoffs is specific to the cultivated species, region, and motive behind the aquaculture activity, so siting decisions should balance these tradeoffs as well (Gentry *et al.* 2017, Rolin *et al.* 2017).

Screening frameworks are one of the most widely used planning tools for characterizing the biological, ecological, and social tradeoffs between prospective aquaculture sites (Nobre *et al.* 2005; Ross *et al.* 2013). Multi-criteria evaluation (MCE) is a core tenet of many screening frameworks, because it is a technique that allows for high-level comparisons and overviews within a Geographic Information Systems (GIS) platform (Nobre *et al.* 2005). In MCE, values are assigned to factors with different importance, and then the criteria scores are aggregated to obtain a final suitability rank (Brigolin *et al.* 2011; Silvia *et al.* 2011; Meaden & Aguilar-Manjarrez 2013; Beard *et al.* 2020). The values can be assigned in a variety of ways, but the most ubiquitous strategies are Boolean overlay (BO) and weighted linear combination (WLC), with the former being well-suited for initial suitability assessments and the latter providing slightly more specificity (Thomas *et al.* 2019).

Several studies have conducted screening analyses for kelp aquaculture using MCE techniques. For example, Radiarta *et al.* (2011) combined satellite observations of sea surface

temperature and suspended solids, with bathymetric data to identify the most suitable areas for “hanging culture” of *Saccharina japonica* in southern Hokkaido, Japan. Liu *et al.* (2013) built upon the work by Radiarta *et al.* (2013) by estimating sea surface nitrate from satellite measurements of sea surface temperature and chlorophyll *a*, including that prediction in their suitable aquaculture site-selection model for *S. japonica*. More recently, Thomas *et al.* (2019) combined thirteen environmental, economic, and social criteria to identify suitable sites for *Saccharina latissima* on the western coast of Sweden and evaluated the tradeoffs between BO and WLC approaches for assigning values to factors in their model. Each of these studies provides valuable examples of how screening analyses can be tailored for specific kelp species and cultivation regions. Yet, most available literature generally focuses on a single use of macroalgae (van den Burg *et al.* 2016). To our knowledge, there has not yet been a screening analysis conducted for kelp aquaculture that incorporates an additional important aspect influencing tradeoffs between other water users, ecosystem services, and aquaculture activities – namely, the market.

Commercial macroalgal aquaculture is relatively new in Europe and North America (Grebe *et al.* 2019), despite its comparatively long and widespread practice in eastern Asia, Africa, and the Indo-Pacific. As such, the commercialization approach for macroalgae produced in Europe and North America is still largely speculative (van den Burg *et al.* 2016; Grebe *et al.* 2019) and in strong contrast with the narrow focus on food markets (*i.e.*, nori and wakame) and phycocolloids (*i.e.*, carrageenan and agar) that supported the expansion of macroalgal aquaculture throughout the Indian and Western Pacific (Buschmann *et al.* 2017, 2019; Porse and Rudolph 2017). Most aquaculturists or companies currently growing macroalgae in the United States are targeting food or livestock supplement markets, which currently require a relatively

small amount of raw seaweed. Meanwhile, there is intensifying interest in farming macroalgae to obtain a feedstock for bioplastics, textiles, fertilizers, biofuels, and for ecosystem services like nutrient bioextraction which would require colossal amounts of raw seaweed (van den Burg *et al.* 2020). There are also conspicuous differences in the characteristics of macroalgae required for markets of different classes (Seghetta *et al.* 2017; Table 5.1). Macroalgae for direct human consumption, as livestock supplements, or incorporation in pharmaceuticals and cosmetics must have high purity; free of any contaminants that would be harmful to human health (Wells *et al.* 2016), animal health, or specialized manufacturing processes. However, macroalgae destined for use in textile and biofuel production or nutrient bioextraction could potentially have compositional deficiencies or contaminants and still serve its intended purpose. Initial observations of these developing markets suggest that, just like other industries, this gradient in biomass purity and specific compositional characteristics is directly linked to price. As purity of the material increases, the dollar value per kilo of kelp biomass increases and the scale of cultivation required to meet market demands decreases (O'Shea *et al.* 2019; Chopin & Tacon 2020). As investment and market are important considerations of aquaculture siting (Ross *et al.* 2013; Gentry *et al.* 2017), understanding how the differences in these potential markets can influence the optimal siting of seaweed farms is important.

**Table 5.1.** Common characteristics of macroalgal biomass and cultivation arrays grouped into two market classes for the purposes of this study. Adapted from O'Shea *et al.* (2019) and Chopin and Tacon (2020).

End-Use	Biomass needed	Purity	Sale price	Quality Considerations	Scale Considerations
<p><b>“High-end food and feed”</b></p> <ul style="list-style-type: none"> <li>• Pharmaceuticals</li> <li>• Biostimulants</li> <li>• Cosmetics</li> <li>• Organic food</li> <li>• Food</li> <li>• Organic Feed</li> <li>• Non-organic livestock feed</li> </ul>				<ul style="list-style-type: none"> <li>• Absence of bacteria and viruses pathogenic to humans, absence of heavy metals</li> <li>• Low biofouling</li> <li>• Target nutritional content, specific compounds, or other characteristics</li> </ul>	<ul style="list-style-type: none"> <li>• Small hectareage ok</li> <li>• Vertical integration of businesses is likely</li> <li>• Ease of access to fresh, small harvests more important</li> </ul>
<p><b>“Biofuels and bioextraction”</b></p> <ul style="list-style-type: none"> <li>• Soil amendments</li> <li>• Bioplastics</li> <li>• Textiles</li> <li>• Nutrient bioextraction</li> <li>• IMTA</li> <li>• Carbon capture/trading</li> <li>• Biofuels</li> </ul>				<ul style="list-style-type: none"> <li>• High C &amp; N</li> <li>• Target fast growth rates</li> </ul>	<ul style="list-style-type: none"> <li>• Large hectareage needed</li> <li>• Access by large vessels, further offshore or waves ok</li> </ul>

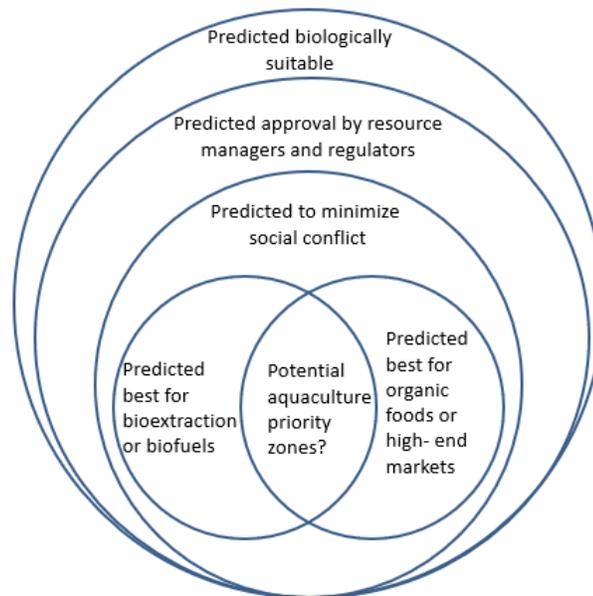
Here, we conduct an MCE using Boolean overlay to explore how the optimal siting and distribution of macroalgal farms may vary according to the end use of the seaweed biomass. For our purposes, optimal sites are predicted to have water characteristics that are within the range supporting rapid growth of the target macroalga, meet all federal siting restrictions, have minimal impact on existing water uses, wild flora and fauna, and ecosystem services.

Specifically, we ask four questions about the theoretical siting of seaweed farms (Fig.5.1):

1. Where is ocean area predicted to be biologically optimal while also meeting state and federal siting criteria?

2. How does the predicted distribution change if we apply a series of social considerations?
3. How does the predicted distribution change if we apply parameters important to seaweed bioextraction or biofuel production?
4. How does the prediction shift if we apply parameters for organic seaweed food products or pharmaceuticals?

Finally, we evaluate the observed patterns in theoretical siting of kelp aquaculture in the context of aquaculture priority areas for seaweed aquaculture zoning and the Western world’s growing interest in macroalgal biomass.



**Figure 5.1.** Conceptual approach for comparing spatial constraints for market-specific seaweed aquaculture.

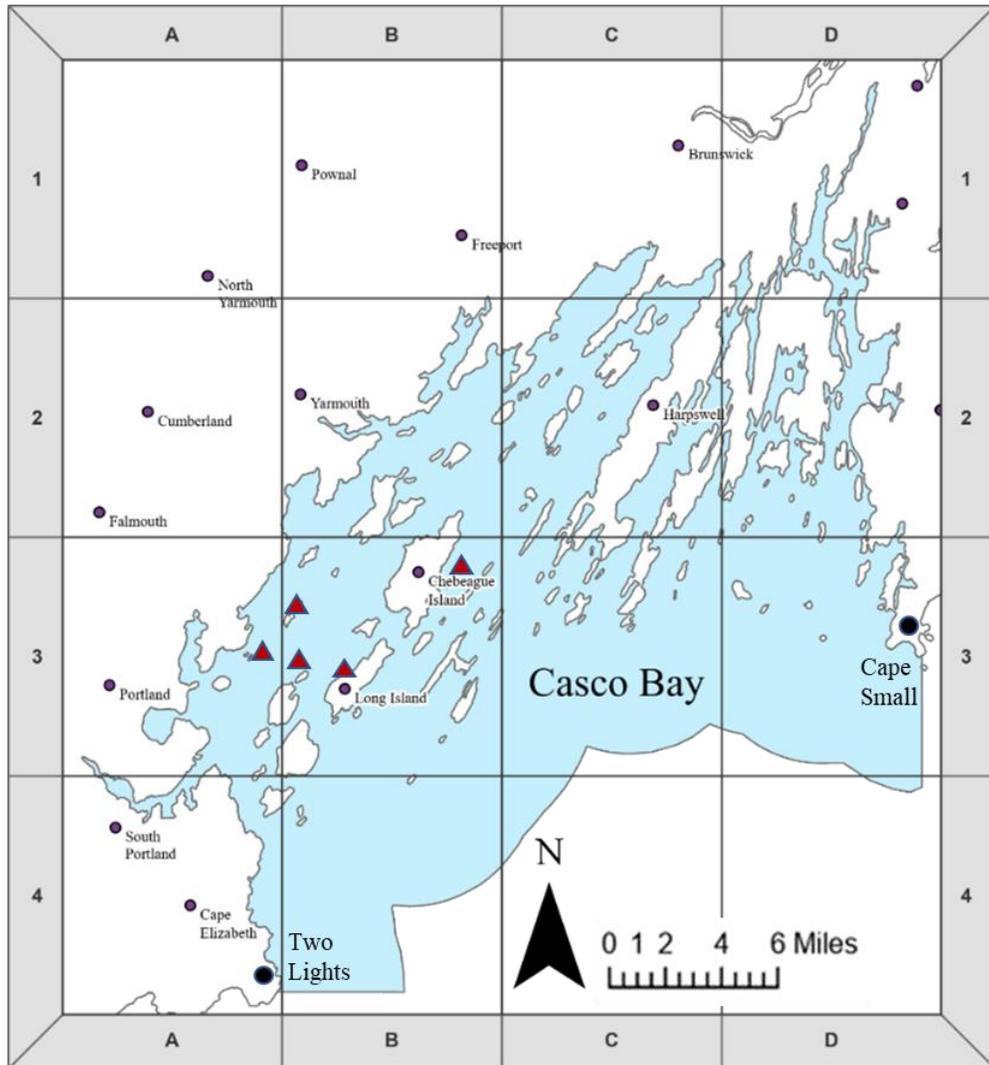
## 5.3 Methods

### 5.3.1 Study site, cultivation system, species, and target months

In the United States, Casco Bay, Maine, is one of the best model systems to observe the growth and evolution of the seaweed farming industry. Casco Bay has 136 islands (most of which are inhabited; USCP 2020), an active fishing and shipping industry, and recreational water users. Portland, the largest city in Maine, is located on the shores of Casco Bay, along with South Portland (4<sup>th</sup> largest city in Maine) and nine other notable cities and towns (US Census Bureau 2019). The Submerged Land Act of 1953 (43 U.S.C. § 1301) gives most coastal states jurisdiction over waters extending three nautical miles from their shoreline, so we applied this distance from Maine's state waters to define the eastward boundary of Casco Bay and trimmed the projection at Cape Small to the east and Cape Elizabeth (Two Lights) to the west (Fig. 5.2).

Casco Bay's productive and cool waters are well-suited for growing temperate macroalgal species from late autumn through late spring (Bricknell *et al.* 2021). In 2009, the first commercial seaweed farm in the U.S. was launched in Casco Bay (Grebe *et al.* 2019). Now Casco Bay has dozens of LPAs and leases permitted for macroalgal aquaculture (MEDMR 2019a,b) and continued expansion is predicted for the coming years. Longline systems are currently the most common seaweed cultivation system in Casco Bay, and throughout the US (Grebe *et al.* 2019). The longline approach uses anchors to secure a horizontal polyethylene cultivation line at the lease site. Surface buoys working against stiff PVC spacers maintain the longline at the desired depth. Small farms may use a single longline or several, whereas larger farms may deploy many more in modules. For the purposes of this study, we assumed a longline cultivation system for all kelp aquaculture activities in the Bay. Additionally, our screening

analysis only considered cultivated kelp, but there are also wild seaweed harvesters who harvest seaweed biomass from Casco Bay.



**Figure 5.2.** Our study site, Casco Bay, Maine. The shaded polygon indicates the extent of Maine State waters (3 nautical miles from shore). Towns and cities are indicated with shaded circles. The bay is bound by Two Lights (Cape Elizabeth) to the west and Cape Small to the east. Locations of sites where new environmental sampling occurred are indicated with red triangles.

### 5.3.2 Input data

The growing group of macroalgal farmers in Casco Bay, and along Maine's coastline, arguably represents the forefront of the domestic seaweed aquaculture industry (Grebe *et al.* 2021). Just like the rest of North America and Europe, the State of Maine is in the process of defining its strategy for aquacultural development. As Casco Bay has a variety of other water users with wide-ranging interests, identifying ocean area for aquaculture activities while minimizing tradeoffs for other water users and the ecosystem is a multi-faceted process influenced by the productivity of the resource, existing infrastructure, established governance systems, and potential conflicts between actors in the space (Puniwai 2014; Johnson *et al.* 2019). We aimed to capture spatial aspects of this process by acquiring and consolidating existing datasets into one common GIS platform. In several instances, we also transformed data layers or created new data layers so that we could include additional criteria in our analyses. All baseline data are classified as either a biological, ecological, or social consideration for ease of reference, although in some cases a criterion could fit into multiple categories (*e.g.*, conservation areas, molluscan shellfish areas). All data processing and spatial analyses were conducted in ArcGIS Pro (ver. 2.6.0) and RStudio Desktop (ver. 1.4.1106).

#### Biological considerations

Biological considerations, such as determining the suitability of sites based on natural conditions and the needs of the species, are an important piece of analyses of aquaculture suitability (McKindsey *et al.* 2006). Biological conditions relevant to seaweed aquaculture include temperature, salinity, nutrients, light availability, wave action, water currents, and grazing pressure (Lobban & Harrison 1997). For this screening model, we targeted optimal biological conditions for *Saccharina latissima*, commonly known as sugar kelp, because it is the

most widely cultivated species in the Gulf of Maine, North America, and Europe (Grebe *et al.* 2019, 2021). *Saccharina latissima*, along with *Alaria esculenta*, is also considered to be one of the most promising macroalgal species for cultivation in the Western Hemisphere (Peteiro *et al.* 2016; Buck *et al.* 2017; Kerrison *et al.* 2018; Bricknell *et al.* 2021). *Saccharina latissima* is also a particularly interesting species to consider with regards to diverse markets for algal biomass because there are concerns about the limitations of its consumption as a food product due to the high iodine content of its tissue (Wells *et al.* 2016). However, *S. latissima* is a great candidate for other uses of algal biomass because it has a high specific growth rate, a life cycle that can be easily manipulated in the lab or nursery, and a tolerance for the warmer winter water temperatures associated with human-induced climate change (Bricknell *et al.* 2020).

Sea surface temperature and salinity data were obtained from monitoring efforts conducted by the Maine Department of Marine Resources (MEDMR) from 1990 - 2019 and Friends of Casco Bay in 2018 and 2019. We used all temperature and salinity observations recorded on sampling dates to calculate a grand mean for temperature and salinity in the month of May. Then we assigned the grand mean value to the monitoring station in a point layer. We elected to use the month of May as the period of focus because this is when most of the kelp cultivated in southern Maine is harvested, and it is also when the quality and yield of cultivated *S. latissima* from the WGoM is highly dependent on ambient environmental conditions (Grebe *et al.* 2019; 2021). We used Inverse Distance Weighting techniques to spatially interpolate between the monitoring stations which produced separate temperature and salinity raster data layers with smoothed predictions between individual sampling points. Then we used values for optimal temperature, salinity from the literature to convert all criteria data layers to Boolean format, where 1 = optimal and 0 = suboptimal (Table 5.2). As a ground-truth for the predicted mean May

water temperatures, we also collected and analyzed temperature and salinity timeseries data from 5 sites centrally located in Casco Bay (Fig. 5.2; Grid Squares A3 and B3). Water temperatures at each site were measured at 15 min intervals using either a Hobo Pendant Temperature/Light Logger Part UA-002-08 or a Hobo Dissolved Oxygen Logger, Part U26-001. Salinity was characterized for three of these sites using grab samples collected from 2 m depth with a Niskin bottle. This water was stored in Whirlpak bags prior to processing and assessed using a refractometer (Cole-Parmer RSA-BR90A; 0 – 90%).

**Table 5.2.** Biological considerations used for screening model inputs, data source and file type, Boolean assignments, and justification for assignments.

<b>Data</b>	<b>Source and File Type</b>	<b>Inside (= 1)</b>	<b>Outside (= 0)</b>	<b>Justification</b>
Sea surface temperature (SST)	FOCB (point); MEDMR (point)	$\geq 5^{\circ}\text{C}$ , $\leq 15^{\circ}\text{C}$	$< 5^{\circ}\text{C}$ , $> 15^{\circ}\text{C}$	Growth of <i>S. latissima</i> sporophytes is optimal at 5 – 15 °C (Fortes and Lüning 1980; Kerrison <i>et al.</i> 2015).
Salinity	FOCB (point)	$\geq 23$	$< 23$	<i>S. latissima</i> can withstand salinities of 23 - 35 with no reduction in growth (Druehl 1967; Bartsch <i>et al.</i> 2008). Stress responses are observed at lower salinities (Gerard 1987).

Ecological considerations

Ecological considerations for siting in Casco Bay are largely dictated by existing siting criteria from the Maine Department of Marine Resources (MEDMR), the state agency tasked with reviewing and approving applications for aquaculture in Maine state waters. The MEDMR’s regulatory framework has been recognized as one of the clearer and functional frameworks in the country (Bernadette 2013). The MEDMR’s aquaculture permitting process requires aquaculture producers to consider potential user conflicts and environmental impacts, stating that “a lease may not unreasonably interfere with riparian owners’ land access,

navigation, fishing, or other uses, support of ecologically significant flora and fauna, or public use or enjoyment within 1000 ft of government managed or conserved beaches, parks, docks, and land, and cannot have an unreasonable impact due to noise or light” (MEDMR 2019d).

Generally, the ME DMR criteria lead to the avoidance of cobble bottoms that provide habitat, breeding grounds and migration corridors, heavily fished areas, and transportation corridors. In addition to these State guidelines, a federal mandate by the US Army Corps of Engineers also stipulates that aquaculture leases must be sited outside delineated eelgrass zones (USACE 2015).

We used written guidance from MEDMR and USACE to convert all criteria data layers to Boolean format, where 1 = feasible and 0 = constrained (Table 5.3).

**Table 5.3** Ecological considerations used for screening model inputs, data source and file type, Boolean assignments, and justification for assignments.

<b>Data</b>	<b>Source and File Type</b>	<b>Inside (= 1)</b>	<b>Outside (= 0)</b>	<b>Justification</b>
Alewife critical habitats	MEDMR (Polygon)	$\geq 0.25$ mi	$<0.25$ mi	MEDMR has identified these lakes, ponds, and flowages that are not above a known natural barrier area as current and potential alewife habitat.
Eelgrass	MEDMR (Polygon)	$\geq 0.25$ mi	$<0.25$ mi	Eelgrass beds are important and sensitive habitat. Aquaculture arrays can shade or compete for space with <i>Zostera marina</i> if sited too close (Skinner <i>et al.</i> 2014; Ferriss <i>et al.</i> 2019). U.S. Army Corps of Engineers requires that “aquaculture be outside delineated eelgrass zones and requires that potential loss of any other benthic vegetation be declared”. For this layer we use Casco-Bay specific eelgrass surveys completed by MEDMR in 2018.
Sediment	Northwest Atlantic Marine Ecoregional Assessment (Polygon)	$< 0.48$ mm	$\geq 0.48$ mm	Experimental and standard leases must show evidence of minimal benthic impacts, because in addition to the protection of eelgrass beds, USACE also requires that the potential loss of any other benthic vegetation be declared (MEDMR). Sand and mud typically have the least amount of benthic flora and fauna (Palma <i>et al.</i> 1999; Lacharité and Metaxas 2017). Lester <i>et al.</i> 2018 also required “developable” sites to have soft bottom habitat.
Elver migratory pathways	MEDMR (Point)	$\geq 0.25$ mi	$<0.25$ mi	Locations where fishing activity for elvers was observed in 1996 and 2011 are fishing grounds and migratory pathways.

Social considerations

Social considerations, both public knowledge of aquaculture and perceptions or real competition with well-established water uses have strong influence over the growth of aquaculture in the United States (Bacher *et al.* 2015; Costello *et al.* 2020). However, social opposition to aquaculture is highly variable across regions and context (Bacher 2015; Froehlich 2017; Hanes 2018; Costello *et al.* 2020). In Maine, Johnson *et al.* (2019) identified marine

farmers, other water users, riparian landowners, coastal residents, and commercial seafood harvesters as actors with influence on aquaculture development and siting. Hanes (2018) further showed that the actors' perceptions of proposed aquaculture activities are influenced by their socioeconomic attributes (Hanes 2018). For example, areas with more amenity tourism (*i.e.*, second homeowners) tend to have more opposition to proposed aquaculture activities than areas that have less (Hanes 2018). To create layers that would likely represent the aquaculture-related preferences of actors in Casco Bay we collected publicly available data layers indicating the distance from shore, location of navigation corridors, sandy beaches, boat launches, conservation areas, elver harvest locations, molluscan shellfish areas, existing aquaculture leases and LPAs. Then we applied a conservative buffer around each polygon, point, or line and converted all criteria data layers to Boolean format, where 1 = optimal and 0 = suboptimal (Table 5.4).

**Table 5.4** Social considerations used for screening model inputs, data source and file type, Boolean assignments, and justification.

<b>Data</b>	<b>Source and File Type</b>	<b>Inside (= 1)</b>	<b>Outside (= 0)</b>	<b>Justification</b>
Navigation corridors	USACE (Polygon)	≥ 0.25 mi	<0.25 mi	MEDMR aquaculture siting criteria prohibit aquaculture installations within established navigation corridors.
Sandy beaches	Maine Geological Survey Coastal Marine Geologic Environments (CMGE) 1976 (Polygon)	≥ 0.5 mi	< 0.5 mi	Disturbance of swimming and picnicking have been previously presented at Maine aquaculture public lease hearings (Hanes 2018). Sandy beaches are likely areas with high concentrations of swimmers and picknickers, so we have added a 0.50 mi buffer along the length of these beaches.
Boat launches	Maine Department of Agriculture, Conservation and Forestry (Point)	≥ 0.25 mi	<0.25 mi	Disturbance of sailing, kayaking and motor boating were previously presented at Maine aquaculture public lease hearings (Hanes 2018). Boat launches are likely areas with high concentrations of boaters, so we have added a ¼ mi buffer around these areas.
Conservation areas	MaineGIS (Polygon)	≥ 0.25 mi	<0.25 mi	Identified and prioritized areas of high biodiversity or critical habitat provided by the Department of Agriculture, Conservation and Forestry, Bureau of Parks and Lands, Land Use Planning Commission, Department of Inland Fisheries and Wildlife, State Planning Office, The Nature Conservancy, New England Forestry Foundation, Maine private land trusts, US Park Service, US Fish and Wildlife, Maine municipal towns, Appalachian Mountain Club.
Molluscan shellfish areas	MEDMR 2010 (Polygon)	≥ 0.25 mi	<0.25 mi	If spatial overlaps between aquaculture and wild fisheries are considered, they can help to address potential interactions between them (Sivas & Caldwell, 2008; Clavelle <i>et al.</i> 2019; Costello <i>et al.</i> 2020; Froehlich <i>et al.</i> 2020). These polygons were reported to the MEDMR as shellfish areas from 2008 - 2010. Shellfish harvesters, town officials, harbor masters contributed information along with MEDMR staff including biologists, specialists, marine patrol officers, and scientists.
Existing aquaculture leases and LPAs	MEDMR 2021 (Point and polygon)	≥ 100 ft	<100 ft	MEDMR stipulates that leases and LPAs cannot be approved if there is already an LPA or lease at that site. A narrow buffer is applied to facilitate navigation between farms and account for potentially diminishing losses of having additional aquaculture in an area with existing arrays.
Distance from shore	Marine Cadastre.gov and Submerged Lands Act of 1953 via NOAA Shoreline data explorer (Line)	≥ 0.5 mi	< 0.5 mi	Establishing a corridor between the coastline and aquaculture installations is likely to reduce potential conflicts with recreational users and the visual impact on viewsheds. Shafer <i>et al.</i> (2010) showed that homeowners prefer that aquaculture is sited further from their home, but that these effects may diminish nonlinearly with distance.

## Bioextraction & Biofuels

Aquaculture installations producing biomass for markets like biofuels and bioextraction must consistently generate large quantities of macroalgal biomass and do so at low-cost (O'Shea *et al.* 2019; Chopin & Tacon 2020). These installations could consist of numerous modules, each spanning 100 meters in length (ARPA-E 2021), which may have a greater impact on viewsheds than small arrays. Thus, identifying ocean area further away from the coastline for these sites will likely reduce social conflicts. However, macroalgal installations in the northeastern U.S. can also benefit from proximity to consistent and elevated dissolved inorganic nitrogen and phosphorus in the effluent from wastewater treatment facilities (WWTFs) (Kim *et al.* 2017) which contributes to the suite of environmental conditions supporting maximum species-specific growth rates (Harrison & Hurd 2001). Additionally, the capital costs of current longline arrays increase fairly linearly with scale if they are designed for relatively shallow sites (< 50 m), but a significant increase in capital costs occurs when these sites are designed for depths greater than 50 m (Bak *et al.* 2020). To create layers that would likely represent the optimal ocean area for growing macroalgae destined for markets like biofuels and bioextraction we collected publicly available data layers indicating the locations of WWTFs in the bay, bathymetry, and the location of the coastline. We applied a polygon buffer around each WWTF and processed the bathymetry and coastline data into rasters for depth and distance from shore to enable these additional relevant considerations. We used observations from the literature to convert all criteria data layers to Boolean format, where 1 = optimal and 0 = suboptimal (Table 5.5).

**Table 5.5** Data used for screening specific to bioextraction and biofuel markets, data source and file type, Boolean assignments, and justification for assignments.

<b>Data</b>	<b>Source and File Type</b>	<b>Inside (= 1)</b>	<b>Outside (= 0)</b>	<b>Justification</b>
Site depth	Northwest Atlantic Marine Ecoregional Assessment (Polygon)	>10 m, < 50 m	≥ 50 m	The technical limit on longlines are depths > 100 m (Kapetsky <i>et al.</i> 2013, Mizuta & Wikfors 2019), but Bak <i>et al.</i> (2020) consider depths ≥ 50 m to present "offshore" or "exposed" conditions.
Anthropogenic nutrients from WWTFs	MEDEP (Point)	≤ 2 mi	> 2 miles	Pollution discharge elimination system facilities are reliable sources of dissolved inorganic nitrogen and phosphorous. Using nitrogen stable isotopes, Grebe <i>et al.</i> (2021) observed that kelp grown in Casco Bay, ≥2 mi from a WWTF did not show evidence of nitrogen use from WWTFs.
Distance from shore	Marine Cadastre.gov and Submerged Lands Act of 1953 via NOAA Shoreline data explorer (Line)	> 0.5 mi = 1, < 3 mi	≤ 0.5 mi, > 3 mi	Further away reduces potential conflicts with recreational users and visual impact and increases the likelihood for approval of large farms which will be required to produce low-cost kelp biomass. Shafer <i>et al.</i> (2010) showed that homeowners prefer that aquaculture is sited further from their homes, but that these effects may diminish nonlinearly with distance. Evans <i>et al.</i> (2017) examined single-family home sales from 2012 - 2014 and found that in Casco Bay, having an aquaculture farm within a 2-mile radius (approximately 14% of all homes in the bay) had no evidence on housing price. Two miles is the upper bound of impact identified by Evans <i>et al.</i> (2017) through a mixture of stakeholder feedback, literature review, and consideration of visibility of a 1ft structure above the water surface. Bak <i>et al.</i> (2020) consider sites > 3 mi to be "offshore" conditions.

### Organic food and feed inputs

Within the State of Maine, the most robust guidance for macroalgae destined for food or feed inputs is provided by the Maine Organic Farmers & Gardeners Association's (MOFGA) Certification Services which presents certification criteria for organic sea vegetables per mandate by the U.S. Department of Agriculture National Organic Program (NOP). Development of the siting criteria was informed by existing criteria from the Canadian Organic Crop Improvement Association and by adapting NOP 205.202 to identify waters of "high ecological quality" away from known sources of radioactive, chemical, or sewage bacteriological contamination (MOFGA Certification Services LLC, 2018). We incorporated MOFGA's siting criteria into the screening model by obtaining publicly available data layers for each of the potential sources of contamination, except for major and small harbors which we created manually using satellite imagery in Google Earth. Then we applied polygon buffers around each potential contamination point, erased those from the Casco Bay mask, converted the remaining polygon into raster, and assigned a Boolean value of 1 = optimal for all pixels in that raster. We also obtained publicly available bathymetry and coastline layers and processed them into rasters for depth and distance from shore to enable these additional relevant considerations. We then used written guidance from MOFGA and observations in the literature to convert all criteria data layers to Boolean format, where 1 = optimal and 0 = suboptimal (Table 5.6). All rasters were compared in a Boolean And operation to obtain a single final layer indicating optimal ocean area for high-end food and feed markets.

**Table 5.6.** Data used for screening specific to organic food and feed markets, data source and file type, Boolean assignments, and justification for assignments.

Data	Source and File Type	Inside (= 1)	Outside (= 0)	Justification
Site depth	Northwest Atlantic Marine Ecoregional Assessment (Polygon)	>10 m, < 50 m	≥ 50 m	The technical limit on longlines are depths > 100 m (Kapetsky <i>et al.</i> 2013, Mizuta & Wikfors 2019), but Bak <i>et al.</i> (2020) consider depths ≥ 50 m to present "offshore" or "exposed" conditions.
Distance from shore	Marine Cadastre.gov and Submerged Lands Act of 1953 via NOAA Shoreline data explorer (Line)	> 0.5 mi	≤ 0.5 miles	Further away reduces potential conflicts with recreational users and visual impact and increases the likelihood for approval of large farms which will be required to produce low-cost kelp biomass. Shafer <i>et al.</i> (2010) showed that homeowners prefer that aquaculture is sited further from their home, but that these effects may diminish nonlinearly with distance.
Nuclear	n/a	n/a	n/a	MOFGA eligible sites must be twenty (20) miles from any nuclear facility (Organic seaweed siting criteria -- 11.2 NOP §205.202 Land Requirements).
Commercial boat building	n/a	≥ 3	< 3 mi	MOFGA eligible sites must be three (3) miles from any commercial boat building facilities. (Organic seaweed siting criteria -- 11.2 NOP §205.202 Land Requirements).
Industrial and wastewater discharge	MEDEP (Point)	≥ 3	< 3 mi	MOFGA eligible sites must be three (3) miles from any industrial wastewater and city/town discharge area. (Organic seaweed siting criteria -- 11.2 NOP §205.202 Land Requirements).
Major harbor and thoroughfare	Maine DACF (Point)	≥ 3	< 3 mi	MOFGA eligible sites must be three (3) miles from any major harbor or thoroughfare (Organic seaweed siting criteria -- 11.2 NOP §205.202 Land Requirements).
Major thoroughfare	USACE (Polygon)	≥ 3	< 3 mi	MOFGA eligible sites must be three (3) miles from any major thoroughfare (Organic seaweed siting criteria -- 11.2 NOP §205.202 Land Requirements).
Small harbors	NOAA ENC Chart US4ME03M (Point)	≥ 0.75 mi	< 0.75 mi	MOFGA eligible sites must be 0.75 mi miles from any major harbor or thoroughfare (Organic seaweed siting criteria -- 11.2 NOP §205.202 Land Requirements).
Minor WWTF outfall	MEDEP (Point)	≥ 0.75 mi	< 0.75 mi	MOFGA eligible sites must be 0.75 mi from any minor WWTF (Organic seaweed siting criteria -- 11.2 NOP §205.202 Land Requirements).
OB discharge or other effluent	MEDEP (Point)	≥ 0.25 mi	<0.25 mi	MOFGA eligible sites must be 0.25 mi from any overboard discharge area or special circumstance ( <i>i.e.</i> , fish farm, small boat builder, etc.)(Organic seaweed siting criteria -- 11.2 NOP §205.202 Land Requirements).

### Additional considerations or data not included

We considered several other potentially relevant data identified by previous aquaculture siting analyses and discussions (Ross *et al.* 2013), but these data were either not publicly available or we determined them to not be applicable (Table 5.7).

**Table 5.7.** Potentially relevant data not included in the screening model and justification.

<b>Data</b>	<b>Justification</b>
Nitrate, ammonium, and phosphate	Insufficient spatial resolution of observations during growing season
Lobster zones	One zone for all of model area
Principal boat use	One zone for all of model area: motor boat use
Oil production or pipelines	No active oil production in model area
Seed mussel areas	No designated seed areas in model area
Scallop management areas	Rotate annually
Marine protected areas	No designated MPAs in model area
Marine mammals and turtles	No sightings recorded within model area
Atlantic salmon habitat	No designated spawning or rearing habitat in the tributaries to Casco Bay model area
Piping plover, roseate tern, least tern essential habitat	No designated essential habitat for the species in Casco Bay
North Atlantic right whales	No sightings recorded within model area

### **5.3.3 Scenarios**

We used the Boolean operator in ArcGIS Pro to combine and analyze the relevant layers for each scenario. We began by first running the Boolean operator with biological, ecological, and social criteria layers to areas that would fit these constraints. Then we added in the social criteria layers and re-ran the Boolean operator to obtain a refined map for *S. latissima* regardless of end use.

From there, we incorporated either the Bioextraction & Biofuels layers or the Organic Food layers. We combined all layers in the Boolean operator to explore whether there were any areas that have clearly overlapping or co-located market potential. Lastly, we explored the model's sensitivity to the depth and distance from shore constraints by enabling farms in the Organic

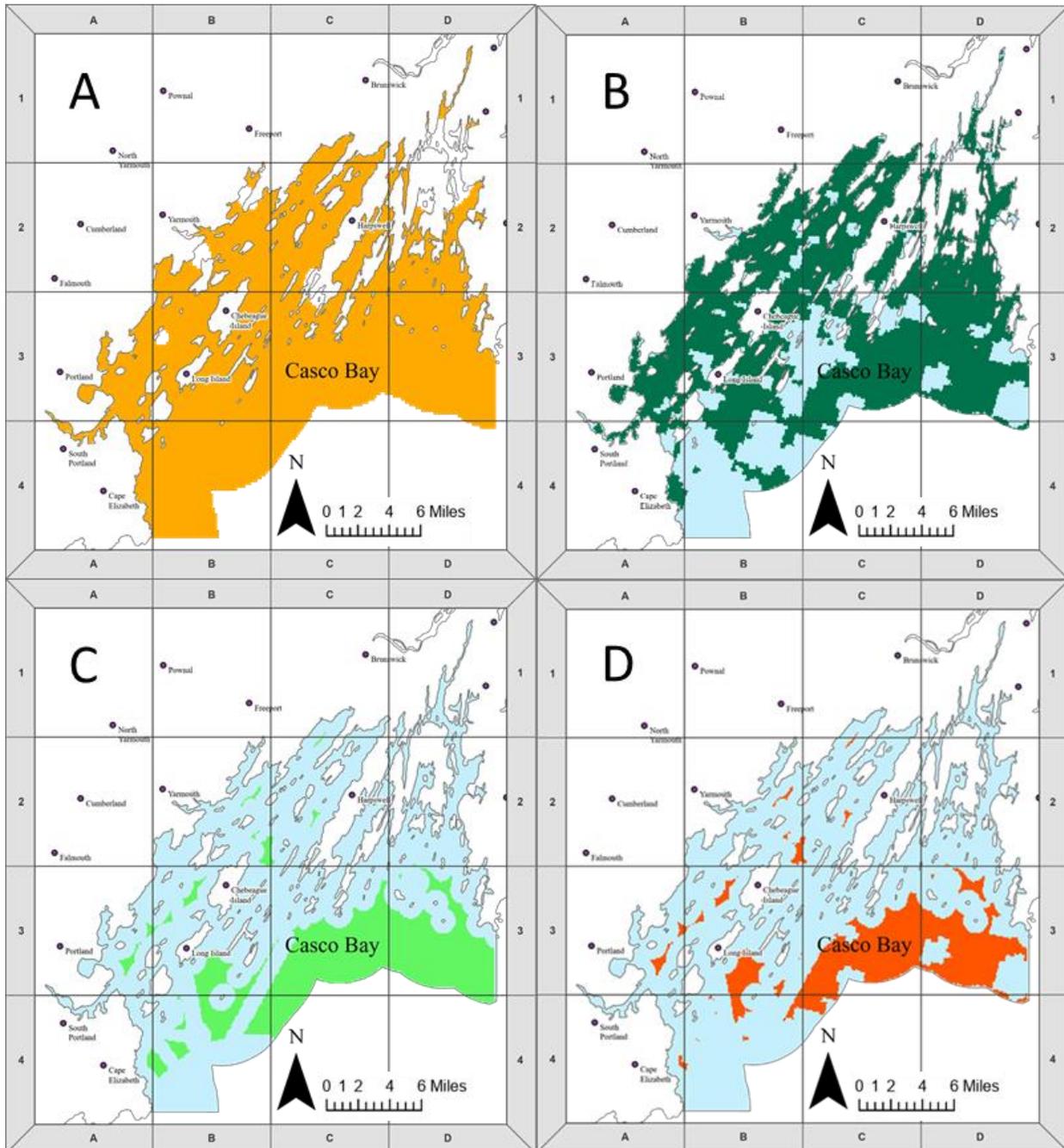
Food Layers to be sited within 0.25 mi of shore and enabling farms in the Bioextraction & Biofuels layers to be sited across ocean areas without seafloor depth constraints.

## 5.4 Results

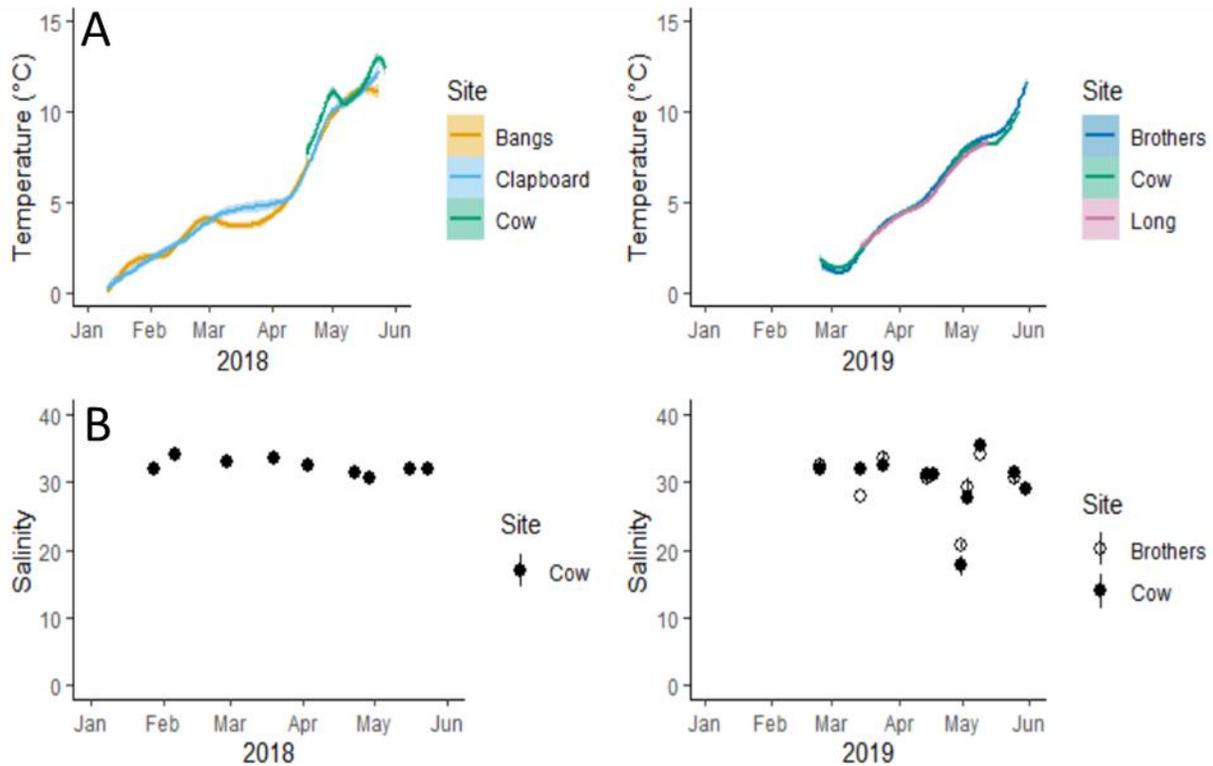
Much of Casco Bay was predicted as biologically optimal for *S. latissima* in the month of May (Fig. 5.3, A). Mean May temperatures across the Bay are predicted to be within the 5 - 15°C range and the temperatures measured at the aquaculture sites in 2018 and 2019 support this general prediction (Fig. 5.4). Some areas near river mouths are predicted to have low mean salinities for the month of May which resulted in several concentrated areas being predicted as suboptimal (Grid Squares B2 – B4). The salinities observed at our monitoring sites in 2018 and 2019 demonstrate how steep declines in salinity can occur during the May period of consideration (Fig. 5.4). The ecological criteria were more restrictive, predicting that areas where longline kelp aquaculture would have the least impact on migratory fish species of concern, eelgrass beds, and benthic fauna were located throughout the Bay (Fig. 5.3, B). The social siting criteria, the most restrictive of the three screening categories, predicted that areas where longline kelp aquaculture would have the least impact on navigation, recreation, conservation, molluscan and riverine fisheries, and viewsheds were primarily located in Grid Squares B4, B3, C3, and D3 (Fig. 5.3, C).

When the biological, ecological, and social suitability criteria were combined in a Boolean overlay, small areas predicted to meet the screening criteria were located throughout the Bay, but the largest contiguous areas predicted to be optimal were in the easternmost region (Grid Squares C3 and D3; Fig. 5.4, D). When we applied the additional criteria for nutrient bioextraction or organic seaweed cultivation in a Boolean overlay with the biological, social, and

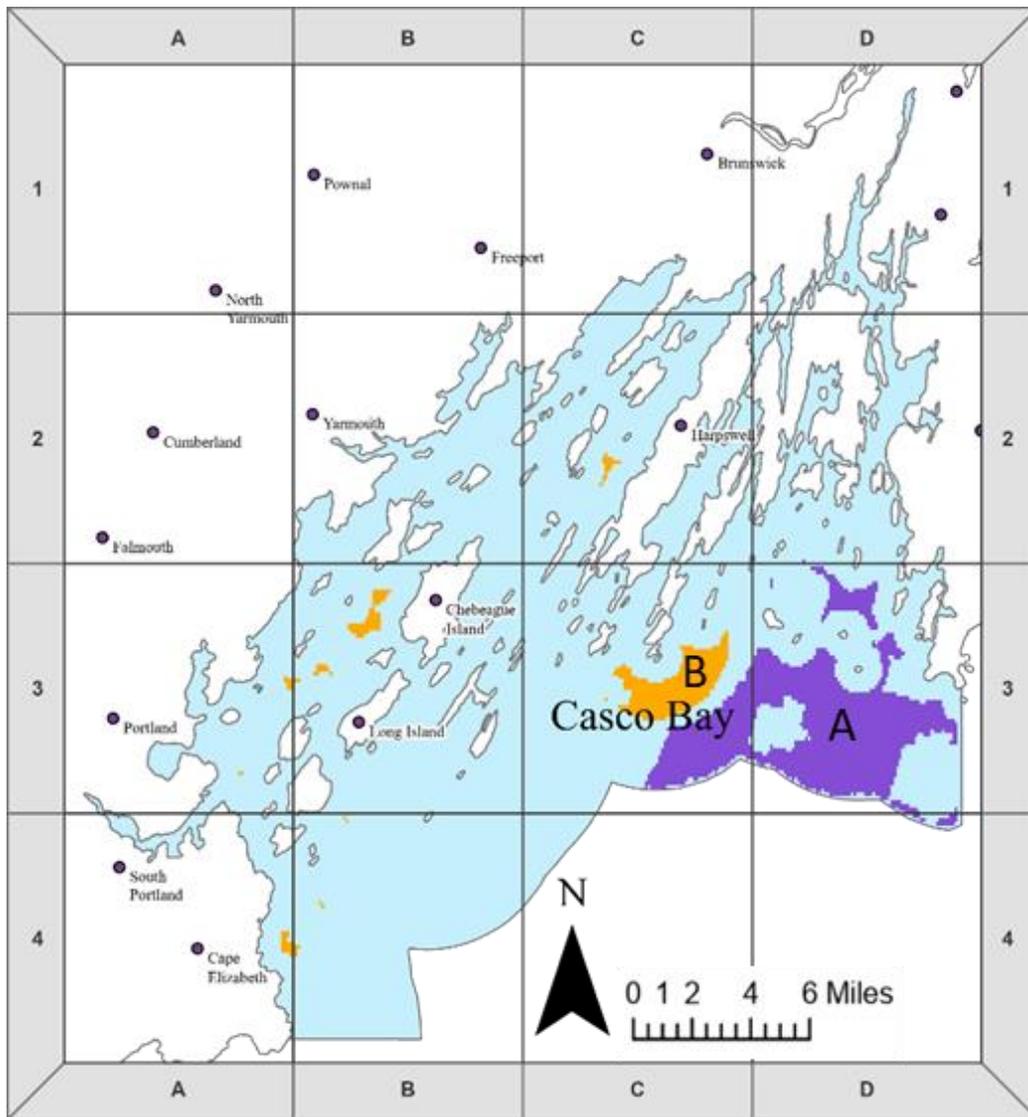
ecological criteria, the optimal ocean area was further reduced within the already identified Grid Squares C3 and D3 (Fig 5.5).



**Figure 5.3 (previous page).** Ocean area (shaded) that is predicted to meet biological, ecological, or social screening criteria. A) Orange shading shows ocean area predicted to be optimal for *S. latissima* using our biological screening criteria. B) Dark green shading shows area predicted to be optimal for longline seaweed aquaculture using our ecological screening criteria. C) Light green shading shows area predicted to be optimal for longline seaweed aquaculture using our social screening requirements. D) Red shading shows ocean area satisfying biological, ecological, and social screening requirements.



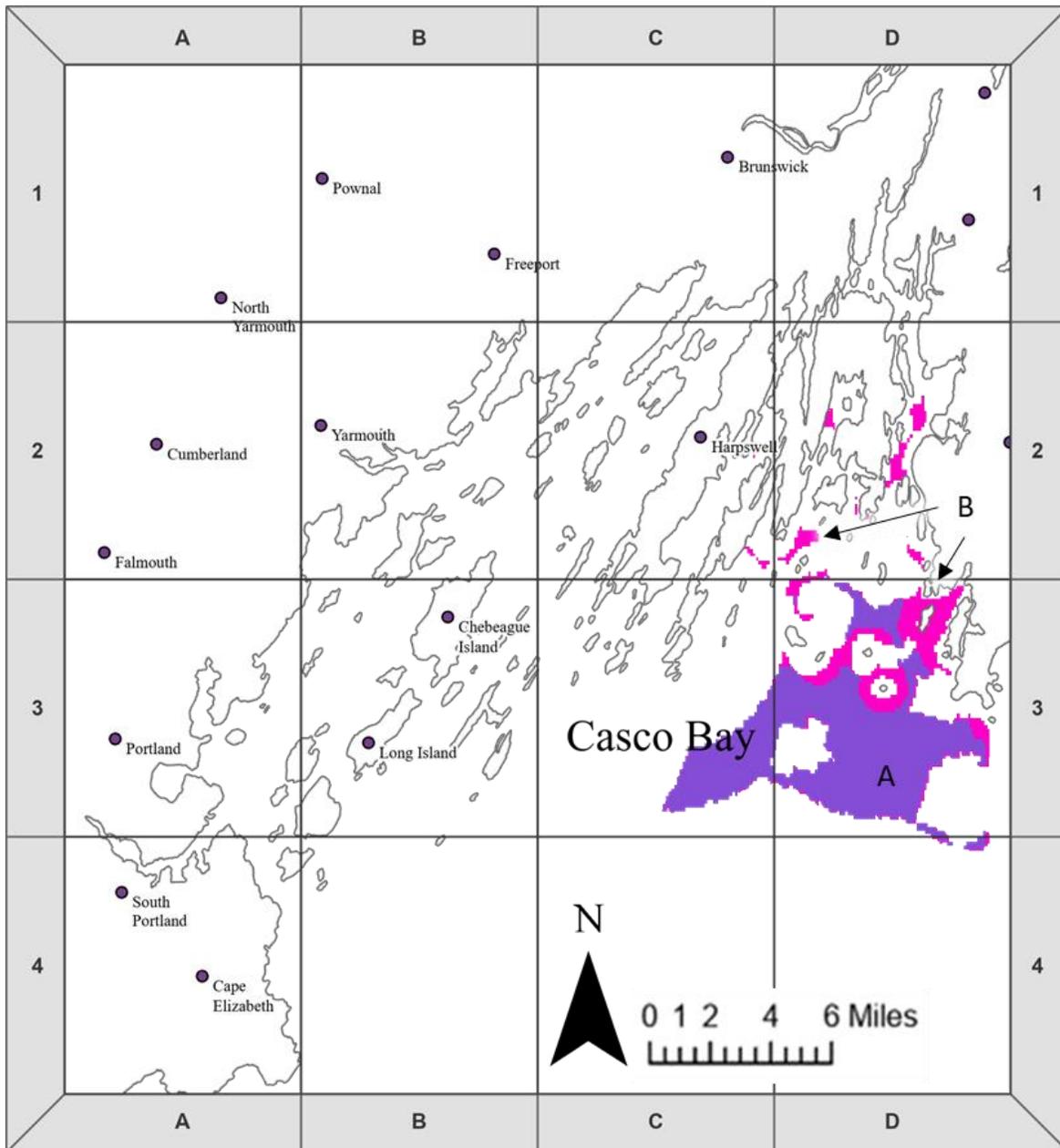
**Figure 5.4.** Daily water temperatures (A) and salinity (B) measured at Bangs Island, Brothers Island, Clapboard Island, and Cow Ledge from January – June 2018 and 2019. Loggers were suspended approximately 2 m below the water surface. Salinity was quantified by water grab samples collected from 2 m below the water surface.



**Figure 5.5.** Ocean area predicted to be optimal for specific markets. A) Purple shading shows ocean area which may be optimal for producing *S. latissima* destined for high-end food and feed markets. B) Orange shading shows ocean area which may be optimal for producing *S. latissima* destined for biofuel and bioextraction markets.

We explored an alternative scenario for both market types: A modification of the depth criterion for the bioextraction and biofuel markets and a modification of the distance from shore buffer for

the high-end food and feed markets. Initially, a 50 m depth limit was applied to all arrays to account for the limitations of low-cost design and installation of longline arrays. When this limit was removed from the biofuel and bioextraction criteria, there was no shift in predicted optimal area for this market. This is because the deeper ocean area in Casco Bay is further than 2 mi from any WWTF and being within that range was a separate criterion for the biofuel and bioextraction markets. Initially a 0.5 m buffer was applied to all potential ocean area to reduce the likelihood of impact on coastal water uses and viewsheds. When this was removed from the Boolean Overlay for high-end food and feed markets, additional optimal ocean area was predicted in Grid Squares D3 and D2 (Fig. 5.6).



**Figure 5.6.** Increased optimal ocean area for high-end food and feed markets obtained when coastal buffer criterion is removed. A) Predicted optimal ocean area for arrays when coastal buffer restricting siting within 0.5 mi of shore is active. B) Predicted optimal area gained when buffer is relaxed.

## 5.5 Discussion

As additional criteria were applied in the screening analysis, the ocean area predicted to be optimal was generally reduced and shifted eastward to grid squares furthest away from coastal development, river mouths, conserved lands, and recreation centers like boat launches and harbors. Grid squares C3 and D3 appear to be the best location within Casco Bay for a potential AOA and AZA because they have a large quantity of ocean area predicted to be optimal for both high-end food and feed markets and bioextraction and biofuel markets. In the following subsections we further discuss the considerations with high influence on the predictions, the limitations of this approach, and possibilities for future efforts to build on this work.

### 5.5.1 High-end food and feed markets

The prioritization of cultivation sites to produce sugar kelp meeting MOFGA's organic sea vegetable requirements resulted in eastward shifting of zones classified as highly promising for *S. latissima* aquaculture. This shift occurred because some of the organic buffers exclude a sizable area of Casco Bay. When the coastline buffer was relaxed to enable siting within 0.5 mi of the coastline, this resulted in the consideration of additional ocean area in Casco Bay that met MOFGA's criteria. Relaxing this buffer is meant to mimic an increased social acceptance of aquaculture impacts to viewsheds and soundscapes. Evans *et al.* (2017) showed that in Casco Bay the willingness to accept viewshed and soundscape impacts is already higher than other locations in the State. If this increased acceptance can be maintained for small arrays, this will benefit producers. Grebe *et al.* (in review) showed the value of small harvests throughout the spring to mitigate sloughing, biomass loss to storms, and potentially extract a price premium for early-season kelp and frequent harvests are facilitated by having an accessible site closer to shore.

In the United States there is currently no specific guidance from federal agencies regarding the siting of seaweed aquaculture for food or feed. Instead, states have been left to determine and implement regulations with few regional studies specifically exploring the relationship between water quality and food and feed safety. To provide some direction for aquaculturists, several states have restricted seaweed aquaculture to shellfish harvesting areas, but this is misguided, because macroalgae and shellfish are functionally very different in their interactions with pathogens and harmful algal blooms. The MOFGA criteria used in this analysis does not currently apply phyecological knowledge or regional oceanographic knowledge either, but the National Organic Standards Board, the rule-making entity advising the NOP, is in the process of reviewing these standards with the goal of refining the criteria based on the best available science around marine water quality and seaweed harvesting.

The forecasted impacts of climate change, including increasing interannual variability with temperature and precipitation in the Gulf of Maine (Bricknell *et al.* 2020), may also result in a shift of optimal area for aquaculture activities targeting high-end food and feed markets. These shifts in optimal area could occur from changes in physical conditions at a site resulting in marked differences in the characteristics of the macroalgal biomass produced. If terrestrial agriculture becomes more challenging in the future climate scenarios, there may also be an increased social acceptance of aquaculture activities producing food and feed inputs within the GOM.

### **5.5.2 Bioextraction and biofuels**

Much less ocean area is considered optimal for *S. latissima* cultivation when the biofuel and bioextraction criteria are applied because the rule that the site must be within 2 mi of a nutrient point source works against the social buffers that prioritize aquaculture development

further away from coastlines and activity centers like boat launches. While proximity to a point source of nutrients like WWTFs makes sense when *S. latissima* is grown for low-purity-high-biomass markets, it could be adjusted in accordance with other goals, for example, the strategic cultivation of seaweeds as a method for managing effluent from fed aquaculture systems like open-water salmon pens (Goldman *et al.* 1974; Ryther *et al.* 1975; Neori *et al.* 1996, 2004; Chopin *et al.* 1999, 2001, 2012). Presently there are no ocean or land-based fed aquaculture systems in the Casco Bay region, but there are two land-based recirculating aquaculture systems for salmon under construction in nearby Bucksport and Belfast. If fed aquaculture systems were established in Casco Bay, this could result in a change of siting strategy for at least a portion of the operations growing kelp for biofuel or bioextraction markets.

The cost of production, and therefore the location of seaweed processors or buyers, will also become increasingly important with the scale of production required for biofuels and bioextraction. The current cost of seaweed production is too high to fulfill these larger markets (van den Burg *et al.* 2016; O'Shea *et al.* 2019). Suurs (2002) and van den Burg *et al.* (2016) showed that the cost of unloading and loading is the greatest contributor to overall cost of transportation in the macroalgal biomass supply chain; indeed, it is almost comparable to the cost of moving the seaweed from farm to harbor, and four times as much as transporting it to the processing facility. Thus, if a biomass processing facility were established along the Casco Bay Coastline that facilitated unloading of harvested seaweed biomass, that could result in a shift of optimal area for large arrays, because it would lower the total cost of production.

#### **5.4.3 Potential Aquaculture Opportunity Areas**

The co-located ocean area predicted to be optimal for both high-end food and feed and biofuel and bioextraction markets identifies a portion of Casco Bay that could be considered for

an Aquaculture Opportunity Areas or Allocated Zone for Aquaculture. The U.S. National Oceanic and Atmospheric Administration (NOAA) describes Aquaculture Opportunity Areas (AOAs) as geographic “areas that are environmentally, socially, and economically appropriate for commercial aquaculture” (NOAA 2021). NOAA’s process for establishing AOAs uses spatial analysis and public input to determine the exact location of an area that has optimal ocean conditions for aquaculture and minimal potential user conflicts and aquaculture operations within the AOAs are required to comply with all applicable federal and state regulations. North Carolina, Florida, and Virginia currently have established AOAs (Campbell et al. 2021). Allocated zones for aquaculture (AZAs) are areas where aquaculture development has been prioritized in the marine spatial planning process (Clavelle *et al.* 2019). Maine does not currently have either of these predefined aquaculture areas; instead, initial siting choice is entirely determined by the lease applicant, and Evans *et al.* (2017) highlight that this distinction has resulted in largely decentralized spatial pattern of aquaculture areas in the state. However, given the recent growth of the aquaculture industry, the establishment of AOAs or AZAs can both encourage critical cooperation between small-scale aquaculture producers on the grounds of co-located sites (Campbell *et al.* 2021) and define the extent to which aquaculture can encroach on other water uses (Clavelle *et al.* 2019). If AOAs or AZAs are established in Maine, locating one in the identified area which is predicted to support seaweed aquaculture for multiple markets and minimize impacts to the environmental and other water users would be advised.

The co-located ocean area predicted to be optimal for both market categories in Grid Square C3 may also be indicative of a zone particularly well-suited for cultivation of kelp destined for an advanced seaweed biorefinery. In the biorefinery processing model, the same macroalgal biomass can be used to produce several intermediate products (Bikker *et al.* 2016;

Van den Burg *et al.* 2016). This concept is gaining considerable traction within biochemical fields as the development key to a profitable seaweed value chain (van den Burg *et al.* 2016) and *S. latissima* produced in the identified zone would meet MOFGA's organic criteria while also benefitting from the economies of scale made possible by larger cultivation arrays. The development of new uses for seaweed biomass, or greater adoption of existing uses could also warrant a new iteration of market-based screening analysis for *S. latissima* in Casco Bay.

When concentrated siting of macroalgal farms in AOAs or AZAs is brought forth for consideration by the MEDMR, the potential social and regulatory benefits of this approach must be balanced with the anticipated ecosystem services supporting aquaculture production. Marine spatial planning tools like AOAs or AZAs can likely mitigate many of the potential environmental impacts from large-scale kelp cultivation (Lester *et al.* 2018), by excluding critical habitat areas, sensitive benthic flora like eelgrass, known migration corridors and spawning grounds. However, water circulation that supplies macroalgal arrays with fresh nutrients could be reduced if several larger arrays with dense biomass are sited closely together (Zhang *et al.* 2004; Shim *et al.* 2014; Park *et al.* 2018). The risk of disease and pests spreading from one cultivation array to another is also elevated when spacing between farms is reduced (Salama & Murray 2011; Buschmann *et al.* 2014). Mitigating these unintended impacts is likely possible with AOAs or AZAs in Casco Bay, but it will require diligent and extensive monitoring of the environmental conditions at these locations.

#### **5.5.4. Strong influence of social considerations**

Social considerations had the strongest influence on the results of the screening analysis. The application of conservative buffers around areas with high social importance prevents much of the western Casco Bay from being designated as optimal area for seaweed aquaculture with an

undefined market (Fig. 5.3). This pattern is consistent with the work presented by Hasselström *et al.* (2018) who conducted an in-depth assessment of the potential impact of 2 hectares of *S. latissima* aquaculture along the Koster archipelago on the western Swedish coastline. They concluded that the externalities of small-scale seaweed aquaculture in that region appear to be mostly social impacts like potentially negative impacts on space and waterways, recreation, aesthetic values, and natural heritage. The exclusion of much of western Casco Bay is also consistent with the summary of state-wide stakeholder concerns that Hanes (2018) provided after synthesizing the historic aquaculture lease hearings. When combined, 53% of all concerns raised at these hearings were related to boating or aesthetics (Hanes 2018), so by buffering around boat launches, sandy beaches, and conservation areas there is a much better likelihood that proposals to site seaweed farm outside of those areas will not be subject to these concerns.

Our analysis used rigid relationships to predict suitability and theoretical shifts in siting according to end-uses of kelp. As such, it was difficult to capture aspects of conflicts and compromise between stakeholders that can be situation specific. For example, Maine's extant aquaculture lease application and review process is one of the key factors enabling the recent growth of seaweed aquaculture in the region (Grebe *et al.* 2019). Mandatory scoping sessions and public hearings are included in Maine's lease application process which facilitates conversation and potential compromise between concerned citizens, government officials, and aquaculture practitioners (Hanes 2018; Johnson *et al.* 2019). This formalized process provides the structure to support compromises in siting to be addressed at the site-level scale. For example, Hanes (2018) reports on a lease hearing in which a proposed lease that was highly controversial for actors of several backgrounds was ultimately approved after the size of the lease and water access through the lease was modified by the applicant after receiving feedback

during their hearing. He further concludes that aquaculturists and MEDMR find ways to adapt proposed aquaculture activities to the scenic and recreational priorities expressed by the public (Hanes 2018). Screening analyses have a limited ability to capture negotiation like this; using our approach, this site would have likely not have been identified as an optimal area due to one or more potential conflicts, but Campbell *et al.* (2021) suggest that including components of community suitability and values in screening analyses could help. For example, future screening analyses aimed at identifying specific parcels predicted to be optimal for *S. latissima* could build on the work we present here by collecting additional data on community preferences and values with a Bayesian Belief Network, as several studies have previously shown (Schmitt & Brugere 2013; Coccoli *et al.* 2018). Then those value systems could be used to assign values in a weighted linear combination MCE. This would likely result in an expansion of the ocean area determined to be socially optimal because the sites could be considered on a spectrum rather than binarily sorted (Thomas *et al.* 2019).

Screening analyses are also limited in their ability to capture the cumulative effect of aquaculture activities or the positive feedback loops that can develop as stakeholders become more familiar with aquaculture activities. Several studies show that the public's perception of aquaculture is influenced by lack of knowledge of the practice (Bricknell & Langston 2013; Bronnmann *et al.* 2017; Rickard *et al.* 2020), concern about local environmental impacts unrelated to aquaculture development (Froehlich *et al.* 2017), and prior experiences, or lack thereof, with aquaculture (Evans *et al.* 2017; Hanes 2018). When marine spatial planning efforts for Casco Bay are initiated in the future, engaging stakeholders in an interactive mapping platform or simulation game (as demonstrated in Verutes & Rosenthal 2014 or Gangnery *et al.* 2021) may help to capture these aspects of compromise and cumulative development. Li *et al.*

(2020) showed that estimated opportunity costs were different between static and dynamic methods, highlighting the necessity to include dynamic costs as an extra source in decision-making.

New macro policies, whether directly or indirectly pertaining to marine resource use or aquaculture, could also result in a significant discordance between forecasted and actual optimal areas for seaweed aquaculture siting in Casco Bay. Gephart *et al.* (2020) highlight that macro policies stemming from globalization and economic growth play a huge role in the establishment of aquaculture. For example, macro policy changes like the 2020 initiative for expanded aquaculture exploration in the US conveys a message that aquaculture development has been recognized as a need at a federal level (Fairbanks 2019) which may ultimately influence perceptions of aquaculture at a community or individual level.

## **5.6. Conclusion**

The U.S. seaweed aquaculture industry is poised to move past proof-of-concept and into routine production of seaweed biomass for specific markets. However, the optimal siting of seaweed farms might vary depending on the target market for this biomass. In this study, we present the first exploration of how market-specific siting may result in distinct predictions for optimal ocean area by using *S. latissima* aquaculture in Casco Bay, ME as an example. We co-located physical data in a GIS-based screening analysis, beginning with a baseline screening analysis that included conservative biological, ecological, and social constraints as a baseline and then added in market-specific constraints for either high-biomass-low-purity markets or low-biomass-high-purity markets. When more conservative baseline constraints are applied, the results indicate that most of the optimal area for kelp aquaculture is in the easternmost portion of the bay. This distribution pattern persists when organic food and feed market constraints are

applied, and when biofuel and bioextraction market constraints are applied, but in both cases the optimal area is reduced. The model predicts a clear collocation of optimal ocean area for both market types which indicates that is could be an ideal location for an aquaculture opportunity area. Ultimately this analysis suggests that the distribution patterns of optimal areas for kelp aquaculture may not be market-specific when conservative baseline siting constraints are applied. However, if social acceptance of small-scale aquaculture is higher than assumed, or if it increases in the future, the ocean area predicted to be optimal for *S. latissima* destined for high-end food and feed markets could potentially be more evenly distributed across the bay. Future studies can contribute to, or build on, our approach by collecting additional biophysical data throughout the winter growing season, incorporating additional criteria for specific markets, and by further assessing stakeholder willingness to accept seaweed aquaculture installations of various sizes.

## CHAPTER 6

### CONCLUSION

The work presented in this dissertation generated new scientific knowledge regarding the siting, management, production yields, and ecosystem services of *S. latissima* in the Western Gulf of Maine. Specifically, I offer four intersectional studies of kelp aquaculture that were designed with principles of socioecological systems and industry relevance in mind. Their significance may be described as: the first study of bioextraction in the Gulf of Maine, the first time the Ecosystem Approach to Aquaculture has been applied to a non-fed species, the most comprehensive reporting of *S. latissima* morphological and compositional responses to distal-end trimming, and the first publicly available site screening for seaweed aquaculture that includes market-specific constraints .

Key findings from each chapter can be summarized as:

- **Chapter 1:** The Ecosystem Approach to Aquaculture can, and should, be applied to new kelp aquaculture in the region. Specific attention should be given to protecting the genetic diversity of wild kelp beds and cultivar development using strain selection, developing best management practices and climate resiliency within the industry, and integrating wild and farmed kelp management.
- **Chapter 2:** On a hectare-to-hectare basis, the nitrogen bioextraction by kelp farming in the region far exceeded the nitrogen loading from riverine or atmospheric sources. The amount of N removed can be maximized by harvesting in early May. Ambient environmental conditions at the sampling sites were significantly different from one-another, highlighting the importance of pilot-

studies for bioextraction (and reinforcing the value of the DMR's aquaculture regulations allowing for limited purpose agreements).

- **Chapter 3:** Distal-end trimming had a significant effect on *S. latissima* stipe morphology, blade morphology, and increased late-season production yields. Two distinct size classes were observed, and each size class responded differently to the trimming treatment. Distal-end trimming appears to be a strategy that can help to retain cultivated kelp biomass later into the season by reducing the risk of dislodgement or breakage from storms. The practice may also offer economic benefits for farmers if the trimmed biomass can be sold for a price premium.
- **Chapter 4:** The ocean area predicted to be optimal for seaweed aquaculture can shift when market-specific constraints (*e.g.*, composition, food safety, biomass produced) are applied to siting criteria for kelp aquaculture. If resource managers can consider the uniqueness of these locations in aquaculture zoning efforts this may better serve the budding U.S. seaweed aquaculture industry. In Casco Bay, societal preferences, and not biological needs or ecological conservation measures, are predicted to have the strongest influence on optimal siting of *S. latissima* aquaculture.

I hope that the result of my work can benefit multiple stakeholders. Findings from these efforts may benefit coastal resource managers like the Maine Department of Marine Resources or the United States Army Corps of Engineers by providing informed recommendations for priority management measures for seaweed aquaculture. Regionally ground-truthed estimates of nitrogen removed by *S. latissima* harvest in the Gulf of Maine could be incorporated into cost-

benefit analyses of nitrogen mitigation or extraction measures and may be used as a baseline estimate for future nutrient trading programs in the region. The identified additional data needs for kelp aquaculture screening models may be of interest to the National Oceanic and Atmospheric Administration that is tasked with developing suitability analyses for aquaculture along both U.S. coasts. Most importantly, I hope that some of these findings will benefit existing and prospective seaweed producers as we continue to seek insight regarding where to site operations, when to harvest seaweed crops, which markets (food, energy, offsets, etc.) offer the best revenue generating opportunities, and how to continue developing this practice in an environmentally and socially sustainable manner.

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## **BIOGRAPHY OF THE AUTHOR**

Gretchen Grebe was born in Denver, Colorado and graduated from the Colorado Rocky Mountain School in 2005. She attended Bates College in Lewiston, ME and graduated in 2009 with a Bachelor of Arts in Latin American and Environmental Studies. After several years working in K -12 science education, Grebe enrolled in the Bren School of Environmental Science & Management at the University of California, Santa Barbara where she specialized in Water Resources Management and Eco-Entrepreneurship. She graduated from Bren in 2013 with a master's degree in Environmental Science and Management. Grebe became interested in aquaculture and marine resources during her first year at Bren and built a business plan for an integrated multi-trophic aquaculture system as a component of her group master's thesis. Following her time at Bren, Grebe worked as a watershed scientist and the feed program director for a marine non-profit in B.C.S, Mexico. In the latter role, she collaborated with fishing communities to develop small businesses based in marine aquaculture and recapture of fisheries waste.

In 2016, Grebe started a doctoral program in Aquaculture and Aquatic Resources at The University of Maine. During her first four years in the program she was a fellow in the Sustainable Ecological Aquaculture Network (SEANET) working out of the Byron Marine Ecology Lab at the University of New England Biddeford Campus. Grebe volunteered as a guest aquaculture educator with the Island Institute's Kelp for Kids Program, a visiting researcher at the Hurricane Island Foundation, and a scientific diver in training during her doctoral work. She also provided service to her academic community as a frequent grant reader for the UMaine Graduate Student Government, manuscript reviewer for a handful of peer-reviewed journals, and

by serving two years on the UMaine School of Marine Sciences Professional Development Committee.

In the fall of 2019, Grebe published the second chapter of this dissertation, “An ecosystem approach to kelp aquaculture in the Americas and Europe” in *Aquaculture Reports*. In the spring of 2021, she published the third chapter of this dissertation, “The nitrogen bioextraction potential of nearshore *Saccharina latissima* cultivation and harvest in the Western Gulf of Maine” in the *Journal of Applied Phycology*. At the time this dissertation was submitted, the fourth chapter, “The effect of distal-end trimming on *Saccharina latissima* morphology, composition, and productivity” was in press with the *Journal of the World Aquaculture Society*. Grebe is a member of the World Aquaculture Society, the International Phycological Society, the Phycological Society of America, and the Oceanography Society. She is a candidate for the Doctor of Philosophy in Aquaculture and Aquatic Resources from the University of Maine in May 2021.