Ecophysiology and Taxonomy of Saccharina latissima forma angustissima (Laminariales, Phaeophyceae) From the Gulf of Maine, USA

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Ecophysiology and Taxonomy of *Saccharina Latissima* Forma *Angustissima*  
(Laminariales, Phaeophyceae) From the Gulf of Maine, USA

Simona Augyte, PhD  
University of Connecticut, 2017

Abstract

The overarching theme of this doctoral dissertation was to resolve the taxonomic status of an endemic narrow-bladed kelp, *Saccharina latissima* forma *angustissima* (Laminariales, Phaeophyceae), which has a very restricted distribution of 8 nautical miles in the Gulf of Maine, USA. Since the kelp only grows on ledges and islands exposed to high ocean swells, it was unknown if phenotypic plasticity alone was driving its morphology or if the kelp was a distinct genotype (a population with heritable traits). I incorporated lab and fieldwork to discriminate genetic divergence of this kelp, investigated temperature and light requirements of the gametophytic and juvenile sporophytic stages, and its potential use for sustainable aquaculture. The final objective was to tease apart existing relationships of parapatric speciation, where gene flow is limited by the extreme habitat.

In Chapter 1, I used a multi-locus genetic approach to answer questions about the phylogenetic placement of *S. latissima f angustissima*. The results revealed the need for a new combination and status elevation to *Saccharina angustissima* comb. nov. & stat. nov. (Collins) Augyte, Yarish & Neefus. In Chapter 2, I examined the ecophysiological temperature and light tolerance of its early developmental stages, specifically looking at the response of gametophytes and juvenile sporophytes. For Chapter 3, I worked with two aquaculture companies in the Gulf of
Maine to domesticate and commercially cultivate *S. angustissima* at two farm sites and provide data on morphometric traits, biomass yields, blade tissue analysis and ecosystem services. In the last Chapter 4, using microsatellite data, I investigated the population genetic structure of *S. latissima* and *S. angustissima* in across four sites in the Northwest Atlantic and found some genetic differentiation between the two species as well as between other *S. latissima* populations in Long Island Sound and the Gulf of Maine.

In conclusion, directional selection has propelled this unique kelp to persist and colonize an extreme ecological niche and speciate. This reproductive isolation has led to incipient speciation of a *Saccharina* sp. in the Northwest Atlantic. Finally, as a result of my efforts there has been the domestication of a new kelp crop in New England.
Ecophysiology and Taxonomy of *Saccharina latissima* forma *angustissima*

(Laminariales, Phaeophyceae) from the Gulf of Maine, USA

Simona Augyte, PhD

B.S. Humboldt State University, 2008

M.S. Humboldt State University, 2011

A Dissertation

Submitted in Partial Fulfillment of the

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Doctor of Philosophy Dissertation

Ecophysiology and Taxonomy of *Saccharina latissima* forma *angustissima*
(Laminariales, Phaeophyceae) From The Gulf Of Maine, USA

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Mano brangiem tėvams, Ritai ir Rimvydui, dėkoju kad tikėjote ir skatinote mane; ir mano dukrytėi, kad atrastum savo kelia kaip aš atradau savo.

To my beloved parents, Rita and Rimvydas, for believing in me; and to my daughter, may you find your path as I have found mine.
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CHAPTER 1

Speciation in the exposed intertidal: the case of *Saccharina angustissima* comb. nov. & stat. nov. (Laminariales, Phaeophyceae)

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Abstract
Saccharina latissima is a perennial kelp with a circumboreal distribution from the north Pacific to the north Atlantic coasts. Our study aimed to elucidate the taxonomy of the morphologically distinct Saccharina latissima forma angustissima (Collins) A. Mathieson found in the low intertidal on exposed islands and ledges of Casco Bay, Maine, USA. To identify genetic divergence between the two morphotypes, S. latissima and S. latissima f. angustissima, we used a multi-locus phylogenetic approach including nuclear encoded ITS, mitochondrial cox1 and cox3, and plastid encoded rbcL gene sequences. Genetic analysis of the individual markers and combined dataset using SVDquartets resulted in P-distance values for all markers of < 1% suggesting low divergence between the two forms. However, there was as much or more genetic divergence between S. latissima and S. latissima forma angustissima as there were between other taxonomically accepted species of Saccharina spp. To investigate sexual compatibility between the two forms, we made reciprocal crosses of the gametophytes and observed sporophyte formation. All crosses were successfully grown to the sporophyte juvenile stage, suggesting that the two are reproductively compatible in vitro. It is unknown if the two populations freely hybridize in the field. Lastly, we compared wave action, the ecological factor most likely driving the unique morphology, at exposed sites with S. latissima f. angustissima and protected sites with S. latissima. The mean wave force at the exposed site was over 30 times higher in magnitude than at the protected site at 160.04 (±32.58) Newtons (N) and 4.75 (± 6.75) N, respectively, during the summer. The significant differences in morphology, the lack of specimens with intermediate morphologies, and the results of a common garden experiment suggests that the morphological differences in S. latissima f. angustissima are heritable with a genetic basis. Therefore, on the basis of our molecular evidence coupled with ecological and hybridization studies, we are elevating Saccharina angustissima (Collins) Augyte, Yarish &
Neefus *comb. nov. & stat. nov.* from its former designation of *Saccharina latissima* forma *angustissima* (Collins) A.Mathieson.

**Key Words:** Hybridization, Hydrodynamic forces, Laminariales, Phaeophyceae, *Saccharina angustissima*, Taxonomy

**Introduction**

Several studies on members in the order Laminariales (Phaeophyceae) have identified phenotypic plasticity as a driver of the great range of morphological variation seen in thallus shapes as well as macroalgal production and physiology when exposed to strong wave exposure (Gerard & Mann 1979, Sjøtun & Fredriksen 1995, Hurd 2000, Blanchette *et al.* 2002, Fowler-Walker *et al.* 2006). In the rocky intertidal zone, macroalgae can adapt their shape and size to be more flexible and streamlined to lessen the hydrodynamic forces from breaking waves (Carrington 1990; Gaylord & Denny 1997; Boller & Carrington 2007). Typically, an increase in wave exposure has been linked to a decrease in kelp blade width (Philibert 1990; Fig. 1). Transplant experiments of kelps from exposed environments with rapid water movement to protected ones with slow flow, can result in a shift in morphological development from narrow, thick, flat blades to wider, thinner, and more undulate blades (Sundene 1961; Gerard & Mann 1979; Koehl *et al.* 2008). Larger blade size maximizes surface area for light harvesting and nutrient uptake compared to narrower and thicker blade size reducing drag and preventing breakage when exposed to high water currents (Hurd *et al.* 2014). Furthermore, smooth macroalgal thalli experience lower drag than ruffled thalli but also lower photosynthetic rates (Carrington 1990). The strong selection pressure of water motion over small spatial scales has been shown to promote genetic and phenotypic divergence among kelp populations including the

Phenotypes are regulated by gene expression in response to certain environmental cues. Phenotypic plasticity may allow species to maximize their fitness to survive in a broader range of environments (Price *et al.* 2003; Auld *et al.* 2010). As selection acts on phenotypes, novel traits may emerge as adaptive responses to environmental cues. Populations may become genetically isolated, and produce fixed differences causing a shift to a nonplastic phenotype (West-Eberhard 2005; Pfennig *et al.* 2010). The capacity of macroalgae to adjust the shape of their thalli to overcome various physical stressors is an important adaptive strategy allowing them to survive and exploit a range of niches (Roberson & Coyer 2004; Fowler-Walker *et al.* 2006; Wernberg & Vanderklift 2010). Intraspecific morphological variation in response to various environmental conditions is common in both macro- and microalgae (Blanchette 1997; Lürling 2003; Demes *et al.* 2009; Leliaert *et al.* 2014; Verbruggen *et al.* 2014).

The temperate kelp *Saccharina latissima* is a large Pacific-Atlantic species complex with broad morphological and physiological plasticity with a strong genetic component (Bartsch *et al.* 2008). The center of evolutionary diversity of the genus *Saccharina* is in the Northwest Pacific including Japan, Korea and portions of the South China Sea (Bolton 2010). In the western North Atlantic, *S. latissima* is a common kelp with its southern distributional limit in Long Island Sound (Egan & Yarish 1988).

*Saccharina latissima* forma *angustissima* (Collins) A.Mathieson is an endemic narrow-bladed morphotype found only in the most wave exposed habitats of mid coastal Maine. It was first described by Frank S. Collins (1880) as *Laminaria agardhii* Kjellman, and was later amended as *Laminaria agardhii* forma *angustissima* (Collins 1911; Mathieson *et al.* 2008). The
binomial *L. agardhii* has since been designated a synonym for *L. saccarina* (Wilce 1965; Philibert 1990) and was finally reclassified as *Saccharina latissima* (Lane *et al.* 2006). *S. latissima* f. *angustissima* reportedly occurs in only two counties (Sagadahoc and Cumberland) of southern Maine spanning 8 nautical miles, on flat ledges and vertical cliffs exposed to strong surf (Fig. 1; Mathieson *et al.* 2008). The maximum tidal range in Casco Bay is 4.2 m. *Laminaria digitata* and *Alaria esculenta* are two other species that are found growing at the lower boundary of the *S. latissima* forma *angustissima* populations (Philibert 1990, pers. observation). The upper boundary of the narrow-bladed kelp is set by extensive monospecific-stands of the red alga, Irish moss (*Chondrus crispus*) and intermixed with the kelp beds are extensive populations of blue mussel (*Mytilus edulis*).

A two-year kelp aquaculture cultivation study was conducted using meiospores from *S. latissima* forma *angustissima* at several open-water sites in coastal Maine (Augyte *et al.*, *In Press*). Juvenile sporophytes were grown to maturity on long-lines for 6 months. The wave-energy at both farm sites was low compared to what the kelp population experiences in the field. Furthermore, sporophytes of the common sugar kelp, *S. latissima*, with parental meiospores obtained from subtidal populations from Casco Bay, Maine, were grown alongside the *S. latissima* f. *angustissima* sporophytes and the resulting lengths and widths of the two morphologies were compared at harvest. The results of this common garden experiment showed that the sporophyte *S. latissima* f. *angustissima* blade retained its length to width ratio and did not become wide like the sporophytes of *S. latissima*. However, some characters, including thickness of the blade were lost while ruffles formed on blade edges. These results confirmed that environmental cues alone were not wholly responsible for the unique morphology in *S. latissima* f. *angustissima* and suggested a genetic basis. Similarly, Philibert (1990) made preliminary observations on *S. latissima* f. *angustissima* and based on habitat, morphology and transplant
experiments concluded that it was a genetically distinct form. His work further suggests that the two morphotypes remained distinct after growing in identical laboratory culture conditions for 15 weeks.

Current algal species delimitation is centered on molecular data combined with other supporting evidence including morphology, ecology or physiology (Mann 2010; Balakirev et al. 2012; Leliaert et al. 2014). The cytochrome c oxidase subunit I (COI-5P) gene has been successfully used to test species boundaries and resolve groupings of mitochondrial haplotypes of various kelp populations (Lane et al. 2007; McDevit & Saunders 2009; Macaya & Zuccarello 2010; Marins et al. 2012). Similarly, the RuBisCo spacer has been used to resolve the phylogeny of members of class Phaeophyceae (Yoon et al. 2001; Boo et al. 2011; Fraser et al. 2009; Leliaert et al. 2014). Previous studies show that Saccharina latissima can easily hybridize with other kelp genera including with Eisenia arborea, Nereocystis leutkeana, and Lessoniopsis littoralis (Druehl et al. 2005) however, it was found that, after 20 weeks, the sporophytes were stunted or morphologically deformed. Bolton et al. (1983) found a lack of pre-zygotic barriers with several species of Saccharina crossed from the Pacific and Atlantic Oceans including with S. ochotensis and S. longicurris, before the latter was synonymized with S. latissima (McDevit & Saunders 2010). Because of the recent (15-35mya) divergence events as well as similar reproductive phenology for genera of the Alariaceae, Laminiriaceae and Lessoniaceae, intergeneric hybridization readily occurs between these family members. However, laboratory produced kelp hybrids fail to yield viable meiosporangia on adult sporophytes (Lewis & Neushul 1995). As kelps easily hybridize among different genera, we hypothesized that our kelp would produce successful reciprocal crosses between the two forms.

The phenotypic differentiation of the narrow-bladed kelp along with strong physical isolation and potential reproductive isolation from the common sugar kelp could be driven by
local adaptation in response to the drag forces imposed by waves. The small blade size of the *S. latissima* f. *angustissima* population combined with its adaptation to these extreme conditions make it an ideal candidate for a rapidly evolving novelty. Therefore, the present study aimed to clarify the taxonomic position of *S. latissima* f. *angustissima* from southern Maine based on 1) morphological measurements, 2) phylogenetic analyses of four common algal barcode molecular markers, 3) an ecophysiological experiment testing sexual compatibility, and 4) quantification of hydrodynamic forces associated with the wave exposed sites compared to sheltered sites. In the present study, the high wave environment was hypothesized to be driving this unique kelp morphotype.

**Methods**

Specimens were collected on June 14th, 2013 and again on October 8th, 2014 of *S. latissima* f. *angustissima* from Giant’s Staircase, Bailey’s Island, Harpswell, Maine (43°43’22.81” N, 69°59’39.36” W) and regular *S. latissima* from Land’s End, Bailey’s Island, Harpswell, Maine (43°43’01.84” N, 70°00’17.29” W). Field collected specimens were kept in plastic bags over ice and transported in a cooler to the UCONN Stamford Seaweed Biotechnology Laboratory for DNA extraction. Kelp specimens were measured for length and width of blades and stipes as well as thickness of blades. A small piece of each thallus was excised and placed in silica gel for DNA extraction. The specimens were then pressed onto acid free herbarium paper and deposited into the UCONN George Stafford Torrey herbarium in Storrs, CT (accession #s CONN00209762 - CONN00209779).

A multi-locus approach was used to analyze the genetic variability of *Saccharina latissima* forma *angustissima* as compared to other *Saccharina* spp. DNA sequences from four loci were studied, including mitochondrial (*cox1* = cytochrome oxidase subunit I and *cox3* = cytochrome
oxidase subunit III), nuclear (ITS = internal transcriber spacer 2 and 5.8S rRNA gene) and chloroplast (rbcL = large subunit of RuBisCo) markers.

Genomic DNA was extracted from dried kelp blade tissue following protocol of the DNeasy Plant Mini kit (Qiagen, Hilden, Germany) with a slight modification of an initial soak in 400 μL acetone for 10 min. Subsequently, the acetone was discarded and the tissue was air-dried. DNA amplification was done by Polymerase Chain Reaction (PCR) following protocol of Apli-Taq Master Mix (New England BioLabs). For rbcL, the primer pair KL2 and KL8 was used following the thermal profile by Lane et al. (2006). The nuclear ITS primers and thermal profile from Tai et al. (2001) were used with modifications as per Lane et al. (2006). Finally, for mitochondrial cox3, primers C3F34 and R20 were used according to Boo et al. (2011) following PCR thermal protocol as in Lee et al. (2009). For cox1, the CO1-5P region was amplified with the primer pair GAZR2 and GAZF2 (Lane et al. 2007) using the thermal profile by McDevit & Saunders (2009). PCR products were purified with a QIAquick PRC purification kit (Qiagen, Hilden, Germany) and sequenced at the UCONN Biotechnology Core facility (Storrs, CT).

Genome Compiler 2.2.86 (freely available program package, acquired by Twist Bioscience) was used to check and trim raw sequences. Additional sequences of the same genus were retrieved from the GenBank nucleotide database (Table S1). Laminaria digitata was added as an outgroup. Sequences were aligned with MUSCLE (Edgar 2004), as implemented in MEGA v6.06 (Tamura et al. 2013). Alignments were deposited in GenBank.

To assess the variation between the two morphotypes and other closely related species, each dataset was compared using several techniques. First, pairwise uncorrected p-distances were estimated in MEGA v6.06 (Wang et al. 2014). Second, phylogenetic relationships were inferred using maximum likelihood and Bayesian approaches. Maximum Likelihood analyses were performed in RAxML v8 (Stamatakis et al. 2008) with 10,000 bootstrap replicates. For each gene
region, the best model for maximum likelihood was selected using MEGA v6.06 using the Akaike Information Criterion (with the fewest parameters). The best selected model was T92+I for both ITS and rbcL, HKY+I for cox1, and TN93+G for cox3. Bayesian Inference was performed with MrBayes v3.2.4 (Huelsenbeck & Ronquist 2001) using 5 million generations and the selected models. The trees were sampled every 1000 generations. The initial 10,000 trees were discarded as burn-in, and the remaining trees were used to compute a consensus phylogeny and to determine posterior probability values at the nodes. Final phylogenies from RAxML and MrBayes data were displayed using FigTree v1.3.1 (Rambaut 2009). Lastly, a species level phylogenetic analysis was performed using multi-locus data under the coalescent model using SVDquartets in PAUP* 4.0 (Swofford 2002; Chifman & Kubatko 2014). We did this by combining data of rbcL and ITS together and then again combining data from cox3 and cox1 sequences. All the sequences could not be used because the same specimen for one marker did not have data available for the other three markers.

A hybridization experiment was set up to test whether the two morphologically distinct forms were potentially reproductively isolated or if they could produce sporophytes. After collection of reproductive blades, spores released from sorus tissue were used to establish separate male and female gametophyte cultures that were used to make crosses using standard protocols following Redmond et al. 2014. The strips of sorus tissue were scraped gently and cleaned of epibionts by immersion in a dilute iodine solution, rinsed, and then wrapped in damp paper towels. The sorus tissue was stored overnight at 10°C in darkness. The following day, sorus tissue was re-immersed in sterile seawater to stimulate release of the meiospores. After removing the spent sori, the spore-filled seawater was filtered through 35-80µm filters to remove potential contaminants and debris. Spore concentrations were determined with a hemocytometer under a compound microscope, and adjusted to a spore cell concentration of 4000 - 6000 cells per mL.
These meiospores were allowed to settle onto cover slips left overnight in a settling chamber in PES/2 enriched seawater.

Kelp gametophytes were isolated and sexed based on filament width, where female filament cells are larger than male cells, as well as the presence of oogonia and antheridia. These filaments were grown under 12:12hr light: dark at 10°C over a period of 3 months during which time the culture medium was changed bi-weekly and the filaments were periodically chopped up to increase biomass. The reciprocal and selfing crosses were made by mixing males and females of the two kelps into deep-well petri dishes containing 250 mL of autoclaved seawater with 1/2PES nutrients. Trials were run in 10°C at 12:12hr light: dark cycle at 40 μmol photons m⁻² s⁻¹. Final observations were made at 2 months and presence or absence of sporophyte formation was recorded.

Maximum wave velocity and force comparisons of exposed and protected sides of ledges were quantified by deploying dynamometers (Bell & Denny 1994) at Bailey’s Island, Harpswell, Maine. These simple devices, in conjunction with a mathematical model, provided direct measurements at the attachment position during the period of deployment and allowed for among-site estimates (Bell and Denny 1994; Robels et al 2010). The dynamometers (n=3 at each site) were attached to rocks at mean low water at two separate sites of Bailey’s Island; at the very exposed site of Giant’s Staircase with known populations of S. latissima forma angustissima and at the protected site of Land’s End with known populations of S. latissima. Readings were taken over 4 consecutive low tides in late August of 2015. Maximum water velocity (in m s⁻¹) and drag force (measured in Newtons) were calculated using the formulas provided in Bell & Denny (1994). Using R software 3.3.1 (R Development Core Team 2008), we ran a 2-way ANOVA to test the effects of location (exposed vs. sheltered) and time (4 low tide readings) on the amount of drag force as the response variable.
Results

Morphological observations

Samples collected in June of 2013 from Harpswell, Maine revealed that the narrow-bladed kelp is morphologically distinct from other common kelps in the western North Atlantic including *Saccharina latissima*, *Alaria esculenta*, and *Laminaria digitata*. Comparisons between the common *S. latissima* show that *S. latissima f. angustissima* blades were on average over 10-20 times narrower (Table 1, Figs 2, 3). Measurements taken of 125 samples revealed that the average blade length is 180cm (± 96cm), blade width is 1.6cm (± 0.7cm), stipe length is 9cm (±7cm) and blade thickness is 1.1mm (±0.25mm). Besides being exposed to strong surf and high current velocities, this phenotype also grows in the very low intertidal where it is further exposed to desiccation stress during spring tides.

Phylogenetic analyses

For *rbcL*, alignments were based on a total of 1017bp after trimming raw sequences. The consensus tree topology was identical between MrBayes (Fig. 4) and RAxML analyses. The tree branches were very short, providing little species level resolution. The narrow form (*S. latissima f. angustissima*) was sister to broad form of *S. latissima* and to *S. chichorioides* with low bootstrap support. Although the tree topology suggests that there is some divergence between the two forms of *S. latissima*, the p-distance was low at 0.6% (Table 2).

The two mitochondrial markers showed congruence in the phylogenetic tree topologies. Both the *cox3* (640 bp in length) and *cox1* (619 bp in length) showed little divergence between the two morphologies and had p-distances of 0.48 % and 0.18%, respectively (Table 2, Figs 5, 6). In the *cox1* phylogenetic tree, the clade with high support (1) included the four species; *Saccharina coriacea, S. chichorioides, S. latissima*, and *S. latissima forma angustissima*. It is interesting to note that on the *cox3* phylogenetic tree, one sequence of *S. latissima* (KM675818.1) from China
appeared as sister to the *S. latissima* clade that included *S. latissima*, *f. angustissima* and *S. coriacea*.

The nuclear ITS-based p-distances were very low (0.05%) between the two forms of *Saccharina latissima* (Table 2, Fig. 7). The tree topology based on ITS sequences (404 bp in length) shows support for a clade that includes both *S. latissima* forms. Interestingly, *S. gyrata* (AF319021.1) and *S. nigripes* (AY857893.2) were placed within that clade as well.

Results of the SVDquartets analysis (Fig. 8) reveals that the species tree of *rbcL* and ITS has moderate support for the clade of *S. latissima* and *S. latissima f. angustissima*. The tree based on mitochondrial data supports a clade composed of the two forms of *S. latissima* as well as *S. coriacea* (Fig. 9). However, it is interesting to note that some other taxonomically accepted *Saccharina* spp. are not well resolved in this tree. For example, in the *rbcL* + ITS tree (Fig. 8) there is moderate bootstrap support (53%) for a clade that shows low resolution for *S. japonica*, *S. japonica var. ochotensis*, *S. angustata*, *S. longissima*, and *S. japonica var. religiosa* (Yotsukura et al. 2008). The second tree suggests a polytomy between the above mentioned species in addition to *S. diabolica* and *S. longipedalis* (Fig. 9).

*Hybridization*

Successful crosses were completed between *S. latissima* and *S. latissima f. angustissima*. Blades were observed with all the reciprocal and selfed crosses. Juvenile kelp sporophytes were observed that grew ~ 0.5cm in length.

*Wave energy comparisons*

The maximum wave force recordings confirmed higher water velocities and forces impacting the kelps at the exposed versus the protected sites, with a significant relationship between drag force and location (*F*₁,₂₂ = 179, *p*<0.000, Fig. 10). More than half of the data for the protected site were at 0 m s⁻¹ at the time of measurement, meaning there was not enough water
velocity to make an impact on the recording device. The exposed sites had mean water velocities of 18.12 (±3.89) m s\(^{-1}\) while the protected site mean was 2.22 (±3.25) m s\(^{-1}\). The mean force at the exposed site was over 30 times higher in magnitude than at the protected site at 160.04 (±32.58) N and 4.75 (± 6.75) N respectively.

**Discussion**

Plasticity yields specific morphological polyphenic traits, multiple discreet phenotypes from a single genotype, and is a starting point for rapid evolution (Schlichting & Smith 2002). If, over time, a population subject to strong selection pressure becomes specialized and later becomes reproductively isolated, an ecotype may form that no longer maintains a plastic response (Schlichting & Smith 2002). This sort of divergent selection on ecological traits can result from sympatric populations that inhabit separate niches inside close geographic areas and over time become locally adapted (Rundle & Nosle 2005; Leliaert et al. 2014).

The four markers used in this study indicate some level of separation between the broad form of *S. latissima* and the narrow *S. latissima* f. *angustissima*; and, all of the phylogenetic trees indicate as much or more divergence between the two as there are between other species of taxonomically accepted *Saccharina* spp. For example, the ITS marker shows no divergence between *S. latissima* and *S. latissima* f. *angustissima*, but this is expected as other studies report as few as 5-7 base pair differences for *S. latissima* and closely related species (Lane *et al.* 2006; 2007). Yotsukura *et al.* (2005, 2010) also show low divergence between *S. angustata* and *S. longissima* from the ITS region.

McDevit and Saunders (2009) report 3-6% divergence within the genus of Saccharina based on the mitochondrial COI marker. In this study, only 0.18 % divergence was observed between *S. latissima* and *S. latissima* f. *angustissima* for the mitochondrial markers used. While
these values are low, in cases with divergent selection and local adaptation, neutral markers can be uninformative for the assessment of rapid phenotypic divergence (Nosil et al. 2009, Leliaert et al. 2014). All phylogenetic trees, including the ones generated with SVDquartets, provide support for a close relationship between *S. latissima* and *S. latissima f. angustissima*, while other taxonomically accepted species in the genus show similar levels of divergence. With enough time, we can expect to see substantially more genetic divergence between the two populations. Furthermore, the unique population/ecotype is not geographically widespread and intermediate morphologies are not evident between the distinct populations. The range boundaries of *S. latissima f. angustissima* are clearly delimited to a few wave facing ledges and islands in Casco Bay (this study, Mathieson et al. 2008, Mathieson & Dawes 2017). The extreme example is Bailey’s Island, where on the side that faces incoming swells, dense low intertidal kelp beds of *Saccharina latissima* forma *angustissima* are found while on the protected side, only the typical *S. latissima*. Furthermore, there are some noticeable differences in the timing of sporogenesis - for *S. latissima* it occurs in both spring and summer, while it has only been observed in the fall for *Saccharina latissima* forma *angustissima*.

The fact that the entities are persistently morphologically distinct and that in a common garden experiment, *Saccharina latissima* forma *angustissima* stayed true to its parental population exhibiting traits adapted to a wave-bashed environment even when grown in calm conditions (Augyte et al. 2017). The narrow morphotype was conserved over two consecutive growing seasons for the duration of the cultivation study (Augyte et al. 2017) and in subsequent years as farmers continue to grow it and provide feedback on the differences observed in texture, thickness, and taste (S. Redmond, pers. communication). For example, significant differences were found between the average length to width ratios ($p < 0.001$) of the two cultivated kelp; *S. latissima f. angustissima* was at 85.89 ($±20.23$) - 56.14 ($±5.02$) cm, while *S. latissima* was 9.54
(±2.24) - 12.9 (±1.10) cm for the two farms, respectively (Augyte et. al. 2017). This research shows that some of the traits enhancing fitness on the wave exposed coast are heritable with a genetic basis for the observed morphotype.

Our combined morphological and molecular analyses and results of the common garden cultivation study (Augyte et al. 2017) provide evidence that *S. latissima* f. *angustissima* adapted to an extreme habitat with high exposure to oceanic swells and has undergone some level of speciation and has a genetic basis driving its unique morphology. These results further suggest that the differences in environment, specifically the extreme habitat niche has led to barriers to gene flow between the Saccharina *latissima* forma *angustissima* population reproductively isolated from other *S. latissima* populations. These pre-zygotic barriers to gene-flow have allowed the unique kelp to diverge, colonize and persist the new habitat. Consequently, the molecular evidence presented in this study, coupled with ecological and hybridization studies, provides support to elevate *Saccharina latissima* forma *angustissima* (Collins) A. Mathieson to the new status designation of *Saccharina angustissima* (Collins) Augyte, Yarish & Neefus *comb. nov.* & *stat. nov.*

The fact that the two forms can successfully hybridize under laboratory conditions is another indication that the two are closely related, yet not necessarily conspecific. Many previous studies demonstrate that kelp easily hybridize with congeneres and even with members of the same family (Lewis & Neushul 1995; Kraan & Guiry 2000; Liptack & Druehl 2000; Druehl *et al.* 2005). Furthermore, without genetic testing, it is impossible to elucidate whether the blades observed in this experiment were sporophytes from hybridization or the products of parthenogenesis, androgenesis or apogamy (Liptack & Druehl 2000). Although few kelp hybrids have been observed in natural populations, many kelp species occur sympatrically suggesting that in the field, prezygotic barriers exist to preserve species integrity (Tellier *et al.* 2011). On the
ledges where *S. angustissima* occurs, the timing of sexual reproduction is similar as for *S. latissima*, with peak sorus production occurring in the fall (Philibert 1990). The critical reproductive barrier then is the different wave-exposure types corresponding to the two morphotypes.

Macroalgal photosynthesis and nutrient uptake are profoundly influenced by wave motion (Kregting *et al.* 2013) and considerable variation in wave energies is found even along short segments of coastline (Robles *et al.* 2010). Water flow rates and wave velocities experienced by macroalgae in the subtidal are greatly reduced compared to the forces experienced on exposed rocky coasts (Gaylord & Denny 1997). The known distribution for *S. angustissima* is the low intertidal on islands and flat horizontal platforms or ledges severely impacted by breaking waves (Mathieson *et al.* 2008; Mathieson & Dawes 2017). As expected, we found large differences in wave forces at the exposed sites compared to the protected sites. In stormy conditions in the surf zone, seaweeds may experience flow rates of 25 m s\(^{-1}\) and accelerations of >400 m s\(^{-2}\) (Harder *et al.* 2006). Maximum wave forces have been measured for the stipe of the large (> 4 m) kelp *Durvillaea antarctica* (Chamisso) Heriot of around 300 N and water velocities of 30 m s\(^{-2}\) (Stevens *et al.* 2002). Forces of 101 - 234 N were documented over the course of a few tidal cycles. It is also interesting to note that measurements were taken on relatively calm days in the summer in August. In the winter, sites with high wave exposure would be expected to have greater water forces and velocities compared to days in the summer (Blanchette 1997). Similarly, the wave velocity recordings at our site during the low wave energy season were underestimates for the differences in flow speed among sites, as we would expect higher wave exposure during the fall season or during storms. In the winter, the forces are so great that the blades can be torn off from their stipes (personal observ.).
It is still unclear why this unique population does not take up residence on numerous other sites along the coast with similar exposure habitats, especially since _S. latissima_ has a circumboreal distribution while _S. angustissima_ is only known to span a radius of 8 nautical miles in Maine. As part of future work, we will utilize microsatellite markers to examine population level divergences and identify potential hybridization events in field populations.

**Taxonomic treatment**

The formal combination and new status is presented below:

*Saccharina angustissima* (Collins) Augyte, Yarish & Neefus **comb. nov. & stat. nov.**


HOMOTYPIC SYNONYM: *Saccharina latissima* forma *angustissima* (Collins) A.Mathieson in Mathieson et al. 2008.

LECTOTYPE (designated here): Phycotheca Boreali-Americana no. LXXXIII (1905), NY No. 02243824 collected by Frank Collins, July 18\(^{th}\), 1903.

ISOTYPES: NY No. 02243826, 02243815

TYPE LOCALITY: Bailey Island, Casco Bay, Maine, USA. 43°43’22.81” N, 69°59’39.36” W)

Description: Blade length up to 4.5 m, strictly narrow 1-5 cm wide, arising from hapterous base, usually coalesced with multiple terete stipes of 9 cm in length, up to 36 cm long. Thalli are annuals with peak sorus production in October thru late November.

GENBANK ACCESSION NUMBERS: (waiting for accession #s)
HABITAT: Dense beds found on flat, rocky ledges and vertical cliffs within the low intertidal to shallow subtidal zones (+0.5 to –0.5 m) of very exposed coast and off-shore islands. Rare; distribution spans 8 nautical miles.

Acknowledgements
Special thanks to R.T. Wilce for bringing the *S. latissima* f. *angustissima* mystery to our attention; L. Santoferrara for assistance with interpreting phylogenies; J. Gilbert and C. Ludwig for lab support; B. and G. Tobey for field assistance; and A. Mathieson, A. Lamb, and P. Lewis for reviews of manuscript. Funding for this research was provided by The Maine Aquaculture Innovation Center (Project No. 15-11), The Francis Rice Trainer Endowment Fund to the Dept. of Ecology and Evolutionary Biology and the Connecticut Museum of Natural History, The Ronald Bamford Fund to the Dept. of Ecology and Evolutionary Biology, The Carl Schaefer Fund for Student Research Award, The University of Connecticut Graduate School Doctoral Dissertation Fellowship, Sigma Xi Grants-in-Aid of Research, and The Woman’s Seamen’s Friend Society of Connecticut.

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TABLE 1. Origin of specimens used in phylogenetic analysis including GenBank accession numbers. New sequences generated in this study are indicated in bold.

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Figure 1. Map of *Saccharina latissima* forma *angustissima* range with insert showing known distribution in Casco Bay in the Gulf of Maine, circled on insert. Insert from Nauticalchartsonline.com.
Figure 2. Representative specimens of the genus; *Saccharina latissima* forma *angustissima*, top, from exposed site, and *Saccharina latissima*, bottom, from wave-sheltered site.
Figure 3. *Saccharina latissima* forma *angustissima* from habitat. (Photo credit: Sarah Redmond)
Figure 4. Bayesian phylogenetic tree obtained with MrBayes based on rbcL DNA sequences of *Saccharina* (alignment = 1017 bp in length). *Laminaria digitata* was used as an outgroup. Nodal supports are maximum likelihood (ML) bootstrap values ($\geq 50\%$; left), and Bayesian inference (BI) posterior probability ($\geq 0.50$; right). Scale bar represents units of length in units of expected substitutions per site.
Figure 5. Phylogenetic topology based on sequences of the mitochondrial *cox1* marker obtained with MrBayes. DNA sequences of *Saccharina* (alignment = 619 bp in length). Nodal supports are maximum likelihood (ML) bootstrap values (≥ 50%; left), and Bayesian inference (BI) posterior probability (≥0.50; right). Scale bar represents units of length in units of expected substitutions per site.
Figure 6. Phylogenetic topology based on sequences of the mitochondrial *cox3* marker obtained with MrBayes. DNA sequences of *Saccharina* (alignment = 640 bp in length). Nodal supports are maximum likelihood (ML) bootstrap values (≥ 50%; left), and Bayesian inference (BI) posterior probability (≥0.50; right). Scale bar represents units of length in units of expected substitutions per site.
Figure 7. Phylogenetic topology based on sequences of the nuclear ITS (internal transcriber spacer) region obtained with MrBayes. DNA sequences of Saccharina (alignment = 404 bp in length). Nodal supports are maximum likelihood (ML) bootstrap values ($\geq 50\%$; left), and Bayesian inference (BI) posterior probability ($\geq 0.50$; right). Scale bar represents units of length in units of expected substitutions per site.
Figure 8. SVDquartets bootstrap consensus species tree based on 13 *Saccharina* taxa and 1 outgroup (*L. digitata*) on *rbcL* and ITS markers, total of 1422 bp made with evaluating 20,000 random quartets performing bootstrapping, with 10,000 number of replicates with seed at 475839. Bootstrap support values are indicated on tree nodes.
Figure 9. SVDquartets bootstrap consensus species tree based on 11 *Saccharina* taxa and 1 outgroup (*L. digitata*) was built with sequences from *cox3* and *cox1* markers, for a total of 1259 bp made with evaluating 20,000 random quartets performing bootstrapping, with 10,000 number of replicates with seed at 475839. Bootstrap support values are indicated on tree nodes.
Figure 10. Average forces experienced at two sites with varying degrees of wave exposure. Mean maximum wave forces (N) recorded at two sides of Bailey’s Island, the exposed site (Giant’s Stairs) and the protected site (Land’s End) over 4 consecutive low tides.
The impact of global climate change on the early growth stages of the kelp *Saccharina angustissima* (Laminariales, Phaeophyceae)

Simona Augyte

**Abstract**

Anthropogenic disturbances, including coastal habitat modification and climate change are threatening the stability of one of the most diverse and productive global ecosystems, namely kelp beds. To test the effect of different temperature, range 3-17 °C, and irradiance, range 10-200 μmol photons m$^{-2}$ s$^{-1}$, conditions on the microscopic gametophyte and juvenile sporophyte stages of the kelp, *Saccharina angustissima*, we deployed two separate experimental treatments using a temperature gradient table. The first set of experiments combined a temperature range of 7-18 °C with irradiance 20, 40 and 80 μmol photons m$^{-2}$ s$^{-1}$, respectively. The second set combined a temperature range of 3-13 °C with irradiance 10, 100, and 200 μmol photons m$^{-2}$ s$^{-1}$, respectively. Over two separate 4-week trials, we monitored gametogenesis, the early growth stages of the gametophytes, and early sporophyte development of this kelp. Gametophytes grew best at temperatures of 8-13°C at the lowest light levels of 10-μmol photons m$^{-2}$ s$^{-1}$. Light had a significant effect on both male and female gametophyte growth only at the higher temperatures tested. Temperatures of 8-15°C and photon fluence levels of 10-100 μmol photons m$^{-2}$ s$^{-1}$ were
ideal for sporophyte growth. Sporophyte and male gametophyte growth was reduced at the highest temperature tested, 17 °C. Our findings provide a framework for continued open-water cultivation work of this economically important kelp. The threats of warming, coupled with the restricted distribution of *S. angustissima* in the western North Atlantic place it at risk of disappearing from its current range.

**Introduction**

Large subtidal kelps are critical to near shore ecosystems. Kelps form habitat structure, nursery grounds, food for invertebrates and pelagic organisms, and buffer the shoreline from storms (Steneck et al. 2002; Smale et al. 2013). Macroalgal detritus fuels various food webs and is an important nutrient source herbivores and detritivores (Krumhansl and Scheibling 2012; Duggins et al. 2016). In economic terms, these ecosystem services have been valued at billions of dollars annually (Kim et al. 2015; Rose et al. 2015; Krumhansl et al. 2016). Several kelp species are economically important for food and alginates (Kim et al. 2015; Kim et al. 2017; McHugh 2003; Wells et al. 2016).

Sea-surface temperature (SST) is a primary factor driving macroalgal biogeographic distributions in the marine intertidal and, along with light and nutrient requirements, confine macroalgae to the shallow, photic zones (Valentine 1966; Lee and Brinkhuis 1988; Lüning 1990; Adey and Steneck 2001; Steneck et al. 2002). In the past few decades, studies have shown the retreat of Northern Hemisphere kelp beds northward with small fluctuations in SST (Pereira et al. 2011, Moy and Christie 2012; Wernberg et al. 2016). Even more studies show kelp declines due to warming (Diez et al. 2012; Harley, et al. 2012; Bartsch et al. 2013; Voerman et al. 2013; Krumhansl et al. 2016, Park et al. 2017). In the western North Atlantic, kelps in the order Laminariales reach their upper thermal tolerance limits in Long Island Sound (LIS) (Egan and
Yarish, 1988). Only 4 kelp species are found in LIS, including *Saccharina latissima*, *Laminaria digitata*, *Chorda filum* and *C. tomentosa* (Van Patten 2006; Schneider et al. 1979). From LIS to more northern latitudes, kelp species diversity increases with the addition of *Alaria esculenta* and *Agarum clathratum*. Both the LIS and Gulf of Maine regions are currently impacted by non-native herbivores that are threatening kelp beds (Steneck et al. 2002).

Kelps have a two-stage alternation of heteromorphic generations with one large prominent sporophytic phase giving rise via meiosis to a microscopic filamentous gametophytic phase. The haploid meiospores are highly susceptible to abiotic stresses, since they are surrounded by only a plasma membrane, not a protective cell wall (Lee and Brinkhuis, 1988; Egan et al. 1989; Bartsch et al. 2013). The microscopic gametophytic filaments that develop are either males that produce sperm or females that produce eggs. For *Saccharina* spp. in particular, the female gametophytes are relatively large and the eggs stay attached, releasing a pheromone to attract the flagellated male sperm, which then swims to and fertilizes the eggs (Lüning, 1990; Redmond et al. 2014; Luthringer et al. 2014). Microscopic stages are critical for maintenance of the population after annual sporophytes decay (Pereira et al. 2011). When a large disturbance causes mass destruction, it is the microscopic stages that permit fast colonization of the disturbed areas in the case of perennial populations, such as the giant kelp, *Macrocystis pyrifera* (Ladah and Zertuche-Gonzaléz, 2007). According to Kain (1969), *S. latissima* juvenile sporophyte development was inhibited at 17 °C and the upper temperature limit for growth ranged between 18-19 °C. Similarly, Redmond (2013) showed that the gametophyte stages of populations of *S. latissima* from the western North Atlantic have an upper critical thermal limit of 22°C, while gametogenesis was suppressed at temperatures above 17 °C. Ecotypic differentiation may allow adult sporophytes at the southern end of their distribution, in LIS, to tolerate more heat stress than more northerly populations in the Gulf of Maine (Gerard and Du Bois, 1988).
The light saturation point of growth for *Saccharina latissima* juvenile sporophyte blades has been shown to be about 50-70 μmol photons m\(^{-2}\) s\(^{-1}\), with optimum photon flux density 110 μmol photons m\(^{-2}\) s\(^{-1}\) as the juvenile sporophytes approach 6 weeks old (Kain 1969; Egan et al. 1989). Reports for the same species indicate that 1-2 year old fronds show photoinhibition at 250 μmol photons m\(^{-2}\) s\(^{-1}\) (Fortes and Lüning, 1980). Weincke & Fisher (1990) showed that *Laminaria digitata* adult sporophyte growth was light saturated between 55 and 105 μmol photons m\(^{-2}\) s\(^{-1}\). Gametophytes grew best at low light levels of 5-20 μmol photons m\(^{-2}\) s\(^{-1}\) (Lee and Brinkhuis, 1988) to 25-50 μmol photons m\(^{-2}\) s\(^{-1}\) (Egan et al. 1989).

This study aimed to identify ideal temperature and light conditions for the early development stages of a rare and potentially economically important kelp species *Saccharina angustissima* (Collins) Augyte, Yarish & Neefus from Casco Bay, in the Gulf of Maine. Although closely related, this kelp is phenotypically and genetically distinct from *S. latissima* and is found spanning only 8 nautical miles in the low intertidal on ledges and islands exposed to high wave forces (Mathieson et al. 2008; Mathieson and Dawes 2017; Augyte et al. Accepted).

**Material and methods**

In this study, two independent experiments tested the effects of temperature and light on the early life stages of *Saccharina angustissima*, specifically gametogenesis, gametophyte growth, and early sporophyte development. The first set of experiments (in 2014) used temperature treatments between 7±1 °C and 18±1 °C and photon fluence rates of 20, 40 and 80 μmol photons m\(^{-2}\) s\(^{-1}\). The second set of experiments (in 2015) had five different temperature treatments between 3±1 °C to 13±1 °C, and 3 photon fluence rates set at 10, 100, and 200 μmol photons m\(^{-2}\) s\(^{-1}\). Two separate experiments were run to assess a wider range of photon fluence rates. The photoperiod
was set to 12:12 light: dark cycle in both experiments. Two experiments were run because of the limited access to the collection site and the limited availability of sporogenous tissue.

Sorus tissue was collected from mature blades at low intertidal from Bailey’s Island, Harpswell, ME (43°43.32’ N, 69°59.46’ W) during the extreme low tides of October, 2014, and October, 2015. Fall is the peak reproduction period of *S. angustissima* when about 85-95% of the blades are covered with sorus tissue (Philibert, 1990). The sorus tissue was cut, cleaned with an iodine wash and allowed to dry overnight. The following day the tissue released meiospores when placed in sterile seawater. The resulting concentration of the spore solution was, on average, 135,000 zoospores mL\(^{-1}\). Two drops of this spore solution (~27,500 spores) was pipetted onto glass cover slips and allowed to germinate in a moisture chamber at 10 °C in complete darkness, following Egan et al. (1989). After settlement of 48 hours, the cover slips were placed into 300 mL crystalline Pyrex dishes with 250 mL of sterilized seawater enriched with 0.5x Provasoli’s Enriched Seawater (PES) medium, which was replaced weekly. Temperature measurements were taken with a digital thermometer (Traceable, Control Co., Webster, TX) and light was measured with a Li-Cor LI-1000 (Li-Cor, Inc., Lincoln, NE, USA) photometer. Each week, for a total of 4 successive weeks, a cover slip was randomly chosen from the treatment dish and photographed with a camera (PixeLINK, NY, USA) attached to a compound microscope (\(n = 100\), for each treatment). Cover slips were then discarded. Images of spore germination, gametophyte and sporophyte length and size were analyzed using ImageJ software (Abramoff et al. 2004).

Germination was recorded as presence/absence data with the observation of germ tube formation, indicating that the meiospore had settled and was developing into a gametophyte. The female gametophytes were measured by counting the number of cells in each filamentous multicellular structure in week 3 of the experimental treatment. The male gametophytes were measured by taking the length of the longest part of each filamentous multicellular structure at
week 3 of all the experimental treatment. Length was used instead of number of cells because the individual male cells were small and indistinguishable within the multicellular filaments. Finally, total length of juvenile sporophytes was measured at week 3 and again at week 4 of each experiment.

Separate data analyses were carried out for the two independent experiments. The effects of light and temperature on the measured responses (germination, size and length) were analyzed with a two-way ANOVA using the generalized linear models with R software (R Core Team 2013) with the following formula:

\[
\text{glm (germination} \sim \text{temp} \times \text{light, data=x}).
\]

For count variables for germination, a Poisson distribution was specified. A stepwise multiple comparison test, the Tukey’s honest significant difference (HSD) test, was run to identify which levels of the explanatory variables were significant. Significance was defined as p-value < 0.05. Significant differences between means of variables tested are indicated by different letters on the graphs.

Results

Germination rates

For the higher temperature experiments (from 2014), germination rates were over 90% for all treatments except for the temperatures 12 °C and 15 °C exposed to the highest light levels of 80 μmol photons m\(^{-2}\) s\(^{-1}\) (Fig. 1A, \(P = 0.036\)). Only the lowest irradiance level at 10 μmol photons m\(^{-2}\) s\(^{-1}\) was found to have a significant effect on germination (both with \(P < 0.001\)).
In the second set of experiments (from 2015), 3 °C, 8 °C and 10 °C temperatures were found to have a significant effect on germination (Fig. 1B, \(P < 0.001\)). Irradiance only had a significant effect at the lowest light level of 10 \(\mu\text{mol photons m}^{-2}\) s\(^{-1}\) \((P < 0.001)\).

**Female gametophyte size**

For the higher temperature experiments (from 2014), the 20 \(\mu\text{mol photons m}^{-2}\) s\(^{-1}\) treatment as well as temperatures of 13 °C and 17 °C were found to have a significant affect on growth (Fig. 2A, \(P < 0.001\)). A significant interaction was found between the effect of the 13 °C temperature and highest level of irradiance on female gametophyte size. The highest mean cell numbers were found in treatment 17 °C at 40 \(\mu\text{mol photons m}^{-2}\) s\(^{-1}\) with 3.3 (± 0.6) cells (Fig. 2A).

In the second set of experiments (from 2015), a significant interaction was found between the effects of light and temperature on female gametophyte size. Overall, female gametophytes grew more than in the first set of experiments. The three highest temperatures (8 °C, 10 °C and 13 °C) had a significant effect on female gametophyte size (Fig. 2B, \(P < 0.004\)), as did the highest light treatment (200 \(\mu\text{mol photons m}^{-2}\) s\(^{-1}\), \(P < 0.001\)). The largest female gametophytes at 12.2 ± 4.3, 17.6 ± 3.2 and 19.5 ± 5.3 cells were found at temperatures 8 °C, 10 °C, and 13 °C, respectively, at the lowest light levels of 10 \(\mu\text{mol photons m}^{-2}\) s\(^{-1}\) (Fig. 2A).

**Male gametophyte size**

For the higher temperature experiments (from 2014), only the highest temperature (17 °C) were found to have a significant effect on male gametophyte size (Fig. 3A, \(P < 0.001\)). A significant interaction was found between the effects of light and temperature on male gametophyte size. The largest males were found at 15 °C at 40 and 80 photons m\(^{-2}\) s\(^{-1}\) with values of 111.6 ± 13.9 \(\mu\text{m}\) and 91.0 ± 17.0 \(\mu\text{m}\), respectively. The three lower temperature treatments grew smaller gametophytes with an average range of 12.6-22.9 \(\mu\text{m}\) for all three light conditions.
The second set of experiments (from 2015), again a significant interaction was found between the effects of light and temperature on male gametophyte size. Temperatures of 8 °C, 10 °C, and 13 °C were found to a significant effect on male gametophyte size at week 3 (Fig. 3B, \( P < 0.001 \)), as was the highest light conditions (200 photons m\(^{-2}\) s\(^{-1}\), \( P < 0.001 \)). At 8 °C, there was a spike in the length of gametophytes grown at 10 \( \mu \text{mol photons m}^{-2}\text{s}^{-1} \) of 135 ± 13.4 μm. The other longest lengths were 75.6 ± 6.6 μm and 85.6 ± 8.4 μm at 10 °C and 13 °C temperatures, respectively. At the highest light intensity of 200 μmol photons m\(^{-2}\) s\(^{-1}\), gametophytes were only observed in two temperature treatments of 8 °C and 13 °C and grew to a length of 16.3 ± 5.1 μm and 31.0 ± 13.1 μm., respectively.

Sporophyte growth

For the first set of experiments (from 2014), some sporophyte development was already observed during week 2. By week 3, all temperatures except for the highest one (17 °C) were found to have a significant effect on sporophyte length (Figs. 4A & 7, \( P < 0.001 \)) as was the lowest light level (20 μmol photons m\(^{-2}\) s\(^{-1}\), \( P < 0.001 \)). By week 4, all temperature treatments, except for 9 °C were found to have a significant effect on sporophyte length (Fig. 4B, \( P < 0.001 \)). A significant interaction was found between the effects of light and temperature on sporophyte size. The longest sporophytes were measured at 40 μmol photons m\(^{-2}\) s\(^{-1}\) for the 12 °C and 15 °C conditions with lengths of 557 ± 39 μm and 536 ± 40 μm, respectively (Fig. 4B). At 17 °C, only a few sporophytes were produced under the lowest light levels, while the two highest light conditions produced only gametophytes.

For the second set of experiments (from 2015) in week 3, only the 5 °C temperature treatment had a significant effect on sporophyte length (Fig. 5A, \( P < 0.015 \)). A significant interaction was observed with the two highest temperatures (10 °C and 13 °C) at light levels of 100 μmol photons m\(^{-2}\) s\(^{-1}\). Only gametophytes, no sporophytes, were observed at 10 °C and 13 °C.
for 10 and 200 μmol photons m$^{-2}$ s$^{-1}$ and at 3 °C for 200 μmol photons m$^{-2}$ s$^{-1}$. In week 4, all temperature treatments, except for 8 °C were found to have a significant effect on sporophyte length (Fig. 5B, $P < 0.001$) as did the lowest light treatment of 10 μmol photons m$^{-2}$ s$^{-1}$ ($P < 0.030$). There were no sporophytes, only gametophytes, observed for the light treatments at 200 μmol photons m$^{-2}$ s$^{-1}$. Also, data is not available for week 4, temperature treatment 10 °C at 100 μmol photons m$^{-2}$ s$^{-1}$.

**Discussion**

*Gametogenesis and gametophyte growth*

Kelp meiospores and gametophytes are particularly vulnerable to fluctuations in environmental conditions (Egan et al. 1989; Lüning 1990; Müller et al. 2008; Zhang et al. 2013) and it is therefore critical to kelp physiology and biogeography to understand how these microscopic phases respond in light of changing climate scenarios. Furthermore, previous work shows that *Saccharina latissima* female gametophytes may be more sensitive to increases in temperature, and higher temperatures may produce more male than female gametophytes (Lee and Brinkhuis, 1988; Egan et al. 1989). Furthermore, at the highest temperature tested of 17 °C, the growth of male gametophytes and young sporophytes is reduced. Overall, light level had a significant effect on both male and female growth but only at the highest temperatures. Although the results were not consistent from year to year, at the warmer temperatures, it appears that the lower light levels (10 μmol photons m$^{-2}$ s$^{-1}$ and 20 μmol photons m$^{-2}$ s$^{-1}$) promoted greater gametophyte growth. The results presented here show a high percent (90%) of spore germination in temperatures ranging from 7-17 °C in light conditions of 20-40 μmol photons m$^{-2}$ s$^{-1}$. It is unclear why the highest light conditions (80 μmol photons m$^{-2}$ s$^{-1}$) at 12 °C and 15 °C show a decrease in germination and again an increase with the highest temperature. In the second
experiment, the pattern is not as obvious. For example, there was a sudden decrease in germination for temperature treatments 8 °C and 10 °C.

In the low intertidal in Casco Bay, Maine, *Saccharina angustissima* reaches its peak in sorus and thus meiospore production and release in October to November (Philibert 1990), when SST are at 10-13 °C. The coldest temperatures follow during January through April dropping to lows of 4 ± 2 °C during which time gametophytes need to persist as filaments in order to develop into juvenile sporophyte blades. This study showed that at the lowest temperatures (3 °C and 5°C), growth was limited by temperature, regardless of light level. In the field, the kelp populations must have temperature triggers that stimulate growth when SST reach above 8 °C.

Yarish et al. (1990) reported a reduction in gametangia and sporophyte production of *Saccharina longicruris* and development at 5 °C, especially under high photon fluence rates (> 40 μmol photons m$^{-2}$ s$^{-1}$), but inter-annual variation has also been reported (Egan et al. 1989). In this study, the coldest temperatures at 3-5 °C, of both experiments, showed trends of a reduction in size of male and female gametophytes. The one-celled female gametophytes produced an egg that allowed fertilization to occur as sporophytes were still observed in the subsequent week in those conditions. In this study, longer female gametophyte filaments of 18-19 cells were measured in some of the warmer conditions at 10 °C and 13 °C; temperatures that would only be observed in the field starting in May. Our results suggest that if gametophytes persist over the summer, they are able to grow into large filaments. Similarly, Izquierdo et al. (2002) showed that low light levels and temperatures of 10 - 12 °C were ideal for producing female gametophytes of more than 20 cells in the warm temperate species, *Laminaria ochroleuca*. Furthermore, gametophyte development for the cold temperate kelp, *Saccorhiza polyschides*, in Portugal was not observed at very high temperatures of 25 °C (Pereira et al. 2011). Our results confirm that optimal $S$. 

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*angustissima* gametophyte growth in culture conditions is at temperatures ranging 8 - 13 °C with light levels from 10 to 100 μmol photons m\(^{-2}\) s\(^{-1}\).

Kelp gametophytes have the capacity to withstand long periods of darkness and thus have exceptionally low-light demands (Bartsch et al. 2008). Tom Dieck (1992) showed that *Laminaria digitata*, *L. ochroleuca* and *L. hyperborea* gametophytes become fertile at irradiance as low as 2-4.5 μmol photons m\(^{-2}\) s\(^{-1}\), while Lüning (1980) showed that juvenile blades of *Saccharina latissima* was killed at the photon fluence rate of 50–90 μmol photons m\(^{-2}\) s\(^{-1}\). In our experiments, female gametophytes showed some growth and development at the highest light levels (200 μmol photons m\(^{-2}\) s\(^{-1}\)) in all but the 3 °C condition. However, this highest light condition was too bright and detrimental to some of the male gametophytes and these grew only under the temperature conditions of 8 °C and 13 °C. Male gametophytes, therefore, are able to tolerate some high light but only within their optimal temperature conditions.

*Sporophytes*

Largest sporophytes were observed at temperatures 12 and 15 °C for all light treatments. The highest temperature tested of 17 °C showed some sporophyte development in week 3 for all light levels, but by week 4 only at the lowest level. High temperature coupled with high light levels may be detrimental to juvenile sporophytes in the field as well. Within its current restricted distribution, in Casco Bay, ME, temperatures this high are only experienced by fully developed adult kelp sporophytes in late summer. During the summer months of August and September the SST averages are up to 17 ± 3° C, while the air temperature reaches highs of 18 ± 6° C (Fig. 6A-B), meaning the adult sporophytes have to withstand high temperatures for brief periods during emersion. For both experiments, the cold temperature treatments produced sporophytes that grew very small. Literature shows a broad range of optimal temperatures for *Saccharina latissima* sporophyte growth at 10-15 °C (Fortes & Lüning 1980; Yarish et al. 1990). An additional stress
for *S. angustissima* during early spring is exposure to below freezing air temperatures during low tides that may drop to as low as -16 °C (Fig. 6B). The results presented here show that sporophyte development was stunted at the coldest and hottest temperatures tested alluding to an optimal temperature for growth. In this study, the optimal range was found to be 8-15 °C. Specifically, temperature and light conditions for sporophyte development were 12 °C and 15 °C at both 40 and 80 μmol photons m$^{-2}$ s$^{-1}$ and at 8 °C and 13 °C at 80 μmol photons m$^{-2}$ s$^{-1}$.

Light saturation for *S. latissima* sporophytes has been shown at 70 μmol photons m$^{-2}$ s$^{-1}$ (Fortes & Lüning 1980). This study with *S. angustissima* showed similar results, that high light was detrimental to juvenile sporophyte development. While in week 3 some sporophytes were observed at the highest light (200 μmol photons m$^{-2}$ s$^{-1}$) at 5 °C and 8 °C, by week 4, there were no sporophytes observed in any of the temperatures for the highest light levels tested. However, light levels of 100 μmol photons m$^{-2}$ s$^{-1}$ still produced large sporophytes. The intertidal kelp, *S. angustissima* might be better adapted to higher light levels than the subtidal *S. latissima*.

**Conclusion**

It is critical for kelp to be able to acclimate to short-term heat stress and seasonal temperature shifts, especially for kelp near their physiological temperature limits (Wernberg et al. 2010; Bartsch et al. 2013). There is little experimental evidence suggesting that increasing SSTs will allow species to evolve fast enough to stay within current geographic zones because of global climatic change (Parmesan et al. 2006). The data in this study indicates that temperatures above 15 °C inhibit *Saccharina angustissima* sporophyte formation, especially at high irradiance. Increasing SST temperatures might be detrimental to the survival of this rare species of kelp. It grows in a radius of eight nautical miles (Mathieson et al. 2008; Mathieson and Dawes 2017; Augyte *et al.* *in Review*) and thus appears to have limited dispersal capability, and is consequently
limited in its ability to retreat northward if it becomes pushed out of its optimal temperature range. Due to the growing interest in cultivating *S. angustissima* (Augyte et al. 2017) at aquaculture farms in Maine, these results are important for future aquaculture operations.

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Figure 1. Kelp germination rates after settlement exposed to different temperature and light treatments. Legend indicates light level as μmol photons m\(^{-2}\) s\(^{-1}\). (A) Results for the 2014 experiment. (B) Results for the 2015 experiment.
Figures 2. A-B. Effects of temperature and irradiance on cell number (means ± SE) of female gametophytes in week 3 of the experiment with 95% CI. Legend indicates photon fluence rates (μmol photons m\(^{-2}\) s\(^{-1}\)). (A) Results for the 2014 experiment. (B) Results for the 2015 experiment. Tukey’s honest significance test indicated by letter denoting statistically significant comparisons.
Figure 3. Effects of temperature and irradiance on filament length (means ± SE) of male gametophytes in week 3 of the experiment with 95% CI. Legend indicates photon fluence rates (μmol photons m⁻² s⁻¹). (A) Results for the 2014 experiment. (B) Results for the 2015 experiment. Tukey’s honest significance test indicated by letter denoting statistically significant comparisons.
Figure 4. Effects of temperature and irradiance on kelp sporophyte lengths (means ± SE) in treatment 7-17° C during week 3 (A) and week 4 (B), with 95% CI. Legend indicates photon fluence rates (μmol photons m⁻² s⁻¹). Tukey’s honest significance test indicated by letter denoting statistically significant comparisons.
Figure 5. Effects of temperature and irradiance on kelp sporophyte lengths (means ± SE) in treatment 3-13°C during week 3 and 4, with 95% CI. Legend indicates photon fluence rates (μmol photons m⁻² s⁻¹). (A) In week 3, there were no observable sporophytes, only gametophytes in 10°C and 13°C at both light conditions of 10 and 200 μmol photons m⁻² s⁻¹. (B) In week 4, there were no observable sporophytes in light treatment 200 μmol photons m⁻² s⁻¹. Data is not
available for week 4, temperature treatment 10 °C at 100 μmol photons m⁻² s⁻¹. Tukey’s honest significance test indicated by letter denoting statistically significant comparisons.
Figure 6. Monthly (A) seawater and (B) air temperature averages for 2014 for Casco Bay, Maine. Dashed lines represent average high and lows for the months. Data obtained from NERACOOS buoy 44007.
Figure 7. Juvenile sporophytes at week 3 at temp 12 °C at 80 μmol photons m$^{-2}$ s$^{-1}$. 
Figure 8. Sporophyte at week 4 in condition 8 °C at 100 μmol photons m\(^{-2}\) s\(^{-1}\). Rhizoids are visible.
CHAPTER 3

Cultivation of a morphologically distinct strain of the sugar kelp, *Saccharina latissima* forma *angustissima*, from coastal Maine, USA, with implications for ecosystem services

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Abstract
Consumer interest and demand for North Atlantic sourced sea vegetables drives opportunities for aquaculture development in the Northeast US. The unique morphology and desirable culinary traits of the wild narrow-bladed kelp, *Saccharina latissima* forma *angustissima*, were successfully translated into a cultivated crop on two geographically distinct open-water farms in Maine, USA. Environmental conditions, growth, and tissue analysis were quantified. Peak meristematic growth rates for blade length occurred from March through April at $2.85 \pm 0.34$ cm day$^{-1}$. The kelp was harvested from May through June with yields of up to $17 \pm 4.4$ kg m$^{-1}$ of line and plant density of 330 plants m$^{-1}$ of line at the Bristol farm and yields of $13.3 \pm 6.2$ kg wet weight m$^{-1}$ line and a plant density of 400 plants m$^{-1}$ of line at the Sorrento farm. Second season yields at Sorrento were on average $24.1 \pm 6.3$ kg m$^{-1}$ of line. Both farms grew significantly narrower blades of *f. angustissima* than of the sugar kelp, *S. latissima*. Common garden experiments with the two morphotypes identified trait stability for length and width, while blade ruffles, and thickness varied with the environment. Calculations estimating the nutrient bioextraction capability of the cultivated *f. angustissima* kelp harvested in June reveal N removal of 88.7 kg ha$^{-1}$ and C removal of 1666.7 kg ha$^{-1}$ (combined farm site averages). Overall, this unique kelp form has the potential as a new aquaculture crop for the Gulf of Maine while providing several coastal ecosystem services.

**Keywords** Domestication, Kelp aquaculture, Maine, *Saccharina latissima* forma *angustissima*, Nutrient bioextraction
Introduction

Global seaweed aquaculture (24% of world marine aquaculture production by weight) has grown rapidly in recent decades to an annual value of $5.6 billion yr\(^{-1}\) (FAO 2016). About 28.5 million tons of seaweed is harvested annually worldwide with China and Indonesia as the top mariculture producers (FAO 2016). The demand for seaweed in Western markets is expected to increase rapidly because of growing consumer demand for human health benefits from seaweed consumption, new protein sources, fertilizers, biofuels and applications as nutraceuticals, medicine, cosmeceuticals, pharmaceuticals and food additives, as well as important ecosystem service providers (Cornish and Garbary 2010; Holdt and Kraan 2011; Mouritsen 2013; Kim et al. 2014; Hafting et al. 2015).

The USA domestic seaweed industry consists of a traditional wild harvest industry, largely based in Maine (Maine Seaweed Council 2014). The recent development of sea vegetable aquaculture in the Northeast USA represents a potential opportunity to provide economic and ecological benefits while producing high value, sustainable sea vegetables (Kim et al. 2014; Redmond et al. 2014). Growing macroalgae in the cold temperate waters in the Northeast US is both feasible and practical. High productivities have been recorded in various locations at small-scale operations (Yarish et al. 2013; Kim et al. 2014, 2015). Commercial sea vegetable cultivation has been ongoing in Maine since 2010, with sugar kelp, *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders, being the first commercial kelp crop to be grown in the USA. The mild flavor and nutritional value (Maine Coast Sea Vegetables 2016) of the kelp species in Maine make them attractive as new domestic aquaculture crops.

Seaweed aquaculture produces not only an edible commodity for both animals and humans, but also additional ecosystem services to coastal marine ecosystems thereby providing increased economic incentives for the local aquaculture industry (Chopin et al. 2001; Chopin
Coastal eutrophication from point and non-point sources into coastal waterways from sewage effluent, agricultural runoff and atmospheric deposition has been a major concern to coastal resource managers (NRC 2000; Latimer et al. 2014). The addition of large influxes of nutrients is problematic because it often leads to algal blooms, hypoxia, shading of native vegetation, loss of important habitat, fish die-offs and changes in marine biodiversity (Rose et al. 2015). The United States Clean Water Act requires states to first identify and then work to attain water quality standards for designated uses (Stephenson and Shabman 2015).

Aquatic vegetation, or seaweeds, can act like renewable biological nutrient scrubbers and are excellent candidates to take up the excess inorganic nutrients in marine coastal areas (Chopin et al. 2001). The concept of nutrient bioextraction translates into the cultivation of extractive species including shellfish and/or seaweeds in order to remove excess nutrients from urbanized coastal waters (Kim et al. 2015; Rose et al. 2015).

**Unique kelp**

The endemic intertidal kelp, *Saccharina latissima* forma *angustissima* (F.S. Collins) A. Mathieson (Laminariales, Heterokontophyta) has a restricted southern Maine distribution spanning 8 nautical miles of islands, ledges and peninsulas exposed to heavy ocean surf (Mathieson et al. 2008). The common sugar kelp, *Saccharina latissima*, has a vast coastal range throughout much of the Northern Hemisphere including from the high Arctic to the Iberian peninsula and a deep water population off the coast of New Jersey in the North Atlantic, and Korea to Central California in the North Pacific (Egan and Yarish 1988; Lüning 1990; Paulino et al. 2016). *S. latissima* usually inhabits the wave-protected sublittoral, at depths below the low tide (Lüning 1990; Bartsch et al. 2008). The unique narrow-bladed (NB) phenotype, on the other hand, only grows in the vicinity of high ocean swells, in the low intertidal where it is further exposed to emersion stress during spring tides. Emersion has an effect on seaweed growth via changes in
photosynthetic and nutrient uptake rates (Schagerl and Möstl 2011; Kim et al. 2013; Hurd et al. 2014). Furthermore, macroalgae growing subtidally, usually experience water velocities lower than those of the full-force breaking waves in the intertidal (Hurd 2000). The *f. angustissima* blade morphology is uncommon in the western North Atlantic as it is very strap-like and long, as much as 10-20 times narrower than *S. latissima* (Philibert 1990; Mathieson et al. 2008). Ecological and laboratory culture studies show that kelp morphological variation may be attributed to phenotypic plasticity allowing the form to adapt to local conditions, such as differing wave exposure gradients (Gerard and Mann 1979; Johnson and Koehl 1994; Miller et al. 2000; Roberson and Coyer 2004). Thin, broad, undulate blades may be induced to become strap-like when subjected to hydrodynamic forces and mechanical stress imposed by breaking waves and strong currents (Fowler-Walker et al. 2006; Koehl et al. 2008). For example, Gerard and Mann (1979) found that the average thickness of the center of the blade of *S. latissima* (formerly “*Laminaria longicuris*”) was on average 0.73 mm at protected sites compared to 2.35 mm at exposed sites. However, according to Philibert (1990), when grown in culture tanks for 13 weeks, the narrow-blade kelp (*S. latissima* forma *angustissima*) retained its strap-like morphology. These findings suggested that the form maintains its unique morphology over differing environmental gradients, which may indicate an ecotype, or a genetically fixed population. This debate over species vs. ecotype has been going on for a long time in the Laminariales and is complicated by the fact that many members of the order are interfertile (Kain 1979; Bartsch et al. 2008).

Cultivation techniques for the NB kelp, *f. angustissima*, were non-existent and it was unclear how the kelp would perform when cultivated at open-water farm sites and if the unique features would be preserved. Therefore, the main objective of the present study was to investigate growth rates and yield of the NB kelp at two open water farm sites on a submerged longline system in the Gulf of Maine, USA. Furthermore, to better understand if phenotypic plasticity
alone was driving the selection of kelp morphology in the hydrodynamically stressed environment
or if genotypic variation is involved in the persistence of this unique kelp, we cultivated the NB
kelp and the common S. latissima side by side in a common garden experiment on longlines.
Finally, we measured the nutrient bioextraction capabilities of this kelp as another form of
ecosystem service to the local coastal environment.

Materials and Methods

Cultivation sites
The Bristol, ME site (43°55’43”N, 69°34’23”W) is located in Clark’s Cove on the seaward
end of the Damariscotta River estuary watershed in Lincoln County, a full salinity and tidally
influenced lease area of 12 acres. This is a protected site, with less than 1 mile fetch in any direction,
and is relatively shallow at 9-12 m mean low water (MLW) with a mud/silt bottom. The kelp
longlines were located adjacent to a suspended mussel raft on the lease. The Sorrento, ME, site
(44°27’32”N, 68°10’37”W) is located in the northern portion of Frenchman’s Bay in Hancock
County. The site is sheltered from open ocean swells, but exposed to a southwest fetch of
approximately 7 km, experiencing storm generated swells of 1.0-2.5 m, making it a relatively
exposed site. This site is deeper than the Bristol site, at 21-24 m, with a mud/silt bottom.

Production of seedstring
Initial narrow-bladed kelp sorus tissue was collected from a densely populated intertidal
kelp bed on a horizontal platform, Bailey’s Island, ME, USA (43°43.32’ N, 69°59.46’ W) in
Harpwell, Maine, October 8th of 2014 and processed at the University of Connecticut (UCONN)
Stamford Seaweed Biotechnology Lab. Specimens were kept cool on ice for transport to the lab
where the kelp sorus tissue was excised and cleaned, following protocols by Redmond et al. (2014).
The voucher specimens were pressed onto acid free herbarium paper and deposited into The George
Safford Torrey Herbarium, UCONN, Storrs, CT (accession numbers: 273813, 273814). A final cleaning using a 4% iodine wash remove associated protozoans. The sorus tissue was wrapped in paper towels dampened with seawater and placed in in the dark overnight at 10°C. The next morning, the sorus tissue was placed into 2 L beakers filled with sterile 10°C seawater for zoospore release, where meiospore density was quantified using a hemocytometer. Meiospores were added at 5000 (±1000) spores mL⁻¹ of seawater into 2700 mL containers holding PVC tubes (seedspools) wrapped with 80m of 2mm seedstring (Korean type string: Guraron 24, 2mm) and were allowed to settle onto the seedstring overnight in complete dark in 10°C. After 24 hours, seedspools were placed into 75 L aquarium tanks with sterile seawater at 10°C, nutrient additions of PES/2 and light aeration (Redmond et al. 2014). Culture media were changed weekly. Photon fluence rate was provided at 20 μmol m⁻² s⁻¹ the first week and increasing 20 μmol m⁻² s⁻¹ using a Li-Cor LI-1000 (Li-Cor, Inc., Lincoln, Nebraska, USA) photometer every week thereafter to a maximum of 100 μmol m⁻² s⁻¹ by regulating bulb distance to tanks in conditions of 12:12 light:dark. After 1.5 months of growth in the nursery, the gametophyte stages produced juvenile kelp sporophytes of 1-2 mm in length. The seedstring was outplanted on November 23rd - 24th, 2014 by unwinding and wrapping the seedstring onto 2.54 cm thick longlines at two open water farm sites (Fig. 1). At Bristol, ME (Maine Fresh Sea Farms, ~150 m of longline) the longlines were deployed horizontally at two depths of 0.9 m and 1.5 m below the surface. At Sorrento, ME (Maine Coast Sea Vegetables, ~260 m of longline) the longlines were deployed at 1.4 m and 1.8 m depths. The planting depths differed at the two farms because of the slight variation in kelp farm set-up, specifically the minor difference in the placement of buoys. Kelp plants from the same seeded parental populations were grown at both of the farm sites. Over the growing season, and especially after storm events, the kelp lines were monitored to prevent tangling.
Morphometric measurements

We took monthly measurements of total NB kelp length, blade width at the widest part of the blade and stipe length over the growing season (randomly selected plants, N= 30) at both farms. Blade thickness was taken for blades from the Bristol farm site only by using a razor blade to cut at 10 cm above the stipe to blade interface and at the widest part of the blade (N=16) and was measured under a compound microscope.

Plant density measurements were taken before harvest by counting the number of stipes on 10 cm of longline at every 1 m interval over 30 m. Meristematic growth rates were recorded only at the Bristol farm by taking hole punch measurements (N=30) at 10 cm above the stipe blade interface and recording growth with subsequent visits as outlined by Egan and Yarish (1990). The initial hole punches were made in mid-March, 2015 when cultivated plants were 3.5 months old, and the length of blade (stipe not included), was on average 51.5 (±26.74) cm. The plants were flagged with yellow tape at the base of the stipe for easy identification for subsequent hole punches at 4.5 and 5.5 months and the final measurement at 6 months. Percent growth rate was calculated following the formula by Stephens and Hepburn (2016) taking into account initial and final blade lengths over time.

Harvest

The longline cultivated NB kelp was ready for harvest in May through June of 2015 prior to the reduction in growth caused by fouling, reduction in nutrients and the observance of any reproductive material. At final harvest, NB kelp productivity was estimated by measuring the fresh weight biomass of kelp per longline (e.g. kg FW m⁻¹) for samples (N=30) at each farm site (Kim et al. 2015). Additionally, individual kelp plants (N=30) were weighed while wet and subsequently after drying in the drying oven at 55 °C to get the wet to dry ratio.
Second growing season

Some funding was available to support the cultivation operation for a second growing season (2015-2016) of NB kelp at the Bristol and Sorrento farms. The parental plant material for seeding were collected at Harpswell, Maine on October 15th, 2015 and grown on seedstring in the nursery. The longlines were outplanted on November 28th, 2015. The depths of the longlines were at 1.5 m. Yield (kg FW m⁻¹) and plant density were observed at harvest started in mid May and was completed at the beginning of June of 2016.

Nutrient analyses and environmental parameters

Water samples for nutrient analysis were taken monthly starting in January 2014 running through the end of May 2015. Samples were from 1 m depth collected with handmade device of two bottles attached at the end of a measured stick. One of the bottles would fill with seawater, while the other had a plastic end that would be released by pulling a string to fill up at that particular depth. A total of three samples per site were collected with pre-cleaned, acid washed containers and frozen at -20 °C that same day. With one month, samples were filtered through 0.45 µm filter, and transferred into sterile scintillation (Fisherbrand™ 20 mL HDPE) vials and re-frozen. These were stored at -20 °C and thawed prior to analysis. Samples were analyzed by SmartChem® discrete nutrient auto-analyzer (Unity Scientific, Brookfield, Connecticut) for total inorganic nitrogen.

Over the first growing season, when conditions permitted and after the kelp plants were large enough to harvest (>10 cm in length), kelp plants were collected monthly (N=30), processed, and analyzed for tissue carbon and nitrogen. During the second growing season, plants were only collected at harvest (N=10). After measuring fresh weight, the plants were placed in a drying oven for one week at 55 °C. Once dry, tissues were ground up into a fine powder using a tissue grinder (Model MM200 Grinder, Retsch, Haan, Germany). Tissue amounts of 2±0.5 mg
were weighed and nitrogen (N) and carbon (C) percentages were determined using a CHN analyzer (Series II, CHNS/O 2400 Analyzer, Perkin Elmer Analytical Division of E.G. & G, Wellesley, MA, USA). To measure the N and C removal capabilities of the kelp farm, we used values of dry biomass yield m\(^{-1}\) of longline combined with tissue N and C content following the equation by Kim et al. (2014, 2015).

Monthly environmental parameters were measured at both farms. These included salinity and Secchi disk measurements. Additionally, at the Bristol farm, light measurements used a Li Cor LI-185A PAR meter (Lincoln, Nebraska, USA). Temperature sensors (HOBO data logger 64K, UA-002-64) were attached to the longlines at a 1.5 m depth in Bristol and at 1.4 m depth at Sorrento. We measured salinity using a refractometer (#13104190, Reichert Technologies, Keene, NH, USA) at surface.

*Common garden*

To identify plasticity or a genetic basis for the unique morphology of the NB kelp, and to compare the morphologies of the cultivated NB kelp with *S. latissima*, we ran a common garden experiment by growing both types side by side on longlines. By keeping the environmental variables of the farm sites constant, specifically the relatively calm wave and current conditions, we wanted to observe a genetic basis as a driver for the strap-shaped morphology.

At Bristol, the sugar kelp seedspools were seeded from two parental populations; the first one from Casco Bay, Maine outplanted on October 14\(^{th}\) and the second one from Hancock, Maine outplanted on December 27\(^{th}\), 2014. At the Sorrento farm, all the seedstring was from the Hancock, Maine population and was seeded on December 22\(^{nd}\), 2014. The nursery production of seedstring followed the same protocols as that of the NB kelp. A comparison between the two morphologies was made at the time of harvest, May though June, 2015 at both sites, based upon measurements of the mean of blade length and width.

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Statistical analysis

Data analysis was performed using the “R” (v 3.0.0) software and was checked for homogeneity of variance prior to running ANOVA. We evaluated differences between kelp productivity, meristematic growth rates and tissue chemistry over the growing season (January through June) at two farm sites using a two-way ANOVA. We then performed an analysis to understand how factors such as site and parental population explained variation in adult kelp morphology in the common garden experiment. Finally, a two-way factorial design was used to test the differences in water chemistry at both sites over the growing season.

Results

Growing season 2014-2015 – comparison of sites

Total NB kelp length (blade and stipe) was significantly different throughout the growing season (p<0.001) and at the two sites (p<0.001, Fig. 2). At harvest, lengths were higher at the Bristol farm at 229 (± 28.56) cm compared to Sorrento at 151 (± 12.5) cm. Kelp blade width was also significantly different throughout the growing season (p<0.001) at the two sites (p=0.024). At harvest, blade widths at Bristol were on average of 4.67 (± 0.98) cm compared to Sorrento at 2.76 (± 0.21) cm (Fig. 3). Similarly, stipe lengths were significantly longer (p<0.001) at Bristol at 8.59 (± 1.46) cm than at Sorrento at 3.57 (± 0.22) cm throughout the growing season (p<0.001, Fig. 4). At the Bristol farm at harvest, blade thickness was 0.12 (± 0.02) mm at the stipe/blade interface and 0.11 (± 0.01) mm for the widest part of the blade. It was observed that the cultivated blades were less strap-like and had formed blade ruffles.

Meristematic growth rate measurements of the NB kelp taken over the growing season at the Bristol farm revealed highest growth rates from mid-March to mid-April of on average 2.85 (± 0.34) cm day⁻¹ with a daily growth rate of 2.00 (± 0.18) % (p<0.001, Fig. 5). From mid-April to mid May, the kelp had slightly lower rates of growth at 2.59 (± 0.31) cm day⁻¹ at 1.16 (± 0.18) %.
Later in the season, from mid May through the end of May, the kelp only grew 1.16 (± 0.11) cm day⁻¹ at a growth rate of 0.55 (± 0.09) %.

Yields
Overall, final yield was higher at the Bristol farm during the first season. The wet weight of the harvested biomass at Bristol was on average 17 (± 4.4) kg m⁻¹ of line and plant density was 330 plants m⁻¹ of line. At Sorrento, yield was 13.3 (± 6.2) kg wet weight m⁻¹ line and a plant density of 400 plants m⁻¹ of line. The average dry to wet weight ratio for kelp plants was 11.1 (± 2.6) % with no difference observed between sites. During the second growing season (fall 2015 through spring of 2016), the average yields at the Sorrento farm site were 24.1 (±6.3) kg m⁻¹ of line and a plant density of 1100 plants m⁻¹.

Nutrient analyses
Results from both farms show that tissue nitrogen content was highest in late January at 2.95 (± 0.65) % dry weight (DW) in Bristol and 2.57 (± 0.01) % DW in Sorrento, and decreased over the growing season 1.30 (± 0.11) % DW in Bristol and 1.79 (± 0.02) % DW in Sorrento in June (Fig. 6). At harvest during the second growing season, Bristol had tissue nitrogen content values of 1.04 (± 0.12) % DW and Sorrento at 1.40 (± 0.44) % DW. The tissue carbon content increased over the first growing season for the Bristol farm (from 22.48 (± 5.31) to 28.38 (± 2.29) % DW), but decreased for Sorrento (from 36.22 (± 0.22) to 29.35 (± 0.23) % DW), and site was found to be statistically significant (p=0.027) but not month (p=0.275). At harvest during the second season of cultivation at Bristol and Sorrento sites, respectively, had the following tissue carbon content: 30.87 (± 1.26) % DW and 25.46 (± 5.02) % DW. The carbon to nitrogen ratio increased over the first growing season at both sites, and was significantly different for the months and sites tested (both variables p<0.001). In February, the C to N ratio for Bristol was
7.63 and Sorrento was 14.1 while in, Bristol was at 21.9 and Sorrento was at 14.4 going up to 29.5 in July (we do not have a measurement for July at Bristol).

Calculations for kelp harvested in the month of June, 2015, provide the estimated N removal for Bristol and Sorrento, respectively, at 96.9 kg ha$^{-1}$ and 102.4 kg ha$^{-1}$ for the first season and 6.8 kg ha$^{-1}$ and 148.8 kg ha$^{-1}$ for the second growth season. The C removal for Bristol was 2309.1 kg ha$^{-1}$ and for Sorrento, it was 1456.3 kg ha$^{-1}$ for the first season. The second season C removal for Bristol was much lower at 203.8 kg ha$^{-1}$ and Sorrento was at 2697.2 kg ha$^{-1}$.

Environmental parameters

At the time of outplanting the kelp lines in late November, 2014, the water temperature at planting depth was at was 7.9 °C in Bristol and 8.7 °C in Sorrento (Fig. 7). In early February through mid-March, the water temperature dropped to below freezing (maximum low was -1 °C) and coupled with several winter storms, the farms were inaccessible for over one month. Additionally, ice scour had damaged some of the seeded kelp and long lines and moved buoys out of place. At harvest in May through June, the water temperatures were around 11 °C. We combined results of the water analysis for nitrate, including nitrite and ammonium and found total nitrogen to be highest in January at 30.4 μM and 25.3 μM and dropping significantly over the growing season to 11.2 μM and 7.3 μM at Bristol and Sorrento, respectively. (Fig. 8). This pattern was consistent with the results of tissue nitrogen uptake seen over the growing season.

The Secchi disk readings showed that water clarity was around 4-5 m at both farms and then decreased to 2-3 m in Bristol starting in mid-April to late May. The Sorrento farm had a peak value of 8 m in depth in April and dropped down to 5 m in early May (graph not shown). These results revealed that water turbidity was low early in the growing season and over time slightly increased. From refractometer readings taken January through May, the average monthly salinity was 31 (±1) ppt at the Bristol and 33 (±1) ppt at the Sorrento farms.
Sugar kelp vs. narrow-bladed kelp

For the total lengths of *S. latissima*, Bristol had averages of 72.21 (± 18.02) cm compared to the Sorrento farm at 92.95 (± 10.1) cm (Fig. 9A). Sugar kelp widths at the Bristol and Sorrento farms were 8.72 (± 2.4) cm and 7.38 (± 0.83) cm, respectively (Fig. 9B). The lengths of the sugar kelp stipe were 15.29 (± 2.98) cm and 21.52 (± 2.66) cm at the Bristol and Sorrento farms, respectively (Fig. 9C). Comparisons of sugar kelp and NB kelp made at harvest at both farms revealed no intraspecific variation, or differences of each phenotype between the farm sites in terms of their length, width and stipe lengths (p= 0.136, 0.073 and 0.968, respectively, Figs. 9A-C, 10A-B). However, interspecific variation was observed; the two morphologies differed in averages of total length, width and stipe lengths (all three variables p<0.001). Cultivated sugar kelp plants were wider than cultivated NB plants by 5.67 cm and 4.62 cm in Bristol and Sorrento, respectively. Likewise, significant differences were found between the averages of the two morphologies in their ratios of length to width (p<0.001) but not across the two sites (p=0.179, Fig. 11). The L:W ratio for the NB kelp was 85.89 (± 20.23) cm and 56.14 (± 5.02) cm while for the sugar kelp it was 9.54 (± 2.24) and 12.9 (± 1.10) cm for Bristol and Sorrento, respectively.

Discussion

The NB kelp, *Saccharina latissima* forma *angustissima*, was successfully cultivated at two open water farm sites in Maine over two seasons, 2014-2015 and 2015-2016. During the first season, yields were higher at Bristol with 17 (± 4.4) kg m⁻¹ of line than in Sorrento, at 13.3 (± 6.2) kg wet weight m⁻¹ line. During the second season, yields at Sorrento were 24.1 (±6.3) kg m⁻¹ of line. These yields are similar to other studies such as those done in Long Island Sound reporting 18 kg m⁻¹ of line in 2011-2012 and 9.3 kg m⁻¹ of line in 2012-2013 (Kim et al. 2015). In Spain, Peteiro & Freire (2013) measured *S. latissima* yields of 12-16 kg m⁻¹ of line depending on
exposure of site, and in northwest Scotland Sanderson et al. (2012) report *S. latissima* yields of 20-28 kg m$^{-1}$ of lines grown adjacent to fish farm cages. At harvest, blade widths at Bristol were on average of 4.67 (± 0.98) cm compared to Sorrento at 2.76 (± 0.21) cm. Several factors could be driving the difference seen in mean blade widths at Bristol. One possible explanation is the spacing of the plants on the longlines; at Bristol, there were only 330 plants m$^{-1}$ compared to 400 plants m$^{-1}$ at Sorrento.

Numerous studies have shown that algal thallus morphology changes with the hydrodynamic environment where they are found (Gerard and Mann 1979; Klinger and DeWreede 1988; Johnson and Koehl 1994). As seaweeds are sessile, they are unable to move and must adapt to change their morphology and biological structure to persist on wave-swept shores (Demes et al. 2013a; Starko et al. 2015). In the present study, two kelp morphologies from different parental populations were grown in similar conditions at the two open-water farms protected from high ocean swells and currents. During the season and particularly at harvest, it became evident that some characteristics of the unique strap-like morphology, initially adapted to withstand high impact wave bashing, were preserved in the length to width blade ratios of the plants. These results suggest the NB kelp morphology is somewhat genetically fixed and agree with Philibert (1990)’s work. Although his work was inconclusive, Philibert (1990) found that *S. latissima f. angustissima* retained its morphology in laboratory culture. In this study, some other adaptations seen in the wild populations, including the linearity of the blades were lost. Previous studies show that when water flow is low, macroalgal thalli grow thin and wide forming undulations, or ruffles, on the blade margins to enhance the flux on nutrients across the diffuse boundary layer (Hurd 2000). These bullations may also enhance light capture (Hurd et al. 2014). In comparison, kelp thalli grown in areas of high turbulence grow much thicker and narrower, with few to no bullations (Hurd 2000; Roberson and Coyer 2004; Koehl et al. 2008). This
intraspecific tissue variation was observed in algae growing in mechanically stressful environment and may be a critical acclimation to reduce the drag forces imposed by breaking waves (Denny et al. 1985; Gaylord et al. 2008; Demes et al. 2013b). This was consistent with the findings and comparisons of this study. Preliminary field measurements in 2013 (pers. observation) revealed that parental NB kelp blade thickness was on average 1.28 (±0.21) mm as compared to the cultivated NB blade thickness of 0.12 (±0.02) mm. Furthermore, unlike in the parental population, blade ruffles were induced in the NB kelp sporophytes at both the farm sites.

Sugar kelp is a perennial species with a period of maximum growth in the early part of the year followed by a period of reduced growth during the summer (Lüning 1993; Bartsch et al. 2008; Handå et al. 2013). This was highlighted in the seasonal variation of meristematic growth data that showed highest growth rates in mid-March into early May. Growth rates started decreasing in May to the end of the month. Nitrogen in the water column was highest in the winter and became limiting later in the growing season. This coincides with high nitrogen tissue accumulation during the peak times compared to those of late spring. Other studies showed that when light and temperature availability were adequate, S. latissima seasonal growth patterns were dependent upon nitrogen availability (Chapman et al. 1978; Egan and Yarish 1990; Gevaert et al. 2001). Early in the growth season, a lower C:N ratio (Lee and Brinkhuis 1988) coincided with high nitrogen availability in the water, and the kelp cells were actively growing. In the spring, as total dissolved inorganic nitrogen decreases substantially, the kelp used its previously stored tissue nitrogen reserves (Gevaert et al. 2001; Kim et al. 2015). Chlorophyll a data from the Darling Marine Center situated close to the Bristol farm site, revealed that there were three peaks in phytoplankton growth, two in the spring, and the third one in the fall months. The initial bloom in the spring was from March to April and a second larger one occurred in May through June. Phytoplankton are likely outcompeting kelps for nutrient availability (Lüning 1993; Kim et al. 2015).
2015), as indicated in the decrease in nitrogen availability both in the water column and in the measured kelp tissue.

_Ecosystem services and beyond_

Studies have shown that _Saccharina latissima_ is an excellent candidate for the bioextraction of nutrients from coastal waterways in both urbanized estuaries (Kim et al. 2015) and near fish farms (Sanderson et al. 2012). To get an estimation of nutrient bioextraction capabilities for the NB kelp, we pose a hypothetical seaweed farm situation following Sanderson et al. (2012) where per hectare, there are forty 100 m longlines spaced 2.5 m apart. With this scenario, in the present study, the averages over the two years at both farm sites for kelp harvested in June has N removal capacity at 88.7 kg ha\(^{-1}\) and C removal at 1666.7 kg ha\(^{-1}\). The N and C removal rates are similar to previously published studies; Kim et al. (2015) found rates of 38-139 kg ha\(^{-1}\) removal of N and 1100-1800 kg ha\(^{-1}\) removal of C. To make a significant impact in a heavily N rich water body such as Long Island Sound (LIS), Kim et al. (2015) estimated that approximately 1.5% of the whole LIS would have to be cultivated with both _S. latissima_ and _Gracilaria_ sp. in an area of 5,100 ha. This would remove roughly 1.6-2.2 million kg N year\(^{-1}\), or 10% of the LIS target Total Maximum Daily Load (Rose et al. 2015).

**Conclusion**

The results presented here, suggest that _Saccharina latissima_ forma _angustissima_, a morphologically unique kelp, can be domesticated and produce a high yielding product of up to 24 kg m\(^{-1}\) of line. The morphological characteristics that make the NB kelp unique were preserved on the open water farm. These adaptations were useful in the turbulent wave-swept intertidal environment for the reduction of drag forces and prevention of dislodgement and were retained at the open water farm site in calm and sheltered conditions. Seaweed aquaculture is gaining
popularity in the Northeast US and is becoming one of the fastest growing industries (Rose et al. 2015). Results from this study should provide opportunities for a new crop of kelp for Gulf of Maine sea farmers with culinary and ecosystem service applications.

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National Research Council (NRC) (2000) Clean coastal waters: understanding
and reducing the effects of nutrient pollution.
November 2016.

Characterization of 12 polymorphic microsatellite markers in the sugar kelp Saccharina


Figure 1. Map of New England showing locations of open-water farm sites in Maine, indicated in circles.
Figure 2. Narrow-bladed kelp length, stipe included, (means, ±CI, cm, N = 30) over the 2014–2015 cultivation season. Kelp was outplanted at the end of November of 2014. Triangles are Bristol and circles are Sorrento farm.
Figure 3. Narrow-bladed kelp blade width (means, ±CI, cm, N = 30) over the 2014–2015 cultivation season. Triangles are Bristol and circles are Sorrento farm.
Figure 4. Narrow-bladed kelp stipe lengths (means, ±CI, cm, N = 30) over the 2014–2015 cultivation season. Triangles are Bristol and circles are Sorrento farm.
Figure 5. Narrow-bladed kelp meristematic growth rates (means ± 95% CI, cm day⁻¹, N= 30) over 3-month time in 2015 at the Bristol farm. For exact dates, refer to results section
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Figure 8. Seasonal variation for total dissolved inorganic nitrogen (means ± 95% CI, μM) from water samples collected as triplicates at 1 m below surface.
Figures 9A-C The lengths and widths of sugar and narrow-bladed kelps grown at the two farms at the time of harvest (means, ±95% CI, N = 30).
Figures 10A-B. Seaweed farmer, Seth Barker, holding the sugar kelp (A) and the narrow bladed kelp (B) at the time of harvest.
Figure 11. Fig. 11 Sugar and narrow-bladed kelp length to width ratio at the time of harvest at the two farms, Bristol and Sorrento (means, ±95% CI, N = 30).
CHAPTER 4

**Population connectivity of *Saccharina latissima* at the southern limits of its range distribution**

Simona Augyte

**Keywords** population genetic structure, sugar kelp, *Saccharina latissima, Saccharina angustissima*

**Abstract**

The ecologically and economically important sugar kelp, *Saccharina latissima*, is threatened by range retreat at the southern end of its distribution in Long Island Sound. An endemic, closely related species, *Saccharina angustissima*, grows within its distributional limits. The aims of this study were to test the connectivity of *S. latissima* between other populations in the Northwestern Atlantic and to identify hybridization with the unique endemic species using developed microsatellite markers. Allelic richness for individuals ranged from 4.7-5.8 alleles per locus and gene diversity was moderate with the expected heterozygosity range of 0.309 to 0.417. The results obtained from F_{ST} statistics suggest moderate genetic differentiation between the *S. latissima* populations tested (pair-wise F_{ST} values range from 0.030 to 0.100) with most of the observed genetic variation found within individuals. Overall, a slight pattern of isolation-by-distance was observed and the genetic connectivity among distant populations suggests water current regimes
might be driving some of the new recruitment and gene flow observed. *S. angustissima* was found to have high levels of heterozygosity, hinting at outbreeding. Maintaining and protecting the genetic diversity of *S. latissima* at the southern end of its distribution is critical and needs to be implemented into local conservation and management strategies as well as future cultivation efforts.

**Introduction**

Marine organisms living in both the intertidal and subtidal areas are distributed linearly along the coast (Robuchon et al. 2014). Along these linear stretches of coastline, the genetic connectivity of nearshore marine organisms is influenced by several abiotic factors including habitat discontinuities, sea surface temperatures, turbidity, salinity, and hydrodynamic influences such as ocean current transport, and bathymetry (Kruger-Hadfield et al. 2013). In addition to large-scale oceanographic processes, small-scale location specific factors such as tidal height and kelp forest density as well as suitable habitat availability and continuity of coastlines can influence the reproductive capacity of sessile marine organisms (Coleman et al. 2011). Gene flow intensity is highly dependent on geographic relationships between source and recipient populations (Billot et al. 2003) with an expected increase in genetic differentiation over geographic distances with spatially restricted migration (Kimura & Weiss 1964). Rear edge populations, at the margins of their distributional ranges, are arguably disproportionately important for the conservation of phylogenetic history, evolutionary potential and genetic diversity of species (Hampe & Petit 2005, Pereira et al. 2017). Dispersal dynamics of these edge populations is especially critical in determining the response of species under predicted climate change scenarios (Travis & Dytham 2004). Populations living at the edges of their range typically inhabit a more patchy distributed habitat than those found at the center of their range.
Large brown macroalgae of the Order Laminariales (kelp) are important ecosystem players in temperate near-shore coastal communities as they provide three-dimensional habitat, including cover and shelter, and foraging and nursery grounds for numerous fish species (Bodkin 1983; Bartsch et al. 2008; Efird & Konar 2014). Kelp are considered foundation species in many shallow, subtidal, rocky habitats as they fuel food webs and give rise to higher levels of biodiversity (Dayton 1985; Falkenberg et al. 2012; Trebilco et al. 2015). Extensive kelp beds protect the shoreline by buffering against storms (Smale et al. 2013). Kelp habitats are under threat from stressors such as climate change, pollution, harvesting and other human activities that could have major consequences for the structure and function of near-shore coastal ecosystems (Smale et al. 2013; Krumhansl et al. 2016, Pereira et al. 2017).

The demand for seaweed in Western markets is expected to increase rapidly because of growing consumer demand for human health benefits from seaweed consumption, new protein sources, fertilizers, biofuels, and applications as nutraceuticals, medicine, cosmeceuticals, pharmaceuticals, and food additives, as well as important ecosystem service providers (Chopin et al. 2001; Cornish and Garbary 2010; Holdt and Kraan 2011; Kim et al. 2014, 2015). Seaweeds play a distinct role in human nutrition, with a mineral content ten times higher than that of terrestrial plants and a wide range of minerals, trace elements, and bioactive compounds important for proper cell metabolic activity (Cornish and Garbary 2010; Mouritsen 2013; Kim et al. 2014). Commercial seaweed aquaculture, using kelp and other spp. is a growing enterprise in the Northeast US and has been shown to be feasible and practical with high yields recorded at small-scale operations in various locations in LIS and the Gulf of Maine (Kim et al. 2014, 2015, Rose et al. 2015, Augyte et al. 2017). *Saccharina latissima* is also a model species for algal cultivation and domestication in Europe (Azevedo et al. 2016, Peteiro & Freire 2013).
The sugar kelp, *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders is ecologically and economically important in temperate coastal ecosystems. It has a circumboreal distribution and occurs in both the Pacific and Atlantic Oceans (Lüning 1990). The southern limits of its distribution in the western North Atlantic is Long Island Sound (Egan & Yarish 1988). The narrow-bladed kelp, *Saccharina angustissima* (F.S. Collins) Augyte, Yarish, Neefus, a closely related species, is endemic to mid coastal Maine and found only on a few islands and ledges with high wave exposure (Mathieson et al. 2008; Mathieson and Dawes 2017; Augyte et al. Accepted). The gene pool of these two economically important species are poorly understood and needs further investigation both for management, conservation of natural populations, as well as for future cultivation efforts.

For marine macroalgal community dynamics, one of the major obstacles of dispersal range is the viability of spores and gametes (Gaylord et al. 2006). Kelp life histories are heteromorphic with a dominant, diploid sporophyte phase alternating with a microscopic gametophyte phase. Millions of spores are released from a mature kelp blade, develop into gametangia bearing gametophytes, and can then self-fertilize with individuals from the same parental blade or can out-cross with gametes from other kelp blades. In the stressful intertidal environment, selection may favor restricted dispersal like selfing to maintain local adaptation (Robuchon et al. 2014). For example, in a study of two species of *Laminaria* in France, *L. digitata* in the low intertidal had more genetic structure and thus less connectivity than *L. hyperborea* that occupies a wide band in the subtidal (Robuchon et al. 2014). Billot et al. (2003) found genetic differentiation at distances greater than 10km for non-fragmented *L. digitata* forests, even with the absence of definite population boundaries.

The scope of the current study spans from the Gulf of Maine (GoM) to Long Island Sound (LIS). The Gulf of Maine Coastal Current flows southward from southern Nova Scotia, Canada to
Cape Cod, MA, USA and then on to Cape Hatteras, NC and is largely parallel to the coast, increasing larval transport during spring and summer (Figure 1, Pettigrew et al. 2005, Pringle et al. 2017). The coastal current diverges offshore around Cape Cod, resulting in a region of sluggish circulation in the Nantucket Shoals area (Pringle et al. 2017). Under this hydrographic scenario, we would expect gene flow from the north, along the coast of New England, south to Long Island Sound with new genetic material replenishing the populations in the south.

Highly variable molecular markers supplemented by dense sampling regimes within small regions have enabled studies on the effects of landscape on genetic diversity and gene flow (Kruger-Hadfield et al. 2013). Several recent studies have used expressed sequence tag (EST)-derived microsatellite markers to identify genetic diversity within and between populations of *Saccharina latissima* in Europe (Nielsen et al. 2016; Guzinski et al. 2016; Paulino et al. 2016). Both ESTs and simple sequence repeats (SSR) are widely used in population level studies as they are highly polymorphic, reliable and are abundantly found in the genome (Guzinski et al. 2016). The objective of the present study was to use the highly polymorphic microsatellite markers developed by Paulino et al. (2016) to 1) identify the population connectivity of *S. latissima* at its thermal tolerance limits on the Northwestern Atlantic coast, and 2) identify the population genetic structure of a unique intertidal population *S. angustissima* and look for gene flow with surrounding sugar kelp populations. It was hypothesized that there would be significant genetic structuring among populations because of sampling at the southern limits of *S. latissima* distribution as well as in the middle.
Methods

Sample collection and DNA extraction
Thirty kelp samples were collected at each of the four locations in October, 2016 (Figure 1, Table 1) for a total of 120 samples. Black Ledge (BL) was the only site in Long Island Sound, CT. The rest of the sites including Land’s End (LE), Giant’s Staircase (GS) and Hancock (HC) were in the Gulf of Maine, ME. The GS location only had samples of S. angustissima, while S. latissima was collected at BL, LE and HC. The sites LE, and GS were sites in very close proximity, on the same island. The objective was to identify potential gene flow between S. angustissima and S. latissima on that island. These two species are morphologically distinct and grow in significantly different hydrodynamic environments (Augyte et al. Accepted).

Young blade tissue was excised close to the blade and stipe interface and preserved in silica gel. Small pieces (~20mg) of preserved blade tissue were ground in liquid nitrogen with mortar and pestle. DNA was extracted from each sample using the NucleoSpin 96 Plant II kit (Macherey-Nagel, GmbH & Co. KG, Duren, Germany) at the UCONN CORE – Microbial Analysis, Resources, and Services Facility (Storrs, CT). Template DNA concentration was quantified using PicoGreen (Molecular Probes, Eugene, OR).

Microsatellite genotyping
Primer pairs with the forward 5’ end fluorescently tagged (loci SLN32, SLN34, SLN 35, SLN36, SLN54, SLN58, SLN62, SLN314, SLN319, SNL320, SLN510, SLN511) and labeled (Paulino et al. 2016). All loci were amplified alone following Nielsen et al. (2016). Thermal profiles for PCR amplification for each of the 12 microsatellite loci followed the protocol of Paulino et al. (2016) using a ABI Thermocycler (Applied Biosystems, USA). The total volume for PCR was 12.5 µL and contained 5 µL DNA (diluted 1:10), 0.5 µL of each 10 µM primer, and 6.5 µL OneTaq Master Mix (NewEngland Biolabs, USA). Fragment analysis was conducted at the
DNA Analysis Facility on Science Hill at Yale University (New Haven, CT), on a 3730xl DNA Analyzer using GeneScan 500 LIZ dye size standard, both by Applied Biosystems. Alleles were scored and binned manually with Geneious 10.2.3 (Kearse et al. 2012).

Genetic diversity
Deviations from Hardy-Weinberg equilibrium as well as linkage disequilibrium were evaluated in GENEPOP v3.4 (Raymond & Rousset 1995, Rousset 2008). A Markov chain Monte Carlo (MCMC) method of 1000 batches with 10,000 iterations was run. A standard Bonferroni correction (Rice 1989) was adjusted for significance for multiple tests. Allelic richness (A), observed and expected heterozygosity (H₀ and Hₑ; Nei 1987), and inbreeding coefficient (Fₛ; Weir and Cockerham 1984) were calculated for each site for each locus using genotype frequencies in GenAlEx v. 6.41 (Peakall and Smouse 2006, 2012). Analysis of molecular variance (AMOVA) was performed to describe the partitioning of genetic variation within and among populations (Eriksen et al. 2016) and was conducted on GenAlEx v. 6.41 (Peakall and Smouse 2006, 2012) using 999 permutations.

To estimate the genetic differentiation between the four sampling locations, pair-wise F_STS values were computed by calculating the average of the over-population means for the parameters over the 10 loci with 999 iterations (Weir and Cockerham 1984). Principal Coordinate Analysis (PCoA) is a tool for visualizing patterns of genetic relationships and variation in two dimensional space and was performed based on the pair-wise F_STS values. The Mantel test looks at larger geographic scales to test the genetic difference between populations and the Isolation-by-distance hypothesis, where Rₓᵧ>0 means a relationship between populations. This was performed using the geographic “as the crow flies” distances between the four sites and pair-wise population F_STS values with 999 permutations.
A Bayesian approach was used to characterize population structure using the software STRUCTURE 2.3.4 (Pritchard et al. 2000). Several runs were conducted for the four populations (K=3, 4) with a burn-in of 250,000 followed by 500,000 iterations. The final model was chosen with admixture and sampling location used as prior (LocPrior algorithm). Structure Harvester web v.0.6.94 was used to select the best number of clusters (K set from 1-5, n=5) (Earl et al. 2012).

Results

The number of alleles and gene diversity for each microsatellite locus and for each population are presented (Table 2). Ten of the twelve losi were polymorphic across the four sites. SLN319 and SLN511 were fixed at 420bp and 372bp alleles, respectively, and were thus not used in the analyses. All loci amplified well and data from ten polymorphic loci for 120 individuals were used for all statistical analyses.

Deviations from Hardy-Weinberg equilibrium were not detected. However, there was some evidence of linkage disequilibrium following Bonferroni correction (P<0.00125) at the following loci; SLN36 at LE, SLN62 at BL and LE, and at SLN320 for HC.

Multilocus diversity estimates are presented in Table 2. Allelic richness was highest at LE followed by BL with 5.8 and 5.0 average alleles per locus (Table 2, Figure 2). Lowest allelic richness was found at GS and HC at 4.8 to 4.7 alleles per locus, respectively. The number of private alleles for each population were as follows; BL has 0.9, GS had 1.2, LE had 1.4, HC had 0.7 alleles, respectively (Table 2). Expected heterozygosity varied from the lowest 0.309 at the northernmost site HC, to the highest of 0.417 at the southernmost site at BL, with the highest genetic diversity estimate. The inbreeding coefficient, F_{IS}, varied from 0.000 at GS to 0.203 at BL. The other two Maine sites were at 0.063 and 0.157 for H and LE, respectively.
Pairwise $F_{ST}$ values for samples collected across the four sites were significant for all sites (Table 3). The highest differentiation was found between the two sites geographically farthest from each other, BL and HC at 0.100. The two sites on the same Bailey’s Island, GS and LE had the lowest genetic differentiation at 0.030. For the four *Saccharina* populations tested, AMOVA analysis revealed that 6% of the total variation was partitioned among populations, while 22% variation was among individuals and the rest of the 72% accounted for within individual variation (Table 4, $F_{ST} = 0.059$, $p < 0.010$).

PCoA revealed a strong relationship between sites in close proximity, specifically BL and LE, while BL and LE were far away from each other in geographic and 2-D space (Figure 3). Although there was some indication of isolation by distance, this relationship was not found to be significant (Figure 4, regression equation: $R_{xy} = 0.624$, $R^2 = 0.3897$, $p_{Mantel} = 0.181$).

The STRUCTURE analysis revealed that data from BL and GS are distinct whereas the other two Maine sites, HC and LE share some genetic similarities (Figure 5). Specifically, LE is most similar to HC, with slight affinities to BL and GS. The highest support was for 5 clusters, although 4 clusters also had high support.

**Discussion**

Coastlines with kelp beds in LIS and the Gulf of Maine are ecologically important as these support biodiverse assemblages of organisms (Latimer et al. 2014). Temperature is the main driver for seaweed species survivorship, growth, reproduction, and distribution worldwide (Lüning 1990; Müller et al. 2009; Hurd et al. 2014; Latimer et al. 2014; Krumhansl et al. 2016). As seaweeds play important roles as primary producers in many near-shore environments, increasing sea surface temperatures (SSTs) associated with global climate change may have cascading, detrimental effects upon marine trophic structure. There is little experimental evidence
suggesting that increasing SSTs will allow species to evolve fast enough to stay within current geographic zones because of global climatic change (Parmesan et al. 2006). Over the last decade, Norway has experienced a large-scale disappearance of sugar kelp; eutrophication and increases in temperature have been suggested as drivers for the observed changes (Moy and Christie 2012). A recent study of an Australian temperate reef community showed evidence of kelp forest loss at an alarming rate, with warming SSTs driving a regime shift from kelp forest to persistent seaweed turf, thereby altering key ecological processes and suppressing recovery of kelp (Wernberg et al. 2016). Based on a global dataset analysis of kelp change over the past half-century, Krumhansl et al. (2016) found a decline in kelp abundance in over a third of its existing ecoregions. Sugar kelp, specifically, is a cold-adapted species and needs a greater degree of metabolic reorganization for acclimating to higher temperatures than to low temperatures (Heinrich et al. 2012). This means that climate stress will challenge the resilience of sugar kelp, especially at the southern edge of its distribution where it is likely the most vulnerable to temperature increases.

Due to the short longevity of the spores in the environment, in addition to the requirement of close proximity of male and female gametophytes, kelps are considered poor dispersers (Nielsen et al. 2016). Dispersal is guaranteed primarily by the planktonic bi-flagellate haploid spores and perchance by drifting fertile thallus blade fragments that are displaced by storm or wave events (Billot et al. 2003). Among kelp populations, dispersal may be hindered or enhanced by geographic barriers and local current regimes. It was therefore expected that the S. angustissima population at GS, because of the patchy and extremely limited distribution, would be highly inbred. However, results for GS and for S. latissima at HC show very low FIS values (not different from 0) providing little evidence of departure from Hardy-Weinberg equilibrium. A low value of FIS means that random mating commonly occurs within sampled populations (Guzinski et al. 2016). The other two sites, BL and LE, had higher FIS values, meaning that they
had more homozygotes and thus were more inbred. This is surprising for the most northern population LE where because it was less marginal, it was expected to have less inbreeding.

While genetic divergence increases with distance, especially along a continuous habitat such as a stretch of coastline, it is predicted that genetic diversity is greatest near the center of a species’ distribution while species at the edges of the species’ range are expected to show lower diversity (Robuchon et al. 2014). The exception is with populations in areas that were glacial refugia during the last glacial maximum and are now expected to retain low connectivity and high diversity. Under this scenario, we expected that S. latissima populations in Long Island Sound would have low genetic within-population diversity compared to populations in Maine that are in the center of the kelp’s range with more gene flow occurring with populations from the south and from the north. However, both allelic richness and expected heterozygosity (H_e) were highest at the southernmost site at BL and lowest at HC. These results suggest that our southernmost sampling site BL, might be acting as a glacial refugia for kelp populations.

Furthermore, BL, showed moderate genetic differentiation from LE (pairwise FST= 0.053) and GS (pairwise FST= 0.059) and the most from the northernmost sampling population HC (pairwise FST= 0.100). Research by Guzinski et al. (2016) on S. latissima using a different set of microsatellite markers in a study over broader geographic scales in Europe found substantial genetic differentiation among populations (pairwise FST values ranging from 0.077 to 0.562), which is an order in magnitude higher than our highest FST values. This comparison shows that within our study, there is not much genetic divergence between the populations tested.

Similarly, the differences in the spatial structure between the 4 populations revealed a slight pattern of increasing genetic distance with geographic distance. PCoA revealed that the S. angustissima population at GS was most genetically similar to the population LE, the site in its close proximity (pairwise FST = 0.030). The two species are grossly sympatric sharing ancestral
polymorphisms of the microsatellites markers used in this study. Furthermore, these results suggest introgression in parts of the genome of *S. latissima* and *S. angustissima*. Axis 1, accounting for 56% of the variation observed of PCoA, shows distinction in gene flow from the southern to the northern populations. Furthermore, the number of private alleles was highest for the two sites in close proximity; GS and LE with 1.2 and 1.4 alleles, respectively. These results indicate that even though there is close association between the two species at these locations, they are also highly diverged as compared to the other populations tested. Finally, we see high levels of genetic differentiation between clusters obtained using STRUCTURE where LE shows the most mixing with all the clusters, but still is most like HC, while BL and GS are very distinct. Future population genetics work can place these results into a larger context and take into account *Saccharina latissima* distribution not only to make a comparison with the other side of the Atlantic and European coasts but also to the North Pacific.

Overall, our study shows a moderate to low mean $F_{ST} = 0.059$ suggesting that the populations share high gene flow or have become established relatively recently to where high levels of differentiation have not had time to occur. Most of the variation observed was found within individuals meaning high heterozygosity carried within each individual. In this study, within population variation accounted for 22%, while Robuchon *et al.* (2014) had over 88% for several *Laminaria* sp and Nielsen *et al.* (2016) gave values of 83% for *S. latissima*.

In conclusion, this study provides a baseline for the types of gene flow occurring in *S. latissima* populations in the western North Atlantic, specifically from Long Island Sound to the Gulf of Maine. The results of this study have important implications for conservation and management of sugar kelp at the southern end of its distribution in the western North Atlantic. Specifically, higher conservation priority might be given to populations in LIS that might suffer from inbreeding depression. Furthermore, the *S. angustissima* population found in the high wave
habitats was found to have some genetic structuring from the *S. latissima* populations. These results warrant further investigation on a more fine scale sampling of kelp populations in LIS and the GoM. Overall, the study presented can be used for future management and conservation in addition to being useful in seaweed cultivation operations.

**Acknowledgements**

We would like to thank Sarah Redmond for sending samples and Carol Mariani at the Yale DNA Sequencing Facility on Science Hill for sequencing support. Funding was provided by The Women’s Seaman’s Friend’s Society of Connecticut Fund, The Ronald Bamford Fund from the Dept. of Ecology and Evolutionary Biology, and The UCONN Doctoral Dissertation Fellowship from the UCONN Graduate School.
References


Kruger-Hadfield S.A., Roze D., Mauger S. & Valero M. 2013. Intergametophytic selfing and microgeographic genetic structure shape populations of the intertidal red seaweed


Table 1. Location, habitat and GPS coordinates of *Saccharina latissima* collection in Connecticut (CT) and Maine (ME). Thirty samples were collected at each site with name abbreviations used throughout the text.

<table>
<thead>
<tr>
<th>Habitat</th>
<th>Population</th>
<th>Name</th>
<th>Town, State</th>
<th>GPS coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td>open coast</td>
<td>Black Ledge</td>
<td>BL</td>
<td>Groton, CT</td>
<td>41°18’24.70”N, 72°04’12.24”W</td>
</tr>
<tr>
<td>exposed, tidal</td>
<td>Tidal Falls</td>
<td>HC</td>
<td>Hancock, ME</td>
<td>44°31’19.36”N, 68°13’20.36”W</td>
</tr>
<tr>
<td>exposed</td>
<td>Giant's Stairs</td>
<td>GS</td>
<td>Harpswell, ME</td>
<td>43°43’22.81”N, 69°59’39.36”W</td>
</tr>
<tr>
<td>protected</td>
<td>Land's End</td>
<td>LE</td>
<td>Harpswell, ME</td>
<td>43°43’01.84”N, 70°00’17.29”W</td>
</tr>
</tbody>
</table>

Table 2. Population with associated multilocus genetic diversity estimates for *Saccharina latissima* (sites BL, LE, HC) and *Saccharina angustissima* (site GS) for 10 microsatellite loci across 4 populations. N is the number of individuals per sampling site, PA is the number of private alleles, A is allelic richness (mean number of alleles per locus), $H_E$ is the unbiased expected heterozygosity, and $H_O$ is the observed heterozygosity, and $F_{IS}$ is the inbreeding coefficient. SE values are provided as means over all loci. Negative values are provided as 0.

<table>
<thead>
<tr>
<th>population</th>
<th>N</th>
<th>Pa</th>
<th>A</th>
<th>$H_E$</th>
<th>$H_O$</th>
<th>$F_{IS}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL</td>
<td>29</td>
<td>0.9</td>
<td>5.0±0.75</td>
<td>0.417±0.076</td>
<td>0.340±0.074</td>
<td>0.203±0.086</td>
</tr>
<tr>
<td>GS</td>
<td>28</td>
<td>1.2</td>
<td>4.8±0.80</td>
<td>0.369±0.073</td>
<td>0.384±0.077</td>
<td>0.000±0.028</td>
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<tr>
<td>HC</td>
<td>30</td>
<td>0.7</td>
<td>4.7±0.68</td>
<td>0.309±0.063</td>
<td>0.274±0.069</td>
<td>0.063±0.105</td>
</tr>
<tr>
<td>LE</td>
<td>28</td>
<td>1.4</td>
<td>5.8±0.87</td>
<td>0.387±0.082</td>
<td>0.348±0.092</td>
<td>0.157±0.098</td>
</tr>
</tbody>
</table>
Table 3. Genetic difference among populations using 10 loci based on AMOVA (999 permutations). Pair-wise $F_{ST}$ values for each population (of *Saccharina angustissima* and *S. latissima*) are below diagonal. Significant values are highlighted in bold ($P < 0.001$).

<table>
<thead>
<tr>
<th></th>
<th>BL</th>
<th>GS</th>
<th>HC</th>
<th>LE</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL</td>
<td>0.000</td>
<td>BL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GS</td>
<td>0.059</td>
<td>0.000</td>
<td>GS</td>
<td></td>
</tr>
<tr>
<td>HC</td>
<td>0.100</td>
<td>0.061</td>
<td>0.000</td>
<td>HC</td>
</tr>
<tr>
<td>LE</td>
<td>0.053</td>
<td>0.030</td>
<td>0.053</td>
<td>0.000</td>
</tr>
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</table>
Table 4. Results of the AMOVA (Analysis of Molecular Variance) testing the sources of variation observed in the 4 populations sampled. df = degrees of freedom, SS = sum of squares, Est. Var. = estimated variance, % = percentage of the total variance.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>Est. Var.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among Populations</td>
<td>3</td>
<td>31.575</td>
<td>0.132</td>
<td>6%</td>
</tr>
<tr>
<td>Among Individuals</td>
<td>116</td>
<td>303.350</td>
<td>0.503</td>
<td>22%</td>
</tr>
<tr>
<td>Within Individuals</td>
<td>120</td>
<td>193.000</td>
<td>1.608</td>
<td>77%</td>
</tr>
<tr>
<td>Total</td>
<td>239</td>
<td>527.925</td>
<td>2.244</td>
<td>100%</td>
</tr>
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</table>
Fig. 1. Map of four collection sites. Arrows indicate current movement following Pringle et al. 2017.
Figure 2. Allelic richness, (A, left-hand side, y-axis, white squares, ± SE) and expected heterozygosity (Hₑ, right-hand side, y-axis, grey triangles, ± SE) by site.
Figure 3. Principal coordinate analysis of *S. latissima* (at sites BL, HC, and LE) and *S. angustissima* (site GS) using 10 microsatellite loci based on pair-wise $F_{ST}$ values.
Figure 4. Genetic estimates of pair-wise differentiation ($F_{ST}/(1-F_{ST})$) vs. geographic distance across 4 kelp populations using 10 microsatellite loci based on Mantel’s test for isolation-by-distance.

$y = 0.0001x + 0.1418$

$R^2 = 0.067$
Figure 5. STRUCTURE analysis bar plot with populations clustered into 4 groupings. The best model was K=5, with admixture and sampling locations used as prior.