

The relative contribution of *Saccharina nigripes* (Phaeophyceae) to the Bay of Fundy Laminariaceae: spatial and temporal variability

Caroline M. Longtin*, Gary W. Saunders

Centre for Environmental and Molecular Algal Research, Department of Biology, University of New Brunswick, Fredericton, NB E3B 5A3, Canada

ABSTRACT: Previous research on the Laminariaceae in the Maritime Provinces of Canada was completed under the assumption that only 2 species, *Saccharina latissima* (including *S. longicruris*; simple blade) and *Laminaria digitata* (digitate blade), were present in the flora. However, the species *S. nigripes*, which can look morphologically similar to *S. latissima* and *L. digitata*, was recently recorded. We used molecular tools to observe how Laminariaceae community composition varied across fetch distances and years and to determine the relative abundance of *S. nigripes* in the Bay of Fundy intertidal zone. Over 2 years (2010 and 2011) we studied Laminariaceae across a fetch gradient (sheltered to wave-exposed) in 3 regions (Lepreau, Campobello and Grand Manan), with an additional 3 years (2012 to 2014) of data collected from the Lepreau region. Sheltered sites were most similar to each other and were composed of 99% *S. latissima*. *S. nigripes* was rare in its simple-bladed morphology, as well as at sheltered sites, and likely does not affect previous literature on *S. latissima*. Conversely, *S. nigripes* was the dominant digitate kelp in 2010 and 2011 at moderate and wave-exposed sites; however, it was rare from 2012 to 2014, which may be correlated with increased water temperature. Previous studies on the ecology and distribution of *L. digitata* in this region may have included *S. nigripes* in their observations; therefore, further studies on the Laminariaceae in Atlantic Canada must be performed to determine whether the ecology of *L. digitata* and *S. nigripes* differ, as *S. nigripes* is the dominant digitate kelp in certain years.

KEY WORDS: Community structure · Intertidal · Kelp · Laminariaceae · Molecular identity · *Saccharina nigripes* · Wave exposure

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

The physical structure, algal biomass and organisms associated with kelp forests alter local environments and provide beneficial ecosystem services (Steneck et al. 2002). The complex physical structure of kelp forests provides habitat, nursery grounds and food for numerous benthic and pelagic organisms (Dayton 1985, Bologna & Steneck 1993, Steneck et al. 2002, Graham 2004). Kelp communities are affected by many environmental factors including light intensity, type of substratum, sedimentation, nutrient

availability, water velocity, salinity and temperature (Dayton 1985). Temperature tolerance is a major factor in determining the geographic boundaries of kelp species (Steneck et al. 2002); species have different tolerances to high (summer maxima) and low (winter minima) temperatures, which is reflected in their biogeographic range (Bolton & Lüning 1982, van den Hoek 1982, tom Dieck 1993, Merzouk & Johnson 2011, Oppliger et al. 2012, Bartsch et al. 2013). On a more local scale, wave exposure is one of the major factors affecting species distributions (Lubchenco & Menge 1978, Dayton et al. 1984). Wave exposure is

*Corresponding author: longtin.c@gmail.com

an important factor in kelp community structure, as species are distributed based on their ability to tolerate wave exposure (Dayton et al. 1984, Graham 1997, Pedersen et al. 2012), and in some instances phenotypic plasticity can result in a species occupying a wider range of wave exposure by altering its morphology to suit different flow regimes (Gerard & Mann 1979, Koehl & Alberte 1988, Friedland & Denny 1995).

Ecological studies rely on the proper field identification of individuals, which can be complicated by phenotypic plasticity and cryptic diversity, and kelps are no exception (Miller et al. 2000, Roberson & Coyer 2004, Fowler-Walker et al. 2006, Fraser et al. 2009). Phenotypic plasticity creates confusion in 2 ways: (1) by improper species status being given to environmental variants, and (2) the incorrect assumption that morphological differences are merely environmental variants rather than distinct species. For example, phenotypic plasticity in the holdfast and blade morphology of *Macrocystis pyrifera* (Linnaeus) C. Agardh resulted in the past recognition of 4 species; however, genetic analysis by Coyer et al. (2001) and subsequently Demes et al. (2009) led to the collapse of the genus into this single species. Conversely, genetic analysis of the Antarctic bull kelp *Durvillaea antarctica* (Chamisso) Hariot in New Zealand revealed that what were previously observed as wave exposure-driven environmental phenotypes were indeed 2 distinct and reproductively isolated species (Fraser et al. 2009).

Previous research in the temperate waters of Atlantic Canada has focused on 3 species of Laminariaceae: *Saccharina latissima* (Linnaeus) C. E. Lane, C. Mayes, Druehl & G. W. Saunders, *S. longicruris* (Bachelot de la Pylaie) Kuntze and *Laminaria digitata* (Hudson) J. V. Lamouroux (Edelstein & McLachlan 1966, Edelstein et al. 1970, Chapman 1984, Smith 1985, Johnson & Mann 1988). *S. latissima* has a simple blade with wavy margins and is widely distributed in northern temperate and polar waters; it reportedly inhabits the intertidal of sheltered habitats and the subtidal in more exposed habitats (Ballantine 1961, Jones & Demetropoulos 1968, Druehl & Elliott 1996). *S. longicruris* also has a simple blade with wavy margins, but its stipe is long and hollow. *S. longicruris* (as *L. longicruris*) reportedly inhabits sheltered and exposed sites (Gerard & Mann 1979) from Rhode Island to the Canadian Arctic (McDevit & Saunders 2010). *L. digitata* has a digitate blade and is distributed from the temperate Atlantic Ocean to the Arctic Ocean. It is typically found in moderately exposed to wave-

exposed habitats (Ballantine 1961, Jones & Demetropoulos 1968, Druehl & Elliott 1996). However, McDevit & Saunders (2010) recently reduced *S. longicruris* into synonymy with *S. latissima*. They also recently reported an additional species of Laminariaceae in Atlantic Canada—*Saccharina nigripes* (J. Agardh) C. Longtin & G. W. Saunders (Longtin & Saunders 2015). *S. nigripes* (basonym: *L. nigripes*) was first described from Spitsbergen, Norway by J. Agardh (1868) and was subsequently recorded (as *S. groenlandica*) throughout the Arctic (McDevit & Saunders 2010) and Pacific Oceans (Druehl 1968, McDevit & Saunders 2010), but its presence in the western Atlantic Ocean had gone unnoticed (McDevit & Saunders 2010). Genetically verified collections of *S. nigripes* have either a simple 'saccharina-type' blade or a digitate blade (Fig. 1), morphologically similar to *S. latissima* and *L. digitata*, respectively, resulting in the overlooked status of this species in the northwest Atlantic (McDevit & Saunders 2010). As part of reporting *S. nigripes* (as *S. groenlandica*) in the northwest Atlantic, McDevit & Saunders (2010) noted that microscopic observation of mucilage ducts can be used to differentiate between species. In this region, *S. nigripes* is the only species with mucilage ducts in the stipe and blade, while *L. digitata* has mucilage ducts in the blade and *S. latissima* lacks mucilage ducts (McDevit & Saunders 2010, Longtin & Saunders 2015).

In light of the phenotypic plasticity observed in *S. nigripes* and its likeness to the 2 previously reported Laminariaceae species in the region (*L. digitata* and *S. latissima*), we used molecular tools to examine the Laminariaceae community structure in the Bay of Fundy (Canada) intertidal zone and to determine whether the community changes across wave exposures and time. The resemblance of *S. nigripes* to the 2 previously recognized species in this flora also creates uncertainty with respect to previous publications from the Maritime Provinces regarding the biogeography and ecology of *L. digitata* and *S. latissima*, because the data may have incorporated *S. nigripes* as well. The objectives of this study were to determine (1) the relative abundance of *S. nigripes* in the Bay of Fundy intertidal zone, (2) the most common phenotype (simple or digitate) of *S. nigripes* and whether or not morphology is correlated with habitat, (3) Laminariaceae community structure across differing wave exposures and whether *S. nigripes* is more prevalent at certain wave exposures, and (4) whether Laminariaceae community structure changes through time.

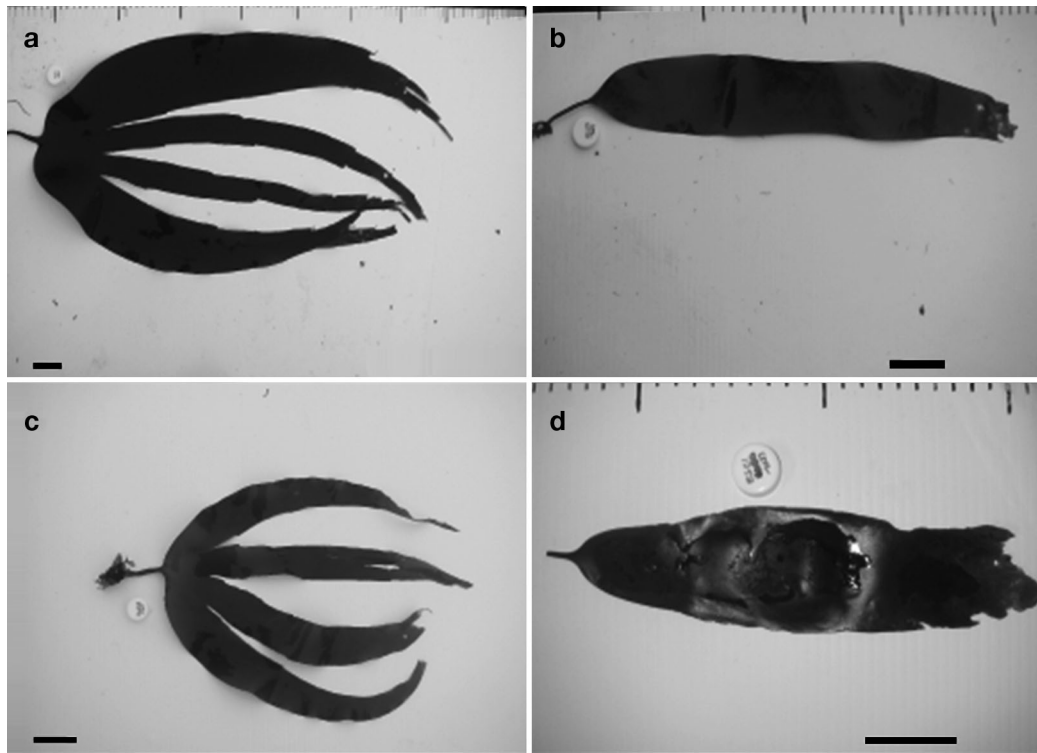


Fig. 1. Gross morphology of genetically verified (a,b) *Saccharina nigripes*, digitate and simple morphologies, respectively, (c) *Laminaria digitata* and (d) *S. latissima*. Scale bars = 5 cm

MATERIALS AND METHODS

Study sites

We sampled sites in the rocky, low intertidal zone (kelp zone) of 3 regions in the southwestern Bay of Fundy (Lepreau, Campobello, and Grand Manan) across a horizontal gradient of increasing fetch distances (i.e. increasing wave exposure) with each site representing a single fetch distance, during the summers of 2010 and 2011 (Fig. 2, Table 1). From 2012 to 2014, only the sheltered (low fetch), moderate (moderate fetch) and wave-exposed (high fetch) sites near Lepreau (Table 1) were sampled to make comparisons of community structure across years and wave exposures. We chose to include only the sites at Lepreau for these comparisons because the fetch gradients at Campobello and Grand Manan did not have representative sites for all exposures. These 2 regions had sites that represented only sheltered and moderate exposures.

Wave exposure was quantified at each site using the modified effective fetch technique developed by Howes et al. (1994). Modified effective fetch was calculated as follows:

$$F_m = [\cos(45^\circ) \times F_{45L} + \cos(0^\circ) \times F_{090} + \cos(45^\circ) \times F_{45R}] / [\cos 45^\circ + \cos 0^\circ + \cos 45^\circ]$$

$$= [(0.707 \times F_{45L} + (1.0) \times F_{090} + (0.707) \times F_{45R}) / 2.414]$$

where:

F_m = the modified effective fetch in km

F_{45L} = the fetch distance in km along 45° left of the shore normal

F_{090} = the fetch distance in km along the direction of the shore normal

F_{45R} = the fetch distance in km along 45° right of the shore normal.

The modified effective fetch was calculated from charts using 3 distance measurements at 45° to the left, 90° and 45° to the right from the study site to the opposite shores. The modified effective fetch index gives an estimate of the average distance in which a wave can collect energy before reaching the shore. We chose this indirect method rather than a direct method because each site was visited for only a short duration, which would make any wave energy values (e.g. dynamometer readings) specific to that time frame (approximately 3 d yr⁻¹). The method of calculating modified effective fetch gives a more general estimate of wave exposure for each site.

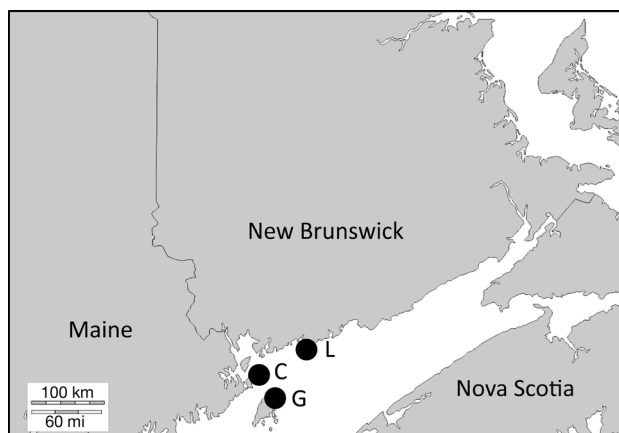


Fig. 2. Study sites at Campobello (C), Grand Manan (G) and Lepreau (L), in the Bay of Fundy, Canada

Table 1. Modified effective fetch index and GPS coordinates for all sampling locations in the Bay of Fundy, Canada. Wave exposures (exposed, moderate or sheltered) are given for the sites at Lepreau

Location	Modified effective fetch (km)	GPS coordinates
Lepreau		
Tim's Point	140.675 (exposed)	45.10047°N, 066.47890°W
Welch Cove	51.558 (moderate)	45.07204°N, 066.47002°W
Maces Bay	2.428 (sheltered)	45.1093°N, 066.4817°W
Campobello		
Lighthouse	34.540	44.95800°N, 066.90086°W
Point on left	5.419	44.95800°N, 066.90086°W
Sheltered Cove	0.812	44.95708°N, 066.90134°W
Grand Manan		
Swallow Tail Light	94.156	44.7518°N, 066.7315°W
Pettes Cove Point	70.361	44.76113°N, 066.74003°W
Pettes Cove	0.203	44.76349°N, 066.74042°W

Field methods

We ran a 100 m transect line parallel to the shore across the low intertidal (kelp zone) at each site in each region. Each year we ran a new 100 m transect line across the low intertidal zone at each site. Along each transect we randomly selected 30 individuals

(macroscopic sporophytes) with either a digitate, simple or indeterminate (juvenile or too eroded to determine) morphology. When we could not find 30 individuals along the transect line, we selected individuals closest to random points along the line. Each individual was photographed in the field and a small sample (1 cm diameter) was collected using a corer for DNA sequencing. Samples for DNA sequencing were preserved in separate vials of silica prior to extraction.

DNA extraction and sequencing

DNA extractions were performed on approximately one-quarter of each sample using a QIAxtractor (Qiagen), following the brown algal extraction protocol developed by Saunders & McDevit (2012). Samples were amplified using the previously published PCR primers P1 and KG4 (Lane et al. 2006) for the internal transcribed spacer region (ITS) of the nuclear ribosomal cistron. The PCR thermal profile and DNA sequencing followed Saunders & McDevit (2012) (samples collected from 2012 to 2014 were sequenced by Genome Quebec); however, $0.27 \mu\text{M}$ amplification⁻¹ of each primer was used for the PCR and only the reverse primer (KG4) was used for sequencing. Sequences were compared to data published in GenBank to determine the species identity for each individual using a Basic Local Alignment Search Tool (BLAST) in Geneious (Biomatters).

Statistical analysis

We compared the proportion of simple *Saccharina nigripes*, digitate *S. nigripes*, *S. latissima* and *Laminaria digitata* across fetch distances using various methods in PRIMER (PRIMER-E + PERMANOVA add-on). All tests were performed on proportion data instead of counts because not all 30 samples for each treatment amplified successfully during PCR.

We performed a 1-way permutational multivariate analysis of co-variance (PERMANCOVA) to determine the effect of fetch as a covariate and region (3 levels) as the independent variable on the Laminariaceae community composition. Site was the lowest sampling unit in this analysis. Species proportion data for each site within a region were averaged over 2 years (2010 and 2011), because data were collected at the same sites with the same fetch distances in each year, and data from each year were similar. In

other words, there was no consistent difference in community structure between years; therefore, the sums of squares for the effect of year would be negative if year were considered as a factor in this design, and we would be unable to perform the PERMANCOVA. PERMANCOVA was performed on the averaged data using a Bray-Curtis resemblance matrix and Type I sums of squares (sequential) calculations to remove the effect of region first, and then test the effect of fetch. The PERMANCOVA had 9823 and 9688 unique permutations for region and fetch, respectively. Data were represented graphically in a 2-dimensional non-metric multidimensional scaling (nMDS) of community structure with bubbles; the bubble size represents the modified effective fetch distance. The overlaid vectors represent the correlations (Pearson correlation coefficient) between species and nMDS axes. The nMDS plot had a stress <0.01 and was, therefore, considered an adequate 2D representation of the higher dimensional situation (Clarke 1993).

To assess the relationship between wave exposure and community composition in a single region (Lepreau) with representative sheltered, moderate and exposed sites, we performed a 1-way analysis of similarity (ANOSIM) on a Bray-Curtis similarity matrix using the Spearman rank correlation method, with exposure (sheltered, moderate and exposed) as the independent variable and year as the unit of replication. A 2-way similarity percentages analysis (SIMPER) without replication was also performed on the Lepreau data to determine which species (or morphologies for *S. nigripes*) contributed most to the wave exposure effect, as well as to determine if there were any differences in Laminariaceae community composition between pairs of years. Differences in community structure at Lepreau across wave exposures and years were visualized in a nMDS plot. The overlaid vectors and stress are as explained above.

RESULTS

Overall, averaging across all fetch distances, regions and years (2010 to 2014) in the Bay of Fundy, *Saccharina nigripes* was approximately 20% more abundant than *Laminaria digitata*, but only about one-third as abundant as *S. latissima*. The data collected at Lepreau over 5 yr indicated that *S. nigripes* was most abundant in its digitate form and very rare in the simple form, with a maximum of only 3 sampled individuals at one site (Lepreau exposed 2010), while the majority of sites had 0 or 1 individual (Table 2).

Fetch distance was a good indicator of Laminariaceae (*L. digitata*, simple and digitate *S. nigripes* and *S. latissima*) community structure at the 3 study regions in the Bay of Fundy in 2010 and 2011 (Fig. 3); kelp community composition varied with fetch distance (PERMANCOVA, Pseudo- $F_{1,5} = 10.65$, $p = 0.015$). Therefore, Laminariaceae communities vary with fetch distance. However, the effect of region (PERMANCOVA, Pseudo- $F_{2,5} = 3.96$, $p = 0.082$) was non-significant. The overlaid vectors on Fig. 3 indicate that sites of greater fetch were similar based on their abundances of *L. digitata* and *S. nigripes* (digitate and simple), while sheltered sites were similar based on their abundances of *S. latissima*. *S. latissima* dominated at sheltered sites, while *L. digitata* and *S. nigripes* were most abundant at sites with greater wave exposure.

Kelp community composition at Lepreau, sampled over 5 yr, was significantly affected by wave exposure (ANOSIM, $R = 0.675$, $p = 0.001$). Sheltered communities were distinctly clustered (Fig. 4). Moderate and exposed communities clustered with respect to their exposure in 2010 and 2011, but 2012, 2013 and 2014 did not cluster as tightly (Fig. 4). Sheltered and exposed sites (ANOSIM, $R = 0.9$, $p = 0.008$) and sheltered and moderate sites (ANOSIM, $R = 0.92$, $p =$

Table 2. Relative abundance (averaged over 2010 to 2014) of *Saccharina nigripes* (digitate, simple and indeterminate), *S. latissima* and *Laminaria digitata* at sheltered, moderate and wave-exposed sites at Lepreau. Relative abundance (proportion) calculated out of a range of 24 to 30 individuals per wave exposure type and year

Wave exposure	Average abundance (proportion) \pm SE				
	Digitate <i>S. nigripes</i>	Simple <i>S. nigripes</i>	Indeterminate <i>S. nigripes</i>	<i>S. latissima</i>	<i>L. digitata</i>
Sheltered	0.01 \pm 0.01	0.01 \pm 0.01	0 \pm 0	0.98 \pm 0.01	0 \pm 0
Moderate	0.23 \pm 0.09	0.02 \pm 0.01	0 \pm 0	0.50 \pm 0.12	0.25 \pm 0.08
Exposed	0.38 \pm 0.10	0.02 \pm 0.01	0.02 \pm 0.01	0.25 \pm 0.12	0.33 \pm 0.10

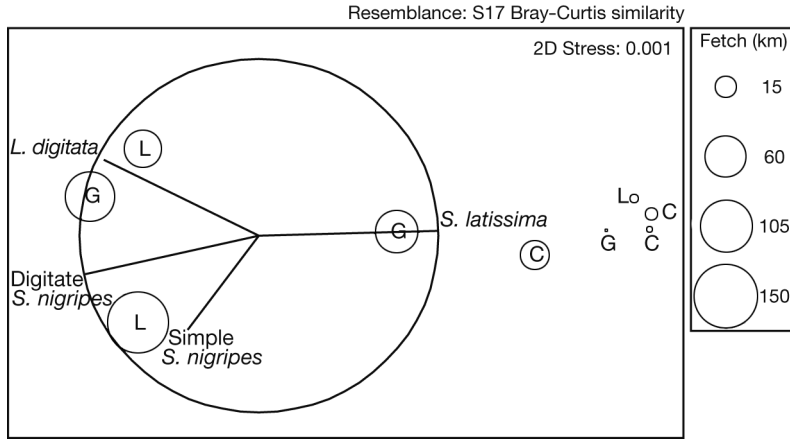


Fig. 3. Non-metric multidimensional scaling (nMDS) bubble plot showing similarities and differences in Laminariaceae (*Laminaria digitata*, *Saccharina nigripes* and *S. latissima*) community structure according to fetch distances at Lepreau (L), Campobello (C) and Grand Manan (G) from averages of data collected in 2010 and 2011, with species vectors overlaid (simple and digitate refer to the 2 morphologies of *S. nigripes*). The vectors overlaid reflect the correlations between a species (or morphology for *S. nigripes*) and the nMDS axes. The vector for each species (or morphology for *S. nigripes*) shows the direction of increased species (or morphology for *S. nigripes*) abundance across the nMDS graph; therefore, sites in that direction on the nMDS graph have a greater abundance of that species (or morphology for *S. nigripes*) than any other sites. The large circle indicates the maximum vector length and $r = 1$ (where r = Pearson correlation coefficient) if the vector is parallel to one of the nMDS axes

(Table 4). The dissimilarity between years was mainly due to *S. nigripes* (% contribution; Table 4), which was high in abundance in 2010 and 2011 but low in abundance from 2012 to 2014. Laminariaceae community composition at the sheltered site was similar in all 5 years, and community composition at moderately exposed and exposed sites was similar in 2010 and 2011, and from 2012 to 2014 (Fig. 4). At the Lepreau sites in 2010 and 2011, digitate *S. nigripes* was more abundant than *L. digitata* at the moderately exposed and exposed site (Fig. 5). However, from 2012 to 2014, *L. digitata* was more abundant than *S. nigripes* at the exposed site, as was *S. latissima* in 2012 (Fig. 5). *S. nigripes* was absent from the moderately exposed site in 2012 and was present in low abundance in 2013 and 2014 (Fig. 5). In all 5 years, *S. latissima* was the dominant species at the sheltered site.

0.008) were significantly different from each other, while moderate and exposed sites (ANOSIM, $R = -0.02$, $p = 0.50$) were similar to each other. Sheltered and exposed sites were the most dissimilar, followed by sheltered and moderate, and moderate and exposed (SIMPER; Table 3). *S. latissima* was an excellent discriminating species between sheltered and exposed sites (i.e. large Diss/SD; see Table 3) and contributed most (50%) to the difference between exposures. Both *S. latissima* and *L. digitata* were good discriminating species between sheltered and moderate sites and contributed most (33 to 50%) to the community difference (Table 3).

Laminariaceae community composition at Lepreau was similar in 2010 and 2011, and from 2012 to 2014 (SIMPER; Table 4). The highest dissimilarity was between 2010 and 2012, followed by 2011 and 2012, 2010 and 2013, 2010 and 2014, 2011 and 2013, and 2011 and 2014

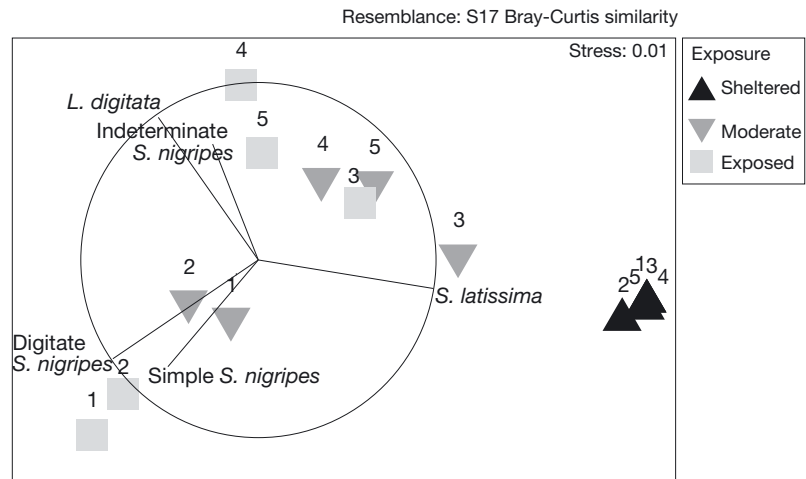


Fig. 4. Non-metric multidimensional scaling (nMDS) plot of Laminariaceae (*Laminaria digitata*, *Saccharina nigripes* and *S. latissima*) community structure at sheltered, moderate and exposed sites at Lepreau (see Table 3) from 2010 to 2014. Numbers denote years: 1 = 2010, 2 = 2011, 3 = 2012, 4 = 2013 and 5 = 2014. The sheltered site data points overlap each other in the graph. The vectors overlaid reflect the correlations between a species (or morphology for *S. nigripes*) and the nMDS axes. The vector for each species (or morphology for *S. nigripes*) shows the direction of increased species (or morphology for *S. nigripes*) abundance across the nMDS graph; therefore, years in that direction on the nMDS graph have a greater abundance of that species (or morphology for *S. nigripes*) than any other years. The large circle indicates the maximum vector length and $r = 1$ (where r = Pearson correlation coefficient) if the vector is parallel to one of the nMDS axes

Table 3. Results for the factor 'exposure' in a 2-way SIMPER analysis of exposure (sheltered, moderate and exposed) and year (2010, 2011, 2012, 2013 and 2014) at Lepreau. Simple and indeterminate *Saccharina nigripes* are not included in this table (though they were included in the analysis) due to their low abundances at all sites. Diss/SD is the average dissimilarity for a given species (or morphology for *S. nigripes*) divided by its standard deviation and is a measure of how well that species (or morphology for *S. nigripes*) discriminates between the 2 communities being evaluated; % contribution is a measure of how much each species (or morphology for *S. nigripes*) contributes to the differences observed

Species	Average dissimilarity	Diss/SD	Contribution (%)
Sheltered vs. exposed	87.20		
<i>S. latissima</i>		6.49	50.06
<i>L. digitata</i>		1.49	25.35
Digitate <i>S. nigripes</i>		1.08	21.43
Sheltered vs. moderate	71.00		
<i>S. latissima</i>		3.53	50.00
<i>L. digitata</i>		4.75	32.68
Digitate <i>S. nigripes</i>		1.02	17.32
Moderate vs. exposed	27.84		
<i>L. digitata</i>		2.02	32.02
<i>S. latissima</i>		1.72	29.11
Digitate <i>S. nigripes</i>		1.96	28.97

DISCUSSION

Saccharina nigripes is a major contributor to intertidal kelp community composition in the southwestern Bay of Fundy. It comprised 23% of all individuals sampled and was more abundant than *Laminaria digitata* (19% of total individuals sampled). Furthermore, in 2010 and 2011, *S. nigripes* was actually ~8 times more abundant than *L. digitata* in the wave-exposed site at Lepreau. Our results suggest that previous literature on the ecology and distribution of *L. digitata* may have overlooked *S. nigripes*, particularly at exposed sites. *S. nigripes* was the dominant digitate kelp at the Lepreau wave-exposed site in 2010 and 2011. However, from 2012 to 2014 it was rare, and *L. digitata* was the dominant kelp. As such, the relative abundance of *S. nigripes* and *L. digitata* appear to fluctuate dramatically between years, and thus abundance of these species in any given year is variable. Recognition of *S. nigripes* in the flora of the Maritime Provinces of Canada requires further research to determine the ecology and distribution of both *S. nigripes* and *L. digitata*.

Laminariaceae community composition varied with fetch distance. Sites with greater fetch distances were composed mainly of digitate individuals, specif-

Table 4. Results for the factor 'year' in a 2-way SIMPER analysis of exposure (sheltered, moderate and exposed) and year (2010, 2011, 2012, 2013 and 2014) at Lepreau. Simple and indeterminate *Saccharina nigripes* are not included in this table (though they were included in the analysis) due to their low abundances at all sites. See Table 3 legend for column descriptions

Species	Average dissimilarity	Diss/SD	Contribution (%)
2010 vs. 2011	7.02		
<i>L. digitata</i>		0.64	33.39
Digitate <i>S. nigripes</i>		2.81	30.93
<i>S. latissima</i>		1.26	20.18
2010 vs. 2012	42.96		
Digitate <i>S. nigripes</i>		1.12	45.91
<i>S. latissima</i>		1.11	31.10
<i>L. digitata</i>		0.63	19.10
2010 vs. 2013	39.47		
Digitate <i>S. nigripes</i>		0.99	45.54
<i>L. digitata</i>		0.83	38.15
<i>S. latissima</i>		0.79	7.82
2010 vs. 2014	37.62		
Digitate <i>S. nigripes</i>		1.11	45.32
<i>L. digitata</i>		0.86	33.36
<i>S. latissima</i>		0.90	15.08
2011 vs. 2012	40.83		
Digitate <i>S. nigripes</i>		1.24	48.57
<i>S. latissima</i>		1.18	35.71
<i>L. digitata</i>		0.63	14.29
2011 vs. 2013	37.33		
Digitate <i>S. nigripes</i>		1.14	48.44
<i>L. digitata</i>		0.87	33.93
<i>S. latissima</i>		0.88	11.61
2011 vs. 2014	35.50		
Digitate <i>S. nigripes</i>		1.26	48.36
<i>L. digitata</i>		0.92	28.64
<i>S. latissima</i>		0.97	19.48
2012 vs. 2013	22.00		
<i>S. latissima</i>		1.15	46.59
<i>L. digitata</i>		1.11	31.06
Digitate <i>S. nigripes</i>		0.84	14.77
2012 vs. 2014	15.33		
<i>S. latissima</i>		1.15	50.00
<i>L. digitata</i>		1.12	28.26
Digitate <i>S. nigripes</i>		1.13	17.39
2013 vs. 2014	9.67		
Digitate <i>S. nigripes</i>		1.06	37.07
<i>S. latissima</i>		1.03	26.72
<i>L. digitata</i>		0.71	25.86

ically *S. nigripes* in 2010 and 2011, and *L. digitata* in 2012, 2013 and 2014. Sites with lower fetch distances were on average 99% *S. latissima*, or simple-bladed individuals. *S. nigripes* was most abundant in the digitate form at sites of greater fetch distances, and as indicated above, did not contribute significantly to the simple-bladed flora.

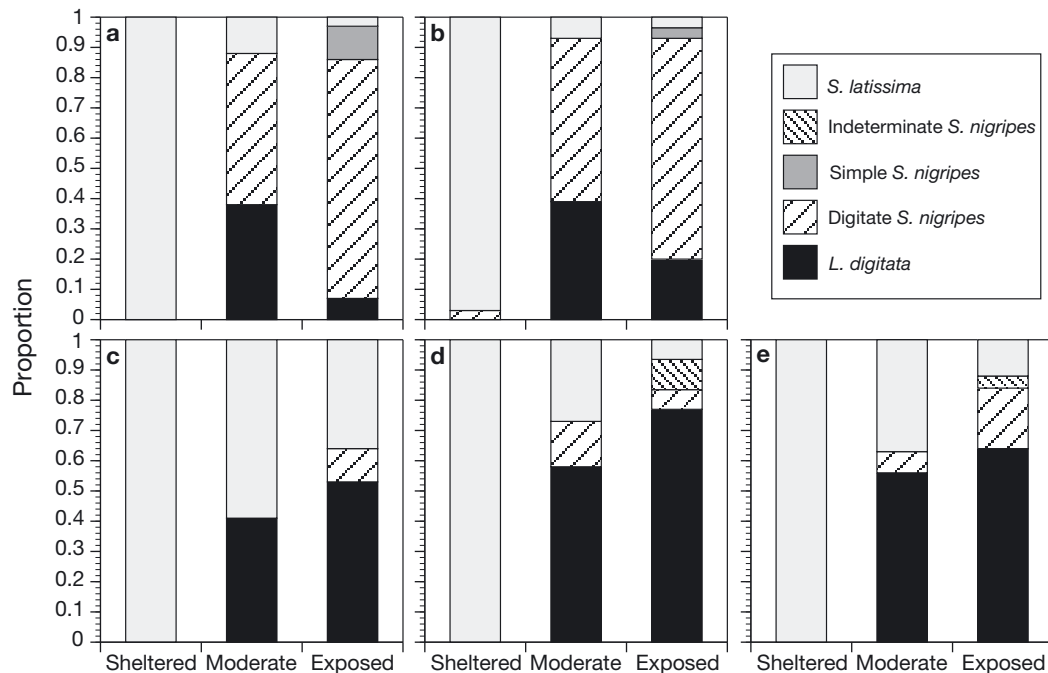


Fig. 5. Average abundance (proportion) of *Saccharina latissima*, different morphologies of *S. nigripes* (indeterminate, simple and digitate) and *Laminaria digitata* at sheltered, moderate and exposed sites at Lepreau in (a) 2010 (b) 2011 (c) 2012 (d) 2013 and (e) 2014. Proportions were calculated out of a maximum of 30 and a minimum of 24 individuals per site and year, because not all samples amplified successfully during PCR

The abundance of *S. nigripes* decreased dramatically from 2011 to 2012 and remained low in 2013 and 2014, resulting in a relative increase in *L. digitata* and *S. latissima* abundance during the sampling period. Wave-driven mortality is common in intertidal macroalgae (Dudgeon & Johnson 1992, Pratt & Johnson 2002); however, *L. digitata* and *S. latissima* populations did not similarly decline (relative to *S. nigripes*), suggesting that heavy storms were likely not the cause of the reduction in *S. nigripes*. Intertidal algae can be limited in distribution due to competition for suitable substrate (Lubchenco 1980, Duggins & Dethier 1985); however, the substrate in the low intertidal zone at our moderate and exposed study sites was not completely covered by organisms at any time, especially in the winter and spring when the Laminariaceae sporophytes recruit (Edelstein & McLachlan 1966, Chapman 1984), which suggests that competition was likely not the main factor in the reduction of *S. nigripes* at our study sites. Salinity is also an unlikely contributing factor because it remained relatively constant at 30 to 33 PSU from 2010 to 2014 in the Bay of Fundy (DFO Canada Atlantic Zone Monitoring Program, www.medsdmm.dfo-mpo.gc.ca/isdm-gdsi/azmp-pmza/hydro/index-eng.html). The one factor that changed consid-

erably from 2011 to 2012 was winter water temperature. Winter sea surface temperature (February to April) in the Bay of Fundy was approximately 4°C warmer in 2012 and 2013 (8°C) compared with 2010 (2 to 5°C) and 2011 (3 to 5°C); however, in 2014 water temperature returned to the 2010 and 2011 levels (2 to 4°C) (Institute of Marine and Coastal Sciences Coastal Ocean Observation Laboratory Rutgers University, http://marine.rutgers.edu/mrs/sat_data/?nothumbs=0&product=sst). Increased temperature may have played a role in the decreased abundance of *S. nigripes*, as it is an important factor in the distribution of both the sporophyte and gametophyte generations of kelp (e.g. Bolton & Lüning 1982, van den Hoek 1982, tom Dieck 1993, Oppliger et al. 2012, Bartsch et al. 2013). For example, the distribution of *L. digitata* is bounded by a southern reproductive boundary at 10°C (warmest temperature that permits fertility in the female gametophytes) and the southern lethal boundary at 19°C (warmest temperature *L. digitata* can be exposed to during the summer) (van den Hoek 1982). Bartsch et al. (2013) recently demonstrated that the narrowest temperature window in *L. digitata* occurs for sporogenesis, where fertility was 100% in temperatures of 1 to 10°C and only 20% at 18 to 19°C. The warmer winter water temper-

atures observed during our study could have negatively impacted sporogenesis, the gametophyte stage or growth of juvenile *S. nigripes*, thereby reducing population size. The upper thermal tolerance for vegetative gametophytes of Pacific populations of *S. nigripes* (as *Laminaria bongardiana*) was between 19 and 22°C, which is slightly lower than upper thermal tolerances observed in *S. latissima* (21°C) and *L. digitata* (22°C), but similar to the temperature tolerance of the Arctic species *L. solidungula* (19 to 20°C) (tom Dieck 1993). Although we know the maximum temperature tolerances of *S. latissima*, *L. digitata* and *S. nigripes* (as *L. bongardiana*) gametophytes, it is unknown whether a specific temperature range is required to trigger the maturation of gametes and subsequent sporophyte development. *S. nigripes* (as *S. groenlandica*) in the Pacific Ocean develops sori in the winter (Druehl 1968), and new recruits become macroscopically visible in March (Druehl et al. 1987). The fact that spores are released in the winter suggests that cool water temperatures may be required for gametophyte or juvenile sporophyte development, as documented in other kelp species (Deysher & Dean 1986, Thornber et al. 2004).

We suggest that the warmer winter sea surface temperatures from 2012 to 2014 affected the abundance of *S. nigripes*, since the initial decline in *S. nigripes* occurred in 2012, when the winter sea surface temperature did not go below 8°C. *S. nigripes* abundance remained low in 2013, when winter water temperatures were comparable to 2012, supporting the notion that *S. nigripes* recruitment may be linked to ocean temperature. Although the winter water temperature decreased in 2014, *S. nigripes* abundance did not increase. It has been demonstrated in California kelp forests that populations can rebound from pre-existing gametophyte banks following destructive events (Carney et al. 2013); however, we did not observe such a rebound for *S. nigripes* in the Bay of Fundy when winter water temperatures returned to normal levels in 2014. If gametophyte banks exist for *S. nigripes* in the Bay of Fundy they may be short-lived (only 1 or 2 yr), resulting in the low abundance of *S. nigripes* observed in 2014. If there is a correlation between the abundance of *S. nigripes* and sea surface temperature, and if water temperature continues to rise, then a contraction in the range with a poleward shift in range is expected.

Acknowledgements. We thank Professor Robert Clarke and Dr. Myriam Barbeau for their assistance with PRIMER and PERMANCOVA analyses. We thank Dr. Dan McDevit for his input in developing this project. This study was made possi-

ble by a scholarship to C.M.L. by the Natural Sciences and Engineering Research Council of Canada (NSERC) and a grant from the New Brunswick Museum, as well as a NSERC grant awarded to G.W.S.

LITERATURE CITED

- Agardh JG (1868) Bidrag till kännedomen af Spetsbergens alger. PA Norstedt & Söner, Stockholm
- Ballantine W (1961) A biologically defined exposure scale for the comparative description of rocky shores. *Field Stud* 1:1–19
- Bartsch I, Vogt J, Pehlke C, Hanelt D (2013) Prevailing sea surface temperatures inhibit summer reproduction of the kelp *Laminaria digitata* at Helgoland (North Sea). *J Phycol* 49:1061–1073
- Bologna PA, Steneck RS (1993) Kelp beds as habitat for American lobster *Homarus americanus*. *Mar Ecol Prog Ser* 100:127–134
- Bolton JJ, Lüning K (1982) Optimal growth and maximal survival temperatures of Atlantic *Laminaria* species (Phaeophyta) in culture. *Mar Biol* 66:89–94
- Carney LT, Bohonak AJ, Edwards MS, Alberto F (2013) Genetic and experimental evidence for a mixed-age, mixed-origin bank of kelp microscopic stages in southern California. *Ecology* 94:1955–1965
- Chapman AR (1984) Reproduction, recruitment and mortality in two species of *Laminaria* in southwest Nova Scotia. *J Exp Mar Biol Ecol* 78:99–109
- Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. *Aust J Ecol* 18:117–143
- Coyer JA, Smith GJ, Anderson RA (2001) Evolution of *Macrocystis* spp. (Phaeophyceae) as determined by ITS1 and ITS2 sequences. *J Phycol* 37:574–585
- Dayton PK (1985) Ecology of kelp communities. *Annu Rev Ecol Syst* 16:215–245
- Dayton PK, Currie V, Gerrodette T, Keller BD, Rosenthal R, Ven Tresca D (1984) Patch dynamics and stability of some California kelp communities. *Ecol Monogr* 54: 253–289
- Demes KW, Graham MH, Suskiewicz TS (2009) Phenotypic plasticity reconciles incongruous molecular and morphological taxonomies: the giant kelp, *Macrocystis* (Laminariales, Phaeophyceae), is a monospecific genus. *J Phycol* 45:1266–1269
- Deysher LE, Dean TA (1986) Interactive effects of light and temperature on sporophyte production in the giant kelp *Macrocystis pyrifera*. *Mar Biol* 93:17–20
- Druehl LD (1968) Taxonomy and distribution of northeast Pacific species of *Laminaria*. *Can J Bot* 46:539–547
- Druehl L, Elliott C (1996) Parks Canada Barkley Sound kelp distribution. Report prepared for Parks Canada, Bamfield Marine Station, Bamfield
- Druehl LD, Cabot EL, Lloyd KE (1987) Seasonal growth of *Laminaria groenlandica* as a function of plant age. *Can J Bot* 65:1599–1604
- Dudgeon S, Johnson A (1992) Thick vs. thin: thallus morphology and tissue mechanics influence differential drag and dislodgement of two co-dominant seaweeds. *J Exp Mar Biol Ecol* 165:23–43
- Duggins DO, Dethier MN (1985) Experimental studies of herbivory and algal competition in a low intertidal habitat. *Oecologia* 67:183–191
- Edelstein T, McLachlan J (1966) Investigations of the marine

- algae of Nova Scotia. I. Winter flora of the Atlantic coast. *Can J Bot* 44:1035–1055
- Edelstein T, Chen L, McLachlan J (1970) Investigations of the marine algae of Nova Scotia. VIII. The flora of Digby Neck Peninsula, Bay of Fundy. *Can J Bot* 48:621–629
 - Fowler-Walker MJ, Wernberg T, Connell SD (2006) Differences in kelp morphology between wave sheltered and exposed localities: morphologically plastic or fixed traits? *Mar Biol* 148:755–767
 - Fraser C, Hay C, Spencer H, Waters J (2009) Genetic and morphological analyses of the southern bull kelp *Durvillaea antarctica* (Phaeophyceae: Durvillaeales) in New Zealand reveal cryptic species. *J Phycol* 45:436–443
 - Friedland MT, Denny MW (1995) Surviving hydrodynamic forces in a wave-swept environment: consequences of morphology in the feather boa kelp, *Egregia menziesii* (Turner). *J Exp Mar Biol Ecol* 190:109–133
 - Gerard VA, Mann KH (1979) Growth and production of *Laminaria longicruris* (Phaeophyta) populations exposed to different intensities of water movement. *J Phycol* 15:33–41
 - Graham MH (1997) Factors determining the upper limit of giant kelp, *Macrocystis pyrifera* Agardh, along the Monterey Peninsula, central California, USA. *J Exp Mar Biol Ecol* 218:127–149
 - Graham MH (2004) Effects of local deforestation on the diversity and structure of southern California giant kelp forest food webs. *Ecosystems* 7:341–357
 - Howes D, Harper J, Owens E (1994) Physical shore-zone mapping system for British Columbia. Resources Inventory Committee, Victoria
 - Johnson CR, Mann KH (1988) Diversity, patterns of adaptation, and stability of Nova Scotian kelp beds. *Ecol Monogr* 58:129–154
 - Jones W, Demetropoulos A (1968) Exposure to wave action: measurements of an important ecological parameter on rocky shores on Anglesey. *J Exp Mar Biol Ecol* 2:46–63
 - Koehl MAR, Alberte RS (1988) Flow, flapping, and photosynthesis of *Nereocystis luetkeana*: a functional comparison of undulate and flat blade morphologies. *Mar Biol* 99:435–444
 - Lane C, Mayes C, Druehl L, Saunders GW (2006) A multi-gene molecular investigation of the kelp (Laminariales, Phaeophyceae) supports substantial taxonomic re-organization. *J Phycol* 42:493–512
 - Longtin CM, Saunders GW (2015) On the utility of mucilage ducts as a taxonomic character in *Laminaria* and *Saccharina* (Phaeophyceae)—the conundrum of *S. groenlandica*. *Phycologia* 54:440–450
 - Lubchenco J (1980) Algal zonation in the New England rocky intertidal community: an experimental analysis. *Ecology* 61:333–344
 - Lubchenco J, Menge BA (1978) Community development and persistence in a low rocky intertidal zone. *Ecol Monogr* 48:67–94
 - McDevit DM, Saunders GW (2010) A DNA barcode examination of the Laminariaceae (Phaeophyceae) in Canada reveals novel biogeographical and evolutionary insights. *Phycologia* 49:235–248
 - Merzouk A, Johnson LE (2011) Kelp distribution in the northwest Atlantic Ocean under a changing climate. *J Exp Mar Biol Ecol* 400:90–98
 - Miller K, Olsen J, Stam W (2000) Genetic divergence correlates with morphological and ecological subdivision in the deep-water elk kelp, *Pelagophycus porra* (Phaeophyceae). *J Phycol* 36:862–870
 - Oppliger LV, Correa JA, Engelen AH, Tellier F and others (2012) Temperature effects on gametophyte life-history traits and geographic distribution of two cryptic kelp species. *PLoS ONE* 7:e39289
 - Pedersen MF, Nejrup LB, Fredriksen S, Christie H, Norderhaug KM (2012) Effects of wave exposure on population structure, demography, biomass and productivity of the kelp *Laminaria hyperborea*. *Mar Ecol Prog Ser* 451:45–60
 - Pratt M, Johnson A (2002) Strength, drag, and dislodgment of two competing intertidal algae from two wave exposures and four seasons. *J Exp Mar Biol Ecol* 272:71–101
 - Roberson L, Coyer J (2004) Variation in blade morphology of the kelp *Eisenia arborea*: incipient speciation due to local water motion? *Mar Ecol Prog Ser* 282:115–128
 - Saunders GW, McDevit DM (2012) Methods for DNA barcoding photosynthetic protists emphasizing the macroalgae and diatoms. *Methods Mol Biol* 858:207–222
 - Smith BD (1985) Recovery following experimental harvesting of *Laminaria longicruris* and *L. digitata* in southwestern Nova Scotia. *Helgol Meeresunters* 39:83–101
 - Steneck RS, Graham MH, Bourque BJ, Corbett D, Erlandson JM, Estes JA, Tegner MJ (2002) Kelp forest ecosystems: biodiversity, stability, resilience and future. *Environ Conserv* 29:436–459
 - Thornber CS, Kinlan BP, Graham MH, Stachowicz JJ (2004) Population ecology of the invasive kelp *Undaria pinnatifida* in California: environmental and biological controls on demography. *Mar Ecol Prog Ser* 268:69–80
 - tom Dieck I (1993) Temperature tolerance and survival in darkness of kelp gametophytes (Laminariales, Phaeophyta): ecological and biogeographical implications. *Mar Ecol Prog Ser* 100:253–264
 - van den Hoek C (1982) The distribution of benthic marine algae in relation to the temperature regulation of their life histories. *Biol J Linn Soc* 18:81–144

Editorial responsibility: Lisandro Benedetti-Cecchi,
Pisa, Italy

Submitted: June 24, 2015; Accepted: November 19, 2015
Proofs received from author(s): January 7, 2016