HHS Public Access

Author manuscript

Phycologia. Author manuscript; available in PMC 2018 January 01.

Published in final edited form as:

Phycologia. 2018 ; 57(1): 32–40. doi:10.2216/17-72.1.

Fine-scale population genetic structure of sugar kelp, *Saccharina latissima* (Laminariales, Phaeophyceae), in eastern Maine, USA

Timothy S. Breton[1,*], **Jeremy C. Nettleton**[2], **Brennah O'Connell**[1], and **Margaret Bertocci**[1]

[1]Division of Natural Sciences, University of Maine at Farmington, 173 High Street, Farmington, ME 04938

[2]Environmental and Biological Sciences Division, University of Maine at Machias, 116 O'Brien Avenue, Machias, ME 04654

Abstract

There is an interest to develop sugar kelp (*Saccharina latissima*) cultivation in the rural, eastern Maine region of the USA. Future farming efforts would benefit from an understanding of the genetic diversity and population structure of kelp, to inform management and conservation, and to identify genetic resources. The purpose of the present study was to characterize the fine-scale population genetic structure of kelp in eastern Maine, using twelve microsatellite loci. A total of 188 samples were genotyped from five sampling locations. Overall, kelp exhibited relatively low genetic diversity and small but significant differentiation among populations (F_{ST} = 0.0157). The greatest genetic difference was detected between two geographically close populations in Penobscot and Frenchman Bays, which is likely due to patterns in the Eastern Maine Coastal Current that may limit meiospore recruitment. The population structure could not be fully explained by an isolation-by-distance model. Fine-scale structuring was also detected among populations along the more continuous, eastern Maine coastline. These differences highlight that sugar kelp populations are finely structured across small spatial scales, and that future management and farming efforts should aim to maintain genetic diversity and assess the culture potential of local populations.

Keywords

Gene flow; Genetic diversity; Kelp; Microsatellite; Polymorphism; Population structure

INTRODUCTION

The sugar kelp *Saccharina latissima* (Linnaeus) C.E.Lane, C.Mayes, Druehl & G.W.Saunders, previously known as *Laminaria saccharina* (Linnaeus) J.V.Lamouroux, is a brown macroalga broadly distributed in both temperate and polar rocky coastal sites in the northern hemisphere, and like many kelp species, plays important roles as nursery habitat, shelter, and substrate for many organisms (Bolton 2010, Devit & Saunders 2010). *S.*

*Corresponding author: timothy.breton@maine.edu.

latissima can grow to immense sizes (>4 m in length) and occurs attached to rocks, mussels, and man-made substrates from the intertidal zone to 26 m depths (Borum *et al.* 2002; Bartsch *et al.* 2008; Mathieson & Dawes 2017). As a biennial or perennial species, this kelp is characterized by rapid, early seasonal growth rates and sporangium formation in the autumn and winter, when daylight and temperature decrease (Lüning 1979; Bartsch *et al.* 2008; Mathieson & Dawes 2017). Meiospore dispersal is limited, and largely driven by water currents (Billot *et al.* 2003; Bartsch *et al.* 2008). Haploid gametophytes mostly settle on hard substrates, or in some rarer cases can develop as endophytes inside other algal hosts, where fertilization takes place and new sporophytes form on the surface (Garbary *et al.* 1999; Lane & Saunders 2005; Bartsch *et al.* 2008).

Saccharina latissima has received considerable economic attention in recent years, due to a variety of uses, including in biofuels, bioremediation, feed supplements, and pharmaceutical products (Forbord *et al.* 2012; Marinho *et al.* 2015; Jahan *et al.* 2017). For example, *S. latissima* and other brown macroalgae contain biologically active phenolic compounds, such as phlorotannins, which are known to exhibit anti-diabetic, anticarcinogenic, and anti-human immunodeficiency virus (HIV) activities (Jormalainen & Honkanen 2004; Thomas & Kim 2011; Cornish *et al.* 2015; Vilg *et al.* 2015). In addition, *S. latissima* contains relatively high quantities of alginates, which function as storage polysaccharides and are used commercially as thickeners and emulsifiers in food industries (MacArtain *et al.* 2007). Consumption of kelp is also associated with several therapeutic and medicinal benefits, due in part to high quantities of alginates and mannitol (MacArtain *et al.* 2007; Kim & Bhatnagar 2011). Another abundant polysaccharide, laminarin, has been investigated in *S. latissima* as an alternative fermentation substrate for efficient bioethanol production (Adams *et al.* 2009). Due to these important uses, the demand for kelp is likely to increase in the future, and may exceed limited supplies from wild harvests and require new production from aquaculture (Peteiro & Freire 2013).

Recent cultivation efforts of *S. latissima* have led to many advances, including successful year-round meiospore induction, identification of optimal growing conditions at the nursery stage, and implementation in integrated multi-trophic aquaculture (IMTA) systems (Forbord *et al.* 2012; Sanderson *et al.* 2012; Rößner *et al.* 2014; Freitas *et al.* 2016). While many of these studies were conducted in Europe, sugar kelp aquaculture has also been investigated in both Canada and the USA (Brinkhuis *et al.* 1984; Troell *et al.* 2009; Redmond *et al.* 2014; Kim *et al.* 2015). Overall, however, seaweed industries in the northeast USA remains largely focused on wild harvests, with some recent efforts in aquaculture (Kim *et al.* 2014 2017; Johnson *et al.* 2014; Redmond *et al.* 2014; Augyte *et al.* 2017). In particular, there is an interest to develop *S. latissima* culture in the rural, economically impoverished region of eastern Maine (Hall-Arber *et al.* 2005), to both diversify local industries and preserve the traditional fishing lifestyle.

The development of a new regional industry in cultivation of *S. latissima* would benefit from a more thorough understanding of local population genetics, for proper management and conservation, as well as the identification of genetic resources and population dynamics, before farming techniques are established. To this end, the purpose of the present study was to characterize the fine-scale population genetic structure of sugar kelp in eastern Maine.

Recently, twelve polymorphic microsatellite loci were identified in *S. latissima* and used to characterize the genetic population structure along a salinity gradient in the North Sea-Baltic transition zone (Nielsen *et al.* 2016). To our knowledge, however, these loci have not been assessed in boreal, northwest Atlantic populations, which also represents an opportunity to further evaluate the genetic diversity of this species.

MATERIALS AND METHODS

Adult sporophyte kelp samples were collected (approximately 40 individuals/site) from five intertidal sites in eastern Maine, spanning approximately 225 km, during the summer and early autumn of 2016 (Fig. 1). The sampling scheme included major bays in the region (Penobscot Bay, Frenchman Bay, and Cobscook Bay), as well as two coastal sites adjacent to the Gulf of Maine (Englishman Bay and Starboard Cove). Sampling was conducted at low tide and in a haphazard manner, to avoid collection of adjacent individuals that could originate from the same gametophyte. Blade fragments (~1 cm^2) were removed from each individual and added to 1.5 ml microcentrifuge tubes with silica gel beads, to preserve and desiccate the samples, until DNA extractions were performed.

Since many brown algae contain abundant, viscous polysaccharides and polyphenols that may interfere with DNA extractions or downstream PCR applications (Snirc *et al.* 2010, Greco *et al.* 2014), genomic DNA was extracted from all kelp samples using a modified, combined cetyl trimethylammonium bromide (CTAB) and sodium dodecyl sulfate (SDS) protocol from Maeda *et al.* (2013). The protocol modifications included a different homogenization method, for ease of use, and several changes in reagent volumes and composition (β-mercaptoethanol and RNase A were not used). Briefly, a dried kelp sample fragment (~5 mm^2) was added to a microcentrifuge tube with autoclaved sand (Sigma-Aldrich, St. Louis, MO, USA), 400 μl of extraction buffer (100 mM Tris-HCl pH 9.5, 50 mM EDTA pH 8.0, 500 mM NaCl), and 29 μl of 20% SDS. Each sample was fully homogenized by hand using sterile pestles (Nettleton *et al.* 2013) and incubated at 65°C for 30–45 min. Potassium acetate (5 M) was added (129 μl) to each sample, incubated at −20°C for 15 min, centrifuged (20,000 × g, 10 min), and the supernatant was transferred to a new tube. Cold isopropanol was added (280 μl), incubated at −20°C for 15 min, centrifuged again, and the DNA pellet was washed twice with 600 μl of cold 75% ethanol. Samples were centrifuged (20,000 × g, 5 min) between wash steps. The DNA pellets were vacuum-aspirated, resuspended in 100 μl of sterile water, and either stored at 4°C overnight or used immediately in successive steps. CTAB buffer (3% CTAB (w/v), 1% polyvinylpyrrolidone (w/v), 1.4 M NaCl, 20 mM EDTA pH 8.0, 100 mM Tris-HCl pH 9.5) was added (500 μl) to each sample, incubated at 65°C for 30 min, combined with 200 μl of 5 M potassium acetate, and incubated on ice for 15 min. Each sample was then centrifuged (20,000 × g, 10 min), the supernatant was transferred, and 650 μl of chloroform:isoamyl alcohol (24:1) was added. Samples were mixed vigorously by hand for 15 sec, centrifuged again, and the top aqueous layer was transferred to a new tube. The chloroform:isoamyl alcohol extractions were repeated twice more, 480 μl of cold isopropanol was then added, and samples were incubated at −20°C for 20 min. Samples were centrifuged again, DNA pellets were washed twice with 75% ethanol, as outlined above, and vacuum-aspirated prior to resuspension in sterile water. Extracted DNA was stored at −20°C.

Microsatellite analysis was performed using twelve loci for *S. latissima* previously characterized by Paulino *et al.* 2016. PCR was conducted in 15 μl volumes using 2 μl of DNA template, 1 × Promega GoTaq Flexi PCR buffer (Promega, Madison, WI, USA), 1.5–2.5 mM $MgCl_2$, 0.2 μg/μl bovine serum albumin, 0.28 mM dNTPs, 0.1–0.3 μM of each primer, and 0.2 U GoTaq Flexi DNA polymerase. Multiplex PCRs were conducted following guidelines in Nielsen *et al.* 2016, except different fluorescent dyes were used to label either the forward or reverse primer: 1) SLN319 (FAM-labeled), SLN320 (FAM), SLN34 (HEX), and SLN35 (HEX), 2) SLN58 (FAM) and SLN62 (HEX), and 3) SLN32 (NED) and SLN36 (HEX). Other loci were used individually in PCR reactions, including SLN54 (HEX), SLN314 (FAM), SLN510 (FAM), and SLN511 (HEX). All PCR cycling conditions were conducted using a C1000 Touch Thermal Cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and followed established protocols by Paulino *et al.* 2016. PCR products were transported to the MDI Biological Laboratory (Salisbury Cove, ME, USA) for fragment analysis in an Applied Biosystems 3130XL capillary sequencer (Applied Biosystems, Foster City, CA, USA). Peaks were scored using PeakScanner 2.0 (Applied Biosystems), and raw scores were sorted manually into allelic bins. The same positive control sample was run on every PCR plate to ensure consistent scoring patterns and normalize interassay variation prior to binning.

Microsatellite data were evaluated for stuttering, large allele drop out, and the presence of null alleles using MICRO-CHECKER (Van Oosterhout *et al.* 2004). Tests for deviations from Hardy-Weinberg equilibrium and linkage disequilibrium were conducted in GENEPOP v3.4 (Raymond & Rousset 1995), using a Markov chain Monte Carlo (MCMC) method of 1,000 or 100 batches, respectively, and 10,000 iterations per test. Significance for multiple tests was adjusted following a standard Bonferroni correction (Rice 1989). Per locus F_{ST} values, a global F_{ST}, and tests of allelic differentiation using Fisher's exact tests (1,000 batches and 10,000 iterations) were also conducted in GENEPOP. Observed and expected heterozygosities (H_o and H_e) and inbreeding coefficients (F_{IS} values) for each locus and population were calculated using GDA (Lewis & Zaykin 2001). Significance of population F_{IS} values were evaluated in GENETIX (Belkhir *et al.* 1996–2004). Number of alleles per locus and allelic richness were calculated using FSTAT 2.9.3 (Goudet 1995). To identify if the sampled kelp populations experienced a recent bottleneck effect, the program BOTTLENECK was used, with 1,000 iterations, all mutation models (88% stepwise mutations and 12% infinite allele mutations), and variance among multiple steps set at 12 (Piry *et al.* 1999). Wilcoxon sign-rank tests for significant heterozygosity excess and allele frequency distributions were used to detect evidence of a recent bottleneck (Luikart *et al.* 1998). The file conversion program CREATE was used to change input data file formats among all statistical tests (Coombs *et al.* 2008).

To identify genetic differences among the five populations, pairwise population F_{ST} values and their significance were evaluated in FSTAT. F_{ST} values were also used in principal component analysis (PCA) in GENALEX 6.503 (Peakall & Smouse 2012), to better visualize the population structure. An isolation-by-distance model for the genetic structure was evaluated using a Mantel test in GENALEX. The correlation between genetic and geographic distance was tested by comparing pairwise linearized F_{ST} values ($F_{ST}(1-F_{ST})$) to the shortest coastal distances between sites (approximately within 5 km of coast).

Geographic distances were calculated using the Measure feature in the ArcGIS Maine Basemap General viewer available from the State of Maine Office of GIS (http://maine.maps.arcgis.com/home/index.html).

To further characterize the population structure, without *a priori* clustering assumptions, a Bayesian approach in STRUCTURE 2.3.4 was used (Pritchard *et al.* 2000; Hubisz *et al.* 2009). The no admixture model with correlated allele frequencies and the LocPrior algorithm was used to infer population structure, since this is better suited to detect overall weak structuring (Hubisz *et al.* 2009; Pritchard *et al.* 2010) likely evident across the fine spatial scale in the present study. Five runs were conducted at each number of assumed populations (K), ranging one through five, with a 300,000 burn-in followed by 200,000 iterations. Data were uploaded to STRUCTURE HARVESTER (Earl & vonHoldt 2012) to determine the optimal number of clusters, using the ΔK method of Evanno *et al.* (2005). Following the identification of the optimal number of clusters, 25 additional runs were conducted in STRUCTURE using this number (K = 3), and the results were averaged using the greedy algorithm in CLUMPP 1.1.2 (Jakobsson & Rosenberg 2007). CLUMPP results were used to generate an averaged bar plot in DISTRUCT (Rosenberg 2004).

To further evaluate differences in population structure evident between F_{ST}- and STRUCTURE-based analyses, analyses of molecular variance (AMOVA) were conducted in ARLEQUIN 3.5.2.2 (Excoffier & Lischer 2010). Populations were grouped into either three (1-Penobscot Bay, 2-Cobscook Bay, and 3-Frenchman Bay-Englishman Bay-Starboard Cove) or four (1-Penobscot Bay, 2-Frenchman Bay, 3-Cobscook Bay, and 4-Englishman Bay-Starboard Cove) clusters, and global AMOVA results were averaged over all loci. Assignment tests in GENECLASS2 (Piry *et al.* 2004) were also performed, to further assess connectivity among populations and identify the ability of each individual to be assigned back to its correct population of origin. The Bayesian approach of Rannala & Mountain (1997), with a 0.05 assignment threshold, was used to calculate the log likelihood of an individual genotype originating from each population. Individuals were assigned to populations based on the greatest log likelihood value. To assess if the observed number of assignments to each population was significantly greater than by chance, chi-square goodness-of-fit tests ($\chi 2$) were conducted. The expected numbers of assignments were calculated by assuming equal proportions to each of the five populations ($n/5$).

RESULTS

Multilocus genotypes were obtained for 188 total samples, with at least 30 individuals from each site. Two microsatellite markers (SLN319 and SLN511) were not polymorphic in the sampled populations (fixed for 419 and 368 bp alleles, respectively) and were removed prior to analyses. In addition, SLN511 exhibited inconsistent scoring patterns among individuals, which likely indicated the presence of null alleles. At all other loci, only six total individuals exhibited missing data at one locus each, with three individuals at SLN35, one at SLN36, and two at SLN54. No evidence of stuttering, large allele dropout, or null alleles was detected at any locus in any population, except for SLN34 in Starboard Cove, where there was a significant homozygote excess ($P < 0.05$). Since an excess was not detected in other populations, however, the possibility of null alleles at SLN34 was not considered in later

analyses. The final data set consisted of 10 polymorphic microsatellite markers and is available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.sp640.

Genetic diversity

No deviations from Hardy-Weinberg equilibrium were detected at any locus in any population ($P > 0.05$), and there was no evidence of linkage disequilibrium between pairs of loci following Bonferroni correction ($P < 0.0011$). The ten microsatellite loci exhibited variable levels of polymorphism (3–17 alleles) and allelic richness varied from 1.4 to 8.7 (Table 1). Observed and expected heterozygosities were also variable and ranged from 0.016 to 0.665 and 0.016 to 0.669, respectively (Table 1). F_{IS} values for each locus were overall small but largest in SLN34 and SLN58 (Table 1). Locus F_{ST} values were also small and ranged from −0.0050 to 0.0170, with a global F_{ST} value of 0.0157. Most loci exhibited significant differentiation across all populations (at the $\alpha = 0.05$ level), except for three loci with extremely low polymorphism (3–4 total alleles) (Table 1).

Genetic diversity was largely similar among the five sampled populations. Mean number of alleles and mean allelic richness ranged from 3.9 to 4.8 and 3.6 to 4.2, respectively (Table 2). Population observed and expected heterozygosities were overall small but largely similar to each other (0.283 to 0.339 and 0.273 to 0.340, respectively) and F_{IS} values were not significantly different from zero ($P > 0.05$) (Table 2). Tests of bottleneck effects exhibited no evidence of heterozygosity excess in any population, under any mutation model ($P > 0.90$), and all allele frequencies exhibited normal L-shaped distributions (data not shown).

Population structure

Significant population structuring was detected by F_{ST} analysis, with 8 out of 10 pairwise comparisons exhibiting significant differentiation following Bonferroni correction ($P < 0.005$) (Table 3). Significant pairwise F_{ST} values ranged from 0.0104 to 0.0316. In addition, the comparison between kelp from Englishman Bay and Cobscook Bay was significant at the $\alpha = 0.05$ level ($F_{ST} = 0.0061$). Only Englishman Bay and Starboard Cove kelp exhibited a nonsignificant pairwise F_{ST} value ($F_{ST} = -0.0020$). The PCA exhibited clear separation between most populations, with no apparent clustering except for Englishman Bay and Starboard Cove. The greatest differentiation was evident between the Penobscot and Frenchman Bay populations (Fig. 2). The population structure could not be fully explained by an isolation-by-distance model, as the Mantel test identified no significant correlation between genetic and geographic distance among the five populations ($P = 0.1290$) (Fig. 3).

STRUCTURE analysis and the ΔK method identified three likely genetic clusters (K = 3). Sampling location information used in the LocPrior algorithm was informative for the population structure, as mean $r = 1.14$ across the 25 runs. The r parameter refers to how sampling locations deviate from the overall population. Large r values (>> 1) indicate that different locations likely have the same ancestry, while small values (near or below 1) indicate that location information was informative to the model and sampling locations differ in their ancestry (Hubisz *et al.* 2009). The three genetic clusters consisted of: 1) Penobscot Bay, 2) Cobscook Bay, and 3) Frenchman Bay, Englishman Bay, and Starboard Cove (Fig. 4). Kelp from Englishman Bay, however, also exhibited some evidence of mixed

membership. Five individuals showed an elevated proportional membership (>59%) to the Cobscook Bay cluster, with two exhibiting >98% membership. In addition, two Cobscook Bay individuals and two Penobscot Bay individuals showed elevated proportional membership to the Frenchman Bay cluster (>52%, and 50%, respectively).

Hierarchical AMOVA, using the three genetic clusters identified by STRUCTURE analysis, showed no evidence of significant genetic variation among the population clusters (P = 0.1020), while significant heterogeneity was present among populations within the Frenchman Bay cluster (P = 0.0112) (Table 4). In contrast, when the four genetic groupings evident by F_{ST} were used instead, significant variation existed among the clusters (P = 0.0064), with the Englishman Bay and Starboard Cove cluster being genetically homogenous (P = 0.6016) (Table 4). Both AMOVAs detected highly significant genetic variation within populations (>98% of total variation, $P < 0.0001$).

Assignment tests also reflected evidence of small genetic divergence among the populations. The overall quality index for the assignment test was 40.66%, with only 45.2% of all individuals correctly assigned to their population of origin. Penobscot Bay kelp exhibited a relatively large correct assignment (71.8%), and its largest incorrect assignment was to Englishman Bay (12.8%) (Table 5). No Penobscot Bay individuals were assigned to Cobscook Bay. Frenchman Bay kelp exhibited a moderate correct assignment (50.0%), with a relatively large proportion assigned to another population in its STRUCTURE cluster (Starboard Cove: 26.7%). No Frenchman Bay individuals were assigned to Penobscot Bay. Englishman Bay individuals were equally assigned back to their original population or to Cobscook Bay (20% each), and a larger proportion was assigned to Starboard Cove (34.7%). Cobscook Bay kelp also exhibited only moderately correct assignments (46.9%), and the largest incorrect assignment was to Englishman Bay (18.8%). Proportional assignments for all populations were significantly greater than expected by chance, except for Englishman Bay individuals ($P > 0.10$), which indicated overall equal assignments across the five populations.

DISCUSSION

Sugar kelp populations in eastern Maine were characterized by significant differentiation among sites but overall low levels of genetic diversity. Although no significant inbreeding was detected, low to moderate levels of polymorphism were evident at most microsatellite loci. In addition, two loci used in the present study were monomorphic across populations. Using the same markers, most individual kelp populations in European waters exhibited larger allelic richness and heterozygosity, irrespective of geographic location (Nielsen *et al.* 2016; Paulino *et al.* 2016). The only previously sampled European population, at these markers, to exhibit somewhat similar heterozygosities to those in the present study was found in the more isolated Vejle Fjord in Denmark (H_o = 0.318, H_e = 0.358), which was within the North Sea-Baltic salinity transition zone and characterized by a small population size and limited connectivity (Nielsen *et al.* 2016). Less genetic diversity was also detected in some populations of other algal species, including the kelp *Laminaria digitata* (Hudson) J.V.Lamouroux and the red alga *Gigartina skottsbergii* Setchell & N.L.Gardner, which were either spatially isolated by habitat discontinuities or in marginal zones of their geographic

distribution, and exhibited significant, recent bottleneck effects (Billot *et al.* 2003; Faugeron *et al.* 2004; Robuchon *et al.* 2014). Kelp populations in eastern Maine, however, did not exhibit a recent bottleneck, and although they were located in boreal waters, were not at the southern edge of their distribution (Mathieson *et al.* 2008; Guiry & Guiry 2017; Mathieson & Dawes 2017). Instead, less genetic diversity in the present study may reflect a relatively recent colonization event in the northwest Atlantic after the last glacial maximum, as evidenced in another brown alga, *Fucus vesiculosis* Linnaeus (Coyer *et al.* 2011). *F. vesiculosis* populations along the Maine coast also exhibited relatively low genetic diversity at microsatellite loci, and were characterized by a high abundance of a single mitochondrial haplotype that implicated a European origin in glacial refugia (Muhlin & Brawley 2009; Coyer *et al.* 2011). Similarly, *S. latissima* populations in western Greenland waters exhibited fewer alleles and heterozygosities than European populations (Paulino *et al.* 2016). Reduced diversity of sugar kelp in eastern Maine, therefore, may reflect some ancestral genetic signature of a colonization and expansion event across the northwest Atlantic, rather than evidence of a marginal or bottlenecked population.

Kelp populations within the region exhibited significant differentiation, but at overall smaller levels than those previously detected in *Saccharina* spp. across broad spatial scales. For example, kelp population comparisons across greater geographic distances in the North and Baltic Seas, as well as in Chinese and Japanese waters, exhibited genetic differentiation an order of magnitude larger ($F_{ST} > 0.1$) than those observed in the present study (Liu *et al.* 2012; Nielsen *et al.* 2016). Small but significant population structuring, however, was still evident across the small spatial scale in eastern Maine waters, including among individuals in the major embayments of Penobscot, Frenchman, and Cobscook Bays. In particular, the greatest level of differentiation was detected between the Penobscot and Frenchman Bay populations, which were geographically closer to each other than several other comparisons, and contributed to an overall lack of evidence for a significant isolation-by-distance model to the population structure. In contrast, isolation-by-distance is relatively common in many kelp species, since meiospore dispersal is both spatially and temporally limited, and female gametophytes are more likely to be fertilized by nearby individuals (Bartsch *et al.* 2008; Alberto *et al.* 2010, 2011; Robuchon *et al.* 2014; Nielsen *et al.* 2016). Other geographical features, however, such as habitat continuity and local ocean currents, also greatly impact population structuring in macroalgae (Alberto *et al.* 2011). The relatively larger differentiation between the Penobscot and Frenchman Bay populations is likely driven by local patterns in the Eastern Maine Coastal Current (EMCC), which flows in a southwest direction along the Maine coast and is deflected offshore, by high outflow, in the vicinity of Penobscot Bay (Pettigrew *et al.* 2005). Meiospore recruitment from eastern locations into Penobscot Bay, therefore, is probably restricted, compared to other sites along the more continuous, eastern Maine coastline. In agreement, STRUCTURE-based analyses identified some evidence of genetic mixing among the Frenchman Bay, Englishman Bay, and Starboard Cove populations. Analyses of molecular variance, though, suggested that they did not represent a single cluster, since significant genetic variation was evident within the grouping. Rather, geographic distance likely contributed to their fine-scale structuring, as there was a larger separation between Frenchman Bay and the other two populations, which were close geographically and more genetically homogenous to each other.

The Cobscook Bay population exhibited significant differentiation from most other sites, likely due to reduced connectivity and partial geographic isolation, since the mouth of Cobscook Bay is connected to the Bay of Fundy and partially separated from the EMCC by the Grand Manan Channel. Some evidence of genetic mixing, however, was detected between the Cobscook and Englishman Bay populations. For example, a few individuals from Englishman Bay exhibited greater proportional membership to Cobscook Bay. In addition, assignment tests identified the Englishman Bay collection as a mixture of individuals from multiple populations, with assignments no greater than expected by chance, and equal individual assignments among itself and Cobscook Bay. This connectivity, however, did not include the centrally located Starboard Cove population, which in contrast, exhibited very small assignment scores to Cobscook Bay and a highly significant pairwise F_{ST} value. In many marine systems, population structure is characterized by asymmetrical transport, due in part to different habitat features and discontinuities among sites (Alberto *et al.* 2011). In the present study, the Englishman Bay site consists of predominately sandy substrate, with kelp sporadically attached to mussels and boulders, while all other locations exhibit more rocky substrates. In addition, Starboard Cove is a relatively sheltered site, compared to Englishman Bay, and receives a greater proportion of freshwater input from the local terrestrial environment. These local features may restrict meiospore recruitment, or perhaps, reduce gametophyte survival from a locally acclimated Cobscook Bay population that is likely exposed to stronger tidal currents and high nutrient levels (Mathieson *et al.* 2009). Kelp populations in other species, for instance, exhibit local acclimation with respect to latitude or habitat (Henkel & Hofmann 2008). More research, however, will be needed to elucidate the possible factors that drive these small, fine-scale differences in populations along the eastern Maine coastline.

The fine-scale genetic structure of kelp in this region is likely strongly influenced by the EMCC southwest current, as well as geographic isolation associated with major bays. Even along the fairly continuous coastline east of Penobscot Bay, some evidence of local differentiation was detected at small spatial scales, which is likely dependent on both habitat features and geographic distances that impact meiospore dispersal. Wild harvesting practices and future kelp cultivation should avoid fragmentation or extirpation of these local populations, especially considering the overall reduced genetic diversity when compared to European *S. latissima* populations. If future kelp aquaculture goals in this region are to be realized, management may be needed to maintain adequate diversity, and further research comparing local populations in culture is possibly warranted.

Acknowledgments

Funding was provided by the Maine Economic Improvement Fund – Small Campus Initiative. We thank Chris Smith from MDI Biological Laboratory for assistance with fragment analyses. Research reported in this project was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103423.

References

Adams JM, Gallagher JA, Donnison IS. Fermentation study on *Saccharina latissima* for bioethanol production considering variable pre-treatments. Journal of Applied Phycology. 2009; 21:569–574.

Alberto F, Raimondi PT, Reed DC, Coelho NC, Leblois R, Whitmer A, Serrão EA. Habitat continuity and geographic distance predict population genetic differentiation in giant kelp. Ecology. 2010; 91:49–56. [PubMed: 20380195]

Alberto F, Raimondi PT, Reed DC, Watson JR, Siegel DA, Mitarai S, Coelho N, Serrão EA. Isolation by oceanographic distance explains genetic structure for *Macrocystis pyrifera* in the Santa Barbara Channel. Molecular Ecology. 2011; 20:2543–2554. [PubMed: 21535280]

Augyte S, Yarish C, Redmond S, Kim JK. Cultivation of a morphologically distinct strain of the sugar kelp, *Saccharina latissima* forma *angustissima*, from coastal Maine, USA, with implications for ecosystem services. Journal of Applied Phycology. 2017; doi: 10.1007/s10811-017-1102-x

Bartsch I, Wiencke C, Bischof K, Buchholz CM, Buck BH, Eggert A, Feuerpfeil P, Hanelt D, Jacobsen S, Karez R, Karsten U, Molis M, Roleda MY, Schubert H, Schumann R, Valentin K, Weinberger F, Wiese J. The genus *Laminaria* sensu lato: recent insights and developments. European Journal of Phycology. 2008; 43:1–86.

Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N., Bonhomme, F. Laboratorie Génome, Populations, Interactions, CNRS UMR 5171. Université de Montpellier II; Montpellier, France: 1996–2004. GENETIX 4.05.2 logiciel sous Windows TM pour la génétique des populations.

Billot C, Engel CR, Rousvoal S, Kloareg B, Valero M. Current patterns, habitat discontinuities and population genetic structure: the case of kelp *Laminaria digitata* in the English Channel. Marine Ecology Progress Series. 2003; 253:111–121.

Bolton JJ. The biogeography of kelps (Laminariales, Phaeophyceae): a global analysis with new insights from recent advances in molecular phylogenetics. Helgoland Marine Research. 2010; 64:263–279.

Borum J, Pederson MF, Krause-Jensen D, Christensen PB, Nielsen K. Biomass, photosynthesis and growth of *Laminaria saccharina* in a high-arctic fjord, NE Greenland. Marine Biology. 2002; 141:11–19.

Brinkhuis BH, Mariani EC, Breda VA, Brady-Campbell MM. Cultivation of *Laminaria saccharina* in New York Marine Biomass Program. Hydrobiologia. 1984; 116:226–282.

Coombs JA, Letcher BH, Nislow KH. CREATE: a software to create input files from diploid genotypic data for 52 genetic software programs. Molecular Ecology Resources. 2008; 8:578–580. [PubMed: 21585837]

Cornish ML, Critchley AT, Mouritsen OG. A role for dietary macroalgae in the amelioration of certain risk factors associated with cardiovascular disease. Phycologia. 2015; 54:649–666.

Coyer JA, Haorau G, Costa JF, Hogerdijk B, Serrão EA, Billard E, Valero M, Pearson GA, Olsen JL. Evolution and diversification within the intertidal brown macroalgae *Fucus spiralis/F. vesiculosis* species complex in the North Atlantic. Molecular Phylogenetics and Evolution. 2011; 58:283–296. [PubMed: 21111835]

Devit DC, Saunders GW. A DNA barcode examination of the Laminariaceae (Phaeophyceae) in Canada reveals novel biogeographical and evolutionary insights. Phycologia. 2010; 49:235–248.

Earl DA, vonHoldt BM. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources. 2012; 4:359–361.

Evanno G, Regnaut S, Goudet J. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology. 2005; 14:2611–2620. [PubMed: 15969739]

Excoffier L, Lischer HEL. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources. 2010; 10:564–567. [PubMed: 21565059]

Faugeron S, Martínez EA, Correa JA, Cardenas L, Destombe C, Valero M. Reduced genetic diversity and increased population differentiation in peripheral and overharvested populations of *Gigartina skottsbergii* (Rhodophyta, Gigartinales) in southern Chile. Journal of Phycology. 2004; 40:454–462.

Forbord S, Skjermo J, Arff J, Handå A, Reitan KI, Bjerregaard R, Lüning K. Development of *Saccharina latissima* (Phaeophyceae) hatcheries with year-round production of zoospores and juvenile sporophytes on culture ropes for kelp aquaculture. Journal of Applied Phycology. 2012; 24:393–399.

Freitas JRC, Salinas Morrondo JM, Cremades Ugarte J. *Saccharina latissima* (Laminariales, Ochrophyta) farming in an industrial IMTA system in Galicia (Spain). Journal of Applied Phycology. 2016; 28:377–385.

Garbary DJ, Kim KY, Klinger T, Duggins D. Red algae as hosts for endophytic kelp gametophytes. Marine Biology. 1999; 135:35–40.

Goudet J. FSTAT (version 1.2): a computer program to calculate F-statistics. Journal of Heredity. 1995; 86:485–486.

Greco M, Sáez CA, Brown MT, Bitonti MB. A simple and effective method for high quality co-extraction of genomic DNA and total RNA from low biomass *Ectocarpus siliculosus*, the model brown alga. PLoS One. 2014; 9:e96470. [PubMed: 24867404]

Guiry, MD., Guiry, GM. World-wide electronic publication. National University of Ireland; Galway: 2017. AlgaeBase. http://www.algaebase.org; searched on 29 May 2017

Hall-Arber, M., Dyer, C., Poggie, J., McNally, J., Gagne, R. New England's fishing communities. MIT Sea Grant College Program; Massachusetts: 2005. p. 426

Henkel SK, Hofmann GE. Differing patterns of hsp70 gene expression in invasive and native kelp species: evidence for acclimation-induced variation. Journal of Applied Phycology. 2008; 20:915–924.

Hubisz MJ, Falush D, Stephens M, Pritchard JK. Inferring weak population structure with the assistance of sample group information. Molecular Ecology Resources. 2009; 9:1322–1332. [PubMed: 21564903]

Jahan A, Ahmad IZ, Fatima N, Ansari VA, Akhtar J. Algal bioactive compounds in the cosmeceutical industry: a review. Phycologia. 2017; 56:410–422.

Jakobsson M, Rosenberg NA. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics. 2007; 23:1801–1806. [PubMed: 17485429]

Johnson RB, Kim J-K, Armbruster LC, Yarish C. Nitrogen allocation of *Gracilaria tikvahiae* grown in urbanized estuaries of Long Island Sound and New York City, USA: a preliminary evaluation of ocean farmed *Gracilaria* for alternative fish feeds. Algae. 2014; 29:227–235.

Jormalainen V, Honkanen T. Variation in natural selection for growth and phlorotannins in the brown alga, *Fucus vesiculosis*. Journal of Evolutionary Biology. 2004; 17:807–820. [PubMed: 15271080]

Kim, S-K., Bhatnagar, I. Physical, chemical, and biological properties of wonder kelp – *Laminaria*. In: Kim, S-K., editor. Advances in food and nutrition research. Vol. 64. Academic Press; Massachusetts: 2011. p. 85-95.

Kim J-K, Kraemer GP, Yarish C. Field scale evaluation of seaweed aquaculture as a nutrient bioextraction strategy in Long Island Sound and the Bronx River Estuary. Aquaculture. 2014; 433:148–156.

Kim J-K, Kraemer GP, Yarish C. Use of sugar kelp aquaculture in Long Island Sound and the Bronx River Estuary for nutrient extraction. Marine Ecology Progress Series. 2015; 531:155–166.

Kim J-K, Yarish C, Hwang EK, Park M, Kim Y. Seaweed aquaculture: cultivation technologies, challenges, and its ecosystem services. Algae. 2017; 32:1–13.

Lane CE, Saunders GW. Molecular investigation reveals epi/endophytic extrageneric kelp (Laminariales, Phaeophyceae) gametophytes colonizing *Lessoniopsis littoralis* thalli. Botanica Marina. 2005; 48:426–436.

Lewis, PO., Zaykin, D. [18 May 2016] Genetic data analysis: computer program for analysis of allelic data. Version 1.1. 2001. Available at: http://phylogeny.uconn.edu/software/

Liu F, Yao J, Wang X, Repnikova A, Galanin DA, Duan D. Genetic diversity and structure within and between wild and cultivated *Saccharina japonica* (Laminariales, Phaeophyta) revealed by SSR markers. Aquaculture. 2012; 358–359:139–145.

Luikart G, Allendorf FW, Cornuet J-M, Sherwin WB. Distortion of allele frequency distributions provides a test for recent population bottlenecks. Journal of Heredity. 1998; 89:238–247. [PubMed: 9656466]

Lüning K. Growth strategies of three *Laminaria* species (Phaeophyceae) inhabiting different depth zones in the sublittoral region of Helgoland (North Sea). Marine Ecology Progress Series. 1979; 1:195–207.

MacArtain P, Gill CIR, Brooks M, Campbell R, Rowland IR. Nutritional value of edible seaweeds. Nutrition Reviews. 2007; 65:535–543. [PubMed: 18236692]

Maeda T, Kawai T, Nakaoka M, Yotsukora N. Effective DNA extraction method for fragment analysis using capillary sequencer of the kelp, *Saccharina*. Journal of Applied Phycology. 2013; 25:337–347.

Marinho GS, Holdt SL, Birkeland MJ, Angelidaki I. Commercial cultivation and bioremediation potential of sugar kelp, *Saccharina latissima*, in Danish waters. Journal of Applied Phycology. 2015; 27:1963–1973.

Mathieson AC, Hehre EJ, Dawes CJ, Neefus CD. An historical comparison of seaweed populations from Casco Bay, Maine. Rhodora. 2008; 110:1–102.

Mathieson AC, Dawes CJ, Hehre EJ, Harris LG. Floristic studies of seaweeds from Cobscook Bay, Maine. Northeastern Naturalist. 2009; 16(Monograph 5):1–48.

Mathieson, AC., Dawes, CJ. Seaweeds of the northwest Atlantic. University of Massachusetts Press; Massachusetts: 2017. p. 816

Muhlin JF, Brawley S. Recent vs relic: discerning the genetic signature of *Fucus vesiculosis* (Heterokontophyta: Phaeophyceae) in the Northwestern Atlantic. Journal of Phycology. 2009; 45:828–837. [PubMed: 27034212]

Nettleton JC, Mathieson AC, Thornber C, Neefus CD, Yarish C. Introduction of *Gracilaria vermiculophylla* (Rhodophyta, Gracilariales) to New England, USA: estimated arrival times and current distribution. Rhodora. 2013; 115:28–41.

Nielsen MM, Paulino C, Neiva J, Krause-Jensen D, Bruhn A, Serrão EA. Genetic diversity of *Saccharina latissima* (Phaeophyceae) along a salinity gradient in the North Sea-Baltic Sea transition zone. Journal of Phycology. 2016; 52:523–531. [PubMed: 27151230]

Paulino C, Neiva J, Coelho NC, Aires T, Marbà N, Krause-Jensen D, Serrão EA. Characterization of 12 polymorphic microsatellite markers in the sugar kelp *Saccharina latissima*. Journal of Applied Phycology. 2016; 28:3071–3074.

Peakall R, Smouse PE. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update. Bioinformatics. 2012; 28:2537–2539. [PubMed: 22820204]

Peteiro C, Freire O. Biomass yield and morphological features of the seaweed *Saccharina latissima* cultivated at two different sites in a coastal bay in the Atlantic coast of Spain. Journal of Applied Phycology. 2013; 25:205–2013.

Pettigrew NR, Churchill JH, Janzen CD, Mangum LJ, Signell RP, Thomas AC, Townsend DW, Wallinga JP, Xue H. The kinematic and hydrographic structure of the Gulf of Maine Coastal Current. Deep-Sea Research II. 2005; 52:2369–2391.

Piry S, Luikart G, Cornuet J-M. BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. Journal of Heredity. 1999; 90:502–503.

Piry S, Alapetite A, Cornuet J-M, Paetkau D, Baudouin L, Estoup A. GeneClass 2: a software for genetic assignment and first-generation migrant detection. Journal of Heredity. 2004; 95:536–539. [PubMed: 15475402]

Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics. 2000; 155:945–959. [PubMed: 10835412]

Pritchard, JK., Wen, X., Falush, D. [18 May 2016] Documentation for structure software: version 2.3. 2010. Available at: http://pritch.bsd.uchicago.edu/structure/html

Rannala B, Mountain JL. Detecting immigration by using multilocus genotypes. Proceedings of the National Academy of Sciences USA. 1997; 94:9197–9201.

Raymond M, Rousset F. GENEPOP (version 1.2) population genetic software for exact tests and ecumenicism. Journal of Heredity. 1995; 86:248–249.

Redmond, S., Green, L., Yarish, C., Kim, J., Neefus, C. [26 May 2017] New England seaweed culture handbook. 2014. Available at: http://digitalcommons.uconn.edu/seagrant_weedcult/1

Rice WR. Analyzing tables of statistical tests. Evolution. 1989; 43:223–225. [PubMed: 28568501]

Rößner Y, Krost P, Schulz C. Increasing seaweed crop yields through organic fertilisation at the nursery stage. Journal of Applied Phycology. 2014; 26:753–762.

Robuchon M, Le Gall L, Mauger S, Valero M. Contrasting genetic diversity patterns in two sister kelp species co-distributed along the coast of Brittany, France. Molecular Ecology. 2014; 23:2669–2685. [PubMed: 24787679]

Rosenberg NA. DISTRUCT: a program for graphical display of population structure. Molecular Ecology Notes. 2004; 4:137–138.

Sanderson JC, Dring MJ, Davidson K, Kelly MS. Culture, yield and bioremediation potential of *Palmaria palmata* (Linnaeus) Weber & Mohr and *Saccharina latissima* (Linnaeus). Aquaculture. 2012; 354–355:128–135.

Snirc A, Silberfeld T, Bonnet J, Tillier A, Tuffet S, Sun J-S. Optimization of DNA extraction from brown algae (Phaeophyceae) based on a commercial kit. Journal of Phycology. 2010; 46:616–621.

Thomas NV, Kim S-K. Potential pharmacological applications of polyphenolic derivatives from marine brown algae. Environmental Toxicology and Pharmacology. 2011; 32:325–335. [PubMed: 22004951]

Troell M, Joyce A, Chopin T, Neori A, Buschmann AH, Fang J-G. Ecological engineering in aquaculture – potential for integrated multi-trophic aquaculture (IMTA) in marine offshore systems. Aquaculture. 2009; 297:1–9.

Van Oosterhoult C, Hutchinson WF, Wills DPM, Shipley P. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes. 2004; 4:535–538.

Vilg JV, Nylund GM, Werner T, Qvirist L, Mayers JJ, Pavia H, Undeland I, Albers E. Seasonal and spatial variation in biochemical composition of *Saccharina latissima* during a potential harvesting season for western Sweden. Botanica Marina. 2015; 58:435–447.

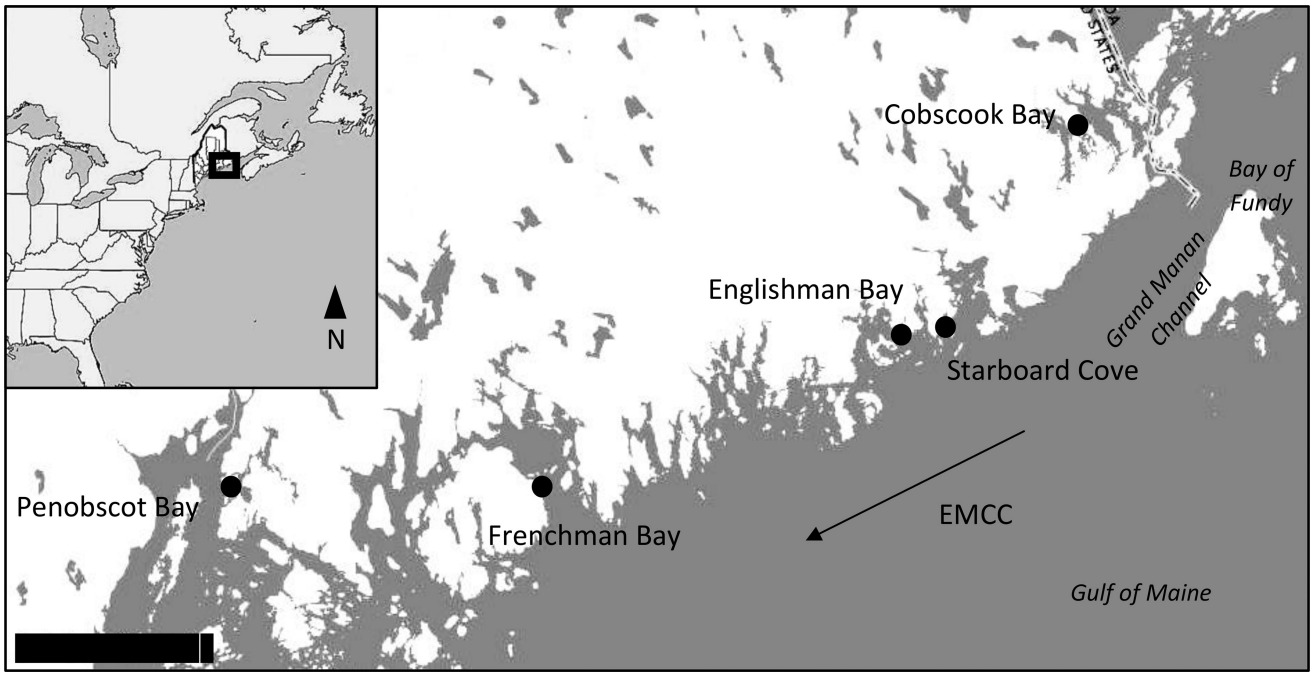

Fig. 1.

Map of sampling locations in eastern Maine. Scale bar refers to 30 km, and the arrow refers to the direction of the Eastern Maine Coastal Current (EMCC) in the region.

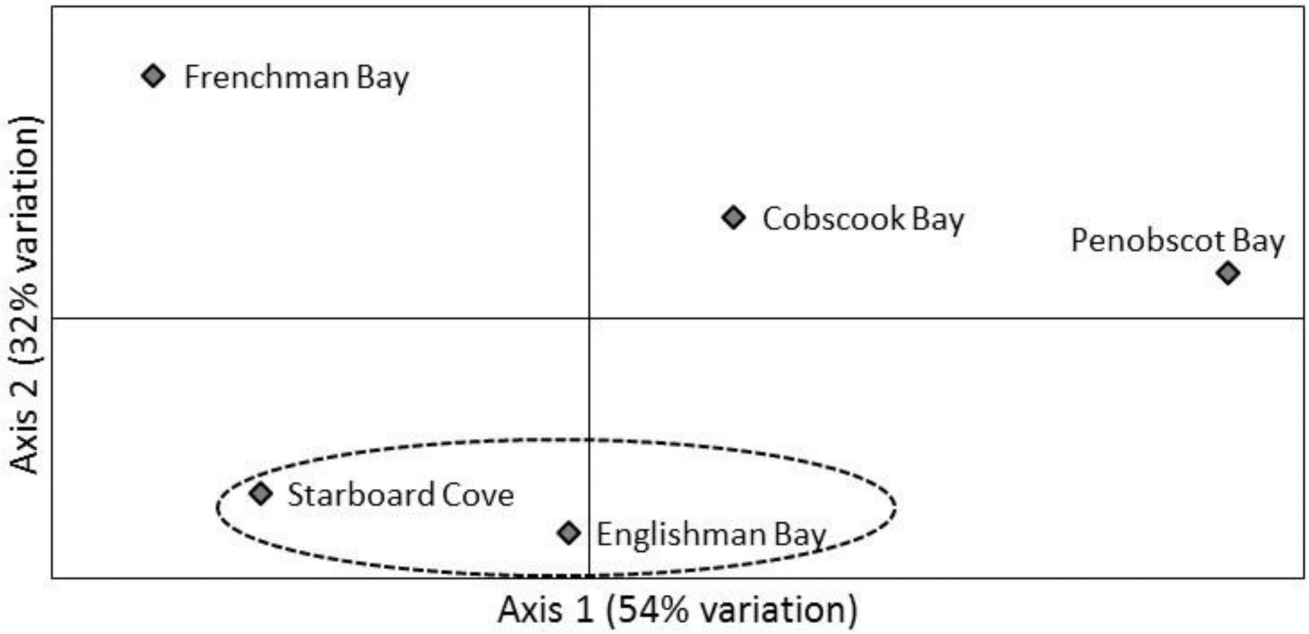

Fig. 2.

Principal component analysis of pairwise F_{ST} values for all sampled populations. The dotted circle encloses populations with a negative, non-significant ($P > 0.05$) pairwise value.

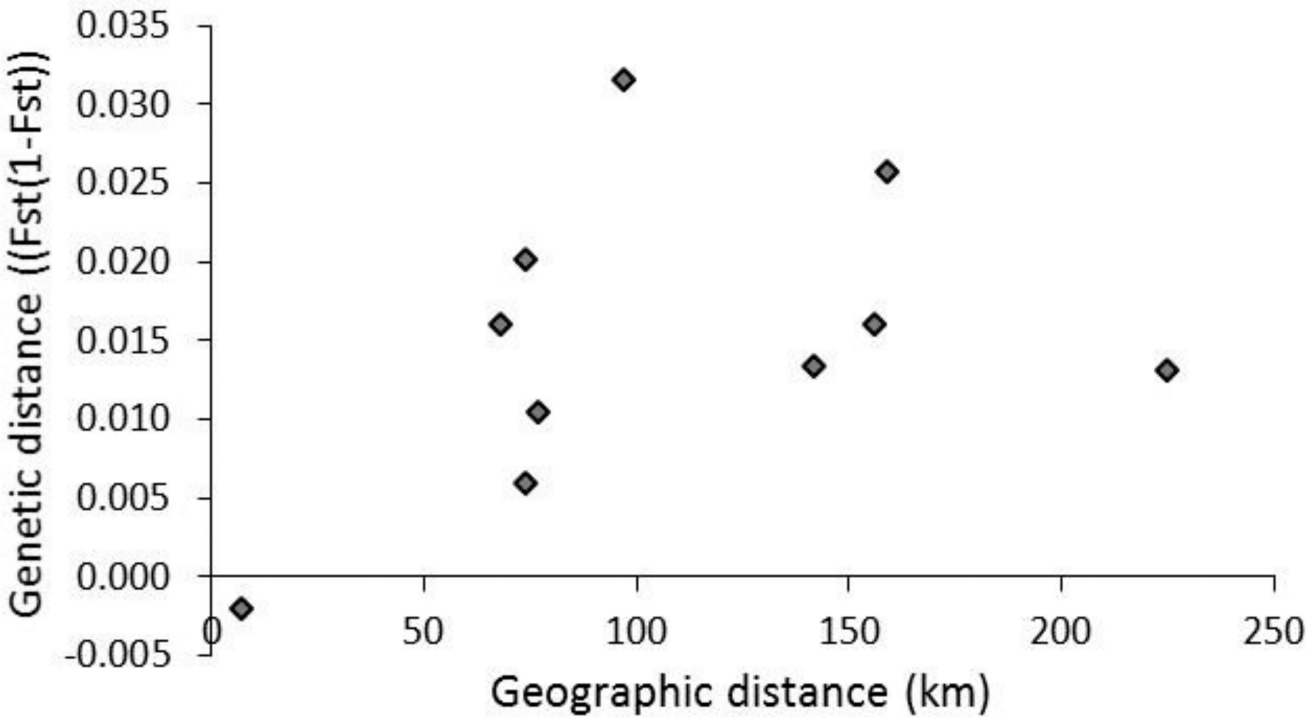

Fig. 3.
Mantel test of isolation-by-distance across all sampled kelp populations ($P = 0.1290$).

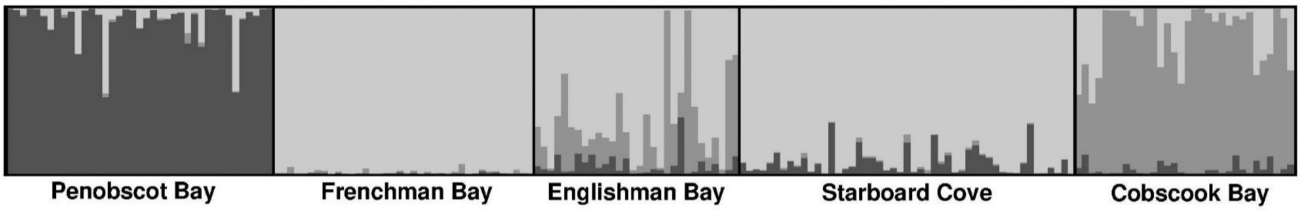

Fig. 4.

Genetic clustering identified through STRUCTURE analysis. Each vertical bar represents an individual kelp sample and its proportional membership to three clusters (light gray, gray, and dark gray). Black lines separate different populations.

Table 1

Number of alleles, allelic richness (AR), observed (H_o) and expected (H_e) heterozygosities, F_{IS}, F_{ST}, and allelic differentiation *P*-values for 10 microsatellite markers.

Locus	Alleles	AR	H_o	H_e	F_{IS}	F_{ST}	*P*
SLN32	17	8.7	0.665	0.699	0.049	0.0170	**<0.0001**
SLN34	4	3.3	0.223	0.253	0.118	0.0032	0.2033
SLN35	8	4.6	0.195	0.202	0.039	0.0300	**0.0001**
SLN36	8	5.1	0.326	0.338	0.036	0.0035	**0.0353**
SLN54	4	2.5	0.075	0.079	0.041	0.0049	0.1488
SLN58	6	3.4	0.096	0.123	0.220	0.0203	**<0.0001**
SLN62	10	6.3	0.293	0.320	0.086	0.0092	**0.0005**
SLN314	6	4.9	0.484	0.494	0.020	0.0388	**0.0001**
SLN320	3	1.4	0.016	0.016	−0.004	−0.0050	0.7900
SLN510	7	4.7	0.612	0.571	−0.072	0.0062	**0.0165**
All	7.3	4.5	0.298	0.309	0.035	0.0157	**<0.0001**

Bold indicates significance at the $\alpha = 0.05$ level.

Table 2

Geographic locations, sample sizes (*n*), mean number of alleles, mean allelic richness (AR), observed (H_o) and expected (H_e) heterozygosities, and F_{IS} values for all populations.

Site name	Latitude	Longitude	*n*	Mean alleles	Mean AR	H_o	H_e	F_{IS}[1]
Penobscot Bay	44.387673	−68.795717	39	3.9	3.6	0.283	0.273	−0.037
Frenchman Bay	44.389029	−68.199764	38	4.3	4.2	0.339	0.340	0.002
Englishman Bay	44.605977	−67.467015	30	4.0	4.0	0.294	0.288	−0.024
Starboard Cove	44.606107	−67.396495	49	4.8	4.1	0.288	0.302	0.048
Cobscook Bay	44.883288	−67.129206	32	4.4	4.2	0.288	0.326	0.116

[1] F_{IS} values were not significant at the $\alpha = 0.05$ level.

Table 3

Pairwise F_{ST} values across 10 microsatellite markers for all kelp populations studied.

	Penobscot Bay	Frenchman Bay	Englishman Bay	Starboard Cove
Frenchman Bay	**0.0316**			
Englishman Bay	**0.0164**	**0.0202**		
Starboard Cove	**0.0260**	**0.0104**	−0.0020	
Cobscook Bay	**0.0133**	**0.0134**	0.0061*	**0.0161**

Bold indicates significance following standard Bonferroni correction ($P < 0.005$), while the asterisk indicates significance at the $\alpha = 0.05$ level.

Table 4

Analysis of molecular variance (AMOVA) averaged over 10 loci, using the five populations grouped into either three or four clusters. Df refers to degrees of freedom, while SS refers to sum of squares.

Source of variation	df	SS	% variation	F-statistic	*P*-value
Three clusters[1]					
Among clusters	2	8.15	0.93	0.0093	0.1020
Among populations within clusters	2	5.34	0.96	0.0097	**0.0112**
Within populations	371	565.56	98.11	0.0189	**<0.0001**
Four clusters[2]					
Among clusters	3	12.20	2.01	0.0180	**0.0064**
Among populations within clusters	1	1.30	−0.20	−0.0021	0.6016
Within populations	371	565.56	98.20	0.0201	**<0.0001**

[1]Clusters: 1) Penobscot Bay, 2) Frenchman Bay, Englishman Bay, and Starboard Cove, and 3) Cobscook Bay

[2]Clusters: 1) Penobscot Bay, 2) Frenchman Bay, 3) Englishman Bay and Starboard Cove, and 4) Cobscook Bay

Bold indicates significance at the $\alpha = 0.05$ level.

Table 5

Percent assignment of all individuals in each population either: 1) back to their correct population of origin, or 2) incorrect assignment to a different population. *P* refers to probability thresholds associated with chi-square ($\chi 2$) values of each population test.

	% assignment					
Population	**Penobscot Bay**	**Frenchman Bay**	**Englishman Bay**	**Starboard Cove**	**Cobscook Bay**	***P***
Penobscot Bay	71.8	5.1	12.8	10.3	0.0	< **0.0001**
Frenchman Bay	0.0	50.0	10.5	26.3	13.2	< **0.0001**
Englishman Bay	16.7	6.7	20.0	36.7	20.0	> 0.10
Starboard Cove	18.4	16.3	24.5	34.7	6.1	< **0.05**
Cobscook Bay	12.5	9.4	18.8	12.5	46.9	< **0.01**

Bold indicates significance at the $\alpha = 0.05$ level.