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Geographic Variation in Intraspecific Differentiation of a Marine Primary Producer

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Geographic Variation in Intraspecific Differentiation of a Marine Primary Producer

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Ecology & Evolutionary Biology

by

Kylla Marie Benes

Dissertation Committee:
Associate Professor Matthew E.S. Bracken, Chair
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2016

DEDICATION

To Grampa Ed, who taught me the joy and wonder of nature, right from his backyard.

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Contribution of Authors

The text of chapter 4 in this dissertation is a reprint of the material as it appears in the Journal of Phycology. Matthew E.S. Bracken, a co-author listed in this publication, directed and supervised research which forms the basis for the thesis/dissertation.

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An interdisciplinary study of intraspecific differentiation, including population genetics, ecophysiology, evolutionary ecology, and spatial ecology.

PUBLICATIONS

Benes KM and MES Bracken. 2016. Nitrate uptake varies with tide height and nutrient availability in the intertidal seaweed *Fucus vesiculosus*. *Journal of Phycology* doi:10.1111/jpy.12454

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ABSTRACT OF THE DISSERTATION

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By

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Doctor of Philosophy in Ecology & Evolutionary Biology

University of California, Irvine, 2016

Associate Professor Matthew E.S. Bracken, Chair

Steep environmental gradients offer the opportunity to study organismal adaptation to local conditions. Yet these local-scale gradients are often nested within latitudinal gradients, which could mediate neutral and selective processes on the local scale. I used a classic study system, a gradient in tidal elevation on temperate rocky shores, to test for geographic variation in intraspecific differentiation across tide heights in a marine primary producer. The seaweed *Fucus vesiculosus* is a foundation species on rocky shores throughout the temperate North Atlantic Ocean. In the Gulf of Maine in particular, *F. vesiculosus* occurs from the lower to upper intertidal zones along the entire coastline, spanning local and latitudinal gradients in abiotic and biotic stressors. I conducted a series of observations and experiments across the species' intertidal distribution at sites along the Gulf of Maine coastline to: 1) identify patterns of genetic diversity and structure from tide height to regional scales, 2) test for inter-population differences in adaptation to tide height, and 3) determine if traits important to ecosystem-level processes were associated with local adaptation.

Molecular studies using microsatellite markers showed genetic variation was significant across multiple sampling scales. Patterns of inbreeding (i.e., F_{IS}) revealed spatial variation in

isolation. Genetic differentiation (i.e., F_{ST}) was attributable to distance in between-site comparisons, but by environment in between-tide height comparisons. Reciprocal transplant experiments, between the upper and lower edges of the intertidal distribution of *F. vesiculosus*, were conducted at sites throughout the Gulf of Maine. Relative growth rates of *F. vesiculosus* showed adaptive phenotypic differentiation in the northeastern Gulf of Maine, countergradient variation in the central gulf, and environmentally-driven responses in the south. Importantly, observations of nutrient physiology demonstrated that nutrient uptake and allocation of tissue nitrogen towards growth were influenced by adaptation in the northeast and driven primarily by environment in the south. By combining molecular and physiological approaches, I have identified geographic variation in genetic and environmental constraints of organismal physiology and population-level processes. Given the important bottom-up role of seaweeds as providers of food and habitat, this variation could have important consequences for the associated rocky intertidal community.

Chapter 1

GENERAL INTRODUCTION

A primary goal in ecology is to understand how large-scale processes influence processes on the local scale (Levin 1992). Environmental heterogeneity at multiple spatial scales can generate genetic and phenotypic intraspecific variation. However, adaptation to environmental conditions depends on both population dynamics (i.e., gene flow and dispersal) and the intensity of natural selection (Linhart and Grant 1996, Lenormand 2002). If large-scale environmental factors mediate the characteristics of populations at local scales, then among-populations differences in within-population diversity may arise (Hastings and Harrison 1994). In ecosystem engineers or foundation species, this variation can have important effects on community and ecosystem-level processes (Hughes et al. 2008, Whitlock 2014).

Rocky intertidal systems are an ideal setting to test the effects of regional environmental differences on intraspecific variation at local scales. Regional-scale differences in temperature and productivity (e.g., chlorophyll a) can influence community structure by modifying local-scale processes such as colonization and predation (Menge 2003). At the local level, a steep gradient in environmental conditions occurs from the low tide line, where the community is more often submerged under water, to the high tide line, where the community is more often exposed to the air. Studies on rocky shores have repeatedly demonstrated the importance of abiotic stress high on the shore (i.e., emersion stress) and biotic stress (e.g., herbivory and competition) low on the shore, as drivers of diversity and abundance along the tidal gradient (Menge and Branch 2001). In intertidal systems, seaweeds often play a bottom-up role in shaping community structure by providing nutrients to higher trophic levels and creating habitat for associated

organisms. Studies of adaptation in seaweeds have demonstrated genetic and phenotypic differentiation across large spatial (i.e., regional) scales (Breeman 1988, Gerard and Du Bois 1988, Bergstrom and Kautsky 2005) and along local environmental gradients (Innes 1988, Williams and Di Fiori 1996, Scott et al. 2001, Roberson and Coyer 2004, Hays 2007, Coyer et al. 2011).

In the Gulf of Maine, which extends from Cape Cod to Nova Scotia in the western North Atlantic Ocean, strong regional environmental differences persist despite a high degree of seasonal variation in abiotic conditions. The northeast region of the gulf is typically cooler and more nutrient rich relative to the southern region (Apollonio 1979). Furthermore, off-shore currents flow southward, and variation in strength and proximity to shore may limit gene flow across latitudes (Xue et al 2000, Pettigrew et al. 2005). This latitudinal variation in both abiotic and biotic factors along the Gulf of Maine coastline has been used to study regional-level differences in phenotypic plasticity (Trussell, 2000), species interaction strength (Kordas and Dudgeon 2010), adaptation/acclimation to climate change scenarios (Sorte et al. 2011), and community structure (Bryson et al. 2014).

Rocky intertidal communities of the Gulf of Maine (and the temperate North Atlantic Ocean in general) are dominated by several species of brown seaweed which serve as habitat and food for the associated community (Luning 1990). *Fucus* species are a primary contributor of biomass and nutrients on Gulf of Maine rocky shores (Topinka et al. 1981). Compared to other *Fucus* species, *F. vesiculosus* occupies the greatest tidal range (K. Benes *personal observation*) and has been shown to be a preferred food source for herbivores (Barker and Chapman 1990, Denton and Chapman 1991), and its recruitment is strongly influenced by snail grazing (Lubchenco 1983). Additionally, extensive genetic surveys of *F. vesiculosus* in Europe suggest

genetic variation along the intertidal gradient is possible (Billard et al. 2010). This variation may be indicative of local adaptation in *F. vesiculosus* due to emersion time along the intertidal gradient. How genetic structure and adaptation on rocky shores changes among regions and what the consequences are for intertidal community and ecosystem processes remains unknown. Using the latitudinal gradient in environmental conditions in the Gulf of Maine and the local-scale elevational stress gradient along rocky shores, I specifically addressed the following hypotheses in this dissertation:

Hypothesis 1: Genetic diversity and differentiation will vary across multiple spatial scales, from tide height to regions, in *F. vesiculosus*.

Hypothesis 2: Phenotypic differentiation between intertidal zones will vary among *F. vesiculosus* populations in the Gulf of Maine.

Hypothesis 3: Variation in nutrient physiology of *F. vesiculosus* at local and regional-scales will be associated with patterns of phenotypic differentiation.

Studies that have compared intraspecific differentiation across the intertidal gradient at multiple sites have found stronger local adaptation at sites where populations experience greater abiotic stress (e.g., 'warm' sites or at sites where individuals are distributed higher on the shore [Schimdt and Rand 1999, Hays 2007]). Therefore, I hypothesized that *F. vesiculosus* would exhibit greater genetic and phenotypic differentiation at southern sites where thermally stressful

conditions are relatively more frequent and ambient nitrate availability is on average lower than elsewhere in the Gulf of Maine.

In chapter two I used a nested sampling design and microsatellite loci to measure genetic variation in *F. vesiculosus* between tide heights, among sites, and among regions (*Hypothesis 1*). Quantification of genetic diversity, inbreeding, and genetic differentiation revealed differences in isolation and differentiation between tide heights. These differences varied among sites and were correlated with physical and environmental factors associated with the intertidal gradient rather than region. In chapter three I quantified relative growth rate and nitrogen use efficiency of *F. vesiculosus* across the intertidal gradient. Utilizing a classic reciprocal transplant experimental design, I identified among region variation in phenotypic differentiation in these traits across the intertidal gradient (*Hypothesis 2 & 3*). Lastly, in chapter four I used a series of observations and experiments to identify drivers of variation in nutrient uptake and identify a possible mechanism underlying patterns uncovered in chapter three (*Hypothesis 3*). Nutrient uptake of *F. vesiculosus* was found to vary with ambient nutrient availability and showed evidence of differentiation between tide heights in some regions of the Gulf of Maine.

Chapter 2

POPULATION STRUCTURE AND GENE FLOW ACROSS MULTIPLE SPATIAL SCALES IN A WIDELY DISTRIBUTED SEAWEED

Abstract

Contrary to theoretical predictions, species with continuous distributions and long-range dispersal potential do show population structure and differentiation even at fine-spatial scales. This can be due to random or selective processes generating spatial or temporal variation in population genetic make-up. We sampled the widely distributed intertidal seaweed, *Fucus vesiculosus*, at the upper and lower edges of its intertidal distribution throughout the Gulf of Maine, a region of this species' distribution that was previously assumed to lack large scale patterns of population structure and diversity. Analyses using seven microsatellite loci revealed regional variation in allelic richness and gene diversity and significant isolation-by-distance between sites. However, observed heterozygosity and inbreeding varied at smaller spatial scales (i.e., tide height) suggesting variation in population-level processes across the intertidal distribution of *F. vesiculosus*. Further, comparisons of upper intertidal samples, between sites throughout the Gulf of Maine, exhibited low differentiation but significant isolation-by-distance. In contrast, comparisons between lower-edge samples had the highest levels of differentiation but lacked significant isolation-by-distance. This suggests that asymmetric gene flow towards the upper edge of this species' intertidal distribution may allow for fine-scale structure along the shore with the potential for genetic drift or selective forces to influence structure at the lower edge.

Introduction

Species with continuous distributions and long-range dispersal potential (e.g., wind-dispersers) are generally assumed to lack population genetic structure and differentiation due to high rates of gene flow (Slatkin 1987). However, studies of species with such characteristics have shown significant genetic structure within and among populations contrary to theoretical predictions (e.g., Johnson and Black 1984, Hogan et al 2010, Iacchei et al. 2013, Teixeira et al. 2016). Fine-scale, temporal and spatial heterogeneity in population-level processes can result in 'chaotic genetic patchiness' – unexpected, and seemingly unpredictable, patterns in population structure (*sensu* Johnson and Black 1984). Neutral processes such as random spatial or temporal variability in the composition of recruits cause lowered effective cohort size into a population, leading to genetic drift and eventual genetic differentiation within populations. Similarly, natural selection can generate patchiness in genetic structure if there is pre- or post-settlement variation in survivorship of particular genotypes due to temporal or microgeographic variation in environmental quality (see discussions in Johnson and Black 1984 and Hogan et al. 2010).

In marine ecosystems, large-scale ocean currents and latitudinal environmental variation can serve as barriers to dispersal (Kinlan and Gaines 2003, Siegel et al. 2003, O'Connor et al. 2007). Even if long-distance dispersal and migration occur, coastline topography and nearshore currents along with temporal and spatial heterogeneity in environmental conditions or population processes can generate genetic structure on the local-scale (Hedgecock 1994, Larson and Julian 1999, Marshall et al. 2010). Rocky intertidal shores in particular provide an opportunity to investigate how variation in population diversity and structure can change over multiple spatial scales. Environmental heterogeneity occurs predictably along the intertidal gradient but also at

smaller scales among microhabitats (Helmuth and Hofmann 2001). Additionally, rocky shores are distributed along latitudinal gradients that vary in exposure to ocean current and other environmental variables. Variation in gene flow, dispersal potential, genetic drift, life-histories, and selection within and among rocky intertidal populations has been implicated in generating geographic and local-scale patterns in intertidal invertebrates and seaweeds (e.g., Innes 1988, Johannesson et al. 1995, Williams and Di Fiori 1996, Schmidt and Rand 1999, Engel et al. 2005, Sotka et al. 2004, Krueger-Hadfield et al. 2013).

Fucus vesiculosus is a dominant member of rocky intertidal shores throughout the temperate North Atlantic Ocean (Lüning 1990). Genetic diversity of *F. vesiculosus* within much of the western North Atlantic is relatively low due to recent colonization of previously glaciated sites by a single haplotype from a glacial refugium near southwest Ireland (Muhlin and Brawley 2009, Coyer et al. 2011b). Rafting and long-distance transport of detached reproductive individuals via oceanic currents is hypothesized to be the driving force in the maintenance of equilibrium and limited differentiation between populations from Connecticut, USA northward in this region (Muhlin and Brawley 2008, 2009). However the ability to detect structure, especially within the Gulf of Maine, may be hampered by the limited number of sampled sites and microhabitats in previous studies (Muhlin and Brawley 2009, Coyer et al. 2011b).

Variation in short- and long-range dispersal of *Fucus vesiculosus* likely drives high levels of inbreeding and spatial-autocorrelation in relatedness at some, but not all sampling locations (Muhlin and Brawley 2008, Teixeira et al. 2016). Coastal topography and near-shore currents can generate unpredicted patterns of differentiation in this species, with some observations revealing greater differentiation between sites separated by only a few kilometers compared to sites hundreds to thousands of kilometers away (Muhlin and Brawley 2008). Genetic structure

due to recent expansion into brackish subtidal habitats (Tatarenkov et al. 2007) suggests the potential for adaptive genetic variation in this species as well. Lastly, variation in genetic structure along the intertidal gradient due to hybridization with congeners that are overlapping or adjacent to *F. vesiculosus* within a shore has been documented (Engel et al. 2005, Billard et al. 2010).

Fucus vesiculosus occurs continuously in the Gulf of Maine, spanning several degrees latitude that vary significantly in temperature, nutrient availability, and exposure to dominant ocean currents (Apollonio 1979, Townsend et al. 1987, Pettigrew et al. 2005, Benes and Bracken 2016). On rocky shores, *F. vesiculosus* occurs across nearly the entire intertidal gradient, leading to large differences in exposure to aerial conditions between individuals across its intertidal distribution (Appendices A and B, Benes and Bracken 2016). We took advantage of the interaction between these large-scale and local-scale environmental gradients, sampling *F. vesiculosus* at the upper- and lower-most edges of its intertidal distribution, at sites from the northeastern to southern Gulf of Maine. Through this sampling scheme we aimed to 1) identify the spatial-scale of genetic diversity and inbreeding, 2) describe genetic differentiation and structure between populations in a low diversity system, and 3) compare the magnitude of differentiation and gene flow across different spatial scales.

Materials & Methods

Specimen Collection and Preservation

We conducted surveys and collected individuals from nine sites spread across three regions of the Gulf of Maine in order to investigate the population structure of *Fucus vesiculosus*

at multiple spatial scales we (Appendices A and B). Surveys were conducted from June to August 2010. All surveys took place during spring tides such that the maximum possible tidal gradient was surveyed starting at or below mean lower-low water (MLLW). At each site, four transects were laid perpendicular to shore (i.e., from low to high intertidal heights) at randomly chosen locations along a 50-meter line. Beginning at the waterline, 0.25 x 0.25m quadrats were placed every 3-meters and the identity and abundance of all species and the tide height of each quadrat was recorded. The number of quadrats surveyed was dependent on the tidal amplitude and shoreline slope at each site. Vegetative apical tissue from up to six individuals of *F. vesiculosus* (> 5 centimeters) was collected from each quadrat. Tissue samples were cleaned of epiphytes and preserved on silica gel as voucher specimens and stored until DNA extraction and amplification were performed. For the current study, we selected individuals from the upper- and lower-most quadrats (i.e., the edges of the species' intertidal distribution) to compare population diversity and structure of *F. vesiculosus* across multiple spatial scales in the Gulf of Maine: the intertidal gradient, sites within regions, and among regions.

DNA Extraction and Amplification

Individual, dried *F. vesiculosus* samples (10-15 mg) were ground to a fine powder using a mixer mill (MM 300; Retsch, Hann, Germany) and total genomic DNA was extracted with the Nucleospin® 96 plant kit (Macherey-Nagel, Düren, Germany) and eluted with a total of 200µL elution buffer. Extraction was according to the manufacturer's instruction except that cell lysis was performed at room temperature for 1 hour. Samples were amplified at ten microsatellite loci (L20, L58, L94 [Engel et al. 2003]; F09, F19, F36, F42, F58 [Coyer et al. 2009]; Fsp 1, Fsp 2 [Perrin et al. 2007]) under the following PCR conditions: 5µL DNA template diluted 1:25, 1x

buffer, 1.5mM $MgCl_2$, 250µM dNTP, 100nM fluorescently-labelled forward primer (6-FAM, NED, PET, or VIC [Applied Biosystems, ThermoFisher Scientific]), 150nM unlabeled forward primer, 250nM reverse primer, and 1.0 units of Taq (Go Taq Flexi, Promega) in a 15µL reaction mix. The PCR program was 95°C for 5 minutes, followed by 35 cycles of 30 seconds at 95°C, 30 seconds at T_m, then 40 seconds at 72°C, and a final extension of 72°C for 10 minutes. PCR products were multiplexed before analysis on 3730*xl* DNA Genetic Analyzer (Applied Biosystems, Grand Island, NY) by Yale University's DNA Analysis Facility. The T_m for each locus and multiplexes are provided in the supplementary material Table 2.1.

Table 2.1. Information for loci used in this study. PCR amplification was performed per locus and multiplexed prior to fragment analysis. Loci F36, L94, and Fsp2 did not reliably amplify and showed some evidence of polyploidy, so were not included in the final data set. For all other loci, annealing temperature (T_m), number of alleles identified (N_a), allele size range, observed heterozygosity (H_o), gene diversity (H_e), and the inbreeding coefficient (F_{IS}) overall *Fucus vesiculosus* samples are provided. Departure from HWE are identified by and asterisk (*$P < 0.05$).

Locus*	5' Label	Multiplex Group	T_m (°C)	N_a	Size Range (bp)	H_o	H_e	F_{IS}	Reference
F09	VIC	A	58	4	174-182	0.159*	0.161	-0.087	Coyer et. al. (2009)
F19	6-FAM	A	55	12	180-222	0.428*	0.519	0.102	Coyer et. al. (2009)
F21	PET	A	58	4	188-214	0.810*	0.523	-0.591	Coyer et. al. (2009)
F36	PET	B	55	-	-	-	-	-	Coyer et. al. (2009)
F42	PET	C	58	5	182-194	0.503	0.507	-0.098	Coyer et. al. (2009)
L20	NED	A	55	9	124-175	0.389*	0.482	-0.017	Engel et. al. (2003)
L58	VIC	C	58	4	118-124	0.049	0.045	0.094	Engel et. al. (2003)
L94	6-FAM	C	58	-	-	-	-	-	Engel et. al. (2003)
Fsp1	NED	C	58	8	140-158	0.551*	0.576	-0.080	Perrin et. al. (2007)
Fsp2	6-FAM	B	58	-	-	-	-	-	Perrin et. al. (2007)
Mean Over Loci				6.57	-	0.412	0.402	-0.097	
Standard Error				1.19	-	0.025	0.079	0.088	

Microsatellite Analyses

Microsatellite chromatograms were scored blindly, with respect to sample identification, and independently by two researchers (KMB & SAKH). Allele sizes for *F. vesiculosus* samples were visualized and scored by-hand using proprietary software similar to GeneMapper (A. Strand *personal communication*). Allele sizes between researchers were compared and, when discrepancies between scores were identified, individual samples were re-scored or re-amplified as needed. Loci L94, F36, and Fsp 2 unreliably amplified and showed some evidence of polyploidy and therefore were removed from the data set. The remaining seven loci (L20, L58, F09, F19, F21, F42, and Fsp 1) were used for all analyses described below. Final sample sizes used in analyses, after removing individuals with more than one missing observation, can be found in Tables 2.2 and 2.3.

The presence and identity of repeated multilocus genotypes (MLGs) was first determined using the 'Multilocus Matches' function in GenAlEx 6.5 (Peakall & Smouse 2006). *F. vesiculosus* in the western Atlantic is a sexually reproducing alga and repeated MLGs were not expected but could arise from: i) errors in sampling in which the same genet was sampled repeatedly (i.e., repeated MLGs would be found within the same quadrat), ii) a low number of loci with a low allelic diversity (i.e., increasing the probability of amplifying repeated MLGs across genets), and/or iii) a high degree of linkage among alleles resulting in repeated MLGs across genets. It should be noted that asexual populations of *F. vesiculosus*, which reproduce via adventitious branching, have been identified in the Baltic Sea (Tatarenkov et al. 2005). Asexuality in these populations is thought be a response to the hyposaline conditions in the Baltic Sea, which reduce longevity and motility of fucoid gametes (Serrão et al. 1996). To the best of our knowledge, these conditions do not occur at our study sites and asexuality has not

been identified in *F. vesiculosus* populations in the western North Atlantic. We used GENCLONE 2.0 (Arnaud-Haond & Belkhir 2007) to calculate the probability of repeated MLGs occurring from different sexual events ($P_{sex} > 0.05$) or being clones of the same genet ($P_{sex} < 0.05$). We used FSTAT 2.9.3 (Goudet 1995) to examine the presence of linkage disequilibrium between pairs of loci overall and within each sample. Lastly, the presence and frequency of non-amplified alleles, or null alleles, was determined using Micro-checker 2.2.3 (Van Oosterhaut et al. 2004).

Deme Identification

We did not, *a priori*, know how *F. vesiculosus* was structured across sampling levels; therefore, we conducted a cumulative pooling analysis to determine the appropriate level that represents panmictic units (i.e., deme or population). When individuals from the same deme are included in the calculation of inbreeding, F_{IS} should be equivalent to zero (range: -1.0 to +1.0). As individuals from separate demes are pooled together, F_{IS} is expected to significantly increase (Goudet et al. 1994, Guillemon et al. 2008). We calculated Weir & Cockerham's (1984) F_{IS} within quadrats, tide heights, sites, regions, and overall using the program FSTAT 2.9.3 to identify the level which represents the appropriate deme structure for *F. vesiculosus* in the Gulf of Maine. We did this for all loci pooled and separately to identify abnormal behavior of any particular locus.

Using all loci, mean (filled dots) F_{IS} was < 0.00 and there was wide variation around estimates (Fig. 2.1A). However, this appeared to be driven largely by locus F21 which showed high level of heterozygotic excess and little change across all sampling levels (Fig 2.1B). Once locus F21 was excluded from the pooling analysis, the predicted pattern of increasing F_{IS} with

higher hierarchical sampling levels was revealed (Fig. 2.1C). A significant increase in F_{IS} was noted from tide height to site sampling level, suggesting that tide height was a true population unit. We chose to take a conservative approach and run population diversity and differentiation analyses at both tide height and site pooling levels due to the small overlap in the 95% CL with 0 for site-level F_{IS}.

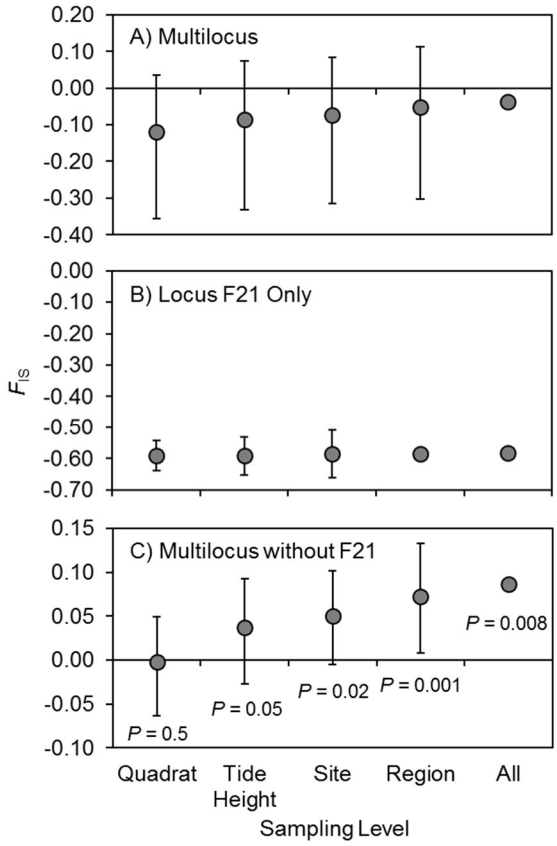

Figure 2.1. Results of a cumulative pooling analysis to determine the appropriate sampling level that represents the true population unit (i.e., deme or panmictic unit [Goudet et al. 1994]). Theory predicts that F_{IS} should increase significantly as individuals from multiple demes are pooled into a few or single population. (A) Mean $F_{IS} \pm$ CL (error bars = bootstrapped 95% confidence limits) using all loci. (B) Locus-specific F_{IS} of locus F21 which showed high levels of heterozygotic excess and little change across all sampling levels. (C) Multi-locus F_{IS} with F21 removed. Significant departures from equilibrium for each pooling level based on randomization tests are provided.

Genetic Diversity

Standard population indices of the number of alleles (N_A), unbiased allelic richness corrected for sample size (A), observed (H_o) and gene diversity (expected heterozygosity, H_e), and inbreeding coefficient (F_{IS}) were calculated for each locus and overall loci. N_A, A, and F_{IS} were calculated using FSTAT 2.9.3 (Goudet 1995). The probability of randomizations being lower or higher than observed F_{IS} values were calculated (2,520 randomizations). H_o and H_e were calculated using GenAlEx 6.5 (Peakall & Smouse 2006). Chi-square tests for significant departures from Hardy-Weinberg equilibrium (HWE) were performed using the package 'poppr' (Kamvar et al. 2014) implemented in R v. 3.3.1 (R Core Team 2016).

To compare diversity indices across the spatial scales represented in our sampling design we performed nested analyses of variance (ANOVA). We compared the variance of each index separately among regions, sites within regions, and tide heights within sites. Variance components for each factor and the percent variance explained were then calculated. The percent variance of the total was calculated by adding the variance due to loci to the residual (error) variance. The level of replication was the locus (n= 7 per tide height per site per region) and locus was treated as a random factor. ANOVAs were performed using the base package in R v. 3.3.1 (R Core Team 2016).

Population Structure

To quantify differentiation between samples, we calculated all possible pair-wise F_{ST}-values (Weir & Cockerham 1984) between sites and between tide heights (i.e., upper and lower distributional edges) from each site. F_{ST}-values were calculated using FSTAT 2.9.3 (Goudet 1995) and significant differentiation was assessed using permutations assuming HWE within

populations and significance levels were corrected for multiple tests (site: 720 permutations, $P \leq$ 0.00139; tide height: 3060 permutations, $P \leq 0.00033$). Calculating F_{ST} at the level of tide height allowed us to examine patterns in gene flow across different spatial scales and microhabitats. We first compared differentiation by spatial scale (i.e., among regions, sites within regions, and tide heights within sites) and then by type of tide height comparisons (i.e., upper v. upper, lower v. lower, and upper v. lower). We compared F_{ST}-values among spatial scales, tide height comparisons, and their interaction using a two-way ANOVA performed using the base package in R v. 3.3.1 (R Core Team 2016).

The presence of isolation by distance was assessed using genetic distances ($F_{ST}/[1- F_{ST}]$) and log-transformed geographic distances in meters (Rousset 1997). For within site (tide height) comparisons, we used the maximum vertical distance observed between the upper and lower edges of *Fucus vesiculosus'* intertidal distribution at each site (Appendix B). For among site pair-wise comparisons, we used Euclidean distances. Meters were used to avoid negative distances after transformation due to the shorter distances between tide heights. Mantel tests were performed for site-level F_{ST} and tide height-level F_{ST}. Because we found significant differences in F_{ST} among tide height comparison categories (see Results *Population Structure* below), we controlled for these differences using a partial Mantel test. Additionally, we carried out separate Mantel tests for each tide height comparison category (i.e., upper v. upper, lower v. lower, and upper v. lower) to examine variation in isolation by distance among these categories. Mantel tests were conducted using FSTAT 2.9.3.

Individual Assignment

To determine the assignment of individuals to particular sampling locations, we utilized a log-likelihood method (Paetkau et al. 2004) implemented in the GenAlEx 6.5 (Peakall and Smouse 2005). Under this test, the log-likelihood of an individual being a member of a particular sampling location is calculated based on the expected genotype frequencies of a population assuming random mating (calculated without the individual being assigned). For a given individual, this calculation is done for all sampling locations and the location with the highest log-likelihood is determined as the location to which the individual is assigned to have the greatest fidelity. We then calculated the proportion of individuals that were assigned to the location in which they were collected ('Self') or another location.

Results

Microsatellite Analyses

We found 253 unique multilocus genotypes (MLGs) and 26 of these were repeated MLGs. Repeated MLGs were found at each sampling site, at a rate of 5-38% of the total number of samples. Five of the nine sampling sites had 1 to 2 MLGs each that were suspected as possible clones (i.e., ramets) of the same genet ($P_{sex} < 0.05$). Within populations there was no evidence of linkage disequilibrium between loci pairs. There was no evidence of null alleles at loci F21 and L58 in either site-level or tide height-level analyses (Tables 2.2 and 2.3). The frequency of null alleles at other loci were low to moderate (site range: 0.009 to 0.210, tide height range: 0.008 to 0.324) and were scattered across sites.

Genetic Diversity

Multilocus F_{IS} values showed no significant departures from HWE for all sites in the site-level analysis and all but one site (SO C Lower) in the tide height-level analysis (Table 2.2 and 2.3). However, at six of the seven loci (F09, F19, F21, F42, Fsp1, and L20) observed heterozygosity (H_o) showed significant departures from expected (H_e) at various sites. Locus Fsp1 showed heterozygotic excess and deficits (depending on location) at least at one site per region. H_o were significantly lower than expected at some sites in the northeast and central regions at loci F09, F42, and F19, and in the southern region at locus L20. Locus F21 showed strong significant heterozygote excess at nearly all sites. Importantly, with few exceptions, these patterns were consistent between site-level and tide height-level calculations further suggesting that tide height is an appropriate panmictic unit to analyze population diversity and structure in *Fucus vesiculosus* (Fig. 2.1).

The mean number of alleles (N_a) varied significantly among sites within regions (Table 2.4). However, after adjusting for sample size, region explained 92% of the variation in allelic richness with the highest richness occurring in the central Gulf of Maine (*A*; Fig. 2.2A and Table 2.4). Gene diversity (H_e) paralleled patterns of allelic richness (Fig. 2.2B) but there were no significant explanatory variables for patterns of observed heterozygosity (H_o; Fig. 2.2C). Relatedness (F_{IS}) varied significantly between tide heights within sites but after correcting for multiple tests, no post-hoc significant differences within sites were identified (Fig. 2.2D). At sites NE C, CE C, CE B, SO C, and SO B F_{IS} was generally lower at the lower edge compared to upper edge of *F. vesiculosus'* intertidal distribution. In contrast, at NE A and CE A F_{IS} was generally higher at the lower compared to upper edge.

Table 2.2. Population diversity indices by site. Samples sizes are given under each site. Significance denoted by asterisks (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

		Northeast			Central			Southern		
		A	B	C	A	C	B	C	A	B
Locus		36	36	29	35	20	37	38	33	22
F09	N_{ll}			0.151						
	A	2	1	2	2	3	2	2	2	2
	Na	1.806	1	1.974	1.995	3	1.908	1.954	2	2
	Ho	0.056	0.034	0.00***	0.171	0.381**	0.081	0.105	0.273	0.500
	He	0.054	0.000	0.098	0.157	0.381	0.078	0.100	0.278	0.375
	F	-0.014	NA	0.659	-0.079	0.07	-0.029	-0.042	0.034	-0.313
F19	N_{ll}		0.156*	0.035	0.031	0.210*	0.019		0.058	0.104
	Na	5	3	5	7	3	5	5	4	3
	A	4.111	2.994	4.069	5.783	3	4.081	3.831	3.212	2.909
	Ho	0.611	0.414*	0.333	0.543**	0.286	0.568	0.368	0.455	0.273
	He	0.609	0.448	0.457	0.611	0.505	0.551	0.316	0.529	0.361
	F	0.01	0.269	0.111	0.126	0.489	-0.015	-0.152	0.156	0.265
F21	N_{ll}									
	Na	3	4	2	3	3	3	3	2	3
	A	2.556	3.111	2	2.571	3	3	2.526	2	2.909
	Ho	0.861***	0.897**	0.917	0.771**	0.667**	0.730	0.816***	0.909***	0.818*
	He	0.502	0.521	0.499	0.497	0.526	0.584	0.502	0.500	0.522
	F	-0.709*	-0.754*	-0.789*	-0.543*	-0.211	-0.237	-0.615	-0.815*	-0.552
F42	N_{ll}						0.204*		0.045	
	Na	3	3	4	3	2	3	4	2	2
	A	2.806	2.806	3.379	2.995	2	2.792	3.558	2	2
	Ho	0.417	0.552	0.444	0.600	0.524	0.270***	0.579	0.455	0.636
	He	0.372	0.415	0.474	0.525	0.495	0.503	0.537	0.483	0.483
	F	-0.105	-0.057	-0.147	-0.128	-0.1	0.473*	-0.064	0.075	-0.295
Fsp1	N_{ll}								0.012	0.040
	Na	5	3	5	4	5	6	5	4	2
	A	4.359	2.917	4.595	3.82	5	4.866	4.477	3.697	2
	Ho	0.528**	0.655*	0.500***	0.543	0.667*	0.649	0.579	0.515***	0.364
	He	0.543	0.488	0.605	0.582	0.587	0.617	0.599	0.556	0.397
	F	0.042	-0.01	-0.066	0.082	-0.074	-0.038	0.047	0.09	0.106
L20	N_{ll}	0.009	0.034				0.073	0.061	0.135*	0.157*
	Na	4	5	6	4	4	7	6	5	4
	A	3.777	3.917	5.062	3.391	4	5.917	4.611	4.82	4
	Ho	0.278	0.414	0.306	0.543	0.476	0.595	0.395***	0.333*	0.273*
	He	0.295	0.312	0.404	0.477	0.458	0.680	0.474	0.468	0.445
	F	0.073	0.034	-0.006	-0.123	0.012	0.14	0.18	0.302	0.407
L58	N_{ll}									
	Na	1	2	3	2	2	2	1	2	1
	A	1	1.917	2.597	1.571	2	1.541	1	1.848	1
	Ho	0.000	0.103	0.083	0.029	0.143	0.027	0.000	0.061	0.000
	He	0.000	0.080	0.099	0.028	0.133	0.027	0.000	0.059	0.000
	F	NA	-0.029	-0.024	0	-0.056	0	NA	-0.016	NA
	Mean Ho	0.393	0.369	0.438	0.457	0.449	0.417	0.406	0.429	0.409
	Mean He	0.339	0.323	0.377	0.411	0.441	0.434	0.361	0.410	0.369
	Multilocus Fis	-0.144	-0.127	-0.147	-0.098	0.018	0.053	-0.111	-0.029	-0.086
	Multilocus Fis w/o F21	0.006	0.058	0.001	-0.006	0.066	0.122	0.014	0.134	0.031

Table 2.3. Population diversity indices by tide height. Samples sizes are given under each sample. Significance denoted by asterisks (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

		Northeast						Central						Southern					
		A		B		C		A		C		B		C		A		B	
Locus		Upper 21	Lower 15	Upper 18	Lower 18	Upper 20	Lower 9	Upper 20	Lower 15	Upper 9	Lower 11	Upper 17	Lower 20	Upper 20	Lower 18	Upper 16	Lower 17	Upper 15	Lower 7
F09	N_{11}					0.172				0.219									
	A	1.333	1.467	1	1	1.737	1	1.9	1.467	2.739	2	1.661	1.35	1.737	1.389	1.438	1.999	2	2
	Na	2	2	1	1	2	1	2	2	3	2	2	2	2	2	2	2	2	2
	Ho	0.048	0.067	0.000	0.000	0.050**	0.000	0.250	0.067	0.111*	0.545	0.118	0.050	0.150	0.056	0.063	0.471	0.143	0.667
	He	0.046	0.064	0.000	0.000	0.139	0.000	0.219	0.064	0.290	0.397	0.111	0.049	0.139	0.054	0.061	0.415	0.133	0.444
	F	0	0	-	-	0.655	NA	-0.118	0	0.652*	-0.333	-0.032	0	-0.056	0	0	-0.103	0	-0.474
F19	N_{11}	0.012		0.028	0.230*	0.040	0.022		0.168*		0.324		0.036				0.141	0.115	
	Na	5	3	3	3	4	3	6	5	2	3	4	4	5	3	3	3	3	2
	A	3.618	2.967	2.855	2.623	2.694	2.778	3.947	4.022	2	2.636	3.192	3.308	2.99	2.265	2.437	2.412	3	1.862
	Ho	0.619	0.600	0.444	0.222**	0.400	0.444	0.700	0.333**	0.444	0.091*	0.529	0.600*	0.450	0.278	0.563	0.353	0.429	0.200
	He	0.628	0.580	0.468	0.426	0.446	0.475	0.631	0.556	0.444	0.492	0.462	0.604	0.375	0.245	0.506	0.493	0.561	0.180
	F	0.039	0	0.078	0.5	0.129	0.123	-0.084	0.429	0.059	0.831	-0.116	0.032	-0.175	-0.104	-0.08	0.312	0.308	-0.077
F21	N_{11}																		
	Na	3	2	3	3	2	2	3	2	3	2	3	3	3	2	2	2	2	3
	A	2.333	2	2.389	2.389	2	2	2.35	2	2.961	2	2.944	2.836	2.35	2	2	2	2	2.467
	Ho	0.857**	0.867**	1.00***	0.833*	0.950***	0.778	0.800	0.733*	0.778**	0.545	0.824	0.650*	0.800	0.833**	0.938***	0.882**	0.571	0.933**
	He	0.509	0.491	0.526	0.508	0.499	0.475	0.516	0.464	0.586	0.397	0.593	0.574	0.516	0.486	0.498	0.500	0.490	0.531
	F	-0.671*	-0.75	-0.895*	-0.624	-0.900*	-0.6	-0.531	-0.556	-0.273	-0.333	-0.362	-0.108	-0.531	-0.7	-0.875*	-0.752*	-0.091	-0.742
F42	N_{11}					0.033						0.187	0.199*	0.008			0.071	0.069	
	Na	3	3	3	2	3	3	3	3	2	2	2	3	4	4	2	2	2	2
	A	2.332	2.191	2.633	1.962	2.347	2.778	2.731	2.862	2	2	1.999	2.583	2.699	2.778	2	1.999	2	2
	Ho	0.571	0.200	0.556	0.333	0.400	0.889	0.550	0.667	0.556	0.545	0.235	0.300***	0.500	0.667	0.563	0.353	0.429	0.733*
	He	0.459	0.184	0.512	0.278	0.434	0.537	0.476	0.571	0.401	0.496	0.415	0.545	0.503	0.552	0.498	0.415	0.500	0.464
	F	-0.221	-0.05	-0.056	-0.172	0.103	-0.62	-0.13	-0.134	-0.333	-0.053	0.458	0.47	0.031	-0.179	-0.098	0.179	0.217	-0.556
Fsp1	N_{11}		0.113						0.139	0.072		0.202*		0.032			0.107	0.069	
	Na	4	4	3	2	5	2	3	4	5	3	6	4	4	5	3	3	2	2
	A	2.662	3.448	2.764	2	3.833	2	2.835	3.688	4.333	2.636	3.647	3.25	3.416	3.805	2.692	2.661	2	1.969
	Ho	0.571	0.467	0.444*	0.556	0.650***	0.667	0.600	0.467	0.556	0.727	0.353	0.900*	0.550	0.611*	0.625	0.412***	0.429	0.333
	He	0.454	0.620	0.434	0.494	0.656	0.444	0.540	0.629	0.642	0.541	0.606	0.611	0.581	0.617	0.557	0.552	0.500	0.278
	F	-0.237	0.279	0.004	-0.097	0.035	-0.455	-0.086	0.29	0.192	-0.301	0.442	-0.452	0.079	0.039	-0.091	0.282	0.217	-0.167
L20	N_{11}			0.029					0.011	0.022		0.110	0.018	0.104	0.036	0.194*	0.072	0.231	0.085
	Na	4	3	4	4	6	2	3	3	3	4	6	6	3	5	5	5	4	4
	A	3.035	1.933	2.739	2.562	3.598	1.961	2.344	2.724	2.778	3.394	4.151	4.274	2.58	3.391	3.402	3.619	4	3.053
	Ho	0.381	0.133	0.333	0.278	0.500	0.222	0.550	0.533	0.444	0.455	0.471	0.700***	0.350***	0.444	0.188*	0.471*	0.286	0.267*
	He	0.396	0.127	0.366	0.250	0.475	0.198	0.411	0.540	0.475	0.384	0.599	0.718	0.466	0.475	0.375	0.529	0.602	0.344
	F	0.062	-0.018	0.117	-0.083	-0.027	-0.067	-0.314	0.047	0.123	-0.136	0.243	0.05	0.273	0.093	0.524*	0.141	0.579	0.258
L58	N_{11}																		
	Na	1	1	2	2	3	2	2	1	1	2	1	2	1	1	2	1	1	1
	A	1	1	1.389	1.633	1.7	1.778	1.35	1	1	1.964	1	1.35	1	1	1.692	1	1	1
	Ho	0.000	0.000	0.056	0.111	0.100	0.111	0.050	0.000	0.000	0.273	0.000	0.050	0.000	0.000	0.125	0.000	0.000	0.000
	He	0.000	0.000	0.054	0.105	0.096	0.105	0.049	0.000	0.000	0.236	0.000	0.049	0.000	0.000	0.117	0.000	0.000	0.000
	F	-	-	0	-0.03	-0.013	0	0	-	-	-0.111	-	0	-	-	-0.034	-	-	-
	Mean Ho	0.435	0.333	0.405	0.333	0.436	0.444	0.500	0.400	0.413	0.455	0.361	0.464	0.400	0.413	0.327	0.448	0.438	0.420
	Mean He	0.356	0.295	0.337	0.294	0.392	0.319	0.406	0.403	0.406	0.420	0.398	0.450	0.369	0.347	0.398	0.320	0.373	0.415
	Multilocus Fis	-0.2	-0.095	-0.173	-0.104	-0.086	-0.341	-0.207	0.043	0.041	-0.034	0.122	-0.007	0.253	-0.368*	-0.141	0.018	-0.06	-0.161
	Multilocus Fis w/o F21	-0.08	0.103	0.029	0.062	0.091	-0.273	-0.136	0.158	0.121	0.012	0.25	0.016	0.056	-0.029	0.027	0.173	0.324	-0.254

Table 2.4. Results from the nested ANOVAs testing for variance in diversity indices across different hierarchical sampling levels. Separate analyses were conducted for the number of alleles (N_a), allelic richness (A), gene diversity (H_e), observed heterozygosity (H_o), and relatedness (F_{IS}).

	F-value[†]				
Factor	N_a	A	H_e	H_o	F_{IS}
Region	1.90	4.95**	6.17**	0.55	2.03
Sites within Region	2.51*	0.56	0.39	0.43	0.05
Tide Height within Site	1.47	0.64	0.76	0.87	2.44*
Error Variance	0.68	0.27	0.01	0.02	0.06

[†]Significance: *$P < 0.05$, **$P < 0.01$

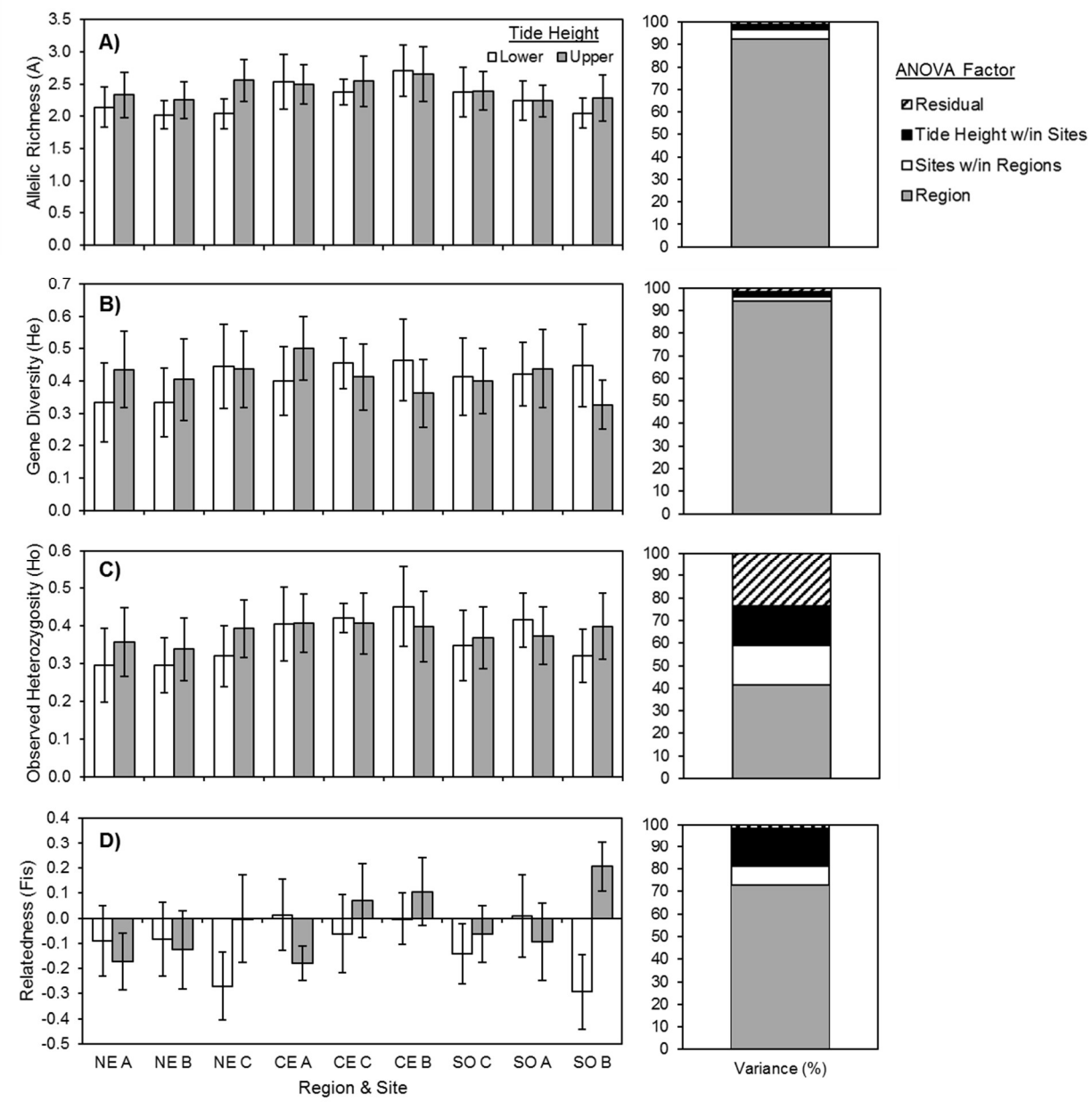

Figure 2.2. For each diversity index, mean ± SE (right panel) and percent variance explained by hierarchical sampling levels from the nested ANOVA (left panel). (A) Allelic richness (unbiased estimation corrected for sample size), (B) gene diversity (expected heterozygosity), (B) observed heterozygosity, and relatedness (*F*is). Mean values are given for each site (ordered by latitude from north to south) by tide height (lower: unfilled bars, upper: grey filled bars). See Table 1 for ANOVA results.

Table 2.5. Pair-wise F_{ST} values for all possible (A) site comparisons and (B) tide height comparisons. Significant differentiation between two samples is shown in bold and italics, significance thresholds were adjusted for multiple comparisons (sites: 720 permutations, $P \leq 0.00139$; tide height: 3060 permutations, $P \leq 0.00033$).

A)

	NE_A	NE_B	NE_C	CE_A	CE_C	CE_B	SO_C	SO_A	SO_B
NE_A	0								
NE_B	0.002	0							
NE_C	0.012	0.003	0						
CE_A	0.014	***0.025***	***0.010***	0					
CE_C	***0.040***	***0.031***	0.007	***0.024***	0				
CE_B	***0.048***	***0.040***	***0.030***	***0.033***	***0.017***	0			
SO_C	***0.041***	***0.024***	0.007	0.011	***0.024***	***0.036***	0		
SO_A	***0.081***	***0.078***	***0.047***	***0.062***	0.010	***0.053***	***0.070***	0	
SO_B	***0.081***	***0.062***	***0.046***	***0.057***	0.021	***0.056***	***0.037***	***0.040***	0

B)

	NE_A_Upper	NE_A_Lower	NE_B_Upper	NE_B_Lower	NE_C_Upper	NE_C_Lower	CE_A_Upper	CE_A_Lower	CE_C_Upper	CE_C_Lower	CE_B_Upper	CE_B_Lower	SO_C_Upper	SO_C_Lower	SO_A_Upper	SO_A_Lower	SO_B_Upper	SO_B_Lower
NE_A_Upper	0																	
NE_A_Lower	0.0278	0																
NE_B_Upper	-0.0036	0.037	0															
NE_B_Lower	0.0171	0.017	0.023	0														
NE_C_Upper	0.0155	0.030	0.016	0.011	0													
NE_C_Lower	-0.0023	0.048	-0.004	0.010	0.009	0												
CE_A_Upper	-0.0050	0.024	0.015	0.023	0.002	0.014	0											
CE_A_Lower	0.0186	0.072	0.030	***0.064***	0.016	0.033	0.004	0										
CE_C_Upper	0.0247	0.039	0.028	0.017	-0.010	0.010	0.015	0.043	0									
CE_C_Lower	0.0532	0.115	0.067	0.071	0.045	0.022	0.035	0.047	0.035	0								
CE_B_Upper	0.0284	0.056	0.032	0.016	***0.016***	0.025	***0.029***	0.031	-0.009	0.040	0							
CE_B_Lower	0.0405	***0.109***	0.048	0.080	***0.045***	0.043	***0.046***	***0.035***	0.016	0.050	0.009	0						
SO_C_Upper	0.0127	0.057	0.012	0.027	-0.002	0.010	0.001	-0.004	0.020	0.042	0.024	***0.047***	0					
SO_C_Lower	0.0351	0.099	0.022	0.052	0.014	0.010	0.032	0.008	0.035	0.035	0.028	0.042	-0.008	0				
SO_A_Upper	0.0218	***0.074***	0.028	0.032	0.011	-0.007	***0.024***	***0.047***	0.006	0.010	0.028	***0.037***	0.026	0.024	0			
SO_A_Lower	***0.1107***	***0.190***	***0.127***	***0.168***	***0.109***	0.094	***0.096***	***0.122***	0.078	0.030	***0.118***	***0.076***	***0.127***	***0.121***	0.047	0		
SO_B_Upper	0.0249	0.097	0.034	0.059	0.009	0.006	0.023	0.041	-0.035	0.006	0.009	-0.014	0.036	0.034	-0.021	-0.001	0	
SO_B_Lower	***0.0999***	***0.188***	***0.072***	***0.152***	***0.108***	0.078	***0.099***	***0.104***	***0.108***	0.067	***0.116***	***0.114***	***0.075***	***0.060***	0.099	***0.124***	0.0979	0

Population Structure

Pair-wise F_{ST}-values revealed significant differentiation between sites throughout the Gulf of Maine (Table 2.5). At the site-level, 75% (27 out of 36) of comparisons were significant whereas at the tide height-level 24% of pair-wise comparisons were significant. While there were fewer significant F_{ST}-values at the tide height-level, the highest estimates of differentiation were more then 2-fold site-level differentiation estimates (0.190 compared to 0.081). Analysis of F_{ST} by categories of tide height and spatial comparison revealed significant differences among tide height comparison categories (ANOVA; $F_{2,146} = 27.60$, $P < 0.0001$) but not spatial comparison categories (ANOVA; $F_{2,146} = 2.64$, $P = 0.07$). F_{ST} was significantly higher between lower-lower and upper-lower pair-wise estimates compared to upper-upper estimates (Fig. 2.3A). Importantly, this pattern was not dependent on spatial scale (ANOVA, 'Tide Height x Spatial Comparison' interaction; $F_{2,146} = 0.75$, $P = 0.48$).

Isolation by distance was identified with both site-level and tide height-level estimates of genetic distance (Table 2.6). Even after accounting for different categories of tide height comparison, genetic differentiation increased with geographic distance (Fig. 2.3B, Table 2.6). However, separate tests for each category showed isolation by distance was significant in upper-upper comparisons but not lower-lower or upper-lower comparisons (Table 2.6).

Individual Assignment

Overall, the majority of individuals were assigned to locations other than where they were collected from (Table 2.7). However, self-assignment patterns (i.e., assignment to the location of collection) corroborated differentiation patterns. At the site-level, self-assignment increased from northeastern to southern sites. When the data set was divided by tide height the

proportion of self-assigned individuals was greater (up to 9-fold) at the lower-edge of the intertidal distribution at all sites except NE C (Table 2.7).

Table 2.6. Mantel test of correlation (r) between genetic ($F_{ST}/[1-F_{ST}]$) and geographic (log [meters]) distance. Slope of the relationship and R^2 from regression are also shown. Tests were performed for pair-wise differentiation between sites and between all possible comparisons by tide height. By tide height, a partial mantel test was performed to account for variation due to category of tide height comparison. Separate tests were also performed for each of these categories: lower-lower, upper-lower, and upper-upper. Significant isolation by distance is shown in bold italics ($P < 0.05$).

Test	*r*	*slope*	R^2	*P*-value
By Site	0.44	0.020	0.20	***0.004***
By Tide Height	0.18	0.008	0.030	***0.025***
Lower-Lower	0.26	0.025	0.068	0.11
Upper-Lower	0.21	0.006	0.042	0.067
Upper-Upper	0.36	0.009	0.13	***0.039***

Table 2.7. The proportion of individuals assigned to the population in which they were sampled ('Self') or some other population either within the same site or region, or different region from the sample location. Data are separated between site-level analysis and tide height-level analysis. Mean (±SE) are given for each category.

	Site-Level			Lower-Limit Samples				Upper-Limit Samples			
Site	Self	Same Region	Different Region	Self	Same Site	Same Region	Different Region	Self	Same Site	Same Region	Different Region
NE A	15.8	36.8	47.4	40.0	0.0	46.7	13.3	4.3	17.4	30.4	47.8
NE B	40.0	7.5	52.5	42.9	4.8	19.0	33.3	15.8	10.5	36.8	36.8
NE C	0.0	34.4	65.6	8.3	0.0	33.3	58.3	10.0	10.0	35.0	45.0
CE A	41.5	7.3	51.2	33.3	9.5	9.5	47.6	10.0	30.0	10.0	50.0
CE C	30.6	13.9	55.6	10.5	0.0	0.0	89.5	0.0	5.9	23.5	70.6
CE B	51.3	5.1	43.6	30.0	15.0	0.0	55.0	21.1	15.8	10.5	52.6
SO C	31.7	9.8	58.5	16.7	22.2	16.7	44.4	4.3	21.7	8.7	65.2
SO A	47.6	16.7	35.7	50.0	9.1	27.3	13.6	20.0	15.0	10.0	55.0
SO B	48.0	8.0	44.0	60.0	6.7	20.0	13.3	0.0	10.0	10.0	80.0
Mean	*34.0 ± 5.6*	*15.5 ± 4.0*	*50.5 ± 3.0*	*32.4 ± 6.0*	*7.5 ± 2.5*	*19.2 ± 5.1*	*40.9 ± 8.5*	*9.5 ± 2.7*	*15.1 ± 2.4*	*19.4 ± 4.0*	*55.9 ± 4.5*

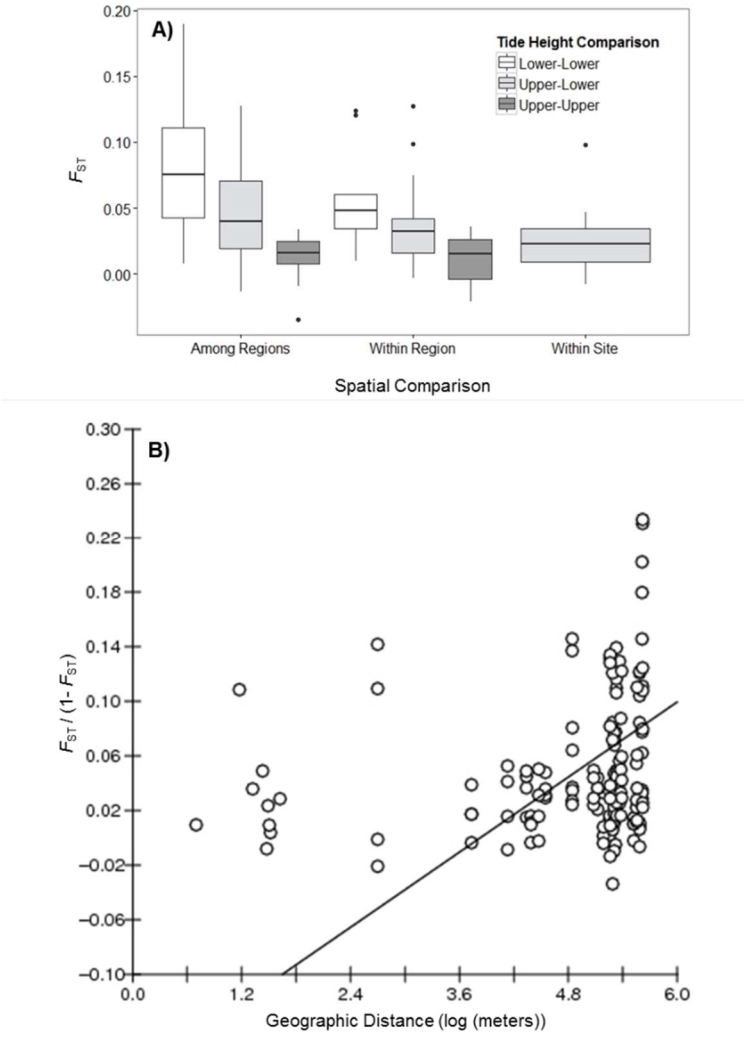

Figure 2.3. Differentiation of *F. vesiculosus* samples. (A) Box-plot of pair-wise *F*st-values (see Table 2.2) categorized by spatial comparison and tide height comparison. (B) Genetic distance (F_{ST} / [1 – F_{ST}]) as a function of geographic distance in meters (log transformed). Mantel test results are presented in Table 3.

Discussion

Widely distributed organisms are typically assumed to lack strong genetic structure and differentiation among populations. However, temporal and spatial variation in neutral and selective processes can generate 'chaotic genetic patchiness' (Johnson and Black 1984), or unexpected patterns in population structure, particularly at the local-scale (Hedgecock 1994, Hogan et. al. 2010). We used microsatellite data to measure population diversity and structure of

an abundant marine macroalga, *Fucus vesiculosus*, at a range of spatial scales (meters to kilometers) that encompassed large- and local-scale environmental gradients. We found predictable patterns of diversity at large (latitudinal) scales but variable patterns of inbreeding and population differentiation at other spatial scales. A combination of oceanic current patterns, shore topography, and spawning and within-site dispersal patterns may explain this genetic patchiness in *F. vesiculosus*.

Genetic diversity, unbiased allelic richness and gene diversity peaked in the central Gulf of Maine, which is expected under an abundant-center model (Eckert et al. 2008). Compared to previous studies of *Fucus vesiculosus* in the Gulf of Maine we uncovered slightly higher allelic richness and diversity likely due to an increased number of sampling sites and tide heights. Two loci in this study (L20 and L58) were also used by Muhlin and Brawley (2008, 2009) in their analysis of population structure and gene flow of *F. vesiculosus* in the northwest Atlantic. Locus L58 was previously found to be monomorphic across sites sampled from North Carolina, USA to Mabou, Nova Scotia, Canada. In the current study we found four unique alleles at this locus, although one was particularly dominant (frequency > 0.92) at all sampling locations. Allelic richness of L20 in previous studies ranged from 3 to 5 alleles per site, whereas our study found 2 to 6. Additionally, the inclusion of markers other than the L-series (Engel et al. 2003) in the current study uncovered greater locus-specific diversity particularly at loci Fsp1 and F19. The greater sampling coverage and diversity of loci may have aided in our ability to detect significant structure in Gulf of Maine *F. vesiculosus* despite high gene flow.

In contrast to diversity, patterns of observed heterozygosity and inbreeding did not show strong regional patterns. Observed heterozygosity in particular was not explained by any sampling unit. There was a general trend towards higher observed heterozygosity in the central

Gulf of Maine. At the tide-height level, there was a trend toward higher observed heterozygosity in the upper intertidal compared to lower intertidal samples at all sites in the northeast Gulf of Maine and one site (SO B) in the southern gulf, but equal or opposite patterns elsewhere. Although not a significant factor, region did explain a large proportion of variation in the inbreeding coefficient (F_{IS}) with values at sites and tide heights in the northeast consistent with outbreeding or excess heterozygosity. However, F_{IS} values were closer to equilibrium at other location in the Gulf of Maine. There was however significant variation in F_{IS} due tide height, as more than half of sampling sites showed a trend toward differences in levels of inbreeding between the upper and lower edges of the species' intertidal distribution. Most multi-locus estimates of F_{IS} were not significantly different from zero suggesting *F. vesiculosus* populations within the Gulf of Maine are at equilibrium.

Levels of differentiation provided insight to the spatial patterns of gene flow in *Fucus vesiculosus* within the Gulf of Maine. Among regions, the greatest levels of significant differentiation (F_{ST}) occurred between northeast and southern sites as predicted by distance (i.e., greatest differentiation between sites with the greatest linear distance between them), followed by comparisons between central and southern sites, and then between northern and central sites. Within regions, there was no differentiation between sites in the northeast but there was significant differentiation between some sites in the central and southern regions, with the later showing the highest within region F_{ST} values. The magnitude of differentiation between our current study sites is comparable to previous estimates within the Gulf of Maine (Muhlin and Brawley 2008, 2009). However, Muhlin and Brawley (2009) reported no significant isolation by distance among populations from Connecticut, USA to Mabou, Nova Scotia, Canada. Our data revealed significant isolation by distance, using both site-level and tide height-level F_{ST} values,

within the confines of the Gulf of Maine. Importantly the magnitude of F_{ST} and isolation by distance patterns depended on the type of tide height comparison rather than type of spatial comparison, which is not predicted if gene flow is uniform across intertidal and latitudinal gradients in this species. The uncovered variability in inbreeding and population structure may be a consequence of 1) large-scale current patterns, 2) local-scale topography and near-shore currents, 3) wrack deposition, and 4) variability in spawning synchrony.

Major circulation in the Gulf of Maine is cyclonic with water flowing southward from the Scotian Shelf into the Gulf and forming the along-shore Gulf of Maine Coastal Current (GMCC). Shore-line topography, seasonal river output, and climatic conditions lead to branching of the GMCC, and spatial and temporal variability in the speed and proximity to the coastline of the GMCC (Xue et al. 2000, Pettigrew et al. 2005). The eastern branch of the GMCC is characterized by relatively strong flow (0.15 to 0.30 ms^{-1}) from near Cutler, Maine (near our NE C sampling site) to Penobscot Bay (an area of major river outflow) compared to the western GMCC that then flows (0.05 to 0.15 ms^{-1}) from Penobscot Bay to Massachusetts Bay (Pettigrew et al. 2005 [see Appendix A]). Strong tidal mixing in the Bay of Fundy and the position of the eastern GMCC entrain particles in the northeast (Xue et al. 2008) which could explain the high gene flow we found within this region. If *Fucus vesiculosus* tends to be retained in the northeast region once detached, this could also explain the strong differentiation between sites in the northeast and elsewhere in the Gulf of Maine. Flow of the western GMCC tends to be pushed off-shore near Cape Ann, separating a portion of the Massachusetts Bay (the location of sites SO A and B [see Appendix A]) from the major currents in the Gulf of Maine (Pettigrew et al. 2005). This may further limit gene flow into and away from the sites in this portion of the southern Gulf

of Maine. Drifter analysis demonstrates a strong decrease in connectivity from north to south, and very little transport from south to other regions in the Gulf of Maine (Manning et al. 2009).

In addition to large-scale currents, local-scale variation in circulation due to coastline topography may generate variable patterns of genetic differentiation. Using orange drifters, Muhlin and Brawley (2008) demonstrated a general southwest coastal current that resulted in drifters dispersing away from eastern sides of peninsulas; whereas on western sides, drifters tended to recirculate near-shore. This pattern was congruent with the observation of greater genetic diversity at sites along western shores of peninsulas. However, counter to expectation, genetic differentiation was strongest between sites on the same side of a peninsula, which suggests localized currents and other processes may have strong influence on gene flow in this species. Our sampling sites varied in direction, but our two closest sites that faced one another (SO A and B) showed significant genetic differentiation, suggesting localized currents may play a strong role in limiting migration between nearby sites.

Ocean current patterns may help explain regional and site-level patterns in F_{ST}, but what about tide height? Genetic differentiation was lowest between upper-upper shore comparison regardless of whether the comparison was within or among regions. If gene flow due to rafting of reproductive individuals is high in the Gulf of Maine, as suggested by the current and previous studies (Muhlin and Brawley 2009), then this pattern by tide height suggests asymmetrical gene flow across the intertidal gradient. Rafting marine organisms tend to be deposited higher on shore (Gómez et al. 2013). Even at locations where wrack may be deposited along the entire intertidal gradient, species-specific patterns in deposition have been noted with fucoid algae being deposited higher on shore (Gómez et al. 2013). Within a site, dispersal between the upper and lower edges of *Fucus vesiculosus'* intertidal distribution may depend on local conditions.

When conditions are ideal, synchronous spawning has been observed to occur after approximately ~2 to 3 hours of submergence on an incoming tide (Berndt et al. 2002). If low shore individuals spawn first and all at once, before upper intertidal individuals, there may be isolation between intertidal zones due to timing of gamete release. But is conditions are not ideal, and spawning happens randomly, there may be increased potential in mixing between zones. Dispersal potential of gametes is predicted to be minimal (Muhlin and Brawley 2008), but zygote dispersal of fucoid algae has been observed following immediate settlement next to parent thalli (Ladah et al. 2008) to greater than 5 meters with potential for both horizontal and vertical dispersal relative to the waterline (Dudgeon et al. 2001).

Dispersal between intertidal zones within a site may be further complicated by variation in spawning synchrony. Models based on climate variables (wave height and cloud cover) predict decreasing synchrony in spawning in populations of *Fucus vesiculosus* from south to north in the Gulf of Maine (Muhlin et al. 2011). This could result in 'pulses' of gamete release in the south but increased variability in gamete release in the north. How synchrony of spawning varies along the intertidal gradient is unknown. Given that the uppermost individuals are more often exposed than submerged and experience extreme and stressful environmental conditions, it would not be surprising if synchrony varied, and perhaps decreased, from the lower to upper edges of the species' intertidal distribution. Hypotheses of asymmetrical dispersal (i.e., within a site from the low to high intertidal zone) and gene flow (i.e., between sites to the upper intertidal zone) require testing but would provide insight to local-scale patterns of diversity and differentiation in this species.

Predicted consequences of lower gene flow and dispersal to the lower intertidal zone, and subsequent isolation, is lowered effective population size and fixation of alleles due to genetic

drift (Slatkin 1987). We only found evidence of the former (*K. Benes* unpublished data) but no wide-spread of fixation of alleles across loci in lower-edge samples. Lack of strong genetic drift suggests other mechanisms drive higher differentiation in these low intertidal populations. Even if spawning is synchronous, temporal variation in which genotypes are contributing gametes, or spatial variation in successful recruitment due to herbivory (Lubchenco 1983), competition (Schonbeck and Norton 1980, Hawkins and Hartnoll 1985), or microhabitat conditions (Brawley and Johnson 1991, Wright et al. 2004) could generate differentiation and structure at smaller spatial scales at the lower intertidal distributional limit. While these may also occur at the upper edge of this species' intertidal distribution, higher gene flow or dispersal to this zone could override potential genetic signals from these other processes.

While the use of putatively neutral microsatellite loci describes the effect of historic and neutral processes, there is evidence of adaptation within *Fucus vesiculosus* populations in Gulf of Maine. First, Gulf of Maine-wide balancing selection at locus F21 (determined from an F_{ST}-heterozygosity outlier test) suggests historic basin-wide selection of particular genotypes (heterozygotes). Second, a decreasing/increasing cline with latitude in alleles 182/191 at locus F42 (in lower intertidal samples only) suggests potential adaptation to large-scale environmental variation (*K. Benes* unpublished data). Lastly, reciprocal transplant experiments revealed adaptation and countergradient variation in growth along the intertidal gradient in the northeast and central, respectively (Chapter 3). Thus, historic and on-going adaptation at different spatial scales could further drive chaotic genetic patchiness if there is or has been differential reproduction or survival of particular genotypes. *F. vesiculosus* would be an excellent candidate for comparing genome-wide patterns in neutral and adaptive loci at multiple spatial scales. Such studies would provide insight into the independent and interactive effects of region and local-

scale environmental variation on dispersal and natural selection. This would further provide a better understanding of how neutral and selective processes, at different spatial scales, shape phenotypic differentiation observed in this species (Benes and Bracken 2016, and K. Benes *personal observation*).

We documented variation in genetic diversity and population structure and isolation by distance in *Fucus vesiculosus* that was previously thought to not exist in Gulf of Maine populations (e.g., Muhlin and Brawley 2009, Coyer et al. 2011a). Seemingly random variation in inbreeding and genetic differentiation is indicative of chaotic genetic patchiness in widely distributed organisms (e.g., Hogan et al. 2010). Circulation patterns and wrack deposition may explain regional variation in differentiation and high gene flow to the upper intertidal limits of *F. vesiculosus.* Higher genetic differentiation between lower-limit populations, without isolation by distance, could be generated by among site differences in adaptation to the local environment such as herbivory, temperature, or nutrient availability.

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Chapter 3

INTRASPECIFIC DIFFERENTIATION ACROSS A STEEP ENVIRONMENTAL GRADIENT VARIES AMONG REGIONS

Abstract

Rocky intertidal shorelines are characterized by steep environmental gradients, where abiotic stress increases and biotic stress decreases at higher tidal elevations. Intraspecific variation across this gradient depends on the relative influences of natural selection and dispersal. However, populations at a given site are nested within larger-scale geographic gradients which could interact with the local-scale gradient to affect rates of dispersal and the strength or mechanism of environmental influence on organisms. Thus, intraspecific differentiation across a steep, local environmental gradient could vary among regions. We combined a reciprocal transplant experiment with genetic surveys to test the hypothesis that intraspecific differentiation across tide heights in the seaweed *Fucus vesiculosus* would vary among regions (northeast, central, and southern) in the Gulf of Maine, USA. Reciprocal transplant experiments between the upper and lower limits of *F. vesiculosus'* intertidal distribution revealed regional differences in adaptation to tide height and variation in response of relative growth and nutrient-use efficiency. Levels of genetic differentiation provided some support for these observations and, importantly, the magnitude of genetic differentiation seems to be driven by biological processes rather than neutral processes (e.g., dispersal distance). Steep, local gradients provide insight into how environmental variation influence organisms and populations. However, environmental variation at large spatial scales may interact with local

gradients to alter the underlying ecological or evolutionary mechanisms that drive intraspecific differentiation.

Introduction

Adaptation to a local environmental gradient can generate genetic or phenotypic intraspecific diversity. However, the degree of adaptation to a gradient depends on both population dynamics (e.g., gene flow and dispersal) and the steepness of the gradient (e.g., the intensity of selection) (Linhart and Grant 1996, Lenormand 2002). These factors may vary among populations resulting in different patterns of adaptation to the local environmental gradient and, consequently, in local intraspecific variation. This variation, in turn, will have emergent effects if local adaptation within a particular species influences community and/or ecosystem-level processes (e.g., primary production, nutrient cycling) (Hughes et al. 2008, Whitlock 2014).

Reciprocal transplant experiments, or common garden experiments, can reveal genetic or environmental influences on phenotype. Alternative patterns of phenotype expression can arise if genotypes vary in their environmental sensitivity (e.g., 'genotype x environment' interactions) or if genotypes are non-randomly distributed across environments (e.g., cogradient [CoGV] or countergradient [CnGV] variation) (Conover and Schultz 1995, Kawecki and Ebert 2004). Many studies have found evidence of local adaptation in which genotypes of local populations outperform genotypes from 'foreign' populations when grown under local environmental conditions (e.g., Chapin and Chapin 1981, Steiner and Berrang 1990, Joshi et al. 2001). Additionally, natural selection can act to generate populations of genotypes that out-perform or

under-perform (e.g., fast or slow growers) generating population differentiation over environmental gradients (e.g., CoGv or CnGV [Chapin and Chapin 1981, Álvarez et al. 2006, Hice et al. 2012]). Whereas differentiation is often thought to occur between geographically disparate or isolated populations, several recent reviews have found little to no relationship between distance and the strength of differentiation between populations (Leimu and Fischer 2008, Hereford 2009, Richardson et al. 2014).

Intertidal rocky shores are characterized by steep environmental gradients that occur over scales of a few to less than 100 meters (Helmuth and Hoffman 2001). Environmental factors are correlated with tide height, and early recognition of this pattern led to seminal experimental work on the causes of zonation among species in this habitat (Baker 1909, 1910). The most stressful abiotic conditions (e.g., desiccation, thermal variation) occur in the high intertidal zone, where marine organisms are exposed to terrestrial conditions for long periods of time. In contrast, biotic factors (e.g., competition, predation) typically limit the distribution and abundance of organisms lower on the shore. Many intertidal organisms occupy distinct intertidal zones due to adaptations to particular environmental conditions and/or species interactions (Menge and Branch 2001).

Intraspecific phenotypic and/or genetic differentiation across intertidal gradients has been documented in the invertebrates *Bembicium vittatum* (Johnson and Black 2008), *Littorina saxatilus* (Johannesson et al. 1995, Pardo and Johnson 2005, Grahame et al. 2006), and *Semibalanus balanoides* (Schmidt and Rand 1999, Schmidt et al. 2000), and in the seaweeds *Chondrus crispus* (Krueger-Hadfield et al. 2013), *Gracilaria gracilis* (Engel et al. 2004), *Silvetia compressa* (Hays 2007), and *Ulva linza* (Innes 1988). Studies that included multiple sites in their investigation have documented among-site disparity in results when there may be potential site-level variation in the strength of selection across the species' intertidal distribution. In the acorn

barnacle *S. balanoides*, greater genetic differentiation at putatively adaptive loci to thermal stress, between upper and lower intertidal barnacles, was found at sites with overall higher temperatures (i.e., greater thermal stress) (Schmidt and Rand 1999). In the seaweed *S. compressa*, there was a greater home height advantage in growth and offspring survival at sites where the gradient in environmental stress between the upper and lower limit was greatest, despite shorter distances and chance for greater dispersal between zones (Hays 2007). These examples demonstrate that the strength of selection along a gradient in intertidal elevation can influence phenotypic and genetic diversity of organisms even when dispersal may be high. Further, the above studies were conducted among sites that spanned less than 100 km; therefore, variation in intraspecific differentiation along the intertidal gradient is hypothesized to differ among even larger spatial scales.

The Gulf of Maine provides a unique opportunity to compare geographic variation in population-level processes given latitudinal gradients in tidal amplitude, temperature, and nitrate availability (Apollonio 1979). This latitudinal environmental variation in the Gulf of Maine influences intertidal community structure (Bryson et al. 2014), species interaction strength (Kordas and Dudgeon 2010), and may also influence population-level processes. Tidal amplitude is 1.6-fold higher in the northeast compared to the southern Gulf of Maine. This increases the linear distance, and may reduce dispersal, between the edges of organisms' intertidal distribution (Appendix B). However, average summer air and water temperatures increase 8^{o}C from the northeast to the southern Gulf of Maine. In the south, the difference in the amount of stress experienced by individuals at the edges of their intertidal distribution is greater due to warmer temperatures and lower nitrate levels (Appendix B) leading to a steeper selective gradient. Thus,

intraspecific differentiation across the intertidal gradient may vary among regions in the Gulf of Maine due to variation in dispersal potential or strength of selection.

Fucus vesiculosus Linnaeus is a common brown seaweed in estuarine and rocky shore communities throughout the temperate North Atlantic Ocean (Lüning 1990). In the Gulf of Maine, it has a wide intertidal distribution occurring from the low to high intertidal zones (Appendix B; Benes and Bracken 2016) and is one of four fucoid seaweed species that are primary space-holders on moderately wave-exposed shores (*K. Benes* personal observation). *F. vesiculosus* is a dioecious broadcast spawner with male and female individuals occurring in ~1:1 ratio in populations (Berndt et al. 2002, *K. Benes* personal observation). Although reproductive individuals can be found year-round, peaks in reproduction occur in spring and fall (Berndt et al. 2002). Gamete release can occur when individuals are emersed or during daytime incoming tides (i.e., while submerged), and eggs are negatively buoyant, resulting in dispersal distances from millimeters to meters at a particular site (Dudgeon et al. 2001, Ladah et al. 2008, Muhlin et al. 2011). Importantly, synchronous spawning in *F. vesiculosus* is dependent on calm, sunny conditions, and climate-based models suggest increased asynchrony in gamete release in the northeast Gulf of Maine. Along with the large tidal amplitude and greater linear distance, asynchrony in gamete release could further limit dispersal across the edges of *F. vesiculosus*' intertidal distribution in this region. However, mature thalli can be detached and transported in off-shore currents resulting in gene flow between sites (Muhlin et al. 2008, Muhlin and Brawley 2009), which could swamp local-level processes and reduce local adaptation and differentiation.

To address the hypothesis that genetic and phenotypic differentiation along local environmental gradients will vary latitudinally, we conducted field observations and experiments at sites within three different regions spanning ~400 km of coastline (Appendix A and B). Using

the brown seaweed *Fucus vesiculosus*, and the intertidal and latitudinal environmental gradients in the Gulf of Maine, we asked the following questions: (a) Does phenotypic differentiation between the limits of *F. vesiculosus'* intertidal distribution change latitudinally? (b) Does adaptation and acclimation to tide height influence nitrogen use efficiency (i.e., allocation of nitrogen towards growth)? and (c) Does genetic differentiation between upper and lower intertidal individuals vary latitudinally? Based on the findings of previous studies (Schmidt and Rand 1999, Hays 2007), we hypothesized we would find greater phenotypic differentiation between tide heights in southern locations because higher temperatures and lower nutrient availability would generate a steeper selective gradient in this region.

Materials & Methods

Study Site and Species

To investigate geographic variation in intraspecific differences across the intertidal gradient, we made observations of genetic differentiation and conducted experiments on phenotypic differentiation in the rockweed *Fucus vesiculosus* L. (Ochrophyta, Phaeophyceae) at sites within three regions of the Gulf of Maine. Sites spanned the latitudinal gradient in tidal amplitude, temperature, and seawater nutrient availability (Appendix B) and were chosen for accessibility and similarity in exposure and community composition (*K. Benes* unpublished data).

Phenotypic Differentiation – Relative Growth

To test for regional differences in phenotypic variation across the intertidal gradient, we conducted a reciprocal transplant experiment and measured growth of *F. vesiculosus*. Experiments were conducted in three regions of the Gulf of Maine, at two sites within each region, corresponding to sites we used in our genetic analyses (Appendix B). Experiments were repeated two to three times at each site (trials) and, due to the logistical constraints of conducting experiments across such a large distance, experiments were conducted at different times across regions and trials (Appendix B).

F. vesiculosus individuals were collected from the upper and lower limits of its distribution (n = 40 individuals per tide height location). Half were placed back into their original ('home') location as a control, and half were transplanted to the opposite ('transplant') intertidal location. This resulted in four possible treatment combinations: upper-to-upper (i.e., upper limit individuals transplanted back to their home tide height), upper-to-lower, lower-to-lower, and lower-to-upper (n = 20 per treatment combination per site per season). Specimens were collected at 1-m intervals, parallel to the shoreline, by scraping individuals off the rocky substratum at the base of their holdfast (i.e., substratum attachment site). Individuals were stored in a cool dark location and transported to flow-thru seawater tables at local marine laboratories (northeast: University of Maine's [UM] Downeast Institute; central: UM Darling Marine Center; southern: Northeastern University's Marine Science Center). All specimens were hydrated in seawater overnight before taking measurements (see below). Following initial measurements, individuals were transplanted back to the intertidal habitat into 25 x 25 cm plots that had been cleared of all organisms. Plots were spaced 1-m apart along the upper-most and lower-most edges of the species' intertidal distribution, and each plot contained two replicates, one upper and one lower

intertidal individual (n = 20 plots per tide height per site per season). Individuals were held in place using a method modified from Hays (2007). Holdfasts were glued to the rock substratum by attaching zip-ties around the stipe and anchoring the ties, and a small portion of the holdfast, into marine epoxy (Z-Spar Splash Zone Compound; Pettit Marine Paint, Rockaway, NJ, USA). Reciprocal transplant experiments were only conducted within sites.

Before and after transplantation, individual wet mass [WM; grams (g)] was recorded and converted to dry mass (DM) using a previously established WM to DM relationship (linear regression; $R^2 > 0.70$; gDM = 0.22 * gWM + 0.64; mean initial size = 3.3 ± 0.11 gDM). We used DM to calculate absolute relative growth rates (RGR; % d^{-1}):

$$RGR = ([(DM_f - DM_i) / DMi] / t) * 100 \quad (1)$$

where DM_f and DM_i were final and initial *F. vesiculosus* dry mass, respectively, and t was the total experimental period in days. Although we began each experiment with balanced replication across treatment combinations, variable weather conditions (e.g., waves, heat stress) resulted in a 0% to 75% loss of replicates and unbalanced replication (see Table 3.1 for final sample sizes).

To account for unbalanced sample size and random effects (sites and trial within sites) we used linear-mixed effects models and type III F-tests (analysis of variance; ANOVA) with Satterthwaite approximation of degrees of freedom to test for the fixed effects of region, transplant height, and home height (Zuur et al. 2009). We used the weighted Akaike Information Criterion (wAIC) to select the best fit model, comparing the full model (i.e., main effects and all possible interactions) to reduced models using a backwards selection approach to removing non-significant model terms. All analyses were conducted using the packages ‘lme4’ and ‘lmertest’ (Bates et al. 2015) in R version 3.3.1 (R Core Team 2016). All other analyses were carried out using the base statistical package in R unless otherwise stated.

Table 3.1. Final sample sizes for each site x treatment combination for growth and tissue nutrient measurements. See Table 1 in main text for site information.

			Home Height			
			Growth		Tissue Nutrient	
Region & Site	Trial	Transplant Height	Upper Limit	Lower Limit	Upper Limit	Lower Limit
NE A	1	Lower Limit	15	15	7	8
		Upper Limit	14	10	12	8
	2	Lower Limit	16	15	16	14
		Upper Limit	12	13	11	9
NE B	1	Lower Limit	17	17	5	13
		Upper Limit	12	8	8	6
	2	Lower Limit	20	17	13	11
		Upper Limit	9	11	7	7
CE A	1	Lower Limit	12	13	8	9
		Upper Limit	6	6	4	4
	2	Lower Limit	14	14	14	12
		Upper Limit	8	12	8	11
CE B	1	Lower Limit	13	10	13	10
		Upper Limit	5	9	5	8
	2	Lower Limit	14	17	13	15
		Upper Limit	9	11	7	7
SO A	1	Lower Limit	16	19	6	7
		Upper Limit	9	7	2	5
	2	Lower Limit	8	11	7	9
		Upper Limit	6	10	6	8
	3	Lower Limit	5	9	3	8
		Upper Limit	4	6	4	5
SO B	1	Lower Limit	10	12	5	7
		Upper Limit	6	12	6	3
	2	Lower Limit	16	10	13	9
		Upper Limit	7	11	6	9
	3	Lower Limit	13	17	12	16
		Upper Limit	7	7	6	6
Total			*294*	*322*	*225*	*239*

Phenotypic Differentiation - Nutrient Use Efficiency

In addition to growth, we investigated variation in nitrogen use efficiency (NUE) associated with differences in growth across tide heights. At the start and end of transplant experiments, a small portion of vegetative tissue (~5% of mass) was removed from each

individual for elemental analysis. Tissue carbon (%C) and nitrogen (%N) of dried and ground *F. vesiculosus* were quantified using an elemental analyzer (FlashEA 1112; Thermo Scientific; Waltham, Massachusetts, USA). Initial and final values of %C and %N were converted to milligrams (mg) based on the dry mass of individual replicates. We then calculated NUE as the amount of biomass produced per unit nitrogen acquired (Bridgham et al. 1995). NUE was determined by calculating the slope of relative growth rate (% d^{-1}) over the change in total nitrogen (nitrogen acquisition; ΔmgN d^{-1}). Calculations were made using *F. vesiculosus* individuals as replicates, and slopes were estimated by region for each treatment combination. Since *F. vesiculosus* can gain and loose mass, our NUE metric represents the amount of biomass lost or gained per change in tissue nitrogen. Therefore, greater NUE (slope values) can represent more biomass produced and/or a greater amount of biomass lost per unit change in nitrogen.

To compare NUE of *F. vesiculosus* during the transplant experiment, we included ΔmgN d^{-1} as a covariate in the linear-mixed effects model for growth described above. This allowed for tests of NUE (% d^{-1} / ΔmgN d^{-1}) across regions, transplant height, home height, and their interactions. We found interactions between fixed effects and ΔmgN d^{-1} (see *Results – Nutrient Use Efficiency*). Therefore, we ran linear models, accounting for the random effects of site and trial within site, to calculate the slope (NUE ± 95% confidence intervals [CIs]), for each unique 'region x transplant height x home height' combination. Over a wide range of nitrogen levels, biomass production is not expected to be linearly related to tissue nitrogen (Pastor & Bridgham 1999). Over our entire data set the relationship was non-linear; however, over the range of growth and nitrogen values for each region x treatment combination the best-fit relationship as determined by *w*AIC was linear (see *Results – Nutrient Use Efficiency*).

Genetic Differentiation

To quantify genetic differentiation between upper and lower limits of the intertidal distribution of *F. vesiculosus*, we sampled individuals from three sites within each of three Gulf of Maine regions from June to August 2010 (Appendix B). At each site, four transects were laid from low to high on the shore, at random locations along a 50 m transect line that ran parallel to the shore. Quadrats (25 cm x 25 cm) were placed on the substratum every 3-m, and the tidal elevation and the abundance and identity of all species were recorded. When *F. vesiculosus* was present, vegetative apical tissue samples (~5 cm) of up to six individuals were collected for genetic analyses. Samples were rinsed in freshwater, patted dry, and preserved in silica gel as voucher specimens and for subsequent DNA extraction. Though *F. vesiculosus* is present throughout the intertidal gradient, we selected samples from the upper- and lower-most quadrats from each transect in which *F. vesiculosus* was present for genotyping. We aimed to genotype 20 individuals per tide height per site, and actual sample sizes are reported in Table 3.2 (n = 8 - 23 per tide height / site / locus). We amplified each sample at seven microsatellite loci: L20 and L58 (Engel et al. 2003); F9, F19, F21, and F42 (Coyer et al. 2009); and Fsp 1 (Perrin et al. 2007). Detailed methods for amplification and scoring of individuals can be found in Benes et al. *in prep*.

Samples were used to identify genetic variation and differentiation between putative upper and lower intertidal populations of *F. vesiculosus.* First, to determine how much variation in genetic structure was attributable to each sampling level (i.e., quadrat- to regional-scale) we conducted a nested-hierarchical analysis of variance using the R-package 'HierFstat' (Goudet 2005). This analysis calculates variance components for each nested sampling level, and significance due to each level, by randomizing genotypes among replicate units within higher

Table 3.2. Sample sizes for microsatellite genetic analyses for each locus by region (northeast [NE], central [CE], and southern [SO]), site (A - C), tide height (lower and upper), and quadrats (Q) within tide heights. See Table 1 for site information.

	Locus						
Region, Site, Tide Height	F09	F19	F21	F42	Fsp1	L20	L58
NE A							
Lower	15	15	15	15	15	15	15
Upper	22	22	23	23	23	23	23
NE B							
Lower	21	21	21	18	21	21	21
Upper	18	19	19	19	19	19	19
NE C							
Lower	12	10	12	12	12	11	12
Upper	20	20	20	20	20	20	20
CE A							
Lower	21	16	21	20	21	21	21
Upper	20	20	20	20	20	20	20
CE C							
Lower	19	12	19	19	19	19	19
Upper	17	9	17	17	17	17	17
CE B							
Lower	20	20	20	20	20	20	20
Upper	19	18	19	19	19	19	18
SO C							
Lower	18	18	18	18	18	18	18
Upper	22	23	23	21	23	23	23
SO A							
Lower	22	18	21	22	22	22	22
Upper	20	16	20	20	20	20	20
SO B							
Lower	15	15	15	15	15	15	15
Upper	10	8	10	10	10	10	9
Grand Total	*331*	*300*	*333*	*328*	*334*	*333*	*332*

sampling levels and comparing computed differentiation (‘*F’*) to the null hypothesis of no differentiation between units (*P*-values based on 10,000 randomizations [Goudet 2005]). Second, we calculated Weir and Cockerham’s (1984) multi-locus F_{ST} and loci-specific F_{ST} values for all possible pair-wise combinations in our data set, allowing for comparison of level of differentiation within a site (i.e., between upper and lower edge *F. vesiculosus*), while accounting for gene flow between sites and among regions, using FSTAT version 2.9.3 (Goudet 2001). We then performed separate correlation tests between within-site F_{ST} and latitude, vertical distance, and difference in submergence time between upper and lower limit *F. vesiculosus*. These tests quantified the relationship between genetic differentiation and geography, potential dispersal distance, and the magnitude of the difference in emersion stress, respectively.

Results

Phenotypic Differentiation – Relative Growth

The relative growth rate (RGR; % day^{-1}) of *Fucus vesiculosus* during the reciprocal transplant experiment differed among regions and growth was highest in the central Gulf of Maine transplant locations (Fig. 3.1 and Table 3.3 [ANOVA; $F_{2, 3.25} = 8.16$, $P = 0.05$]). Between transplant locations, *F. vesiculosus* transplanted to the lower limit of its intertidal distribution had ~1.1% d^{-1} greater relative growth rates compared to individuals transplanted to its upper limit (ANOVA; $F_{1, 603.90} = 107.77$, $P<0.001$); a pattern that was consistent across regions (ANOVA; ‘Region x Transplant Height’, $F_{2, 602.09} = 1.18$, $P = 0.31$). Differences in relative growth rate between home heights depended on region (ANOVA; ‘Region x Home Height’, $F_{2, 600.22} = 5.50$, $P = 0.004$). In the central Gulf of Maine only, *F. vesiculosus* originally from its upper intertidal

limit had ~2.4-fold higher relative growth rates compared to lower limit individuals (Fig. 3.1B; post-hoc test, $P<0.05$).

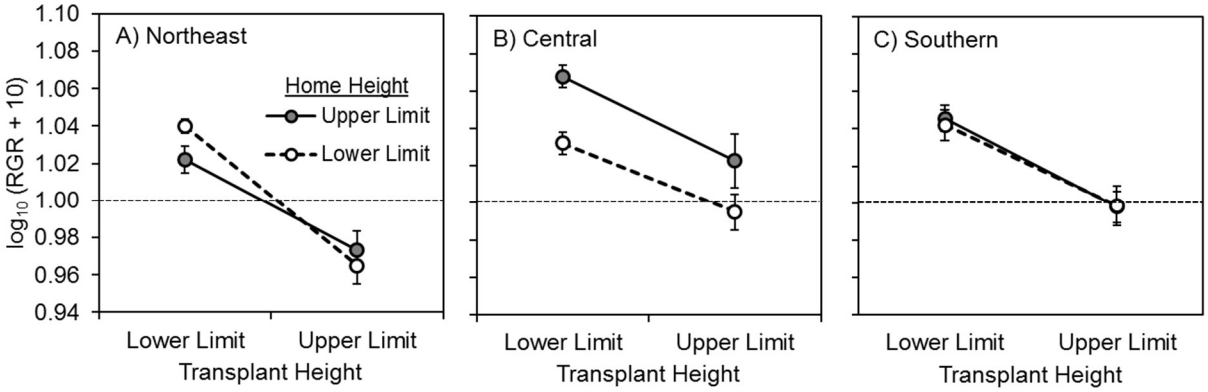

Figure 3.1. Mean (± SE) log-transformed relative growth rate (RGR; % day^{-1}) of *Fucus vesiculosus* during reciprocal transplant experiments in the (A) northeast, (B) central, and (C) southern Gulf of Maine. Dashed line indicates no growth (RGR = 0), mean RGR below this line indicates *F. vesiculosus* senesced during the experiment. RGR was significantly different between home heights in the central Gulf of Maine (post-hoc test; $P < 0.05$).

Table 3.3. Analysis of Variance (ANOVA) results for effects of region, transplant height (TH), home height (HH), and all possible interactions on relative growth rate (RGR; % day^{-1}) of *F. vesiculosus*. A linear-mixed effect model was used to account for the random effects of site and trials within site (N=616). A reduced model, without the 'Region x TH x HH' and 'TH x HH' terms, was the best fit model as determined by weighted Akaike Criterion (wAIC$_{reduced}$ = 0.99). RGR was transformed as $\log_{10}$ (RGR + 10). Initial size differences were accounted for using $\log_{10}(DM_i)$ as a covariate.

Fixed Effects	**MS**	**df_{Num}**	**df_{Den}**	**F-value**	**P-value**
Region	0.025	2	3.25	8.16	**0.05**
Transplant Height	0.328	1	603.90	107.77	***< 0.001***
Home Height	0.005	1	601.36	1.58	0.21
$\log_{10}$ (DM_i)	0.143	1	29.09	47.03	***< 0.001***
Region x TH	0.004	2	602.09	1.18	0.31
Region x HH	0.017	2	600.22	5.50	***0.004***
TH x HH	-	-	-	-	-
Region x TH x HH	-	-	-	-	-

Random Effects	**N**	**Variance**
Trial(Site)	14	2.02E-05
Site	6	2.67E-05
Error		3.04E-03

Phenotypic Differentiation – Nutrient Use Efficiency

Relative growth (% d^{-1}) was positively related to tissue nitrogen acquisition (ΔmgN d^{-1}) (Fig. 3.2 and Table 3.4). Among regions, *F. vesiculosus* in the central and southern Gulf of Maine had more than 2-fold higher NUE compared to *F. vesiculosus* in the northeast (NUE: northeast = 0.66 ± 0.05 %DM mgN^{-1}, central = 1.53 ± 0.10 %DM mgN^{-1}, south = 1.35 ± 0.11 %DM mgN^{-1} [ANOVA; 'Region x $\log_{10}$ [ΔmgN d^{-1} + 10]', $F_{2,444.32} = 48.33$, $P < 0.001$]). Between home heights, *F. vesiculosus* from the upper intertidal zone had 43% higher NUE (1.07 ± 0.07 %DM mgN^{-1}) than *F. vesiculosus* from the lower intertidal zone (0.75 ± 0.05 %DM mgN^{-1}) (ANOVA; 'Home Ht. x [ΔmgN d^{-1} + 10]', $F_{2,443.10} = 20.55$, $P<0.001$). Differences in slopes between transplant heights varied by region (ANOVA; 'Region x Transplant Ht. x [ΔmgN d^{-1} + 10]', $F_{2,443.03} = 5.06$, $P = 0.01$). In the southern Gulf of Maine, NUE was 57% higher in lower limit intertidal transplants (NUE_{Lower}: 1.57 ± 0.15 %DM mgN^{-1}) compared to upper limit intertidal transplants (NUE_{Upper}: 1.00 ± 0.15 %DM mgN^{-1}), but was similar across transplant heights elsewhere in the Gulf of Maine (Fig. 3.2A-F). Importantly, following separate slope calculations for each unique treatment combination, a clear pattern of differences between home heights was evident (two-sample t-test, $P < 0.05$). NUE of upper-limit compared to lower-limit *F. vesiculosus* was greater in the northeast at both transplant heights (Fig. 3.2A, D), in the central region at the lower limit transplant height (Fig 3.2B, E), and in the southern Gulf of Maine in the upper limit transplant height (Fig. 3.2C, F).

Accounting for these differences in NUE (i.e., using ΔmgN d^{-1} as a covariate) revealed an interaction between region, transplant height, and home height (Table 3.4; ANOVA; $F_{2,442.57} = 2.85$, $P = 0.06$). In the northeast Gulf of Maine, relative growth rate at the lower transplant height depended on home height, with individuals originally from the lower intertidal limit gaining

biomass ~4-fold faster than individuals originally from the upper intertidal limit (ANOVA; 'Transplant Ht x Home Ht', $F_{1,145.96} = 2.1$, $P = 0.03$). In the central Gulf of Maine, individuals from the upper intertidal zone had overall higher growth rates than individuals from the lower intertidal zone (ANOVA; 'Home Ht', $F_{1,140} = 15.56$, $P < 0.001$). However, in the southern Gulf of Maine, changes in biomass were driven by transplant height only (ANOVA; 'Transplant Ht', $F_{1,153} = 15.41$, $P < 0.001$). Importantly, these results suggest possible differentiation by home height in the northeast and countergradient variation in the central Gulf of Maine – patterns that are associated with varying levels of nitrogen use efficiency.

Table 3.4. ANOVA results for effects of region, transplant height (TH), home height (HH), the change in tissue nitrogen (ΔmgN d^{-1}), and all possible interactions on the relative growth of *F. vesiculosus*. A linear-mixed effect model was used to account for the random effects of site and trials within sites (N=464). Results below are from the best fit model after removal of $\log_{10}$ (DM_i) and 'Region x HH x ΔmgN d^{-1}' (wAIC$_{reduced}$ = 0.99), 'TH x HH x ΔmgN d^{-1}', and 'Region x TH x HH x ΔmgN d^{-1}' interactions, as compared to the full model (wAIC$_{full}$ < 0.01).

Fixed Effects	**MS**	**df$_{Num}$**	**df$_{Den}$**	**F-value**	**P-value**
Region	0.063	2	445.01	45.90	***< 0.001***
Transplant Height	0.003	1	444.53	2.26	0.13
Home Height	0.041	1	443.24	29.83	***< 0.001***
$\log_{10}$ (ΔmgN d^{-1} + 10)	0.722	1	444.33	529.42	***< 0.001***
Region x TH	0.007	2	443.22	5.30	***0.01***
Region x HH	0.004	2	443.17	3.19	***0.04***
TH x HH	0.004	1	442.56	2.70	0.10
Region x $\log_{10}$ (ΔmgN d^{-1} + 10)	0.066	2	444.32	48.33	***< 0.001***
TH x $\log_{10}$ (ΔmgN d^{-1} + 10)	0.004	1	444.44	3.21	0.07
HH x $\log_{10}$ (ΔmgN d^{-1} + 10)	0.042	1	443.19	30.51	***< 0.001***
Region x TH x HH	0.004	2	442.55	2.85	0.06
Region x TH x $\log_{10}$ (ΔmgN d^{-1} + 10)	0.007	2	443.03	5.06	***0.01***

Random Effects	**N**	**Variance**
Trial (Site)	14	0.00E+00
Site	6	2.20E-05
Residual		1.36E-03

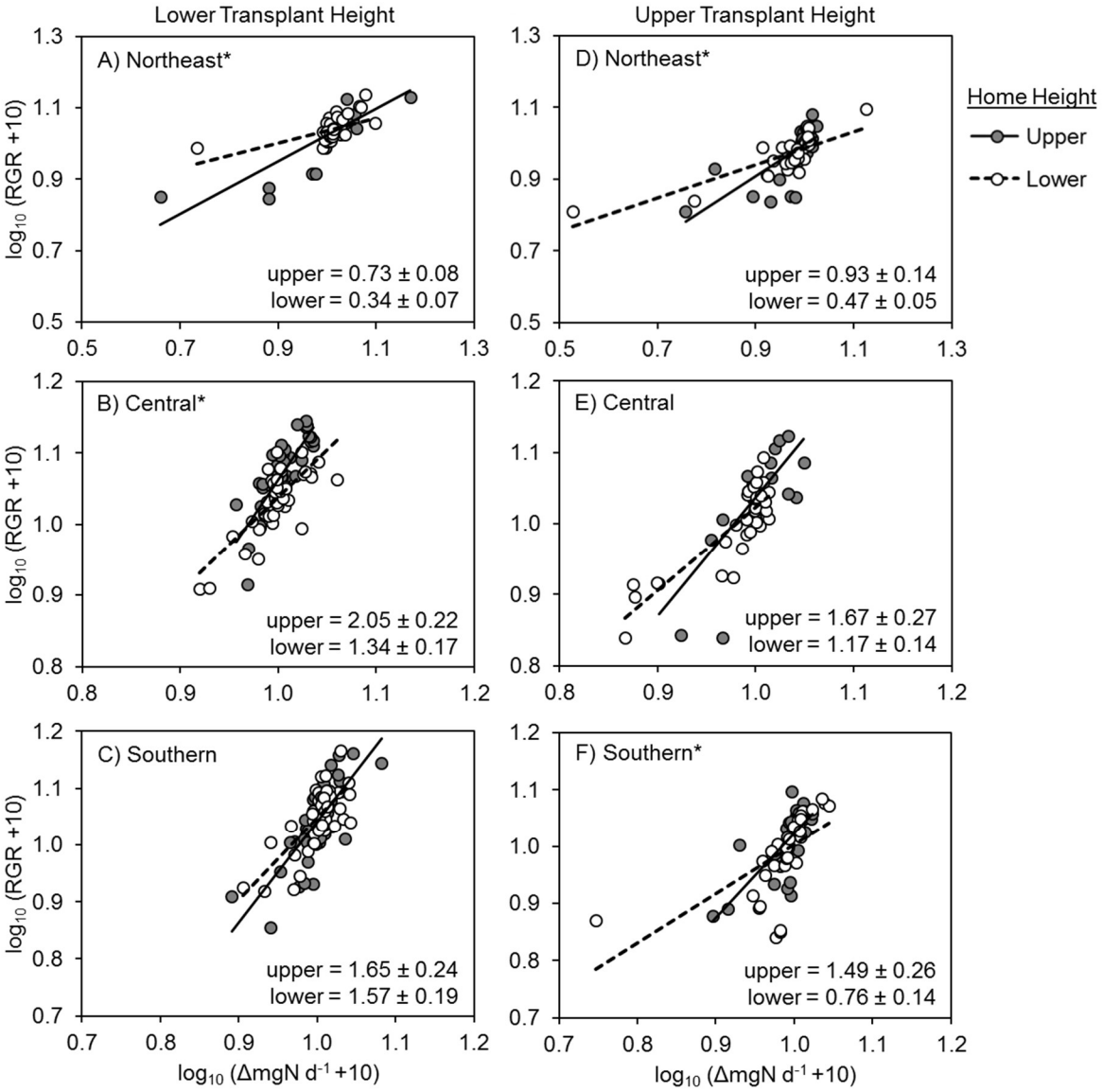

Figure 3.2. The relationship between the change in tissue nitrogen (ΔmgN d^{-1}) and relative growth rate (RGR, % d^{-1}) for individuals transplanted to *Fucus vesiculosus'* lower (A-C) and upper (D-F) intertidal limits within each region. Slopes (± SE), or nutrient use efficiency estimates (= RGR mgN^{-1}; linear-mixed effects model, $P<0.05$), for each home location (lower limit: dashed line and open dots; upper limit: solid black line and grey dots) are given in the lower right hand corner of each panel. Significant differences in nutrient use efficiency between home heights within transplant heights are denoted by asterisks next to the region name (two-sample t-test; $*P < 0.05$). Note axes values vary by region.

Genetic Differentiation

Multi-locus and single-locus hierarchical analyses demonstrated significant genetic structure of *F. vesiculosus* across multiple sampling levels in the Gulf of Maine (Table 3.5). Multi-locus hierarchical analysis showed significant variation among sites within regions, between tide heights within sites, and quadrats within tide heights. Two of the seven microsatellite loci showed significant genetic differentiation between tide heights within sites: F09 and F21, explained 6.70% and 1.22% of variation, respectively (Table 3.5). Loci-specific variation among regions (F21 and F42), sites within regions (F19 and L20), and among quadrats within tide heights (F19, F42, Fsp1, and L20) was also revealed (Table 3.5).

Table 3.5. The percent variation in genetic structure explained by each hierarchical sampling level. Multi-locus and locus-specific estimates are shown and significant values are indicated ($^{\dagger}P \leq 0.06$, $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$). See Table 3.2 for sample sizes.

		Locus						
Test	Multi-locus	F09	F19	F21	F42	Fsp1	L20	L58
Among Regions	0.82	1.81	0.00	0.30*	5.28*	0.00	0.00	0.00
Among Sites w/in Regions	0.78***	0.00	0.72*	0.00	0.00	0.74	4.82**	0.90
Between Tide Heights w/in Sites	1.44*	6.70*	0.33	1.22†	2.83	0.83	1.59	0.00
Among Quadrats w/in Tide Height	2.06***	2.92	1.29**	0.00	2.92†	4.08**	2.60**	10.23
Among Individuals w/in Quadrats	0.00	0.00	0.00	0.00	0.00	0.00	9.33	0.00
Within Individuals	94.90	88.58	97.66	98.48	88.97	94.35	81.65	88.87

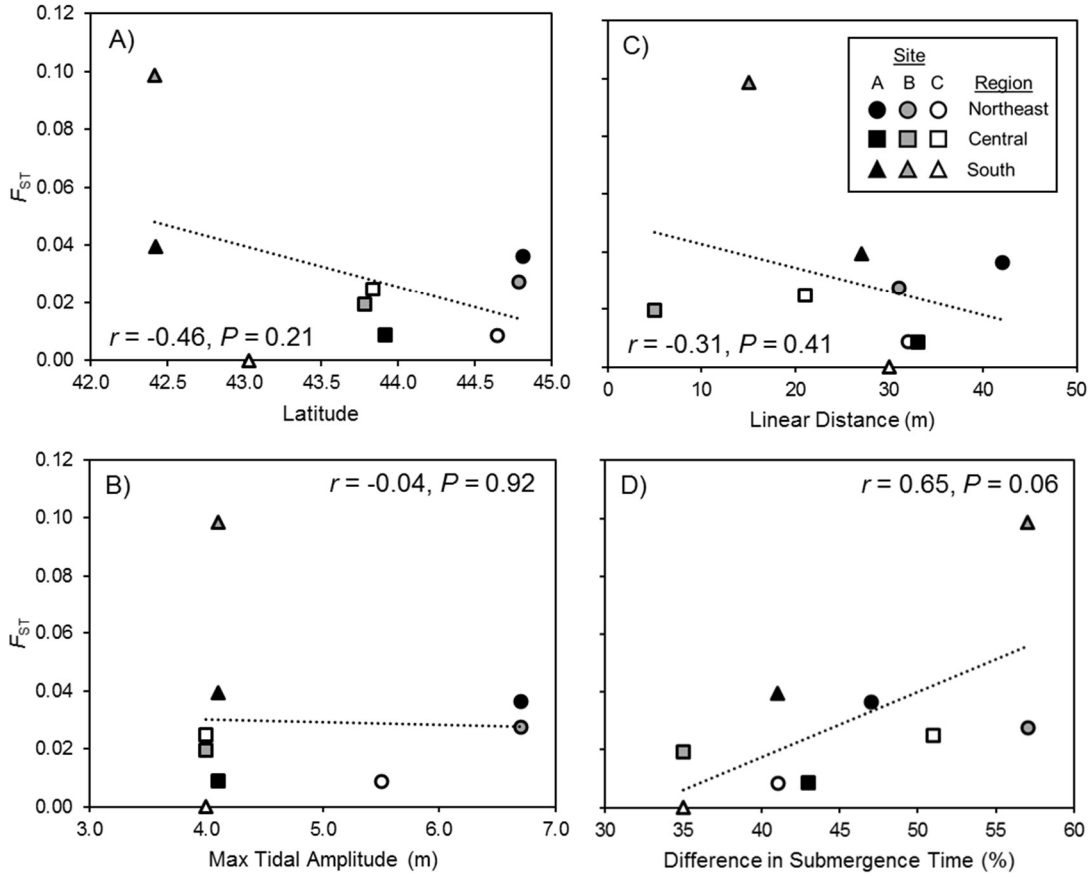

Figure 3.3. Associations between genetic differentiation (F_{ST}) between upper and lower-limit *Fucus vesiculosus* and A) latitude, B) maximum tidal amplitude (meters), C) linear distance (meters) between the upper and lower edges of *F. vesiculosus'* intertidal distribution, and D) the difference in submergence time (%) between upper and lower individuals. Pearson's product-moment correlation (r) and significance (P) are provided on each panel. Different regions are indicated by different symbols and sites within region are differentiated by color (see panel C inset). See table 1 for x-axis values.

No pair-wise differentiation tests (F_{ST}) between tide heights within sites were statistically significant ($P > 0.05$). However, multi-locus F_{ST}-values between tide heights were low to moderate (i.e., $F_{ST} \leq 0.15$) and were comparable to site and region differentiation estimates (i.e., tide height F_{ST}: 0.00 to 0.099 compared to among site and region F_{ST}: 0.00 to 0.19). Locus-specific differentiation between tide heights ranged from 0.00 to 0.27. Multi-locus F_{ST} values between tide heights were not correlated with latitude, tidal amplitude, or linear distance between intertidal distributional edges (Fig. 3.3A-C). However, there was a strong positive association

between F_{ST} and the difference in submergence between upper and lower-limit *F. vesiculosus* (Fig. 3.3D). For loci-specific F_{ST}, differentiation between upper and lower-limit individuals was negatively correlated with latitude at loci F09 and F19 but positively at locus L20 (Pearson Product-moment correlation $P<0.05$). Although most loci were positively correlated with the difference in submergence time, only the correlation with locus Fsp1 was significant (Pearson Product-moment correlation $P<0.05$). As with multi-locus F_{ST}, no locus-specific estimates of differentiation were associated with linear distance between distributional edges of *F. vesiculosus* or tidal amplitude.

Discussion

Rocky intertidal shores are characterized by a steep environmental gradient that imposes abiotic and biotic constraints on organismal to ecosystem-level processes. Intraspecific variation across the intertidal gradient may vary latitudinally if large-scale environmental variation mediates the strength of selection or dispersal across a species intertidal distribution. We found evidence of multiple phenotypic responses to the intertidal gradient that varied by region including adaptation, countergradient variation, and plasticity. The response of growth and nitrogen use efficiency to transplantation were not similar, suggesting different relative environmental and genetic influences on these traits. Here we show the interaction between macro- and micro-geographic variation in abiotic and biotic factors can generate among-population variation in phenotypic and genetic diversity along local environmental gradients. In organisms that are foundation species, this could have important consequences for communities and ecosystems.

Relative growth rates were influenced by regional and local environmental conditions. Among regions, *Fucus vesiculosus* had highest growth rates in the central Gulf of Maine followed by the southern and then northeastern regions. Temperature is a likely driver of these large-scale patterns in growth, with lower seawater temperatures driving lower metabolic rates and overall growth in the northeast. However, the higher temperatures in the southern Gulf of Maine may also impose a higher cost of respiration or drive resource allocation to maintenance and repair such that growth rates are slightly reduced relative to the central Gulf of Maine. Within regions, the steep intertidal gradient also affected growth, with both consistent and region-specific responses to transplant height and home height identified.

Transplant height had a strong and consistent effect on relative growth rates, indicating strong environmental constraints on growth along the intertidal gradient. Transplanting individuals to the upper-limit of the species' intertidal distribution resulted in reduced growth rates relative to individuals transplanted to the lower edge of the distributional limit. Greater durations of exposure to aerial conditions at upper intertidal limits reduces access to dissolved nutrients and may limit net primary productivity and other metabolic processes. If emersion coincides with other stressors, such as extreme temperatures, high desiccation, and changes in salinity, disruptive stress due to aerial exposure can occur, and resources may be allocated towards repairing damaged tissue or maintaining basal functions (see review in Davison and Pearson 1996). Studies comparing interspecific differences in response to emersion show that species living higher on the shore are typically more tolerant and can recover more quickly following emersion (Davison and Pearson 1996). Studies comparing intraspecific variation in seaweed physiology across the intertidal gradient are fewer. In *Fucus gardneri*, absolute rates of photosynthesis and respiration in air and water were found to be similar between individuals at

different tide heights. However, because net productivity is overall higher in water, lower shore individuals are predicted to have overall greater carbon gain compared to individuals higher on the shore due to greater submergence time (Williams and Dethier 2005). To overcome limited resources, upper-limit individuals may have more rapid nutrient uptake rates (Benes and Bracken 2016) or assimilation rates (as measured by enzymatic activity) even during aerial exposure (Murthy et al. 1986). Despite these attributes, the metabolic cost and allocation of resources may limit growth (Chapin 1980), and the relative importance of limitation stress versus disruptive stress in driving growth of intertidal seaweeds is still an open and untested question (Williams and Dethier 2005).

Despite the strong influence of transplant height, effects of home height and interactions between home height and transplant height were detectable and varied among regions. In the northeast, there was a small home-height advantage in relative growth rate at each transplant location that was even more apparent after accounting for variation in tissue nitrogen acquisition. This suggests phenotypic specialization (Lortie and Aarssen 1996) or adaptation (Kawecki and Ebert 2004) to tide height which may be the result of several population-level processes (see below). In the central Gulf of Maine, *F. vesiculosus* originally from the upper distributional limit had overall higher relative growth rates regardless of transplant location. The presence of fast growing genotypes in an environmentally stressful location is indicative of countergradient variation along the intertidal gradient in this region (Conover and Schultz 1995). Importantly, this pattern results in similar rates of growth of individuals at the edges of their distribution, minimizing variation in biomass production and possibly other traits across the intertidal gradient. This could have consequences for the associated community and ecosystem. Lastly, in the south, there were minimal detectable differences in relative growth rates associated with

home height. Environmentally-driven plastic response in growth and other traits may be advantageous in this region with high temperature variation, nutrient-limitation, and other stressors. Alternatively, the climate in the southern Gulf of Maine may constrain phenotypic expression rather than adaptive plasticity.

Physiological traits, other than growth, can vary in their expression following differential environmental and adaptive influences and may provide insight into overall growth. Given that *F. vesiculosus* is an important foundation species on rocky shores, we focused on nitrogen acquisition and use-efficiency (NUE) which could have cascading effects on community and ecosystems as well as organismal physiology. Similarly to growth, we found variation in nitrogen acquisition and NUE attributable to large- and local-scale environments. Nitrogen use was highest in regions where long-term mean ambient nitrate levels are relatively low. The negative relationship between ambient nitrogen availability and NUE is observed frequently in terrestrial plant communities and may result from adaptive responses for overcoming nutrient limitation (Chapin 1980, Vitousek 1982, Bridgham et al. 1995). At the tide height scale, unlike patterns in growth, transplant height did not have as strong or consistent an influence on NUE. Instead, there was among-region variation in both home height and transplant height effects on NUE. In the northeast, *F. vesiculosus* originally from its upper intertidal limit had higher NUE at both transplant heights. Additionally, for both upper- and lower-limit individuals NUE was higher at the upper-limit transplant height in this region. This suggests sufficient nitrogen supply to maintain rates of nitrogen uptake and assimilation, as these processes are positively correlated with ambient nitrate availability in *F. vesiculosus* (Young et al 2007, Benes and Bracken 2016). In the central and southern regions, there were fewer detectable differences in NUE across home heights or transplant heights. Although higher NUE of central Gulf of Maine individuals

originally from the upper-limit is consistent with patterns of growth and suggests that these fast growing genotypes can more efficiently capitalize on higher resources. In contrast, in the central and southern regions, NUE was lower in the upper-limit transplant height suggesting possible allocation of nitrogen to other processes such as heat shock response or photosynthetic pigment production (Chapin et al. 1987).

Intraspecific variation in NUE among regions or across tide heights could be a consequence of morphological or physiological differences that mediate nutrient uptake and assimilation among genotypes (e.g., Li et al. 1991, Hirel et al. 2001). Nutrient uptake in seaweeds occurs via diffusion or active transport directly across the whole thallus; there are no specialized structures such as roots or vascular tissue for nutrient uptake and transport (Hurd et al. 2014). Therefore any morphological adjustments that increase the surface area-to-volume ratio (SA:V) could lead to increases in nutrient uptake rate. SA:V is positively correlated to nutrient uptake (Rosenburg and Ramus 1984), and interspecific variability in hyaline hair production across tide heights has been documented in seaweeds (Hurd et al. 1993). In addition, physiological mechanisms such as greater desiccation enhancement of uptake (Thomas and Turpin 1980, Thomas et al. 1987b), more rapid recovery of uptake following re-emersion (Hurd and Dring 1991), variation in assimilation rates (Murthy et al. 1986), and variation in allocation of nitrogen towards growth can alter NUE. Biomass corrected nitrate flux (i.e., uptake plus assimilation) is higher in upper-limit compared to lower-limit *F. vesiculosus* (Benes and Bracken 2016). Nitrate uptake rates measured in parallel to the current study demonstrated persistently higher uptake by upper-limit *F. vesiculosus* in the northeast and central regions regardless of transplant location. In contrast, nitrate uptake of lower-limit individuals increased when they were moved to higher elevations on the shore (Benes and Bracken 2016). These uptake

experiments were performed on apical tips, so any differences due to whole thallus variation in SA:V or the presence of hyaline hairs were lost. If these traits do vary across tide heights, the different in nutrient uptake may be even greater between upper- and lower-limit *F. vesiculosus*.

Patterns of genetic differentiation of *F. vesiculosus* corresponded, in part, to patterns of phenotypic variation. Genetic variation was attributable to multiple spatial scales, and variation between tide heights within sites was significant overall and at two of the seven loci (Table 3.5). Higher genetic differentiation (F_{ST}) between upper-limit and lower-limit individuals in the northeast at some loci is consistent with the phenotypic differentiation we observed in this region. However, the highest F_{ST} values were found in the south. Importantly, one southern site where differentiation was particularly high had low sample size (Table 3.2) and this may be influencing the high F_{ST} value there. Alternatively, selection in the southern region could be driving genetic differences along the intertidal gradient, but environmental constraints may outweigh genetic influence over phenotypic expression.

Multi-locus F_{ST} was not significantly associated with latitude, tidal amplitude, or vertical linear distance between the limits of *F. vesiculosus'* intertidal distribution. However, F_{ST} increased with greater differences in submergence time between the upper and lower intertidal limits of *F. vesiculosus*. This significant relationship between genetic differentiation and an indicator of the difference in magnitude of stress experienced by upper and lower individuals suggests the potential for selective processes acting at these sites (Fig. 3.3D). Conversely, greater difference in submersion time could increase asynchrony in spawning, reducing dispersal across the species' intertidal distribution. The putatively neutral microsatellite loci used in this study should reflect historic and neutral processes in *F. vesiculosus* populations. However, loci F09 and L20 have been associated with natural selection driven by a salinity gradient over 11.7 km in

northern Norway (Coyer et al. 2011a). Common garden experiments with *F. vesiculosus* adults and offspring and would enhance understanding of the role of neutral and selective processes in shaping among-region variation in local-scale phenotypic diversity.

Species with broad geographic and local distributions face substantial variation in abiotic and biotic factors, and the influence of both environmental and genetic components on traits is not surprising in these widely distributed organisms. Whereas previous studies have demonstrated that the strength of adaptation to the local environmental can vary among populations (e.g. Chapin and Chapin 1981, Hays 2007), our study makes several important contributions. First, we identified that latitudinal and local environmental gradients can *interact* to influence phenotypic and genetic diversity among populations. In particular, local adaptation to tide height in a putatively less stressful region (the northeast Gulf of Maine) was not expected and may be a consequence of limits to gene flow and dispersal at the regional-level. Additionally, the presence of countergradient variation in the central Gulf of Maine is, to the best of our knowledge, the first documentation of such a pattern along the intertidal gradient and adds to a growing number of studies that have identified this on a microgeographic scale (Skelly 2004. Álvarez et al. 2006). Patterns of growth and nitrogen use efficiency in a foundation species indicate that these interactions could have emergent effects beyond the population-level. Processes such as biomass production and nutrient cycling could be constrained by adaptation to tide height in some regions but not others. Lastly, there is growing recognition for the importance of intraspecific variation in communities and ecosystems which has primarily focused on clonal organisms. For sexually reproducing organisms, there is the potential for subastantial turnover in genetic diversity with each generation. Studies that examine how adaptive genetic and phenotypic variation is influenced by and distributed along environmental

gradients could help identify when and where intraspecific variation will be important in these types of organisms.

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Chapter 4

NITRATE UPTAKE VARIES WITH TIDE HEIGHT AND NUTRIENT AVAILABILITY IN THE INTERTIDAL SEAWEED *FUCUS VESICULOSUS*

Abstract

Intertidal seaweeds must cope with a suite of stressors imposed by aerial exposure at low tide, including nutrient limitation due to emersion. Seaweeds can access nutrients only when submerged, so individuals living higher compared to lower on the shore may have adaptations allowing them to acquire sufficient amounts of nutrients to survive and maintain growth. Using a combination of observations and experiments, we aimed to identify intraspecific variation in nitrate uptake rates across the intertidal distribution of *F. vesiculosus* as well as test for acclimation in response to a change in tide height. We replicated our study at sites spanning nearly the entire Gulf of Maine coastline, to examine how local environmental variability may alter intraspecific variation in nitrate uptake. We found that average nitrate uptake rates were ~18% higher in upper compared to lower intertidal *F. vesiculosus*. Further, we found evidence for both acclimation and adaptation to tide height during a transplant experiment. *F. vesiculosus* transplanted from the lower to the upper intertidal zone was characterized by increased nitrate uptake, but individuals transplanted from the upper to the lower intertidal zone retained high uptake rates. Our observations differed among Gulf of Maine regions and among time points of our study. Importantly, these differences may reflect associations between nitrate uptake rates and abiotic environmental conditions and seaweed nutrient status. Our study highlights the importance of long-term variation in ambient nutrient supply in driving intraspecific variation of

seaweeds across the intertidal gradient and local and seasonal variation in ambient nutrient levels in mediating intraspecific differences.

Introduction

Spatial and temporal variation in nutrient availability can limit seaweed growth (e.g., Topinka and Robbins 1976, Chapman and Craigie 1977, Schonbeck and Norton 1979, Wheeler and North 1980) and nutrient content (e.g., Rosenberg et al. 1984, Fujita 1985) and alter the diversity and abundance of seaweed species (e.g., Duarte 1995, Pedersen and Borum 1996, Bracken and Nielsen 2004). Like all intertidal organisms, seaweeds growing on rocky shores must cope with periodic exposure to quasi-terrestrial conditions. Exposure at low tide presents a number of challenges, including temperature stress (Davison and Pearson 1996), desiccation (Dethier et al. 2005), and nutrient limitation (Hurd et al. 2014). The paucity of data on intraspecific differences in seaweeds to withstand nutrient limitation along the intertidal gradient (see Davison and Pearson [1996(Bracken et al. 2011)] for review) limits our understanding of how seaweeds adjust their nutrient physiology in response to simultaneous spatial (i.e., tide height) and temporal (i.e., short-term and seasonal) variation in nutrient supply. Understanding how intertidal seaweeds overcome the profound variability in ambient nutrient supply is critical for determining species abundance and distributions and the nutrient content at the base of marine food webs.

Marine primary producers that are nitrogen (N) deficient or occur in N-limiting environments can compensate with higher uptake rates and/or increased uptake efficiency at low ambient concentrations (e.g., Carpenter and Guillard 1971, D'Elia and DeBoer 1978, Rosenberg

et al. 1984, Fujita 1985, O'Brien and Wheeler 1987). Intertidal seaweeds acquire nutrients while submerged (Hurd et al. 2014), and those living higher on the shore may be more nutrient limited than seaweeds living lower on the shore due to more restricted periods of access during submergence. Accordingly, higher uptake rates, greater total nutrient acquisition during submergence, a greater degree of desiccation enhancement of uptake, and greater nutrient assimilation rates (as measured by enzyme activity) have been found in seaweed species living at higher tidal elevations (e.g., Thomas et al. 1987a, Hurd and Dring 1990, Young et al. 2007a). Similarly, studies examining intraspecific variation in nutrient physiology have shown higher uptake rates by individuals from the upper edges of their intertidal distribution (e.g., Murthy et al. 1986, Phillips and Hurd 2004, Bracken et al. 2011). Additionally, evidence suggests that *Gracilaria pacifica* (Thomas et al. 1987b) and *Porphyra umbilicalis* (Kim et al. 2013) can rapidly acclimate to changes in submergence time (i.e., tide height) via changes in uptake rates and/or enzymatic activity. However, some studies have demonstrated little difference or opposite patterns (i.e., higher rates in individuals from lower on shore compared to higher on shore) in nutrient uptake between individuals at different shore heights (Phillips and Hurd 2003, Bracken et al. 2011). Further, Thomas et al. (1987b) found acclimation to be strongest in *G. pacifica* that was transplanted from their lower- to upper-shore limit. Geographic, local short-term, and/or seasonal changes in ambient nutrient supply could alter the degree of nutrient limitation among seaweeds along the intertidal gradient and may help explain these inconsistent observations of intraspecific differences in nutrient uptake in seaweeds.

Fucus vesiculosus Linnaeus (Ochrophyta, Phaeophyceae) is a conspicuous alga throughout the temperate North Atlantic Ocean (Lüning 1990). Its occurrence in rocky intertidal, estuarine, and brackish subtidal habitats suggests tolerance to a wide range of environmental

conditions and ambient nutrient concentrations. On Gulf of Maine rocky shores in particular, it has a wide tidal distribution occurring from the low (less than1.0-meters above mean lower-low water [MLLW]) to high (greater than2.0-meters above MLLW) intertidal zone. In addition, the Gulf of Maine is characterized by geographic variation in ambient nutrient concentrations. Seasonal changes in surface water turn-over and along-shore currents result in relatively higher average nutrient concentrations in the northeast and seasonal peaks of nutrient availability in the spring and fall throughout the Gulf of Maine (Townsend et al. 1987). Ammonium can be a significant and preferable source of nitrogen for seaweeds (e.g., D'Elia and DeBoer 1978, Phillips and Hurd 2003, Bracken and Stachowicz 2006). In the Gulf of Maine, however, ammonium concentrations are half to two-orders of magnitude lower than simultaneously measured nitrate concentrations (e.g., Holligan et al. 1984, Christensen et al. 1996, Townsend 1998, ammonium range at surface: <0.1 to 0.4 μM) and tissue nitrogen concentrations of *F. vesiculosus* are strongly correlated with ambient nitrate availability (Perini and Bracken 2014). Since empirical evidence suggests that nitrate is an important and potentially limiting source of nitrogen for *F. vesiculosus* in the Gulf of Maine we focused on nitrate availability and uptake for our study.

We evaluated the potential for intraspecific variation and acclimation (i.e., rapid response to environmental change) and/or adaptation (i.e., maintenance of phenotype under changing conditions) in nitrate uptake in response to intertidal elevation among populations of *F. vesiculosus* that experience different long-term, average nutrient levels. Specifically, we tested two main hypotheses: (1) that nutrient uptake rates would be higher in upper shore compared to lower shore individuals and (2) that individuals would acclimate to changes in tide height over a 30-day transplant experiment via changes in nutrient uptake rate. To take advantage of the

natural seasonal and latitudinal variation in ambient nutrient supply in the Gulf of Maine, we conducted observations and experiments across multiple, disparate *F. vesiculosus* populations and at different time points. This allowed us to explore how site-level ambient nutrient concentrations (at the time of field collection) and tidal variation (hours submerged) mediated patterns of nitrate uptake across tide heights and during our transplant experiment.

Materials & Methods

Study Sites and Fucus vesiculosus *Distribution and Collection*

For observations and experiments testing nutrient status and physiology of *Fucus vesiculosus*, seaweed and water samples were collected from sites throughout the Gulf of Maine (Appendices A and B). Sites were chosen based on accessibility and similar wave-exposure and community composition (K. Benes, *unpublished data*). To measure the vertical distribution of *F. vesiculosus* at these sites, transects were laid parallel to the shoreline along the upper and lower edges of the intertidal distribution of *F. vesiculosus*. The tidal elevation of the highest (or lowest) individual at 1-m intervals was recorded (n=20 individuals per transect) relative to MLLW. Maximum tidal amplitude changes latitudinally in the Gulf of Maine, increasing from approximately 4.1-m in the south to approximately 6.7-m in the northeast due to extreme tidal exchange in the Bay of Fundy. Therefore, tidal elevations were converted to the number of hours *F. vesiculosus* was submerged at the lower and upper edges of its intertidal distribution for comparisons. Using data from the nearest locations with published tide heights (Flater 1998), predictions at 5-minute intervals were used to calculate the hours submerged by adding together the number of intervals in a 24-hour period that were at or above a particular elevation, and then

converting the number of intervals to hours or relative submergence time (i.e., 50% = 12 hours submerged per day). Because of the broad intertidal distribution of *F. vesiculosus* in the Gulf of Maine, collections for tissue nutrient content and uptake measurements (see below) were made at low tide during semi-monthly spring tides (i.e., periods of maximal tidal amplitude) to ensure the upper- and lower-most individuals at each site were sampled.

For physiological observations, *F. vesiculosus* individuals were collected haphazardly from the upper and lower edges of its intertidal distribution at each site with a minimum distance of 1-m between each individual. All samples were cleaned of epiphytes and epifauna then chilled and kept in the dark during transport to Northeastern University's Marine Science Center in the southern Gulf of Maine for analyses. Transport lasted from 4 to 8 hours, and southern Gulf of Maine samples were maintained in a cool dark place for at least 4 hours to mimic sample handling from other sites. Portions of vegetative apical tissue (~2 to 3 cm length and ~ 0.5 to 1.5 g wet weight) were then cut from each individual for measurements of tissue carbon and nitrogen concentrations (%C and %N) and nitrate uptake rate (see below for replication and detailed methodologies). Prior to conducting uptake experiments, apical tips were placed in outdoor flow-through seawater tables for a minimum of 24 hours. This holding period was used to fully hydrate samples, allow for tissue healing, and to briefly expose all samples to similar ambient light and ambient nitrate levels following transport and cutting. Apical portions of the thallus are the active growth site (meristem) and contain the greatest tissue %N (Carlson 1991) and have the highest uptake rates (Wallentinus 1984) in *F. vesiculosus*.

Ambient Nutrient Availability and Tissue Nutrient Content

To quantify nutrient availability, 5 replicate water samples (500-mL each) were collected at each site. Samples were filtered (Whatman GF/F) within one hour and frozen for later measurement of ambient nitrate (NO_3^-) and phosphate (PO_4^{3-}) concentrations (μmol L^{-1}) QuickChem FIA 8500 Autoanalyzer; Lachat Instruments; Loveland, Colorado, USA – detection limit: 0.014 μmol L^{-1} nitrate [NO_3^-] and 0.054 μmol L^{-1} phosphate [PO_4^{3-}]). Water samples were collected every 3 to 4 weeks at each site over 2 years (May 2012 – February 2014; no samples December – January and only 1 year at northern Gulf of Maine sites).

To quantify tissue nitrogen (%N of dry tissue) and carbon (%C) in lower and upper shore *F. vesiculosus*, tissue collections were made approximately 3x per year from spring 2012 – spring 2014 (spring, summer, fall) across sites in the Gulf of Maine (n=5 per sampling period / tide height / site). Tissue samples were cleaned of epiphytes, oven dried at 65ºC to constant mass, and then ground to a fine powder using a mixer mill (MM 300; Retsch; Haan, Germany). Approximately 3 mg of dried powdered tissue was used to estimate the %N and %C of *F. vesiculosus* individuals using an elemental analyzer and Aspartic Acid as a standard (FlashEA 1112; Thermo Scientific; Waltham, Massachusetts, USA).

Nitrate Uptake Rates of Upper versus Lower Shore **Fucus vesiculosus**

To test the hypothesis that upper and lower shore individuals would differ in their nitrate uptake rates, we collected individuals of *F. vesiculosus* at the edges of its intertidal distribution at all study sites in May 2012 and measured uptake at four nitrate concentrations. Nitrate uptake rates were measured in 8 1-L chambers using a design modified from Bracken et al. (2011). During the uptake experiment, high water flow (~18 cm/s), saturating light levels (>1000 μmol

photon m^{-2} s^{-1}), and constant temperatures (14.0 ± 0.3 [mean ± SE] ºC) were maintained to maximize nitrate uptake (Hurd et al. 1996, Hurd et al. 2014). Individuals were collected and transported as described above, and nitrate uptake was measured on apical tips within 24 hours following the healing period. Four apical pieces from a single individual were haphazardly assigned to chambers filled with artificial seawater (35‰; Instant Ocean) and, following a 20 minute acclimation period (to chamber conditions), each chamber was spiked with $NaNO_3$ to achieve one of four initial nitrate concentrations: 2, 15, 30, and 50 µmol L^{-1}. After a 5 minute mixing period, water (6-ml) was sampled from chambers every 10 minutes for 50 minutes (n = 6 observations per chamber), and nitrate concentrations were measured as previously described (see *Ambient Nitrate Availability* above). Chambers with obviously spurious data points (e.g., due to problems with the QuickChem Autoanalyzer) were removed from the analysis. The relationship (slope) between time (hours) and nitrate concentration (µmol L^{-1}) was quantified using linear regression to find the rate of uptake (µmol NO_3^- h^{-1} L^{-1}) at each particular nitrate concentration (linear regression, $R^2 > 0.70$, $P < 0.05$). Our measured uptake rates include both 'uptake' (i.e., vacuole filling) and 'assimilation' (i.e., conversion of N into metabolites, etc.) (Pedersen 1994, Taylor and Rees 1999). Compared to other nutrients, such as ammonium and phosphate, there is no strong evidence for an initial 'surge' phase of nitrate uptake in intertidal seaweeds (Thomas & Harrison 1987, Hurd and Dring 1990, Phillips and Hurd 2003). Therefore, we did not include separate measurements to account for different phases or components of uptake.

The rate of uptake (µmol NO_3^- h^{-1} L^{-1}) was divided by the dry tissue mass to calculate biomass-specific uptake rates (V; µmol NO_3^- L^{-1} [g DW]$^{-1}$ h^{-1}) for each apical tip at each initial nitrate concentration (µmol L^{-1}) (i.e., each chamber). Dry tissue mass was determined by

converting wet mass into dry mass using an established relationship determined from samples in the transplant experiment described below (dry mass = wet mass x 0.242; $R^2 = 0.98$, $P < 0.001$). Even though our experimental concentrations included two nitrate concentrations (30 and 50 μmol L^{-1}) that were above those observed in the Gulf of Maine, we found little evidence of saturating uptake rate with higher experimental nitrate concentrations. The lack of saturating uptake rates across experimental nitrate concentrations precluded accurate estimates of traditional uptake kinetic parameters (i.e., Michaelis-Menten model parameters; maximum uptake, V_{max}; half-saturation coefficient, K_s [Berges et al. 1994]). Therefore, we treated our target experimental nitrate concentration (i.e., 2, 15, 30, or 50 μmol L^{-1}) as a fixed factor and analyzed uptake rates using a factorial framework (see *Statistical Analyses*).

Variation in the surface area to volume (biomass) ratio (SA:Vol) and/or the scaling relationship between uptake rate and SA:Vol may be important factors influencing comparisons of uptake rates among species or populations (Hein et al. 1995, Taylor et al. 1998). Since we only used apical portions of thalli for uptake measurements, we chose not to measure SA, as any potential natural variation in SA among sites or tide heights could have been lost in cutting. Data from our field sites showed no difference in the SA:Vol relationship of mid-intertidal *F. vesiculosus* across our study regions (ANCOVA; region x log (SA); $F_{2, 439.25} = 2.2$, $P = 0.11$). Additionally, the scaling relationship between the biomass-specific uptake rate and SA:Vol in our study was not different among regions or tide heights, and there was no significant interactive effect of region and tide height (ANCOVA; $P > 0.2$). We therefore chose to only present biomass-specific uptake rates in our analyses.

Reciprocal Transplant Experiment - Acclimation via Changes in Nitrate Uptake Rates

To test the propensity for seaweeds to acclimate to tide height, we measured the uptake physiology of *F. vesiculosus* before and after a reciprocal transplant experiment. The experiment was conducted at six sites throughout the Gulf of Maine (Appendices A and B) from June to September 2013 to examine potential differences in response that could be due to geographic and local environmental variation. At the beginning of the experiment whole *F. vesiculosus* individuals, separated by a minimum of 1-m, were collected along the upper and lower edges of its distribution using a paint scraper to remove individuals complete with their holdfasts. Individuals were chilled and maintained in the dark during transport (~1-3 hours) to a local marine laboratory (northeast: Downeast Institute; central: Darling Marine Center; south: Marine Science Center). Individuals were placed in indoor flow-through seawater tables overnight for hydration before recording initial biomass (grams [g]; initial average biomass = 25.25 ± 0.03 [mean ± SE] g) and taking ~5% of the biomass (apical tips) of each individual for nitrate uptake measurements. The excised apical tips were kept in the local flow-through seawater tables while the field transplant experiment was established (12 to 24 hours) and then were transported to the southern Gulf of Maine, where nitrate uptake rates were measured (see below).

To establish the field experiment, individuals were transplanted to the intertidal zone either into their home height or opposite height such that there were four treatments: upper – upper (i.e., upper to upper tide height), upper – lower, lower – lower, lower – upper (n = 20 individuals per treatment combination). Twenty plots were established at both the upper and lower edges of the intertidal distribution of *F. vesiculosus*. Plots were cleared of all organisms from a 25 x 25 cm^2 area, and the surrounding fucoid canopy was trimmed so *F. vesiculosus* would not be shaded or abraded. Seaweed individuals were held in place by attaching one zip-tie

around the stipe at the holdfast and looping a second zip-tie through the first to create an anchor. The anchor and a small portion of the holdfast (~0.5-cm) was then submerged into marine epoxy (Z-Spar Splash Zone Compound) affixing it to the rock substratum. Individuals were randomly assigned to plots, and all plots contained two individuals; one from the home location and one from the opposite tide height. Transplants were only conducted within a site, not among sites. After approximately 30 days, individuals were collected from the field and chilled and maintained in the dark during transport to the southern Gulf of Maine for biomass and nitrate uptake measurements of apical tips.

At the beginning and end of the transplant experiment, nitrate uptake experiments were carried out as previously described (see *Nitrate Uptake Rates of Upper versus Lower Shore* Fucus vesiculosus), except that separate individuals were used for each nutrient concentration to allow estimation of population-level nutrient uptake parameters from a larger number of individuals from each experimental treatment (n = 9 to 14 per transplant height / home height / site). Uptake experiments took place 3-9 days after collection from field sites (1-7 days after cutting apical tips), replicates were randomly assigned to nitrate concentrations across days, and nitrate uptake rates did not vary among days (ANOVA, $P > 0.70$).

Environmental Covariates of Nutrient Uptake

Using the factorial variables of region and tide height does not account for among-site variation in ambient nitrate levels and the hours submerged at the time of sample collection. These site-level quantitative variables may influence *F. vesiculosus* nitrate uptake rate and may account for additional variation not included in our factorial analyses. We therefore averaged nitrate uptake rates for each ‘site’ x ‘experimental nitrate concentration’ x ‘tide height’

combination to assess the relationship between uptake and site-level ambient nutrient concentrations on the day of *Fucus* sample collection (i.e., $[NO_3^-]$, $[PO_4^{3-}]$, NO_3^-:PO_4^{3-} ratio) and time submerged (hours) in the 24 hours preceding collection. We did this for the upper versus lower shore experiment and the initial and final measurements of the transplant experiment separately. Additionally, we also examined how the change in nitrate uptake rate (i.e., final mean minus initial mean) was related to the change in ambient nutrient concentrations (i.e., final mean minus initial mean of field site nutrient levels) and change in time submerged over the course of the transplant experiment (see *Statistical Analyses*).

Statistical Analyses

For water samples, tissue samples, and nitrate uptake experiments, we accounted for the random effect of site and non-equal sample sizes using linear mixed effect models with Type III sums of squares and Satterthwaite approximation for denominator degrees of freedom (Zuur et al. 2009) using the package 'lme4' for R (Bates et al. 2015). Analysis of variance (ANOVA) was then conducted to compare response variables across model factors (see below).

Analyses of observations of ambient seawater nutrient levels (i.e., NO_3^-, PO_4^{3-}, NO_3^-:PO_4^{3-}) and tissue nutrients (i.e., %C, %N, and C:N) were conducted separately. Data were compared among regions, sites (a random factor), sample dates, and tide heights (for tissue nutrients only).

To compare nitrate uptake rates of upper and lower *F. vesiculosus*, we tested for the effects of region, site, experimental nitrate concentration, and tide height. For the transplant experiment, we compared nitrate uptake rates among time points (initial and final measurements), regions, sites, experimental nitrate concentration, transplant height, and home

height. Because there was variation in the actual experimental nitrate concentration among chambers (i.e., deviation from target nitrate concentration) that could influence uptake rate, the initial measured nitrate concentration of each chamber was included as a covariate in these models. For all models (i.e., environmental observations and uptake experiments) site was treated as a single random factor, not nested, because there were insufficient degrees of freedom to perform a partially nested analysis. Data were coded such that sites were only associated with their correct region (i.e., no 'site' x 'region' interactions were allowed).

When significant interactions were identified, we conducted post-hoc tests to determine significant differences between interacting levels of factors. Post-hoc tests were carried out using the 'multcomp' package for R (Hothorn et al. 2008), and significance levels were corrected for multiple tests using a Bonferroni adjustment. For presentation of significant comparisons, we present least-square means which were calculated for specified factors while accounting for (holding-constant) variation in all other model factors.

To examine possible influence of site-level nutrients and intertidal elevation (i.e., 'environmental covariates'), we used a multiple linear regression to examine possible covariation in nitrate uptake rates with ambient nitrate and phosphate availability (i.e., NO_3^-, PO_4^{3-}, NO_3^- :PO_4^{3-}) and time submerged (i.e., number of hours in the preceding 24 hours of collection). To determine if submergence time mediated the response to nutrient availability we included two-way interactions between hours submerged and each nutrient predictor variable. Regressions were conducted separately for uptake measurements for each experiment. The target concentration (as a factor) also was included in the model to account for variation due to different experimental nitrate concentrations.

Assumptions of each analysis were checked, and data were transformed as needed. All analyses were carried out using R version 3.2.2 (R Core Team 2015).

Results

Ambient Nutrient Availability and Tissue Nutrient Content

Average nutrient concentrations in the Gulf of Maine were generally low and ranged from 0.04 to 8.30 µmol L^{-1} NO_3^- and 0.07 to 4.06 µmol L^{-1} PO_4^{3-} across sampling dates and sites. NO_3^-, PO_4^{3-}, and NO_3^-:PO_4^{3-} varied significantly among sampling dates (ANOVA; $P < 0.001$), with highest levels typically occurring in spring and fall. On average, ambient NO_3^-

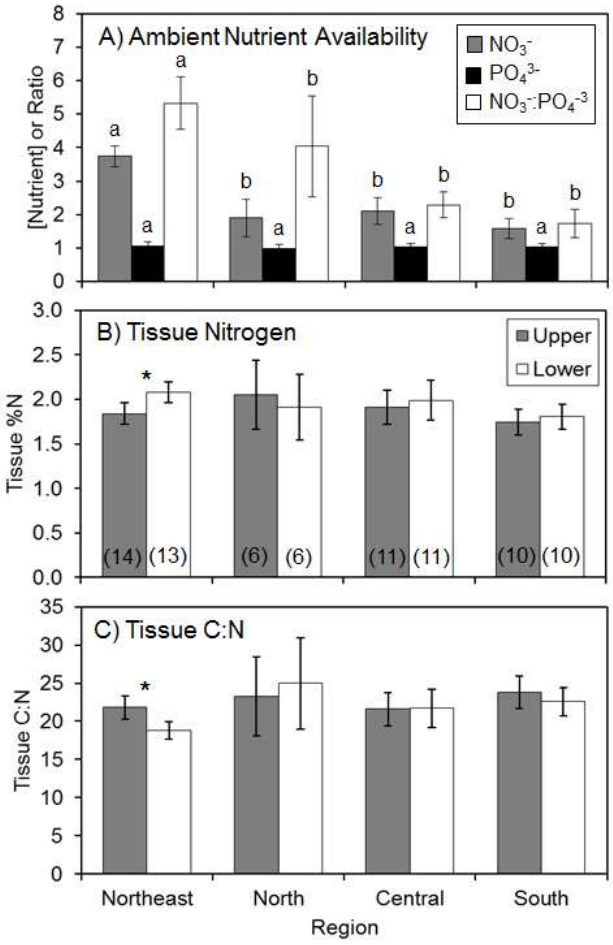

Figure 4.1. Average of long-term water and *F. vesiculosus* tissue nutrient collections across regions in the Gulf of Maine. (A) Mean (± SE) ambient nitrate and phosphate concentrations (µmol L^{-1}) as well as their ratio (northeast (n=32), north (n=16), central (n=27), and south (n=34). Mean tissue (B) nitrogen (%N), and (C) C:N of upper-shore and lower-shore *F. vesiculosus* are presented. Means of all variables are from observations at two sites per region first averaged by sampling date. Post-hoc significant differences ($P < 0.05$) in ambient nutrient availability among regions, for each response variable separately, is shown by differing letters. For tissue %N and C:N, significant differences between upper and lower intertidal values are denoted by an asterisk (*$P < 0.05$).

concentrations were highest in the northeast (ANOVA: $F_{3,55.75} = 12.89$, $P < 0.001$; Fig. 4.1A); however, ambient PO_4^{3-} concentrations did not differ among regions (ANOVA: $F_{3,55.34} = 1.22$, $P = 0.31$). This resulted in overall higher average NO_3^-:PO_4^{3-} ratio in the northeastern compared to other regions in the Gulf of Maine (ANOVA: $F_{3,55.75} = 12.89$, $P < 0.001$).

Tissue %N variation between upper and lower intertidal *Fucus vesiculosus* depended on region (ANOVA: $F_{3,492.76} = 7.93$, $P < 0.001$). On average, ~11% higher tissue N was observed in lower-shore individuals relative to upper-shore individuals in the northeast but not elsewhere in the Gulf of Maine (Fig. 4.1B). Variation in %C between upper and lower-shore *F. vesiculosus* also depended on region (ANOVA: $F_{3,493.56} = 3.01$, $P = 0.03$). In the central Gulf of Maine, we observed relatively lower %C values in upper-shore individuals, a pattern not seen in other regions (Tukey post-hoc test; P=0.049). However tissue C:N was driven by tissue %N, with differences across tide heights found in the northeast Gulf of Maine only (Fig. 4.1C; ANOVA; $F_{3,492.94} = 6.53$, $P < 0.001$). Similar to ambient seawater nutrient levels, tissue %N, %C, and C:N also varied across sampling date, with the highest tissue %N occurring during winter and spring months. Tissue %N was positively related to ambient nitrate concentrations in all regions, but not significantly in the northeastern Gulf of Maine (Pearson Product-moment correlation, $P < 0.05$).

Nitrate Uptake Rates of Upper versus Lower Shore Fucus vesiculosus

Overall, upper-shore individuals had 18% higher nitrate uptake rates than lower-shore individuals (ANOVA; $F_{1,91.38} = 10.35$, $P = 0.02$). However, this difference across tide heights varied by region (ANOVA: 'region x tide height' interaction; $F_{1,91.24} = 3.74$, $P = 0.01$). In the northeast, north, and central Gulf of Maine there was little to no difference in nitrate uptake rates between upper and lower-shore *F. vesiculosus*. In contrast, in the southern Gulf of Maine, upper-

shore individuals had 59% higher nitrate uptake rates than lower-shore individuals (Fig. 4.2). As expected, the nitrate uptake rate increased with the experimental nitrate concentration (ANOVA: $F_{3,83.79} = 10.35$, $P < 0.001$) but there were no significant interactions between experimental nitrate concentration and other main effects (ANOVA: $P > 0.5$).

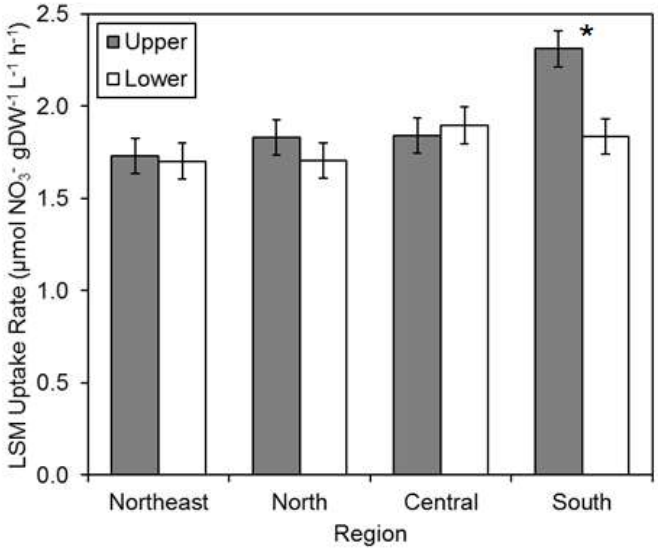

Figure 4.2. Least-square mean (LSM) nitrate uptake rates (µmol $L^{-1} \cdot gDW^{-1} \cdot h^{-1}$) for *F. vesiculosus* at the upper and lower limits of its intertidal distribution in the Gulf of Maine regions (n = 16 per region / tide height). LSMs are of square root-transformed data and error bars are ± SE. Significant differences between upper and lower intertidal uptake rates are denoted by an asterisk (*$P < 0.05$).

Reciprocal Transplant Experiment - Acclimation via Changes in Nitrate Uptake Rates

Nitrate uptake rate varied between the initial and final measurements of the transplant experiment (ANOVA: $F_{1,440.1} = 14.14$, $P < 0.001$) and between 'home' tide heights (ANOVA: $F_{1,440.1} = 23.57$, $P < 0.001$) (Fig. 4.3). However, these differences varied by region. Uptake rate decreased between initial and final measurements by 18% in the northeast (Fig. 4.3A) and 13% in the central regions (Fig. 4.3B) (post-hoc test, $P < 0.05$) but did not change in the southern Gulf of Maine (Fig. 4.3C) (post-hoc test, $P > 0.05$). *F. vesiculosus* that was originally collected from its upper distributional limit had greater nitrate uptake compared to *F. vesiculosus* originally collected its lower distributional limit in the northeast and central Gulf of Maine (25% and 12% higher, respectively; post-hoc test, $P < 0.05$) but not the southern Gulf of Maine (post-hoc test, P

> 0.05). The difference between home heights was independent of time of sampling and transplant height (i.e., no significant 'Time x Home Height' or 'Home Height x Transplant Height' interactions); in the northeast and central Gulf of Maine, *F. vesiculosus* collected from the upper intertidal had higher nitrate uptake at the start and end of the experiment and regardless of whether it was transplanted to the upper or lower intertidal. Additionally, there was a trend toward variation between transplant heights depending on time and region (ANOVA; $F_{2,440.14}$=2.88, P = 0.06). In the northeast Gulf of Maine at the end of the experiment, *F. vesiculosus* transplanted to the upper intertidal had 21% higher nitrate uptake rates compared to individuals transplanted to the lower intertidal (Fig. 4.3A) (post-hoc test, $P < 0.05$).

As in our first experiment, nitrate uptake rate increased with nitrate concentration (ANOVA; $F_{3,440.16}$=135.56, $P < 0.001$). However, this difference depended on time and region (ANOVA; 'Time x Region x Concentration', $F_{2,440.53}$=2.41, P = 0.03) with lower final nitrate uptake rates at 15 and 30 μmol L^{-1} in the northeast and central regions.

Environmental Covariates of Nutrient Uptake Rates

During each of our experiments, ambient nitrate and phosphate levels and NO_3^-:PO_4^{3-} ratios varied significantly among study sites (ANOVA: $P < 0.001$; Table 4.2). During our upper- versus lower-shore experiment in May 2012, the number of hours *Fucus vesiculosus* was submerged was a significant predictor of nitrate uptake rate (Fig. 4.4A; Table 4.2). Importantly, the hours submerged mediated the relationship between nitrate uptake rate and nitrate concentration and NO_3^-:PO_4^{3-} ratio (i.e., significant "[NO_3^-] x Submergence Time" and "NO_3^-:PO_4^{3-} x Submergence Time" interactions). Nitrate uptake rates increased with higher nitrate concentrations 2.8-fold more rapidly in upper-shore compared to lower-shore *F. vesiculosus*

(Fig. 4.4B). The overall response in nitrate uptake rates to changes in NO_3^-:PO_4^{3-} ratio compared to nitrate concentration was an order of magnitude lower, but lower-shore individuals had a 1.8-fold greater increase in nitrate uptake rate with increasing NO_3^-:PO_4^{3-} ratio (Fig. 4.4D). Uptake rates were not related to phosphate concentrations (Fig. 4.4C).

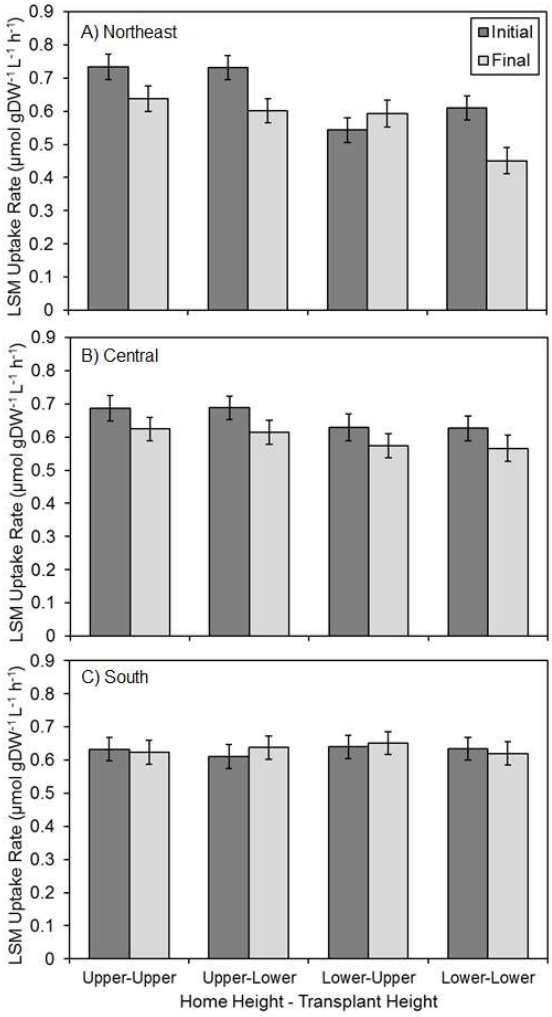

Figure 4.3. Least square mean (LSM) nitrate uptake rates before (initial) and after (final) a 30-day reciprocal transplant experiment between the upper and lower edges of *F. vesiculosus'* intertidal distribution. Transplants were conducted at sites within the (A) northeast, (B) central, and (C) southern Gulf of Maine regions (n=17 - 27 per region / home height / transplant height combination). Error bars are ± SE. Initial and final nitrate uptake rates were significantly different and home tide heights were significantly different in the northeast and central regions only (post hoc test, $P < 0.05$).

Table 4.1. Mean (± SE) nutrient concentrations (μmol L^{-1}) and hours submerged at each field site at the time of collection for each experiment in our study: upper-versus-lower comparison (U v L), at the start of the reciprocal transplant experiment (Transplant – Initial), and at the end of the transplant experiment (Transplant – Final). Hours submerged are based on 5-minute interval tide height prediction data for each site and are based on the sum of intervals in 24 hours preceeding collection. NO sites were not used in the transplant experiment.

		Nutrient Observations			Hours Submerged	
Site	Experiment	$[NO_3^-]$	$[PO_4^{3-}]$	NO_3^-: PO_4^{3-}	Upper	Lower
NE A	U vs L	1.66 ± 0.03	0.31 ± 0.02	5.34 ± 0.32	7.6	19.3
	Transplant - Initial	3.32 ± 0.39	0.58 ± 0.2	5.74 ± 0.72	8.1	16.6
	Transplant - Final	0.48 ± 0.05	0.82 ± 0.02	0.59 ± 0.07	8.1	17.8
NE B	U vs L	0.62 ± 0.07	0.58 ± 0.15	1.26 ± 0.22	9.3	24.0
	Transplant - Initial	2.69 ± 0.27	0.51 ± 0.02	5.31 ± 0.59	9.2	19.1
	Transplant - Final	1.89 ± 0.26	0.99 ± 0.05	1.89 ± 0.15	9.5	21.9
NO A	U vs L	1.38 ± 0.87	1.41 ± 0.23	0.78 ± 0.34	8.3	21.8
	Transplant - Initial	-	-	-	-	-
	Transplant - Final	-	-	-	-	-
NO B	U vs L	0.26 ± 0.03	1.44 ± 0.39	0.22 ± 0.05	12.6	21.8
	Transplant - Initial	-	-	-	-	-
	Transplant - Final	-	-	-	-	-
CE A	U vs L	0.12 ± 0.04	0.38 ± 0.01	0.32 ± 0.10	8.5	21.2
	Transplant - Initial	0.96 ± 0.29	0.95 ± 0.19	1.11 ± 0.38	9.2	19.1
	Transplant - Final	2.02 ± 0.59	0.72 ± 0.02	2.78 ± 0.78	9.5	21.9
CE B	U vs L	0.21 ± 0.11	0.54 ± 0.10	0.49 ± 0.26	6.2	14.8
	Transplant - Initial	1.15 ± 0.50	0.71 ± 0.04	1.56 ± 0.61	6.2	14.9
	Transplant - Final	0.04 ± 0.02	0.47 ± 0.02	0.08 ± 0.04	6.8	15.5
SO A	U vs L	3.15 ± 1.20	1.35 ± 0.18	3.52 ± 1.14	10.1	24.0
	Transplant - Initial	2.54 ± 0.61	1.62 ± 0.11	1.50 ± 0.29	10.2	21.1
	Transplant - Final	0.28 ± 0.12	1.01 ± 0.03	0.27 ± 0.10	11.1	22.9
SO B	U vs L	0.52 ± 0.25	0.95 ± 0.02	0.54 ± 0.26	7.4	22.3
	Transplant - Initial	1.68 ± 0.17	1.58 ± 0.14	1.08 ± 0.13	8.1	19.4
	Transplant - Final	0.58 ± 0.05	0.95 ± 0.10	0.60 ± 0.09	8.8	21.4

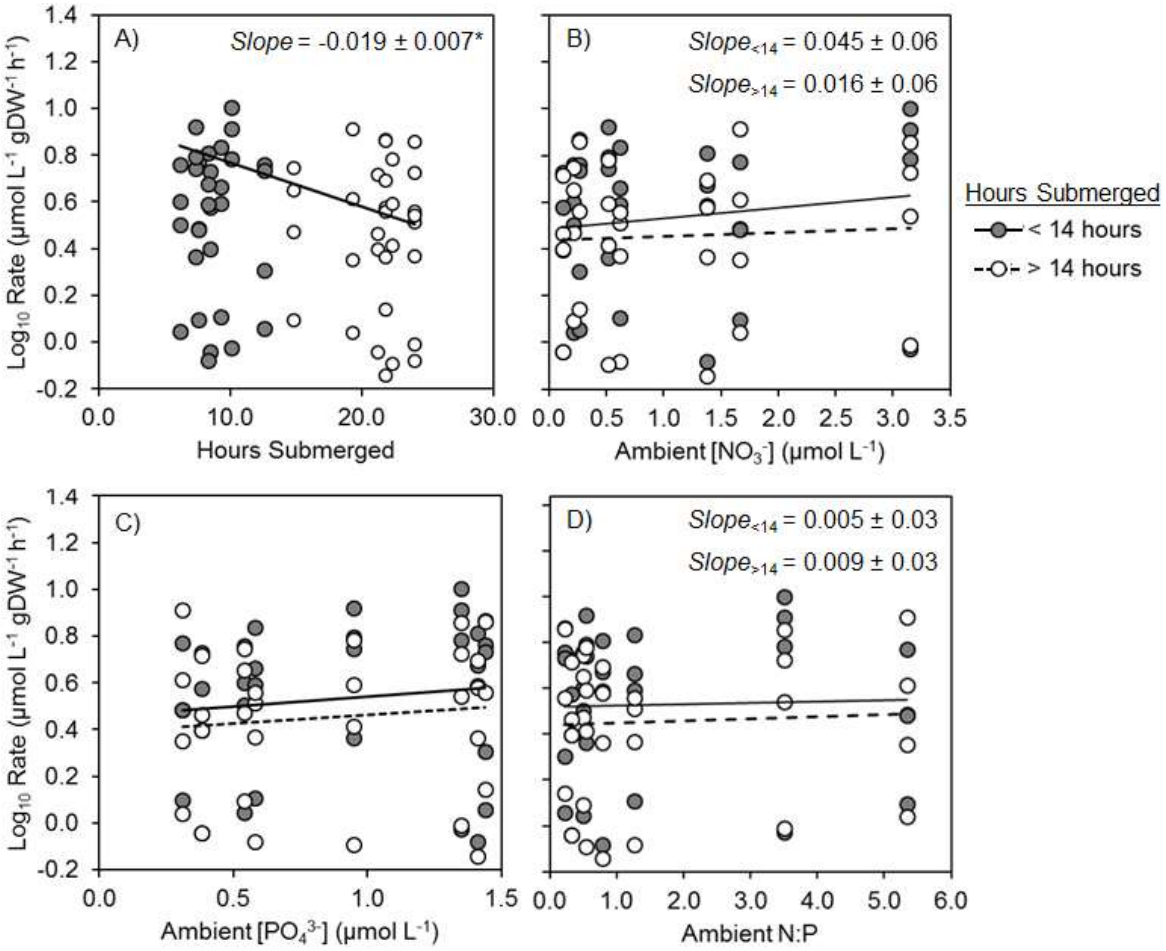

Figure 4.4. Variation in nitrate uptake rate (µmol L^{-1} · gDW^{-1} · h^{-1}) related to (A) hours submerged (B) ambient nitrate concentration (µmol L^{-1}), (C) ambient phosphate concentration (µmol L^{-1}), and (D) NO_3^-:PO_4^{3-} ratio. Significant interactions between hours submerged and nutrient concentrations are shown by splitting the data into observations of upper-shore (less than (<) 14 hours submerged) and lower-shore (more than (>) 14 hours submerged) *F. vesiculosus*. Data for explanatory variables are from the time of sample collection at each site from our upper- versus lower-shore experiment in May 2012 (see Table 4.1). Best fit slopes (± SE) from multiple linear regression analysis (Table 4.2) are given in the in the upper right corner of each panel (*$P < 0.05$).

Table 4.2. Multiple regression parameter estimates for the relationship between $\log_{10}$ transformed nitrate uptake rates ($\mu mol\ gDW^{-1} \cdot L^{-1}$) and explanatory variables: nitrate concentration [NO_3^-], phosphate concentration [PO_4^{3-}], [NO_3^-]:[PO_4^{3-}] ratio, hours submerged, and their interactions. Models also included target nutrient concentration as a fixed factor to account for variation do to different experimental nitrate concentrations. Model results are shown for the upper-versus-lower experiment (Fig. 4.4), initial and final transplant experiment measurements, and change in uptake rate and explanatory variables between initial and final transplant measurements (Fig. 4.5). For the 'change across transplant experiment' model, the difference between initial and final measurements for explanatory variable were used and nitrate uptake rates were not transformed. Statistics of model fit are also given: *F*-value, numerator (df_{num}) and denominator (df_{den}) degrees of freedom, R^2, and *P*-value.

Parameter†	Upper-versus-Lower Experiment	Initial Transplant Experiment	Final Transplant Experiment	Change Across Transplant Experiment
Intercept	0.96 ± 0.12***	0.85 ± 0.17***	1.04 ± 0.13***	-1.81 ± 0.65**
[NO_3^-]	0.18 ± 0.10	-0.17 ± 0.14	0.12 ± 0.18	1.47 ± 0.93
[PO_4^{3-}]	-0.13 ± 0.15	0.17 ± 0.20	-0.065 ± 0.16	-5.81 ± 2.39*
[NO_3^-]:[PO_4^{3-}]	-0.088 ± 0.049	0.13 ± 0.08	-0.104 ± 0.14	-1.27 ± 0.71
Submergence Time	-0.019 ± 0.007*	0.0064 ± 0.013	-0.016 ± 0.010	-0.001 ± 0.068
[NO_3^-] x Sub. Time	-0.012 ± 0.006*	0.01 ± 0.009	-0.014 ± 0.011	-0.095 ± 0.11
[PO_4^{3-}] x Sub. Time	0.015 ± 0.009	-0.01 ± 0.014	0.017 ± 0.012	0.17 ± 0.29
[NO_3^-]:[PO_4^{3-}] x Sub. Time	0.0067 ± 0.003*	-0.0082 ± 0.005	0.011 ± 0.009	0.072 ± 0.085
Model *F*-value	47.69	103.6	113.2	2.75
df_{num}, df_{den}	10, 53	10, 85	10, 83	10, 83
Model R^2	0.88	0.92	0.92	0.16
Model *P*-value	<0.001	<0.001	<0.001	<0.001

†Model Parameter *P*-value *< 0.05, **< 0.01, ***< 0.001

Interestingly, ambient nutrient concentrations and submergence time were not significant predictors of initial and final uptake rates (Fig. 4.5 and Table 4.2); although the overall models explained much of the variation in nitrate uptake rates at both time points (Multiple Linear Regression; Initial and Final Measurements, R^2=0.92). However, the difference in nitrate uptake rate over the 30-day experiment varied significantly with the change in ambient phosphate concentration (Fig. 4.5C) but was not related to changes in ambient nitrate concentration (Fig. 4.5B) or NO_3^-:PO_4^{3-} ratio (Fig. 4.5D). The greatest increases in nitrate uptake rate during the experiment occurred at sites with the greatest decreases in phosphate concentrations (Table 4.1). Although significant interactions between the change in hours submerged and the change in

nutrient concentrations were not identified, such interactions may have been obscured by the large amount of variation in samples that were transplanted back to their home tide height (see Fig. 4.5A). In particular, *F. vesiculosus* from its lower-limit had a much larger variation in response compared to *F. vesiculosus* from its upper intertidal limit. This variation drove a similar response between individuals transplanted to the lower limit and individuals that experienced no change in tide height. In particular, *F. vesiculosus* from the lower limit of its distribution responded to changes in phosphate and NO_3^-:PO_4^{3-} ratio similarly to individuals transplanted to the lower limit of its distribution (Fig. 4.5, see inset graphs). Much of the variation in the change in uptake rate however, was unexplained by our model (Multiple Linear Regression; $R^2 = 0.16$, Table 4.2).

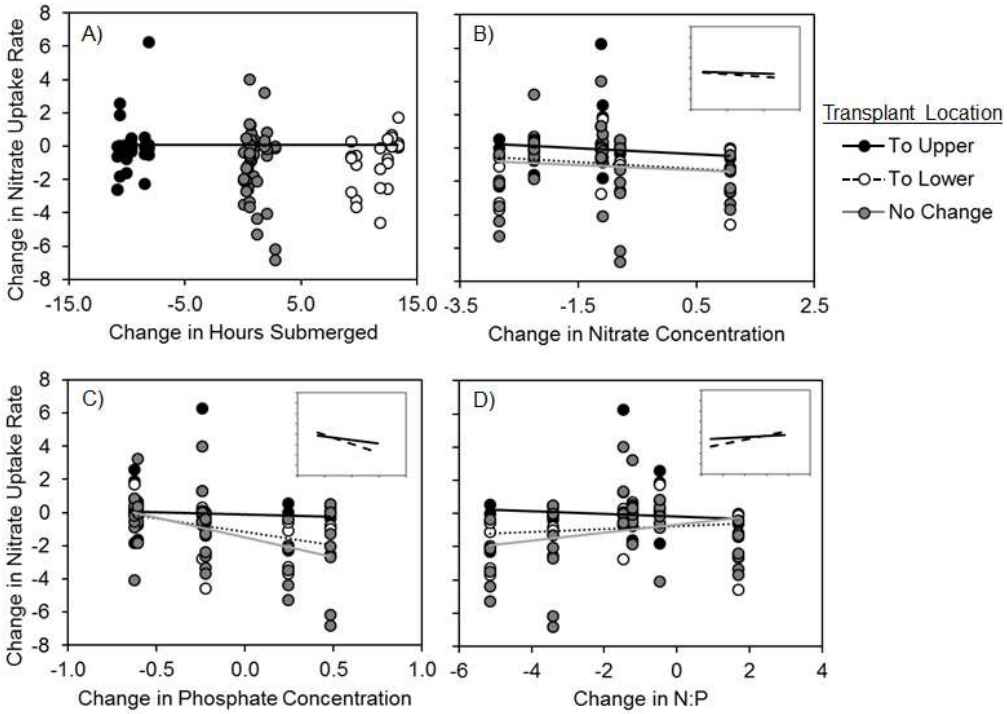

Figure 4.5. The relationship between the change in nitrate uptake rate (µmol L^{-1} · gDW^{-1} · h^{-1}) of *F. vesiculosus* and change in (A) hours submerged, (B) ambient nitrate concentration (µmol L^{-1}), (C) phosphate concentration (µmol L^{-1}), and (D) NO_3^-:PO_4^{3-} ratio. The change in each metric was calculated as the difference between final and initial measurements for the transplant experiment (see Table S2). For interpretation, points and slopes have been identified by transplant treatment: to upper limit, to lower limit, and no change in intertidal location (i.e., transplanted back to home tide height) (see Table 1 for model parameters). Inset graphs show slope for 'no change' transplant category based on home location (upper limit: solid black line, lower limit: dashed line); axis values are same as main graphs.

Discussion

Seaweeds acquire dissolved nutrients when submerged, and intertidal seaweeds living high on the shore may therefore be limited in their access to nutrients. On average, upper and lower intertidal *Fucus vesiculosus* in the Gulf of Maine experience a difference of between 34 and 57% in submergence time, depending on site. However, we observed little difference in %N of seaweeds collected from these two zones. Lack of variability in tissue %N, relative to spatial or temporal variation in ambient nutrient supply, may reflect physiological adaptations that enable seaweeds to acquire sufficient nutrients to for survival and growth (Sterner and Elser 2002). We found that upper-shore *F. vesiculosus* compensates for reduced submergence time via greater nitrate uptake rates, though this pattern was dependent on local nutrient levels, time submerged, and geographic location.

Ambient Nutrient Availability and Tissue Nutrient Content

We found seasonal variation in seawater and tissue nutrient levels similar to a previous study in the southern Gulf of Maine (Perini and Bracken 2014). As expected in this temperate ecosystem, peak nutrient concentrations occurred in spring, and the lowest nutrient concentrations occurred during summer. Variation in ambient nitrate levels was two orders of magnitude higher than variation in tissue %N across sampling dates. Seaweed tissue nutrient content is often observed to be less variable than ambient nutrient supply across cultures (Topinka and Robbins 1976, Rosenberg et al. 1984, Fujita 1985) or temporal (Chapman and Craigie 1977, Wheeler and North 1981, Pedersen and Borum 1996) and spatial (Thomas et al. 1987b, Phillips and Hurd 2003, Kamer et al. 2004) scales. In the Gulf of Maine, intertidal *F.*

vesiculosus tissue %N appears to be more variable across seasons than it is across large spatial scales or tidal distribution (Perini 2013 and *this study*), similar to intraspecific variation in tissue %N along the tidal gradient reported in other species (Thomas 1987a, Phillips and Hurd 2003). *Gracilaria pacifica* at higher tidal elevations were found to have slightly higher tissue %N compared to individuals at lower elevations (Thomas et al. 1987a). Note that only C:N ratios were reported by Thomas et al. (1987a), so similar carbon (%C) levels are assumed. *Stictosiphonia arbuscula* displays temporal variation in the differences in tissue %N between upper and lower shore individuals; summer to fall low-shore *S. arbuscula* has greater %N than high shore individuals, but during the rest of the year tissue %N is similar between zones and even slightly higher in high shore individuals during winter (Phillips and Hurd 2003).

Nitrate Uptake Rates of Upper versus Lower Shore **Fucus vesiculosus**

In the upper-versus-lower experiment, average nitrate uptake rates were higher in *F. vesiculosus* at the upper compared to lower edge of its intertidal distribution, with detectable differences in the southern Gulf of Maine. Intraspecific variation in maximum uptake (V_{max}) at high nutrient concentrations, has been observed to be 1.2- to 26.5-fold higher in upper-shore compared to lower-shore individuals (Phillips and Hurd 2004, Bracken et al. 2011). In contrast, comparisons of uptake ability at low nutrient concentrations using K_s (i.e., half-saturation coefficient) or uptake efficiency (i.e., $\alpha = V_{max}/K_s$), or V_2 (i.e., V at 2 μmol L^{-1}), have generally shown either no difference or a greater ability of low-shore individuals to take up nutrients at low concentrations (Phillips and Hurd 2004, Bracken et al. 2011). These previous results suggest that upper intertidal seaweeds may compensate for less time submerged by increasing uptake rates when ambient nutrient concentrations are high. Although we were not able to calculate kinetic

parameters (i.e., V_{max} or K_s), significant differences in uptake rate between upper and lower *F. vesiculosus* was independent of experimental nitrate concentrations (i.e., no 'Height x Concentration' interaction). These differences were strongest at sites where local ambient nitrate concentrations, at the time of collection, were > 0.5 μmol L^{-1} (see Table 4.1 and *Environmental Covariates* below). This demonstrates a greater ability of upper intertidal individuals to capitalize on *relatively* high nutrient concentrations that are biologically relevant, not just concentrations that may maximize uptake rates and which may be rare at coastal sites in the Gulf of Maine. This further suggests that intertidal seaweeds, particularly upper shore individuals, adjust their nutrient physiology to maximize nutrient uptake when nutrients are readily available and which also may help minimize physiological costs associated with nutrient assimilation (i.e., enzyme production).

Reciprocal Transplant Experiment - Acclimation via Changes in Nitrate Uptake Rates

Similar to our initial observations of *Fucus vesiculosus*, our reciprocal transplant experiment revealed significant differences in nitrate uptake rates of *F. vesiculosus* originally collected from the upper- versus lower-shore (i.e., across 'home' heights). We also found trends in variation in final uptake rates between transplant tide heights in the northeast Gulf of Maine. Similarly, transplants of *Gracilaria pacifica* from the lower to upper edges of its intertidal range exhibited an increase in nitrate reductase activity (NRA) and desiccation-enhanced uptake rates. However, *G. pacifica* transplanted from the upper to lower intertidal maintained high nitrate uptake rates (Thomas et al. 1987a). It is possible that seaweeds can both rapidly acclimate to nutrient-limiting conditions (e.g., lower to upper intertidal transplants) and retain sensitivity to

changing nutrient concentrations after living in a potentially nutrient-limited environment (e.g. upper to lower intertidal transplants).

The effects of both transplant height and home height were most apparent in the northeast and declined to the south (Fig 4.3). During our first experiment regional differences could be attributed to differences local ambient nutrient supply. However, there were no relationships between initial and final uptake rates and nitrate or phosphate concentrations during the transplant experiment. This may be due to seasonal variation in response of uptake rates to ambient seawater and tissue nutrient levels (see *Environmental Covariates* below). The reciprocal transplant experiment was conducted in the summer (i.e., low seawater and tissue nutrient levels), whereas the upper versus lower experiment was conducted in the spring (i.e., high seawater and tissue nutrient levels). Additionally, there could be population differences in the propensity for acclimation and adaptation to tide height underlying our among-region differences in transplant and home height effects on nutrient uptake rates. In the northeast, the maintenance of high uptake rates of *F. vesiculosus* from its upper intertidal limit during the transplant experiment, but changes in uptake rates of *F. vesiculosus* from its lower intertidal limit, is consistent with patterns of specialization or adaptation to intertidal zones (Lortie and Aarssen 1996, Kawecki and Ebert 2004). Overall higher nitrogen availability in the northeast, evidenced by seawater nitrate and tissue %N observations, may allow for greater differentiation and potential adaptation across tide heights in nutrient physiology here. However, more flexible (plastic) nutrient physiologies across tide heights may be an advantage in other regions with higher temporal nutrient variability and longer periods of limiting nitrate concentrations.

Both *Saccharina longicruris* (formerly *Laminaria longicruris*) in the northwest Atlantic (Espinoza & Chapman 1983) and *S. latissima* (formerly *L. groelandica*) in the northeast Pacific

(Druehl et al. 1989) show adaptation and plasticity, respectively, in nitrate uptake rates among nutrient-replete and nutrient-depleted sites. While these are different species, these examples demonstrate that seaweed nutrient physiology can include both acclimation (plastic responses) *and* adaptation (fixed responses) to ambient nutrient levels. The latter may reflect adaptations to long-term nutrient availability (e.g., geographic variation in long-term average nitrate concentrations) or may underlie nutrient demands imposed by constraints of adaptations to other environmental factors. Importantly, differences among populations in adaptation may influence other physiological functions such as amino acid synthesis, soluble N-storage, and N-specific growth rate, with low-N populations exhibiting more efficient use of available nutrients (e.g., higher specific growth rates under low nutrient levels; Espinoza and Chapman 1983, Kopczak et al. 1991).

Environmental Covariates of Nutrient Uptake Rates

Although we found among-region variation at all three time points in our study (i.e., upper-versus-lower experiment, and both before and after the transplant experiment), the direction of variation was not consistent. In our upper-versus-lower experiment, tide height differences were greatest in the south (Fig. 4.2). During the transplant experiment, after accounting for experimental nitrate concentration, differences in uptake rates between tide heights were greatest in the northeast and declined from the central to southern Gulf of Maine (Fig. 4.3). The temporal differences in geographic variation could reflect site-level and/or seasonal differences in nutrient availability or tidal exposure.

Nitrate concentrations, along with time submerged, were significant predictors of nitrate uptake rate during our first study of upper- versus lower-shore *F. vesiculosus*. Individuals at sites

with the highest upper-edge distribution and high nitrate concentrations would be predicted to have the highest nitrate uptake rates, corresponding to observations at sites in the northeast and southern Gulf of Maine (Fig. 4.4, Table 4.2). However, initial and final uptake rates from the transplant experiment were not related to any environmental covariates. Timing (season) of our experiments and corresponding tissue nutrient status may drive these patterns. The estimated critical %N (i.e., the tissue %N below which growth is limited) for *F. vesiculosus* is 1.7% (Pedersen and Borum 1997). The upper-versus-lower experiment took place in May 2012, soon after the spring pulse of nutrients (*this study* and Perini and Bracken 2014) and when tissue %N was on average > 1.7% (range across sites: 1.61- 2.16%) at all but one of our study sites. However, the reciprocal transplant experiment was conducted during summer 2013 during a period of low ambient nutrient levels (*this study* and Perini and Bracken 2014) and when tissue %N was on average < 1.3% (range across sites and time points: 0.92 - 1.70%). Therefore, when *F. vesiculosus* is N-limited, differences in uptake rates across submergence time and/or varying ambient nutrient levels may be minimized. This was corroborated by our observation of greater deviation from target experimental nitrate concentrations ($[NO_3]_{dev}$) after the transplant experiment (i.e., time when tissue %N was lowest) compared to other time points suggesting rapid uptake at the end of the experiment.

The difference between final and initial nitrate uptake rates during this potentially N-limited period were associated with changes in nutrient levels. In particular, decreases in ambient phosphate concentrations were associated with increases in the nitrate uptake rate of *F. vesiculosus* during the experiment. *F. vesiculosus* at sites with the greatest increases in phosphate concentrations had reduced nitrate uptake rates during the transplant experiment. This trend may have been driven by individuals that were from and transplanted to *F. vesiculosus'* lower

intertidal limit (Fig. 4.5 dashed lines on inset and main graphs). Importantly this suggests the potential for co-limitation of nitrate and phosphate on *F. vesiculosus* nitrate uptake, particularly in individuals at the lower limit of its intertidal distribution. Perini and Bracken (2014) found that phosphate uptake efficiency and tissue %P was limited by N-availability in southern Gulf of Maine *F. vesiculosus* but did not show variation in nitrate uptake under different phosphate enrichment levels. Their study was only conducted in the southern Gulf of Maine and, given our data on regional variation in tissue N-status and response to transplantation, there may be geographic variation in co-limitation in this species.

Given that algal nutrient uptake rates are directly related to the concentration of available nutrients, it is not surprising that we found covariation between local ambient nutrient concentrations and nutrient uptake rates during our experiment in spring. Our observations of higher uptake rates at sites with higher nutrient levels is in contrast to theory (Doyle 1975) and experimental studies (e.g., Turpin and Harrison 1979) that demonstrate higher uptake rates or maximum uptake capacity in N-limited primary producers. However, temperate intertidal seaweeds often show the greatest nutrient uptake rates during winter months (Hurd and Dring 1990, Phillips and Hurd 2003, 2004), allowing seaweeds to store excess nutrients (e.g., Phillips and Hurd 2003, Perini and Bracken 2014) when high ambient nutrient availability is decoupled from the growing season (Pedersen and Borum 1996). In *Fucus* species, nitrate reductase activity (NRA), often assumed to be the rate limiting step for nitrate uptake and assimilation, is positively associated with ambient nitrate concentration and is highest during winter when ambient nitrate concentrations and tissue %N are highest (Young et al. 2007a). Further, nitrogen deprivation of *F. vesiculosus* led to a rapid reduction in NRA to ~10% of pre-deprivation levels in just two weeks (Young et al. 2009). Therefore *F. vesiculosus* may require exposure or

'priming' to low or moderate levels of ambient nitrate to increase uptake rates or to maintain NRA, a phenomenon observed in N-deprived kelps and phytoplankton (Turpin & Harrison 1979, Davison and Stewart 1984). This may be a further adaptation of intertidal seaweeds to minimize energy expenditure on active uptake when nutrient levels are low.

General Discussion

Two important factors may have limited our ability to detect larger differences between upper and lower intertidal *F. vesiculosus* and minimized associations between uptake rates and environmental covariates. One possibility is that there is an initial, transient "surge" component of nitrate uptake. Given that surge uptake does not require an investment of energy, it may be an important mechanism by which N-limited seaweeds rapidly adjust to changing nitrate availability (Pedersen 1994). However, whereas surge uptake of ammonium and phosphate has been identified in intertidal seaweeds (Thomas & Harrison 1987, Hurd and Dring 1990, Phillips and Hurd 2003), there is little evidence for surge uptake of nitrate (Thomas and Harrison 1987, Phillips and Hurd 2003) and no intraspecific variation by tide height in nitrate uptake over short (0-15 minutes) compared to longer (15-90 minutes) time intervals (Phillips and Hurd 2003).

A second possibility is that the long period between sample collection and nitrate uptake measurements may have altered short-term physiological changes *F. vesiculosus* used to acclimate to local environmental conditions (i.e., variable nutrient levels, tidal exposure, etc.). Young et al. (2009) found that *F. vesiculosus* held in outdoor flow-thru seawater tanks, as we treated our samples, can maintain similar NRA levels for at least a month suggesting that assimilation-controlled uptake rates should not change greatly over this period. While we expect that there may have been some changes due to transport and physiological adjustment during the

holding period, given that we treated all samples similarly and that our nitrate uptake includes both uptake and assimilation, our measured rates likely represent a conservative estimate of physiological differences between upper and lower intertidal *F. vesiculosus*.

In addition to increased uptake rates, other physiological mechanisms may account for *F. vesiculosus'* maintenance of tissue %N levels across its intertidal distribution. For example, desiccation-enhanced nutrient uptake (Thomas and Turpin 1980, Thomas et al. 1987b) or NRA (Murthy et al. 1986) and more rapid recovery of nutrient uptake (Hurd and Dring 1991) immediately following submergence (i.e., when covered by the incoming tide) has been observed in seaweeds occurring in the upper intertidal zone. Additionally, seaweeds that can rapidly utilize internal soluble N-pools (nitrate or ammonium) may sustain higher uptake rates when submerged, as the concentrations of internal soluble N-pools are inversely related to N-uptake rates (McGlathery et al. 1996). Furthermore, light-independent nutrient uptake (Topinka 1978) allows intertidal seaweeds to acquire nutrients in shaded microhabitats or when high tide occurs at night. N-limited seaweeds may not exhibit diel changes in nitrate uptake rates (D'Elia and DeBoer 1978, Kim et al. 2013), and in *F. vesiculosus* and closely related congeners there is no evidence of diel NRA (Young et al. 2007b). Intraspecific variation in the sensitivity of NRA to ambient nitrate supply may drive the higher uptake rates and underlie the covariance between uptake rates and nitrate concentrations we observed in intertidal *F. vesiculosus.* Intertidal *Ulva lactuca* and *Padina tetrastromatica* show greater NRA with desiccation in upper-shore compared to mid- and low-shore individuals (Murthy et al. 1986). Whether this occurs in *F. vesiculosus* is untested.

Intertidal seaweeds must cope with changes in both water-column nutrient supply and access time to nutrients imposed by rising and falling tides. We found that *F. vesiculosus* can

acclimate to changes in both ambient nitrate concentration and tide height. Additionally, latitudinal variation in nutrient supply may drive among-population differences adaptation and acclimation ability. Seaweeds, such as *F. vesiculosus*, that can adjust their uptake rates according to submergence time and ambient nutrient concentration may have broader intertidal distributions compared to species with less adjustable nutrient physiologies. Physiological studies comparing inter- and/or intraspecific variation in nutrient physiology in response to ambient nutrient supply provide insights into the spatial and/or temporal distribution and abundance of seaweeds (Fujita 1985, Pedersen and Borum 1996, Lotze and Schramm 2000, Bracken and Nielsen 2004) and the nutrient cycling rates of diverse seaweed assemblages (Bracken and Stachowicz 2006, Bracken et al. 2011). Furthermore, studies such this one provide a mechanistic understanding of how primary producers maintain levels of tissue nutrients despite a fluctuating environment.

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Chapter 5

GENERAL CONCLUSIONS

This research highlights the importance of large-scale environmental variation in mediating population-level processes operating at local-scales. I used latitudinal gradients in temperature, nutrient availability, and oceanographic currents in the Gulf of Maine to examine differences in intraspecific variation of a foundation species on rocky intertidal shores. Given the important bottom-up role that seaweeds, in general, and *Fucus vesiculosus*, in particular, play in marine communities and ecosystems, this variation could have important consequences for species interactions and biogeochemical processes in nearshore systems.

Summary of Findings

Previous work suggested that patterns of genetic diversity and structure at multiple spatial scales did not exist in western North Atlantic *Fucus vesiculosus* populations (Muhlin and Brawley 2009). Using new techniques and considering local-scale variation nested within regions and coast-wide patterns, I uncovered previously undiscovered patterns of diversity and structure in these populations. In chapter two, I showed that whereas genetic diversity (i.e., allelic richness [A] and gene diversity [H_E]) varied among regions, mating system patterns (i.e., inbreeding [F_{IS}]) varied across tide heights within sites. Pair-wise comparisons of genetic differentiation (i.e., F_{ST}) revealed isolation-by-distance among sites across the Gulf of Maine and asymmetrical gene flow according to tide height. Upper intertidal subpopulations of *F. vesiculosus* exhibited lower levels of differentiation, indicating greater gene flow towards the upper edge of the species' intertidal distribution. Differences in inbreeding and gene flow

according to tide height suggest that some sites and intertidal subpopulations may be more isolated than others in the Gulf of Maine. At these locations, isolation may allow natural selection to generate adaptive variation in phenotype across the intertidal gradient (Slatkin 1987, Ellstrand 2014).

In the third chapter, reciprocal transplant experiments revealed among-region variation in local adaptation to tide height. In the northeastern Gulf of Maine, a significant interaction between the effects of home height and transplant height on relative growth rates suggested local adaptation to tide height in this region. In the central Gulf of Maine, patterns were consistent with countergradient variation, leading to similar growth rates of *F. vesiculosus* between individuals at either edge of the intertidal distribution. In contrast to the northeast and central regions, southern Gulf of Maine populations showed little evidence of adaptive phenotypic response to tide height. Transplant height was the primary determinant of growth rate in the southern region, indicating a plastic response to environmental heterogeneity. Rates of nitrogen use efficiency (NUE) were primarily influenced by home height. Upper-shore *F. vesiculosus* had 43% higher NUE compared to lower-shore *F. vesiculosus*, which was expected given the limited access time to dissolved nutrients in the upper intertidal zone. This pattern was independent of transplant height in the northeast indicating a possible genetic basis to this and other metabolic processes that could enhance growth or survival of upper-shore individuals in this region.

In the fourth chapter, I identified variation in nitrate uptake attributable to both tide height and local (ambient) nitrate availability. On average, upper-shore individuals had higher nitrate uptake compared to lower-shore individuals. But this was dependent on ambient nitrate and phosphate levels. Measurements of uptake before and after the reciprocal transplant experiment further corroborated the patterns of regional variation in local adaptation found in

chapter three. In the northeastern Gulf of Maine, nitrate uptake rates were higher in upper-shore individuals compared to lower-shore individuals even when transplanted to the lower intertidal zone. Persistently elevated nitrate uptake in upper intertidal individuals in the northeastern gulf may be one mechanism by which this subpopulation maintains higher NUE.

Among Region Variation in Intraspecific Differentiation & Population-level Processes

Taken together, the results of these studies suggest that different mechanisms are acting on *Fucus vesiculosus* populations in different regions, leading to geographic variation in intraspecific differentiation across the intertidal zone. Microsatellite loci used in this study are putatively neutral and therefore may not reflect natural selection that could be influencing other areas of the genome (but see *Study Limitations* below). However, patterns in diversity and differentiation can be used to quantify the extent and direction of gene flow which can limit local adaptation *sensu stricto* (Lenormand 2002). Significant and relatively higher genetic differentiation between sites from different regions suggests a limitation to gene flow over long distances. This regional-level isolation could allow for intraspecific differentiation on the local-scale if gene-flow among populations, which may be adapted to different environmental conditions, is minimized (Slatkin 1987). Variation in levels of inbreeding (i.e., F_{IS}) and differentiation (i.e., F_{ST}) at the local-scale provides further insight into the possible mechanisms driving differentiation across tide heights and are discussed below in the context of phenotypic patterns observed within each region.

In the northeastern Gulf of Maine, the genotype x environment interaction in growth is suggestive of local adaptation. Here, a wide vertical distance between distributional edges combined with dense *Ascophyllum nodosum* stands in the middle of the rocky shore (K. Benes

personal observation) may limit dispersal and increase isolation between intertidal zones, allowing for local adaptation to tide height. However, genetic analyses show no evidence of inbreeding and low genetic differentiation, suggesting high levels of gene flow within and among subpopulations of *F. vesiculosus* in the northeastern gulf. These results are counter to what is expected for local adaptation via isolation as hypothesized above. Instead, the observed phenotypic adaptation may be best explained as a balanced polymorphism, maintained via differential survival of genotypes in each intertidal zone (e.g., Nielsen et al. 2009, Moody et al. 2015).

Similarly, in the central Gulf of Maine, countergradient variation in growth suggests that natural selection may be driving phenotypic differentiation across the intertidal gradient here. There were trends of within-site variation in inbreeding and significant between-site differentiation, suggesting some degree of microgeographic isolation across the intertidal gradient. Interestingly, within-site genetic differentiation (i.e., F_{ST} comparisons between upper- and lower-shore subpopulations) was the lowest in the central gulf relative to other regions. Thus, similar to the northeast region, phenotypic differentiation may be due to post-settlement selection rather than reproductive isolation. However, what alternate mechanisms drive countergradient patterns in the central gulf and genotype x environment patterns in the northeastern gulf remain unclear.

In the southern Gulf of Maine, plasticity was the dominant response, with growth explained entirely by transplant height. This may be due to high dispersal rates homogenizing genotypes across the gradient and/or strong environmental constraints on growth in this region. Genetic differentiation between intertidal zones was highest at these sites, suggesting some level of isolation. The site with the highest levels of differentiation also had low sample sizes due to

low densities of *F. vesiculosus* (K. Benes *personal observations*). Whether high F_{ST} was driven by sample size, or by low density and subsequent genetic drift, needs to be evaluated further. Additionally, whether plasticity is due to high dispersal (and a random distribution of genotypes with respect to tide height) or environmental constraints is unknown and offers an interesting avenue for future research.

Study Limitations

While this study provides strong evidence for among-region variation in population processes and intraspecific differentiation, there are a few important limitations to this research. First the loci used are putatively neutral and best describe historical and current limits to migration, dispersal, and genetic drift (i.e., random, neutral population processes). This allows for understanding patterns of isolation – which is often a requisite for natural selection to occur – but does not typically allow for direct estimation of the influence of selection at the genetic-level (Slatkin 1987). In a closely related congener, *Fucus distichus*, alleles at loci F09 and L20 show strong clines along a ~10-kilometer salinity gradient, suggesting that these loci are associated with regions of the genome under selection. In my work on *F. vesiculosus*, F09 alleles showed latitudinal variation, and L20 showed with-in site variation. Next generation sequencing would allow for observations of neutral and selective processes across the entire genome. *F. vesiculosus* populations in the western North Atlantic Ocean may be an interesting system for such studies as there are likely patterns associated with both latitudinal and intertidal environmental gradients.

Other important limitations of this study are imposed by the use of a single generation (adult) in the phenotype experiments. By using only adult individuals, the presence of maternal or carry-over effects cannot be accounted for, and the ability to identify a strong genetic basis in

phenotype is limited (Rossitter 1996, Kawecki and Ebert 2004). Maternal effects arise when parental environment influences the phenotype of offspring. Maternal effects have been identified in response to abiotic and biotic factors in a number of marine organisms (e.g., Li and Brawley 2003, Allen et al. 2008, Shama et al. 2014, Donelan and Trussell 2015) and can be adaptive if survivorship is enhanced following parental environmental exposure (Mousseau and Fox 1998, Marshall and Uller 2007). Zygotes of *F. vesiculosus* had greater survivorship when females were exposed to thermal stress compared to females kept under benign conditions in laboratory studies (Li and Brawley 2003). If maternal effects are prevalent in wild *F. vesiculosus* populations, it could be a mechanism by which adaptation to tide height occurs. Even under high rates of dispersal, if survivorship of offspring of local ('home-height') parents is higher compared to offspring of parents from other intertidal zones, then differential mortality could lead to observed phenotypic differentiation.

Testing the response of offspring can also provide evidence for the genetic basis of traits. Hays (2007) studied adaptation to tide height in the seaweed *Silvetia compressa* and demonstrated that offspring performed best in the zone where their parents originated. This pattern was observed regardless of parental exposure (i.e., parents exposed to home or foreign tide height) and demonstrated the genetic basis of local adaptation to tide height in this species. I attempted to conduct similar offspring analyses, but high variability in the reproductive state of individuals following the transplant experiment made the comparisons impossible. A common garden experiment in a more controlled laboratory setting would reduce natural variation in environmental factors but could be a good compromise to allow for tests of offspring response to different tidal exposures.

Significance

This dissertation represents an interdisciplinary approach to understanding how large-scale environmental variation can alter intraspecific differentiation at the local-scale. The research presented makes several important contributions to the field of evolutionary ecology. First, few studies have attempted to evaluate variation in mating system and differentiation across the intertidal gradient, and those that have considered variation with tide height have focused on just a few sites and generally found greater inbreeding and isolation of individuals occurring higher on the shore (Engel et al. 2004, Krueger-Hadfield et al. 2013). *F. vesiculosus* displays the opposite pattern, with less inbreeding and isolation high on the shore. Increasing the diversity of species studied improves our understanding of what characteristics (e.g., dispersal potential, reproductive mode) are important to driving microgeographic patterns in genetic structure. The identified isolation-by-distance across large spatial scales but isolation-by-environment between intertidal zones highlights the importance of variation in processes that influence population structure at different spatial scales.

Second, local adaptation studies have revealed interspecific variation in local adaptation (e.g., Yamahira and Conover 2002), variation in strength of adaptation among populations (e.g., Storfer et al. 1999), and intraspecific variation in adaptive responses to *different* environmental gradients (e.g., Chapin and Chapin 1981). I have shown that intraspecific differentiation along the *same* environmental gradient can vary among regions. The importance of large-scale environmental variation on processes at the local-scale is an important issue in ecology (Levin 1992). In marine systems, research has focused on regional variation in local recruitment, community structure, and species distributions (e.g., Menge et al. 2003, Harley and Helmuth 2003, Broitman et al. 2008) and I have demonstrated the importance of large-scale variation for

population-level processes as well. Lastly, increased intraspecific diversity can influence populations and communities (Hughes et al. 2008, Bolnick et al. 2011), is related to the evolutionary potential of populations (Reed and Frankham 2003, Le Rouzic and Carlborg 2008), and represents an important, but often overlooked, component of ecological studies. Differentiation in traits that can influence ecosystem function (i.e., nutrient uptake and NUE) suggest intraspecific variation can lead to higher-order interactions along the intertidal gradient (Hughes et al. 2008, Whitlock 2014).

Fucus vesiculosus is a foundation species on rocky shores throughout the temperate North Atlantic Ocean, and genetically based variation in phenotypes within and among populations could have important higher-order consequences for the associated communities. *F. vesiculosus* populations in the eastern North Atlantic Ocean show genotypic variation in photosynthesis and growth (Rothäuster et al. 2016), induced herbivore defenses (Haavisto et al. 2010), and response to multiple climate stressors (Al-Janabi et al. 2016). Genotypic variation in traits provides the diversity necessary for natural selection to act. Here, I have demonstrated adaptive variation in traits in western North Atlantic populations of *F. vesiculosus*. A genetic component to growth, nitrate uptake, and nutrient resource use-efficiency was evident in some populations that I studied, and phenotypes were differentially distributed along the intertidal gradient. Given that the adaptive response to the intertidal gradient varied among regions, the role of higher-order genetic effects likely also varies among regions. Importantly, these effects would occur over the landscape (or '*seascape*') of the intertidal gradient rather than at the plot scale as has been demonstrated in other marine and terrestrial primary producers (e.g., Hughes and Stachowicz 2004, Crutsinger et al. 2006, Crawford and Whitney 2010 but see e.g., Whitham 1989, Bailey et al. 2004). This study system represents an opportunity to test for associations and

cause-and-effect relationships between population-level processes and community and ecosystem-level processes over a landscape, and the geographic variation in such relationships.

Steep environmental gradients are often used as a tractable system in which to experimentally test how changes in abiotic and biotic factors alter populations, communities, and ecosystem processes. More recently, steep environmental gradients in factors such as temperature and carbon dioxide, have been used as a space-for-time substitute to predict the response of organisms to global climate change in the coming centuries (e.g., Loarie et al. 2009, Kroeker et al. 2011). The results of the current study suggest that environmental variation at the large-scale can interact with abiotic and biotic environmental variation at the local-scale to alter the underlying ecological and evolutionary mechanisms that drive acclimation and adaptation of populations to climate conditions. Accounting for variation in the mechanisms that drive intraspecific differentiation, in response to the environment, improves our basic understanding of how intraspecific diversity arises and our ability to predict how populations will respond to climate change.

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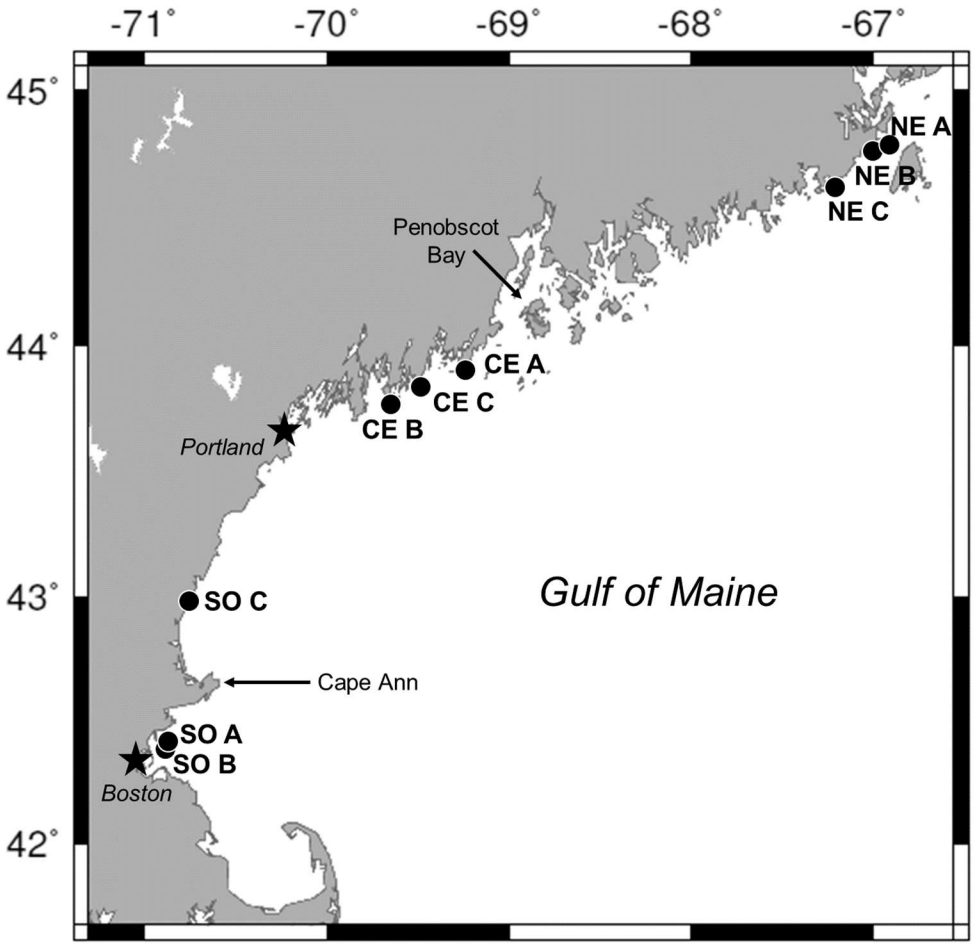
APPENDICES

APPENDIX A. Location of sites used for collections and experiments. Three sites within the northeast (NE), central (CE), and southern (SO) regions were sampled for genetic analyses. Two sites (A and B) within each region were used for phenotype experiments. Detailed information about each collection site can be found in Appendix B.



APPENDIX B. Site-level information for environmental data and *Fucus vesiculosus* population data. *Environmental data:* Temperatures (°C) are shown for July (summer) and January (winter). NO_3^- concentrations (µmol L^{-1}) are from samples taken in 2013 and 2014. Max tidal amplitude is the greatest difference between consecutive high and low tides. *Fucus vesiculosus* data: Linear vertical distance is the maximum distance between upper and lower individuals. Mean elevation is in m above mean lower-low water (MLLW) and was used to calculate % time submerged. Notes: '-' indicates sites where data were not collected. 'n.d.' indicates no data due to datalogger malfunction. All sites were used for genetic sampling. 'A' and 'B' sites were used for reciprocal transplant experiments.

Data were collected at each site to quantify variation in environmental conditions and *F. vesiculosus* distribution and abundance. Temperature (°C) was recorded every 5-10 minutes (n = 1 - 2 loggers per site; TidbiT v2 Temp Logger, Onset Computer Corp., Bourne, Massachussetts, USA) and separated into air and water temperatures by aligning temperature data to published tide height data from the nearest site (Flater 1998). To quantify ambient nitrate levels (µmol L^{-1} NO_3^-), water samples were collected at each site approximately every 3-4 weeks (n = 5 per site per collection date) between May 2012 to February 2014 and analyzed with a QuickChem FIA 8500 Autoanalyzer (Lachat Instruments; Loveland, Colorado, USA – detection limit: 0.014 µmol L^{-1} NO_3^-). More detailed sampling information and data on nitrate levels by sampling date are reported in Benes and Bracken (*in press*).

In summers 2010 and 2012, surveys were conducted at all study sites to quantify the distribution and abundance of *F. vesiculosus*. At each site, four perpendicular transects were laid at random locations along a 50 m transect line from the low to high intertidal zones. Quadrats (25 cm x 25 cm) were placed on the substratum every 3-m, and the tidal elevation and the abundance (percent cover of sessile species and counts of mobile species) and identity of all species were recorded. Tidal elevations for each quadrat were measured with a laser surveyor. Elevation in meters for each quadrat, was converted to the average percent time submerged using tide height predictions (Flater 1998) to determine the daily number of hours a quadrat was under water (i.e., 50% = 12 hours submerged per day). Data collected from surveys was used to determine the elevation, percent time submerged, and the maximum vertical (linear) distance between upper and lower edges of *F. vesiculosus*' intertidal distribution.

	Gulf of Maine Region								
	Northeastern (NE)			Central (CE)			Southern (SO)		
	Quoddy Head State Park, Lubec, Maine (NE A)	Hamilton Cove, Lubec, Maine (NE B)	Little Machias Rd., Cutler, Maine (NE C)	Marshall Point Light, Port Clyde, Maine (CE A)	Pemaquid Point Light, Bristol, Maine (CE C)	Seaside Inn, Newagen, Maine (CE B)	Pulpit Rock Tower, Rye, New Hampshire (SO C)	Forty Steps, Nahant, Massachusetts (SO A)	Dive Beach, Nahant, Massachusetts (SO B)
Latitude & Longitude	44° 48' 48" N 66° 57' 07" W	44° 47' 13" N 67° 00' 34" W	43° 1' 53" N 70° 43' 13" W	43° 55' 07" N 69° 15' 40" W	43° 50' 11" N 69° 30' 27" W	43° 47' 12" N 69° 39' 39" W	44° 38' 30" N 67° 14' 26" W	42° 25' 23" N 70° 54' 28" W	42° 25' 13" N 70° 54' 17" W
Mean Winter Air Temp. (min - max)	-0.7 (-15.8 - 7.9)	-2.4 (-16.4 - 9.2)	-	-1.0 (-14.9 - 7.5)	-	0.7 (-11.1 - 7.1)	-	-0.6 (-11.9 - 11.8)	0.0 (-11.6 - 11.4)
Mean (± SD) Winter Water Temp.	3.8 ± 1.4	3.3 ± 1.4	-	1.4 ± 1.5	-	3.6 ± 0.7	-	3.0 ± 0.9	3.3 ± 0.6
Mean Summer Air Temp. (min - max)	15.4 (9.5 - 32.5)	n.d.	-	19.1 (12.2 - 36.4)	-	18.7 (12.1 - 33.9)	-	24.03 (16.2 - 37.5)	n.d.
Mean (± SD) Summer Water Temp.	10.8 ± 0.9	n.d.	-	15.5 ± 1.6	-	15.5 ± 1.7	-	18.0 ± 1.3	n.d.
Mean [NO_3^-] (min - max)	3.7 (0.5 - 9.3)	3.7 (1.7 -6.7)	-	2.4 (0.2 - 5.6)	-	1.8 (0.04 - 5.9)	-	1.4 (0.05 - 5.3)	1.4 (0.1 - 6.4)
Max Tidal Amplitude (m)	6.7	6.7	5.5	4.1	4.0	4.0	4.0	4.1	4.1
Fucus Linear Vertical Distribution (m)	54	44	32	39	21	14	30	30	15
Upper Limit - Meters Above MLLW ± SE (% time submerged)	3.8±0.02 (31%)	3.4±0.04 (37%)	2.7±0.1 (38%)	2.0±0.1 (37%)	2.4±0.1 (21%)	2.2±0.03 (27%)	1.9±0.1 (37%)	1.8±0.04 (43%)	2.2±0.03 (33%)
Lower Limit - Meters Above MLLW ± SE (% time submerged)	0.8±0.02 (78%)	0.0±0.02 (94%)	0.5±0.04 (79%)	0.4±0.02 (80%)	0.6±0.1 (72%)	0.96±0.1 (62%)	0.51±0.1 (72%)	0.0±0.04 (94%)	0.17±0.03 (90%)
Genetics Sampling Date	June 14, 2010	June 13, 2010	June 15, 2010	July 15, 2010	July 17, 2010	July 16, 2010	August 15, 2010	June 17, 2010	June 18, 2010
Transplant Trial Start Dates	1: August 30, 2011 2: June 24, 2013	1: August 30, 2011 2: June 24, 2013	-	1: July 10, 2013 2: August 16, 2015	-	1: July 10, 2013 2: August 16, 2015	-	1: August 14, 2011 2: August 19, 2013 3: July 30, 2015	1: August 15, 2011 2: August 19, 2013 3: July 30, 2015