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## NITRATE UPTAKE VARIES WITH TIDE HEIGHT AND NUTRIENT AVAILABILITY IN THE INTERTIDAL SEAWEED *FUCUS VESICULOSUS*<sup>1</sup>

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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 *Fucus vesiculosus*. Furthermore, 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.

**Key index words:** *Fucus vesiculosus*; Gulf of Maine; intertidal; latitudinal variation; nitrate; nitrogen; nutrient uptake; phosphate

**Abbreviations:** ANOVA, analysis of variance; C, carbon;  $K_s$ , half-saturation constant; MLLW, mean lower low water; N, nitrogen;  $\text{NO}_3^-$ , nitrate;  $\text{PO}_4^{3-}$ , phosphate;  $V_{\max}$ , maximum uptake rate

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 1994 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

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(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). Furthermore, 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 (Phaeophyceae, Ochrophyta) 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 than 1.0-m above mean lower low water; MLLW) to high (greater than 2.0-m 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: <math>0.1\text{--}0.4\ \mu\text{M}</math>) 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 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: (i) that nutrient uptake rates would be higher in upper shore compared to lower shore individuals, and (ii) that individuals would acclimate to changes in tide height over a 30-d 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 AND METHODS

*Study sites and F. vesiculosus distribution and collection.* For observations and experiments testing nutrient status and physiology of *F. vesiculosus*, seaweed and water samples were collected from sites throughout the Gulf of Maine (Fig. 1 and Table S1 in the Supporting Information). 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 ~4.1-m in the

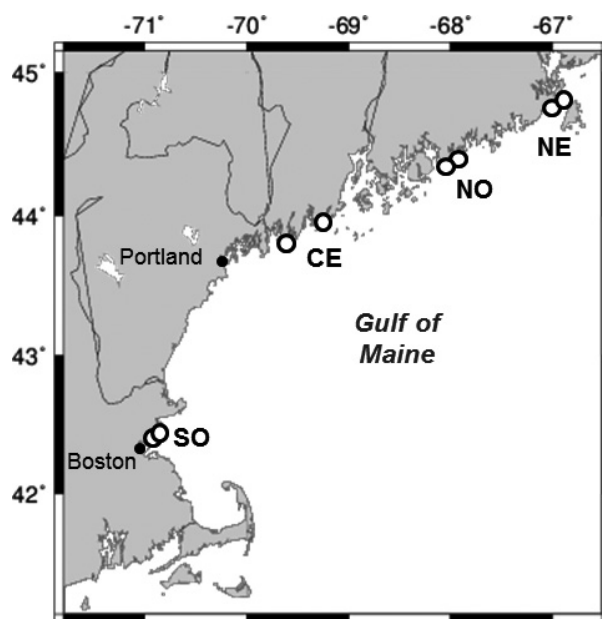


FIG. 1. Study sites in the northeast (NE), northern (NO), central (CE), and southern (SO) Gulf of Maine. Map created using the online Map It tool (USGS/Woods Hole).

south to ~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-min intervals were used to calculate the hours submerged by adding together the number of intervals in a 24-h 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 h 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 h, and southern Gulf of Maine samples were maintained in a cool dark place for at least 4 h to mimic sample handling from other sites. Portions of vegetative apical tissue (~2–3 cm length and ~0.5–1.5 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 h. 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, five replicate water samples (500 mL each) were collected at each site. Samples were filtered (Whatman GF/F) within 1 h and frozen for later measurement of ambient nitrate ( $\text{NO}_3^-$ ) and phosphate ( $\text{PO}_4^{3-}$ ) concentrations ( $\mu\text{mol} \cdot \text{L}^{-1}$ ) (QuickChem FIA 8500 Autoanalyzer; Lachat Instruments; Loveland, CO, USA – detection limit:  $0.014 \mu\text{mol} \cdot \text{L}^{-1}$  nitrate [ $\text{NO}_3^-$ ] and  $0.054 \mu\text{mol} \cdot \text{L}^{-1}$  phosphate [ $\text{PO}_4^{3-}$ ]). Water samples were collected every 3–4 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 ~3× 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 65°C 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, MA, USA).

*Nitrate uptake rates of upper versus lower shore F. 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 eight 1-L chambers using a design modified from Bracken et al. (2011). During the uptake experiment, high water flow (~18  $\text{cm} \cdot \text{s}^{-1}$ ), saturating light levels ( $>1,000 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ), and constant temperatures ( $14.0 \pm 0.3$  [mean  $\pm$  SE]°C) were maintained to maximize nitrate uptake (Hurd et al. 1996, 2014). Individuals were collected and transported as described above, and nitrate uptake was measured on apical tips within 24 h 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-min acclimation period (to chamber conditions), each chamber was spiked with  $\text{NaNO}_3$  to achieve one of four initial nitrate concentrations: 2, 15, 30 and  $50 \mu\text{mol} \cdot \text{L}^{-1}$ . After a 5-min mixing period, water (6 mL) was sampled from chambers every 10 min for 50 min ( $n = 6$  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 ( $\mu\text{mol} \cdot \text{L}^{-1}$ ) was quantified using linear regression to find the rate of uptake ( $\mu\text{mol NO}_3^- \cdot \text{h}^{-1} \cdot \text{L}^{-1}$ ) 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 and 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 ( $\mu\text{mol NO}_3^- \cdot \text{h}^{-1} \cdot \text{L}^{-1}$ ) was divided by the dry tissue mass to calculate biomass-specific uptake rates ( $V$ ;  $\mu\text{mol NO}_3^- \cdot \text{L}^{-1} \cdot [\text{g DW}]^{-1} \cdot \text{h}^{-1}$ ) for each apical tip at each initial nitrate concentration ( $\mu\text{mol} \cdot \text{L}^{-1}$ ; 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  $\times 0.242$ ;  $R^2 = 0.98$ ,  $P < 0.001$ ). Even though our experimental concentrations included two nitrate concentrations (30 and  $50 \mu\text{mol} \cdot \text{L}^{-1}$ ) 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,  $V_{\text{max}}$ ; half-saturation coefficient,  $K_s$  [Berges et al. 1994]). Therefore, we treated our target experimental nitrate concentration (i.e., 2, 15, 30 or  $50 \mu\text{mol} \cdot \text{L}^{-1}$ ) 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  $\times$  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 (Fig. 1; two sites in NE, CE, and SO regions) 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 h) 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 =  $25.25 \pm 0.03$  [mean  $\pm$  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 (12–24 h) 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  $25 \times 25$  cm<sup>2</sup> 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 ~30 d, 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 *F. 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 ( $n = 9$ –14 per transplant height/home height/site). Uptake experiments took place 3–9 d after collection from field sites (1–7 d after cutting apical tips), replicates were randomly assigned to nitrate concentrations across days, and nitrate uptake rates did not vary among days (analysis of variance [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”  $\times$  “experimental nitrate concentration”  $\times$  “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<sub>3</sub><sup>-</sup>], [PO<sub>4</sub><sup>3-</sup>], NO<sub>3</sub><sup>-</sup>:PO<sub>4</sub><sup>3-</sup> ratio) and time submerged (hours) in the 24 h 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 nonequal 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). ANOVA was then conducted to compare response variables across model factors (see below).

Analyses of observations of ambient seawater nutrient levels (i.e., NO<sub>3</sub><sup>-</sup>, PO<sub>4</sub><sup>3-</sup>, NO<sub>3</sub><sup>-</sup>:PO<sub>4</sub><sup>3-</sup>) 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; Fig. S1 in the Supporting Information) 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”  $\times$  “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 (Torsten 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<sub>3</sub><sup>-</sup>, PO<sub>4</sub><sup>3-</sup>, NO<sub>3</sub><sup>-</sup>:PO<sub>4</sub><sup>3-</sup>) and time submerged (i.e., number of hours in the preceding 24 h 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  $\mu\text{mol} \cdot \text{L}^{-1}$   $\text{NO}_3^-$  and 0.07 to 4.06  $\mu\text{mol} \cdot \text{L}^{-1}$   $\text{PO}_4^{3-}$  across sampling dates and sites.  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$ , and  $\text{NO}_3^-:\text{PO}_4^{3-}$  varied significantly among sampling dates (ANOVA;  $P < 0.001$ ), with highest levels typically occurring in spring and fall (Fig. S2 and the Supporting Information). On average, ambient  $\text{NO}_3^-$  concentrations were highest in the northeast (ANOVA:  $F_{3,55.75} = 12.89$ ,  $P < 0.001$ ; Fig. 2A); however, ambient  $\text{PO}_4^{3-}$  concentrations did not differ among regions (ANOVA:  $F_{3,55.34} = 1.22$ ,  $P = 0.31$ ). This resulted in overall average  $\text{NO}_3^-:\text{PO}_4^{3-}$  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 *F. 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. 2B). 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. 2D; 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 (Fig. S3 in the Supporting Information). Tissue %N was positively related to ambient nitrate concentrations in all regions, but not significantly in the northeastern Gulf of Maine (Fig. S4 in the Supporting Information).

**Nitrate uptake rates of upper versus lower shore *F. 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  $\times$  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

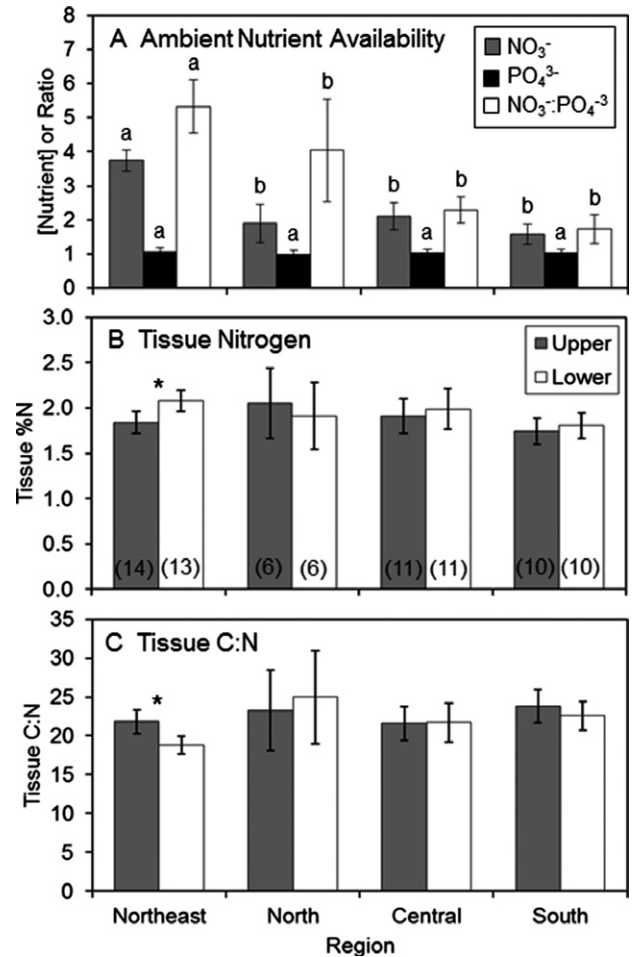


Fig. 2. Average of long-term water and *Fucus vesiculosus* tissue nutrient collections across regions in the Gulf of Maine. (A) Mean ( $\pm$ SE) ambient nitrate and phosphate concentrations ( $\mu\text{mol} \cdot \text{L}^{-1}$ ) as well as their ratio (northeast ( $n = 32$ ), north ( $n = 16$ ), central ( $n = 27$ ), 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 (\* $P < 0.05$ ).

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. 3). As expected, the nitrate uptake rate increased with the experimental nitrate concentration (ANOVA:  $F_{3,83.79} = 10.35$ ,  $P < 0.001$ ; Fig. S5 in the Supporting Information) but there were no significant interactions between experimental nitrate concentration and other main effects (ANOVA:  $P > 0.5$ ).

**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:

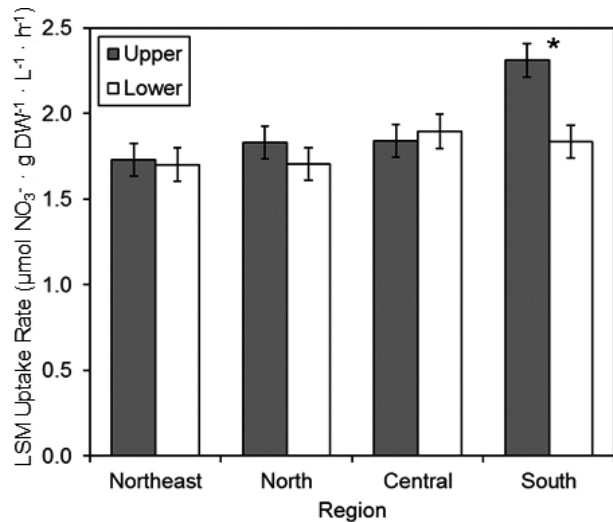


FIG. 3. Least-square mean (LSM) nitrate uptake rates ( $\mu\text{mol} \cdot \text{L}^{-1} \cdot \text{gDW}^{-1} \cdot \text{h}^{-1}$ ) for *Fucus vesiculosus* at the upper and lower limits of its intertidal distribution in the Gulf of Maine regions ( $n = 16$  per region/tide height). LSMs are of square root-transformed data and error bars are  $\pm$ SE. Significant differences between upper and lower intertidal uptake rates are denoted by an asterisk ( $*P < 0.05$ ).

$F_{1,440.1} = 14.14$ ,  $P < 0.001$ ) and between “home” tide heights (ANOVA:  $F_{1,440.1} = 23.57$ ,  $P < 0.001$ ; Fig. 4). However, these differences varied by region. Uptake rate decreased between initial and final measurements by 18% in the northeast (Fig. 4A) and 13% in the central regions (Fig. 4B; post hoc test,  $P < 0.05$ ) but did not change in the southern Gulf of Maine (Fig. 4C; 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 from 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  $\times$  Home Height” or “Home Height  $\times$  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. 4A; post hoc test,  $P < 0.05$ ).

As in our first experiment, nitrate uptake rate increased with nitrate concentration (Fig. S6 in the

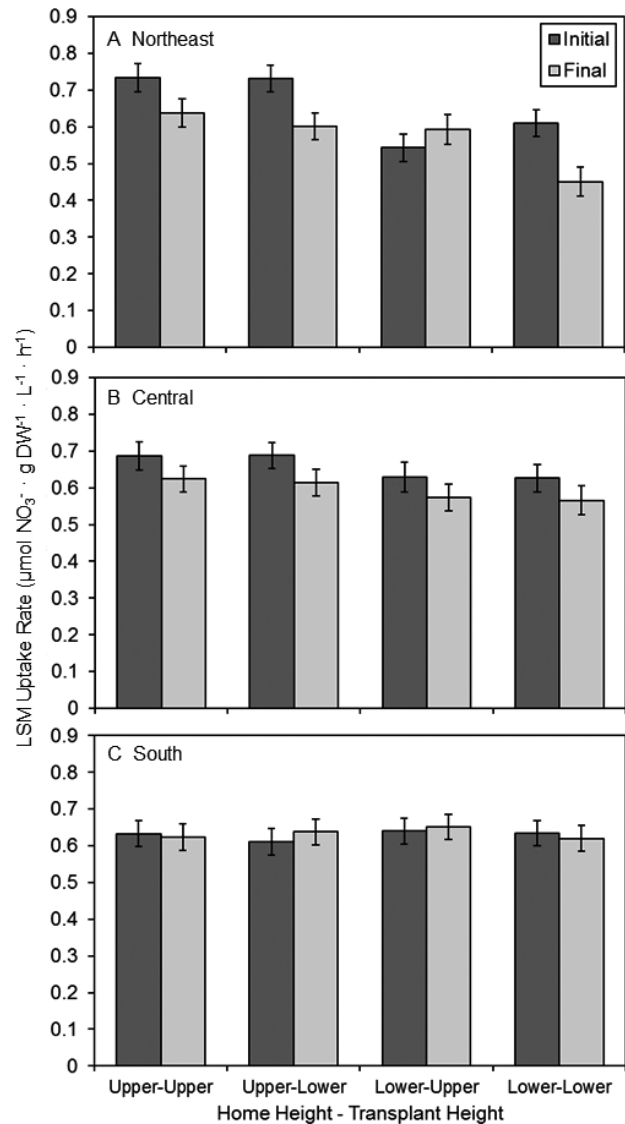
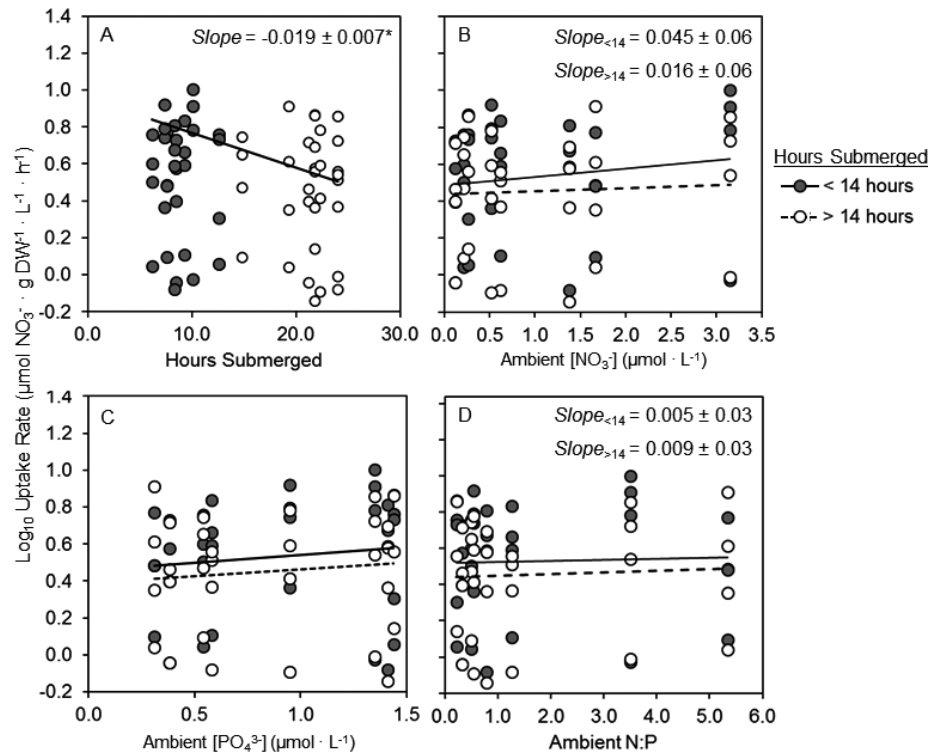


FIG. 4. Least square mean (LSM) nitrate uptake rates before (initial) and after (final) a 30-d reciprocal transplant experiment between the upper and lower edges of *Fucus vesiculosus*' intertidal distribution. Transplants were conducted at sites within the (A) northeast, (B) central, and (C) southern Gulf of Maine regions ( $n = 17$ –27 per region/home height/transplant height combination). Error bars are  $\pm$ 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,  $P < 0.05$ ).

Supporting Information; ANOVA;  $F_{3,440.16} = 135.56$ ,  $P < 0.001$ ). However, this difference depended on time and region (ANOVA; “Time  $\times$  Region  $\times$  Concentration,”  $F_{2,440.53} = 2.41$ ,  $P = 0.03$ ) with lower final nitrate uptake rates at 15 and 30  $\mu\text{mol} \cdot \text{L}^{-1}$  in the northeast and central regions (Fig. S6).

*Environmental covariates of nutrient uptake rates.* During each of our experiments, ambient nitrate and phosphate levels and  $\text{NO}_3^-:\text{PO}_4^{3-}$  ratios varied significantly among study sites (ANOVA:

FIG. 5. Variation in nitrate uptake rate ( $\mu\text{mol} \cdot \text{L}^{-1} \cdot \text{gDW}^{-1} \cdot \text{h}^{-1}$ ) related to (A) hours submerged (B) ambient nitrate concentration ( $\mu\text{mol} \cdot \text{L}^{-1}$ ), (C) ambient phosphate concentration ( $\mu\text{mol} \cdot \text{L}^{-1}$ ), and (D)  $\text{NO}_3^-:\text{PO}_4^{3-}$  ratio. Significant interactions between hours submerged and nutrient concentrations are shown by splitting the data into observations of upper shore (less than (<) 14 h submerged) and lower shore (more than (>) 14 h submerged) *Fucus 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 S2). Best fit slopes ( $\pm\text{SE}$ ) from multiple linear regression analysis (Table 1) are given in the upper right corner of each panel (\* $P < 0.05$ ).



$P < 0.001$ ; Table S2 in the Supporting Information). During our upper versus lower shore experiment in May 2012, the number of hours *F. vesiculosus* was submerged was a significant predictor of nitrate uptake rate (Fig. 5A; Table 1). Importantly, the hours submerged mediated the relationship between nitrate uptake rate and nitrate concentration and  $\text{NO}_3^-:\text{PO}_4^{3-}$  ratio (i.e., significant

“ $[\text{NO}_3^-] \times \text{Submergence Time}$ ” and “ $\text{NO}_3^-:\text{PO}_4^{3-} \times \text{Submergence Time}$ ” interactions). Nitrate uptake rates increased with higher nitrate concentrations 2.8-fold more rapidly in upper shore compared to lower shore *F. vesiculosus* (Fig. 5B). The overall response in nitrate uptake rates to changes in  $\text{NO}_3^-:\text{PO}_4^{3-}$  ratio compared to nitrate concentration was an order of magnitude lower, but lower

TABLE 1. Multiple regression parameter estimates for the relationship between  $\log_{10}$  transformed nitrate uptake rates ( $\mu\text{mol} \cdot \text{gDW}^{-1} \cdot \text{L}^{-1}$ ) and explanatory variables: nitrate concentration  $[\text{NO}_3^-]$ , phosphate concentration  $[\text{PO}_4^{3-}]$ ,  $[\text{NO}_3^-]:[\text{PO}_4^{3-}]$  ratio, hours submerged, and their interactions.

Parameter <sup>†</sup>	Upper versus lower experiment	Initial transplant experiment	Final transplant experiment	Change across transplant experiment
Intercept	$0.96 \pm 0.12^{***}$	$0.85 \pm 0.17^{***}$	$1.04 \pm 0.13^{***}$	$-1.81 \pm 0.65^{**}$
$[\text{NO}_3^-]$	$0.18 \pm 0.10$	$-0.17 \pm 0.14$	$0.12 \pm 0.18$	$1.47 \pm 0.93$
$[\text{PO}_4^{3-}]$	$-0.13 \pm 0.15$	$0.17 \pm 0.20$	$-0.065 \pm 0.16$	$-5.81 \pm 2.39^*$
$[\text{NO}_3^-]:[\text{PO}_4^{3-}]$	$-0.088 \pm 0.049$	$0.13 \pm 0.08$	$-0.104 \pm 0.14$	$-1.27 \pm 0.71$
Submergence time	$-0.019 \pm 0.007^*$	$0.0064 \pm 0.013$	$-0.016 \pm 0.010$	$-0.001 \pm 0.068$
$[\text{NO}_3^-] \times \text{sub. time}$	$-0.012 \pm 0.006^*$	$0.01 \pm 0.009$	$-0.014 \pm 0.011$	$-0.095 \pm 0.11$
$[\text{PO}_4^{3-}] \times \text{sub. time}$	$0.015 \pm 0.009$	$-0.01 \pm 0.014$	$0.017 \pm 0.012$	$0.17 \pm 0.29$
$[\text{NO}_3^-]:[\text{PO}_4^{3-}] \times \text{sub. time}$	$0.0067 \pm 0.003^*$	$-0.0082 \pm 0.005$	$0.011 \pm 0.009$	$0.072 \pm 0.085$
Model <i>F</i> -value	47.69	103.6	113.2	2.75
$d_{f_{\text{num}}}, d_{f_{\text{den}}}$	10, 53	10, 85	10, 83	10, 83
Model $R^2$	0.88	0.92	0.92	0.16
Model <i>P</i> -value	<0.001	<0.001	<0.001	<0.001

<sup>†</sup>Model Parameter *P*-value \* $<0.05$ , \*\* $<0.01$ , \*\*\* $<0.001$ .

Models also included target nutrient concentration as a fixed factor to account for variation to different experimental nitrate concentrations. Model results are shown for the upper versus lower experiment (Fig. 5), initial and final transplant experiment measurements, and change in uptake rate and explanatory variables between initial and final transplant measurements (Fig. 6). 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 ( $d_{f_{\text{num}}}$ ) and denominator ( $d_{f_{\text{den}}}$ ) degrees of freedom,  $R^2$ , and *P*-value.



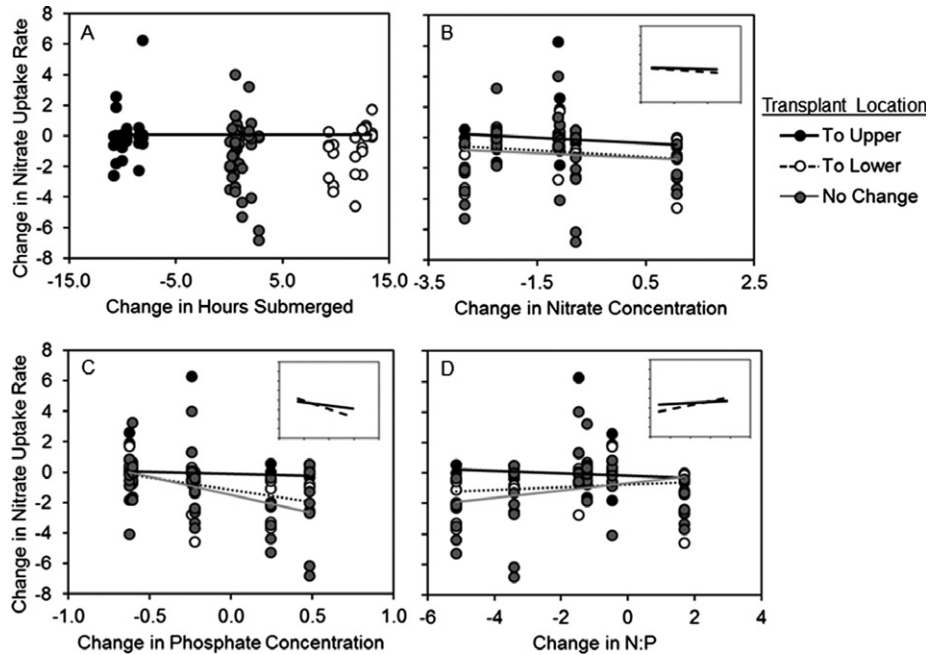


FIG. 6. The relationship between the change in nitrate uptake rate ( $\mu\text{mol} \cdot \text{L}^{-1} \cdot \text{gDW}^{-1} \cdot \text{h}^{-1}$ ) of *Fucus vesiculosus* and change in (A) hours submerged, (B) ambient nitrate concentration ( $\mu\text{mol} \cdot \text{L}^{-1}$ ), (C) phosphate concentration ( $\mu\text{mol} \cdot \text{L}^{-1}$ ), and (D)  $\text{NO}_3^-:\text{PO}_4^{3-}$  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.

shore individuals had a 1.8-fold greater increase in nitrate uptake rate with increasing  $\text{NO}_3^-:\text{PO}_4^{3-}$  ratio (Fig. 5D). Uptake rates were not related to phosphate concentrations (Fig. 5C).

Interestingly, ambient nutrient concentrations and submergence time were not significant predictors of initial and final uptake rates (Table 1); 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. 6C) but was not related to changes in ambient nitrate concentration (Fig. 6B) or  $\text{NO}_3^-:\text{PO}_4^{3-}$  ratio (Fig. 6D; Table 1). The greatest increases in nitrate uptake rate during the experiment occurred at sites with the greatest decreases in phosphate concentrations (Table 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. 6A). In particular, *F. vesiculosus* from its lower limit had a much larger variation in response compared to *F. vesiculosus* from its upper intertidal limit (Fig. S7 in the Supporting Information). 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  $\text{NO}_3^-:\text{PO}_4^{3-}$  ratio similar to individuals transplanted to the lower limit of its distribution (Fig. 6, 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 1).

#### 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 *F. 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 (Figs. S2 and S3). 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 seaweed species (Thomas et al. 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 F. 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  $2 \mu\text{mol} \cdot \text{L}^{-1}$ ), 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  $\times$  Concentration” interaction). These differences were strongest at sites where local ambient nitrate concentrations, at the time of collection, were  $>0.5 \mu\text{mol} \cdot \text{L}^{-1}$  (see Table S2 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 *F. 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 *G. 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). During our first experiment, regional differences could be attributed to differences in 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 (Figs. S2 and S3), 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 and Chapman 1983) and *S. latissima* (formerly *L. groenlandica*) 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, Kocczak 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. 3). 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). 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. 5; Table S2). 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 (Fig. S2; 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 (Fig. S2; 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 ( $[\text{NO}_3]_{\text{dev}}$ ) after the transplant experiment (i.e., time when tissue %N was lowest) compared to other time points (Fig. S1) suggesting rapid uptake at the end of the experiment.

The difference between final and initial nitrate uptake rates during this potentially N-limited period was 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. 6 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). Furthermore, nitrogen deprivation of *F. vesiculosus* led to a rapid reduction in NRA to ~10% of predeprivation levels in just 2 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 and 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, although surge uptake of ammonium and phosphate has been identified in intertidal seaweeds (Thomas and 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 min) compared to longer (15–90 min) 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 include 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 as this one provide a mechanistic understanding of how primary producers maintain levels of tissue nutrients despite a fluctuating environment.

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### Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

**Figure S1.** Mean ( $\pm$ SE) proportional deviation from target nitrate concentration ( $x$ -axis;  $\mu\text{mol} \cdot \text{L}^{-1}$ ) measured at  $t_{5\text{min}}$  during our uptake experiments ( $[\text{NO}_3]_{\text{Dev}} = ([\text{NO}_3]_{5\text{min}} - [\text{NO}_3]_{\text{Target}}) / [\text{NO}_3]_{\text{Target}}$ ). Data shown for (A) upper versus lower experiment in May 2012 and (B–D) initial and final measurements for the transplant experiment. (A)  $[\text{NO}_3]_{\text{Dev}}$  differed between regions depending on target nitrate concentration (ANOVA: “Region  $\times$  Concentration” interaction,  $F_{9,92} = 2.28$ ,  $P = 0.02$ ). (B–D) For the transplant experiment,  $[\text{NO}_3]_{\text{Dev}}$  was greatest at the final time point of our experiment (ANOVA:  $F_{1,441.8} = 88.38$ ,  $P < 0.001$ ), depending on region (ANOVA: “Time  $\times$  Region,”  $F_{1,441.8} = 88.38$ ,  $P < 0.001$ ) and target concentration (ANOVA: “Time  $\times$  Concentration,”  $F_{3,441.9} = 14.90$ ,  $P < 0.001$ ). Upper and lower transplant heights were significantly different (ANOVA:  $F_{1,442.1} = 7.38$ ,  $P < 0.01$ ) which depended on concentration (ANOVA: “Transplant Height  $\times$  Concentration” interaction,  $F_{3,442.43} = 3.61$ ,  $P = 0.02$ ).

**Figure S2.** Mean monthly  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$ , and  $\text{NO}_3^-:\text{PO}_4^{3-}$  (N:P) ratios for the (A) northeastern, (B) northern, (C) central, and (D) southern Gulf of Maine. Data are averages from two sites within each region. Error bars have been left off for clarity.

**Figure S3.** Mean monthly %N and C:N ratios for the (A) northeastern, (B) northern, (C) central, and (D) southern Gulf of Maine. Data are averages across sampling dates from two sites within each region. Error bars have been left off for clarity.

**Figure S4.** Associations between tissue %N and seawater  $\text{NO}_3^-$  at sites in the (A) northeast, (B) north, (C) central, and (D) southern Gulf of Maine. Pearson-product moment correlation values are shown with significant values denoted by an asterisk ( $*P < 0.05$ ). Data for upper (filled circles) and lower (open circles) intertidal *Fucus*

*vesiculosus* are shown but were not treated separately in the analysis. Each point represents average replicate samples of tissue %N and seawater nitrate concentrations ( $\text{NO}_3^- \mu\text{mol} \cdot \text{L}^{-1}$ ) per sampling date and site ( $n = 5$  per point per variable).

**Figure S5.** Mean nitrate uptake rates ( $\mu\text{mol} \cdot \text{L}^{-1} \cdot \text{gDW}^{-1} \cdot \text{h}^{-1}$ ) at four nitrate concentrations ( $\mu\text{mol} \cdot \text{L}^{-1}$ ). Uptake rates are for *Fucus vesiculosus* at the upper and lower limits of its intertidal distribution in the (A) northeastern, (B) northern, (C) central, and (D) southern Gulf of Maine regions ( $n = 4$  per region/tide height/concentration). Error bars are  $\pm\text{SE}$ .

**Figure S6.** Initial (left panel) and final (right panel) mean ( $\pm\text{SE}$ ) nitrate uptake rates ( $\mu\text{mol} \cdot \text{L}^{-1} \cdot \text{gDW}^{-1} \cdot \text{h}^{-1}$ ) at target (experiment) nitrate concentrations ( $\mu\text{mol} \cdot \text{L}^{-1}$ ) for each of the treatment groups from the transplant experiment. Means are shown for the northeast (A and D), central (B and E), and southern (C and F) Gulf of Maine.

**Table S1.** Study site information including location and intertidal elevation of the upper and lower edges of *Fucus vesiculosus* distribution at each site. Proportion of time submerged is provided since tidal amplitude changes latitudinally in the GOM. Proportion is calculated over 1 year of 5-min interval tide height prediction data for each site.

**Table S2.** Mean ( $\pm\text{SE}$ ) nutrient concentrations ( $\mu\text{mol} \cdot \text{L}^{-1}$ ) 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-min interval tide height prediction data for each site and are based on the sum of intervals in 24 h preceding collection. Northern region sites were not used in the transplant experiment.