Eco-physiological and ecological responses of *Fucus* algae to changes in the surrounding environment

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DEDICATION

I would like to dedicate this written dissertation to my wife, Meg, for whom my world was forever changed the day we met. She is my biggest supporter, my closest confidant, and my best friend. Thank you for all that you have done and all that you continue to do. You make me a better scientist, a better husband, a better father, and a better person.
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ABSTRACT OF DISSERTATION

A fundamental goal of global climate change research is to understand how organisms will likely respond to novel environmental conditions. Determining the photosynthetic and eco-physiological response of marine macroalgae to changes in their environment is vital to characterizing the success of these habitat-forming primary producers in the face of climate change. For this dissertation research I used a combination of empirical and theoretical methods to explore the main drivers of ecophysiological performance of Fucus, and how this may translate to patterns in the field under future environmental conditions. In Chapter 1, I examined how changes in seawater temperature affected net photosynthetic production by Fucus gardneri, a northeastern Pacific species. Using empirical measurements, I determined the thermal performance optimum for non-reproductive apical tips of F. gardneri at a saturating irradiance, and the oxygen consumption rate during respiration in the dark. In conjunction with one year of environmental data (e.g., seawater temperature, irradiance, tidal height), I developed a net photosynthesis model, and used this model to explore how photosynthetic production in F. gardneri is affected by the timing of low tide at multiple intertidal elevations. Because macroalgae attain maximal photosynthesis when submerged in shallow water during peak solar radiation, the timing of when high tide occurs was found to have a significant effect on photosynthetic productivity, an observation that has not been made before. In Chapter 2, I evaluated how nutrient enrichment (i.e., nitrogen) affects macroalgal growth and photosynthesis in Fucus vesiculosus, a northwestern Atlantic species. I quantified tissue growth, photosynthetic performance (i.e., photosynthesis versus irradiance curves: P-E curves) and O₂ production for F. vesiculosus individuals incubated in either ambient or nutrient-enriched conditions. Thermal
performance curves were calculated by measuring O₂ production for ambient and nutrient-enriched *F. vesiculosus* over a range of ecologically relevant temperatures (6–30°C). Though previous studies have shown the deleterious effects of nutrient enrichment in coastal habitats, this study demonstrated the potential additive effects on the growth and photosynthesis of a macroalgal primary producer. The findings from this study highlight the importance of quantifying the effects of nutrient enrichment in coastal systems near urban environments, and suggests that in some cases increased nutrients can have positive effects. In Chapter 3, I examined within- and between-site and population differences in nutrient content of *F. vesiculosus* and how these differences might affect grazing by an herbivorous snail *Littorina littorea*. Water and algal tissue samples were collected over the course of one year from three distinct sites (Deer Island, Nahant Island, and Marblehead) in Massachusetts to determine differences in nitrogen and chlorophyll *a* levels within seawater, and tissue nitrogen within *F. vesiculosus*. This study demonstrated the spatial and temporal variability in nitrogen levels in a macroalgal primary producer and grazing pressure from *Littorina littorea*, a dominant herbivore along the greater Boston coastline.

Coastal zones, especially urban coastal areas such as those in the greater Boston area, present organisms with patterns of environmental stressors and drivers that have high temporal and spatial heterogeneity. More fine-scale resolution studies are required in order to describe small-scale, local variability within an environment, and understand how species will tolerate differences in environmental nutrients in the coming decades as urban coastal communities increase in population. Therefore, this dissertation addresses the fundamental knowledge gap of environmental conditions and organism response by describing how temperature, the potentially mitigating effects of environmental nutrients, and spatial differences in grazing pressure affect
the ecological and ecophysiological response of a macroalgal primary producer in the rocky intertidal environment. Collectively, the results of this dissertation emphasize the complex ways in which climatic stressors (temperature) and non-climatic drivers (environmental nitrogen) affect a marine macroalgal species, and how these impacts may affect animals at higher trophic levels. By understanding the mechanisms by which these multiple effects influence ecologically important species such as *Fucus*, we have a better chance of predicting and potentially mitigating some of the impacts of global climate change in urban coastal zones.
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INTRODUCTION

A long-standing goal of marine physiological ecology has been to understand energy allocation in intertidal organisms (Van Haren and Kooijman 1993, Kearney et al. 2010), and to elucidate the ecological impacts of organismal vulnerability to differing environmental conditions (Kooijman et al. 2004, Dethier and Williams 2009). Global climate change has lent a new urgency to these studies. In order to predict ecological responses to what will likely be novel environmental conditions, mechanistic approaches are needed (Nisbet et al. 2000, Helmuth and Hofmann 2001, Helmuth 2009).

To date, most studies on the impacts of climate change have focused on the lethal impacts of weather extremes (Jones et al. 2009), but recent studies have emphasized the importance of sublethal physiological stress (Sokolova et al. 2012, Howard et al. 2013), both in driving fitness and species interactions. Many studies on the sublethal effects of environmental change have examined the influence of temperature on foraging rates (Sanford 2002, Kordas et al. 2011), growth (Almada-Villela et al. 1982), and reproduction (Petes et al. 2008) in marine animals. Sublethal stressors have also been shown to also impact macroalgal species, by affecting photosynthesis, growth, and survival (Bell 1993, Matta and Chapman 1995, Zou et al. 2007). To address the disconnect between observed patterns and the ultimate and proximate determinants of those patterns, a coupling of empirical measurements and mathematical modeling must be adopted.

In order to effectively measure and model biological responses to environmental change, the rocky intertidal system provides a natural gradient of physical factors over a small area. The rocky intertidal zone has been extensively researched because of the high diversity of organisms
and large spatial and temporal variability in temperature that can occur on tidal and diel cycles (Bertness 1981, Paine and Levin 1981, Wethey 1983, Helmuth 1998, Helmuth and Hofmann 2001). Intertidal invertebrates and algae are subject to these large environmental fluctuations, and have physiologically adapted to withstand such stressors. Nevertheless, many intertidal species may be living close to their upper thermal tolerance limits (Somero 2010), and therefore may serve as model organisms for evaluating the early ecological impacts of climate change. This dissertation addresses the fundamental knowledge gap of environmental conditions and organism response by describing how temperature, the potentially mitigating effects of environmental nutrients, and spatial differences in grazing pressure affect the ecological and ecophysiological response of a macroalgal primary producer in the rocky intertidal environment.

The influence of temperature fluctuations has long been evaluated in marine and terrestrial organisms (Paine and Levin 1981, Dromgoole 1987, 1988, Matta and Chapman 1995, Davison and Pearson 1996, Kawamitsu et al. 2000, Helmuth and Hofmann 2001). Temperature is of particular concern for marine intertidal species, which can experience large thermal variation over very rapid time scales. Although the body temperatures of intertidal invertebrates and macroalgae are determined by several factors (i.e., time of day, cloud cover, and tidal height), one of the most significant drivers is exposure to solar radiation, such that organism temperature is often considerably higher than air temperature, and shaded organisms can be markedly cooler than adjacent organisms in the sun. The subsequent variability in body temperature among organisms and species is one of the most influential determinants of organism physiology (Hofmann 1999, Pörtner 2001 2002, Somero 2002, 2005, Anguilletta 2009), leading to changes in behavior, growth, fecundity, and food consumption (Zimmerman and Pechenik 1991, Pörtner et al. 2001, Pincebourde et al. 2008).
In addition to thermal stress driven by solar radiation (Bell 1995), photosynthetic organisms are also strongly affected by levels of irradiation during high tide, leading to potential trade-offs between living in sun-exposed and shaded microenvironments. For example, small changes in tidal height affect the light microenvironment and temperature experienced by intertidal macroalgae, (Helmuth 2002, Alexandridis et al. 2012), affecting photosynthetic capacity, water retention, somatic growth, and reproductive development (Dromgoole 1980, Dring and Brown 1982, Dromgoole 1987, Falkowski and LaRoche 1991, Davison and Pearson 1996, Dethier and Williams 2009). Because macroalgae in intertidal habitats serve as structural refuges and food sources for many species, variation in tissue growth and photosynthetic productivity in different microhabitats has significant ecological consequences.

An increase in environmental nitrogen levels can impact the organisms in many coastal systems that are typically nitrogen-limited (Nordemar et al. 2003). High, chronic levels of nitrogen input from sewage and agricultural fertilizer has severely altered environmental nutrient levels (Nixon 1995, Valiela et al. 1997, Howarth 2008), which has been shown to affect trophic interactions (Levine et al. 1998, Hulot et al. 2000, Harpole et al. 2011, O’Connor et al. 2015). Although most studies have emphasized the downside of nutrient enrichment (Foster and Gross 1998, Smith et al. 1999, Anderson et al. 2002, Nixon and Buckley 2002), it is possible that there is also an upside to increased environmental nitrogen, as it would alleviate nitrogen limitation and allow for increased growth and production in some species (Hemmi and Jormalainen 2002, Bracken 2004, Elser et al. 2007, Galloway et al. 2008, Harpole et al. 2011). Several studies evaluating the role of nutrient enrichment in an environment have focused on estuarine and wetland communities (Valiela et al. 1997, Herbert 1999, Anderson et al. 2002, Howarth 2008), specifically dealing with eutrophic conditions associated to terrestrial runoff (Anderson et al.
However, there is a growing need for research within the rocky intertidal environment dealing with elevated environmental nutrients, particularly exploring the interactive effects of increased nitrogen - a limiting nutrient within this system – with environmental stressors (i.e., temperature). With the projected increase in urban coastal development in the coming century, it is paramount to characterize the success of coastal organisms exposed to nutrient-enriched conditions as terrestrial runoff and nearshore discharge of sewage are expected to increase, thus affecting coastal communities (Heugens et al. 2001, Thornber et al. 2008, Orth et al. 2006, Altieri and Gedan 2014).

This dissertation presents a combination of field and laboratory work coupled with quantitative modeling to explore how changes in environmental conditions affect a macroalgal primary producer in temperate rocky intertidal ecosystems, and evaluate how small spatial scale environmental differences may influence interspecific interactions (i.e., herbivorous grazing). In Chapter 1, I show that the photosynthetic production of a foundational macroalgal species in the northeastern Pacific, *Fucus gardneri*, reaches a thermal optimum at temperatures well above current environmental conditions at the study site. This suggests that with anticipated increases in seawater temperature for this region in the coming century, *F. gardneri* will experience increased yearly net photosynthetic production. In contrast, outputs from this study’s photosynthetic model suggest that yearly production for this alga will either not change or will decrease in central and southern California (respectively) as a result of increased temperatures. Findings from Chapter 1 demonstrate that manipulating exposure timing (i.e., tidal timing) and duration (i.e., tidal elevation) drastically influenced macroalgal yearly average net photosynthesis, an observation that has not been made before.
Recent work from a variety of habitats has shown that the impact of environmental stressors may be counteracted by nutritional state of the environment, implying that local factors such as nutrient delivery can potentially ameliorate (or exacerbate) larger, regional-scale changes (Figueroa et al. 2009, O’Connor et al. 2015). For example, increased available nitrogen can lead to an increase in plant productivity and biomass accumulation (Blanchette et al. 2000, Stachowicz 2001, Bracken 2004, Elser et al. 2007, Harpole et al. 2011). In terrestrial environments, photosynthetic production and leaf litter decomposition rates are directly affected by environmental nitrogen supply (Hunt et al. 1988, Berg and Tamm 1991, Boxman et al. 1995). Nitrogen levels in the environment can be altered or affected by human-mediated nutrient introduction from agricultural practices, changes in terrestrial runoff from increased urbanization and development, and coastal sewage discharge (Nixon 1995, Valiela et al. 1997, Vitousek et al. 1997, Howarth 2008). In marine and coastal ecosystems, nitrogen can be seasonally limiting at high latitudes and chronically limiting at lower latitudes (Sarmiento et al. 1993, Herbert 1999), which directly influences organism photosynthetic production and success in these respective environments. It is well accepted that an increase in environmental nitrogen can increase tissue growth and reproductive success in marine algae (Topinka and Robbins 1976, Van Alstyne and Pelletreau 2000, Hemmi et al. 2005); however, less is known about how nutrient enrichment will affect macroalgal species that are thermally stressed. In Chapter 2, I describe how nutrient-enriched *Fucus vesiculosus* exhibit increased O$_2$ production and tissue growth at higher seawater temperatures when compared to ambient conditions. These findings suggest that nutrient enrichment from coastal run-off may in some cases help to mitigate thermal stress, particularly at higher temperatures. Since most macrophytes are nitrogen limited (Galloway et al. 2004, Elser et al. 2007, Harpole et al. 2011), small increases in available nitrogen are beneficial to tissue

Herbivores serve an important role within their community by facilitating trophic interactions (top-down and bottom-up) that stabilize species populations (Nielsen 2001, Bracken and Stachowicz 2007, Schmitz 2008, Bracken et al. 2014). Additionally, herbivores can change their behavior in response to environmental changes in order to maximize their fitness (Bracken 2004, Iacarella and Helmuth 2011), thus altering inter- and intra-specific interactions. In marine environments, the response of herbivores to their surroundings are evident in zonation (Hawkins and Hartnoll 1985), recruitment to new habitats (Stachowicz 2001), and growth and development rates (Steneck et al. 1991). Organisms within the rocky intertidal are subjected to abiotic (e.g., temperature, desiccation) and biotic (e.g., competition) stressors from both aquatic and terrestrial environments (Hawkins and Hartnoll 1985); therefore, producing a unique array of environmental conditions they must tolerate daily.

The distribution of environmental nutrients differs geographically and temporally in coastal marine systems (Lapointe 1987, Fujita et al. 1989, Wootton et al. 1996, Van Alstyne and Pelletreau 2000); though phosphorus and iron have been shown to contribute to algal productivity in nutrient-poor environments, for northern temperate waters, nitrogen is the most influential nutrient in macroalgal growth and photosynthetic activity (Van Alstyne and Pelletreau 2000, Bracken 2004, Perini and Bracken 2014, Bracken et al. 2015). The carbon/nutrient balance hypothesis (CNBH) predicts that the concentration levels of environmental nutrients (namely nitrogen) influence the allocation of resources in plants and algae to tissue growth or defense against herbivory (Yates and Peckol 1993, Van Alstyne and Pelletreau 2000). When plants and
algae are not light or carbon-limited in nitrogen-rich conditions, then they are predicted to allocate carbon to growth; however, when nitrogen is limited then carbon is allocated towards defense mechanisms in an effort to deter herbivory (Van Alstyne and Pelletreau 2000). Seasonal differences in available nitrogen are observed in rocky intertidal systems, with conditions in warm summer months being more nitrogen-limited as nutrients are readily taken up from the environment by micro- and macroalgal species, and cold winter months being more nitrogen rich attributed to increased storm mixing and terrestrial runoff (Lapointe 1987, Wootton et al. 1996). Therefore, in Chapter 3 of this dissertation, I examine the temporal and spatial variability in environmental nitrogen in seawater and how that corresponds to algal tissue nitrogen levels, and subsequent tissue growth and grazing pressure from a dominant mesograzer in the community. As macroalgae respond to changes in environmental nutrient levels is it important to evaluate how these marine macrophytes may physiologically respond and what the implications of those responses will be on the interaction with other species within the ecosystem. This research further explores the role of the herbivore Littorina littorea grazing pressure on F. vesiculosus over small spatial scales along the greater Boston coastline. Littorina littorea snails serve as a dominant herbivore in the rocky intertidal of New England, ubiquitous in exposed and sheltered habitats and tolerant of a range of environmental conditions (i.e., thermal and salinity stresses) (Newell et al. 1971, Lubchenco 1978, Watson and Norton 1985). Evaluating the interaction of this herbivore and algal food source will help to elucidate how local habitat differences may drive species interactions within the rocky intertidal as coastal development and nutrient enrichment increases in the coming century.

Conclusions
The main objectives of this dissertation were to characterize how environmental conditions—namely temperature—drive algal photosynthetic production, how nutrient enrichment could potentially mitigate the photophysiological response to thermal stress, and describe how local scale processes may be more influential on organism growth and success than large-scale changes in climate. In order to accomplish these study objectives, I have conducted three research projects that described the photosynthetic and growth responses of *Fucus gardneri* and *Fucus vesiculosus* to changes in light, temperature, and nutrient enrichment, and how grazing pressure by littorine snails on *Fucus vesiculosus* differed over a small spatial scale. I used photosynthetic performance curves linked with environmental measurements to characterize the response of macroalgae to multiple environmental conditions. Additionally, I described the variability in nitrogen levels in seawater and in *Fucus* tissue over a local scale in the greater Boston area, and further explained how those levels influenced algal tissue growth and the impact from a dominant mesograzier in the habitat. The findings from this research aim to better characterize the future state of *Fucus* algae with ensuing changes to the natural environment from climate change and human mediated nutrient enrichment. This effort is particularly important since *F. gardneri* and *F. vesiculosus* serve as foundational species for the rocky intertidal in the northeastern Pacific and northwestern Atlantic (respectively).
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CHAPTER I

Temperature-dependent photosynthesis in the intertidal alga *Fucus gardneri* and sensitivity to ongoing climate change

ABSTRACT

Understanding the photosynthetic responses of marine macroalgae to changes in their thermal environment is vital to characterizing the success of these habitat-forming primary producers in the face of climate change. I measured net photosynthesis in apical tips of *Fucus gardneri* collected from the intertidal zone of Friday Harbor, WA over a range of irradiance levels (0-1500 µmol photons m$^{-2}$s$^{-1}$) at 10°, 14°, and 18°C to determine levels of saturating irradiance. I then recorded net photosynthesis at saturating irradiance in tips exposed to seawater temperatures ranging from 6–22°C, as well as dark respiration. Results show that *F. gardneri* at this location has a peak in net photosynthesis at 16–18°C seawater temperature, with significant declines in net photosynthesis at 20° and 22°C. Respiration showed a positive linear relationship with increasing seawater temperature. Using archived seawater temperature, irradiance, and tidal data, I produced a model of net photosynthesis over two years (October 2010–October 2012). Maximal seawater temperatures recorded at FHL rarely exceeded 14°C, suggesting that an increase of +2 and +4°C in seawater temperature would lead to increased net photosynthesis at this site. These results allowed us to develop a predictive model to forecast the net photosynthesis of *F. gardneri* at different intertidal elevations to explore the effects of seawater temperature and irradiance on net photosynthesis. I also examined the effects of the timing of high tide, testing the hypothesis that net photosynthesis will be highest at sites where submersion occurs during peak levels of irradiance. These results suggest that as seawater temperatures increase (up to +4°C above ambient) *F. gardneri* below +1m MLLW tidal elevation will experience increases in net photosynthesis. These analyses also suggest that the effects of environmental change may depend in part on tidal regime, which determines the extent to which algae are submerged during times of day when irradiance is high.
INTRODUCTION

A fundamental goal of global climate change research is to understand how organisms will likely respond physiologically to novel environmental conditions (Chown and Gaston 1999, Somero 2002) and how the influence of climatic factors may be modified by other non-climatic factors (e.g., Mislan et al. 2011). Intertidal and shallow subtidal organisms have long served as model systems for examining the impacts of the physical environment on patterns of distribution in nature (Paine 1994). To this end, many studies have examined the effects of lethal temperatures in setting the local and geographic distribution of intertidal and shallow subtidal organisms (Wethey et al. 2011). A number of recent studies have emphasized the importance of also considering the sublethal effects of environmental change on marine animals (Howard et al. 2013), for example the influence of temperature on rates of foraging (Sanford 2002, Kordas et al. 2011), growth (Almada-Villela et al. 1982), and reproduction (Petes et al. 2008). Comparable studies of macroalgae have been conducted examining the effects of temperature and desiccation on rates of survival, photosynthesis, and growth (Bell 1993, Matta and Chapman 1995, Zou et al. 2007). A principal consideration is the importance of local factors, often non-climatic in nature, in modifying the influence of environmental factors related to climate change. For example, wave exposure and the timing of low tide can significantly affect the risk of thermal stress in intertidal organisms (Mislan et al. 2011), and the vulnerability of invertebrates to thermal stress can be significantly affected by food supply and the presence of other stressors, such as pollution (Howard et al. 2013).

Several studies have shown that, for some populations, small increases in temperature may lead to increases in performance, especially at a species’ poleward distributional limits or to
decreases at their equatorial limits (Somero 2002, Howard et al. 2013). Other studies have suggested that prolonged exposure to sublethal conditions can lead to large-scale mortality due to the cumulative effects of environmental stress on energetics (Woodin et al. 2013). Surprisingly, however, while lethal thermal limits have been relatively well studied for many species, complete thermal performance curves describing the effects of temperature on marine organism performance are comparatively rare (Monaco and Helmuth 2011). In cases where we do have a thorough knowledge of how populations are likely to respond to temperature, studies have shown that local adaptation (Kuo and Sanford 2009) and low genetic diversity (Pearson et al. 2010) can potentially create large differences in thermal tolerance between populations and among closely related species. This lack of information for many ecologically important species limits our ability to predict how changing temperatures likely impact marine ecosystems.

In this study I explored the effects of seawater temperature and irradiance on a common, ecologically important species of marine alga, *Fucus gardneri*. *F. gardneri* serves as a primary producer for the mid-intertidal region, and thus as a food source for littorine snails, isopods, and amphipods, and can produce large algal mats that serve as protection for intertidal invertebrates (Dethier 1982). Given this species’ foundational ecological role in the Northeastern Pacific intertidal zone, an explicit understanding of how *F. gardneri* is likely to respond to ongoing change is paramount. It is well understood that temperature and irradiance influence photosynthesis in fucoids (Williams and Dethier 2005, Nygard and Dring 2008, Kraufvelin et al. 2012). For example, Dethier and Williams (2009) showed that *F. gardneri* photosynthesis, growth, and reproduction were most influenced by seasonal differences in environmental temperature and irradiance levels. Previous studies of brown algae have documented the effects of irradiance levels on photosynthetic capacity (Dring and Brown 1982, Dromgoole 1987, 1988,
Johnson et al. 1998, Williams and Dethier 2005), somatic growth (Lüning 1971, Falkowski and LaRoche 1991, Davison and Pearson 1996, Kübler and Dudgeon 1996, Dethier and Williams 2009, Kim et al. 2011), and reproductive development (Davison and Pearson 1996, Dethier and Williams 2009). Several studies have also evaluated the photosynthetic performance of macroalgae in relation to environmental temperature (Bell 1993, Kübler and Davison 1993, Matta and Chapman 1995, Williams and Dethier 2005, Kim et al. 2011), where many have found an initial positive relationship between photosynthesis and increases in seawater temperature above current field conditions. Research on *Fucus vesiculosus* (Terry and Moss 1981, Middleboe et al. 2006, Nygard and Dring 2008, Alexandridis et al. 2012, Kraufvelin et al. 2012) has demonstrated how light and temperature can influence germination, recruitment, growth, and photosynthesis in this alga. Although previous research has evaluated the photosynthetic activity of the Pacific species *F. gardneri* (previously *F. distichus*) to environmental change (Johnson et al. 1974, Quadir et al. 1979, Williams and Dethier 2005), these studies evaluated photosynthesis under a fairly narrow temperature range (e.g., summer temperatures).

Our study expands upon previous work examining *F. gardneri* photosynthesis in order to characterize how this species will likely respond to a range of seawater temperatures including those predicted in the near future. As previously described for other algal species (Dromgoole 1988, Bell 1993, Williams and Dethier 2005), I hypothesized that increased seawater temperature would generally increase net photosynthesis in *F. gardneri* up to some (previously unidentified) optimal temperature, but beyond this thermal optimum productivity would decline (Davison and Pearson 1996). I further quantified patterns of seawater temperature in the intertidal zone at FHL to determine how close environmental conditions at this site currently are relative to this species’ thermal optimum and how future increases in seawater temperature will
likely affect *F. gardneri* populations. Finally, I evaluated the potential role of the tidal regime in driving sensitivity to environmental change. While it is well recognized that the timing of low tide determines the frequency by which organisms are exposed at low tide to extreme aerial conditions of temperature and desiccation during the hottest parts of the day (Orton 1929, Helmuth et al. 2002), a less explored corollary for intertidal algae is that maximal levels of photosynthesis are likely to occur when high tides occur mid-day when irradiance levels are highest. The primary goals of this research were to (a) determine the photosynthetic performance curve for *F. gardneri* with increasing temperature at a saturating irradiance and in the dark; (b) develop a generic net photosynthesis model that can be used to predict net photosynthesis (P_net) under a range of future temperature and irradiance combinations at FHL; (c) to explore the relative importance of the timing of high tide in driving sensitivity to environmental change; and (d) evaluate whether the current southern range limit of *F. gardneri* is likely to be set by temperature.

**METHODS**

*Study Location and Algal Collection*

All algal specimens were collected from the mid-intertidal region (~0.5 – 1 m above Mean Lower Low Water, MLLW) along the coastline near FHL between May and August 2012. This tidal elevation harbors dense *Fucus* algal mats as well as herbivorous grazing littorine snails, isopods, and amphipods (Dethier 1982, Dethier et al. 2005). All algal specimens collected were free of grazers and epiphytes, and had minimal tissue damage to the thallus or ‘wing’ regions.
Seawater temperatures collected from October 2010 – October 2012 (http://depts.washington.edu/fhl/fhl_wx.html) showed that seawater temperature at FHL rarely drops below 7°C, and rarely exceeds 14°C at 1.7 m depth (Figure 1); though surface waters can exceed 14°C on warm summer days. Future climate models suggest an average increase in sea surface temperature of 2.3°C by the year 2090 for the North Pacific region (IPCC 2007). Maximal water temperatures near the equatorial edge of this species’ southern range (Monterey Bay, CA) are approximately 17.4°C from 2010 to 2012 (www.ndbc.noaa.gov). I therefore selected a temperature range of 6 to 22°C for these experiments, to encompass temperatures experienced by this species over much of its range, both now and in the near future.

All *F. gardneri* were maintained in a flow-through seawater table where they were kept for 24–72 h before photosynthetic measurements were conducted. Experiments were conducted on the apical tip, the growing region, which was cut from a whole algal thallus 24 h prior to photosynthesis measurements to avoid wounding response. At the end of the experiment, the dry weights of all algal tissues were measured (dried to constant weight at 80°C) in order to standardize all photosynthetic measurements.

*Photosynthetic measurements of Fucus gardneri*

Net photosynthesis of the apical tips (~2 cm²) of *F. gardneri* was measured using a Hansatech DW3 chamber system (Hansatech, Norfolk England) with a Clark-type oxygen electrode (Maberly 1992, Bell 1993). This 10mL closed volume chamber was temperature-controlled by a recirculating water jacket connected to a chiller, and a 300-W quartz-halogen lamp projector was used as an external light source and light levels were manipulated using
neutral density filters. All seawater used in the experiments was filtered through a 10-micron filter bag, and the seawater was replaced for each individual alga measured.

A preliminary set of experiments was conducted to quantify the saturating irradiance for *F. gardneri* apical tips by recording photosynthesis as a function of irradiance (PI curves) at 10°, 14°, and 18°C ± 0.1°C. These seawater temperatures are representative of ambient (recorded temperature), high, and extreme (respectively) summer conditions at FHL. The PI curves were generated using a range of irradiance levels from 0 to 1500 µmol photons m⁻² s⁻¹. Specimens were dark acclimated for 1 h before respiration measurements. Apical tips (n=3 per temperature) were non-reproductive (0.021±0.005 g dry wt, n=9) to avoid tissue that did not contribute to photosynthesis (Suppl. Figure 1). Oxygen production was calculated based on rate of change in O₂, normalized by dry oven weight of the algal tissue.

A curve was fit to all points in each of the three PI curves using the following equation (Jassby and Platt 1976):

\[
\text{P}_{\text{net}} = \text{P}_{\text{gross,max}} \times \tanh \left( \alpha \times \frac{I}{\text{P}_{\text{gross,max}}} - \text{R}_d \right)
\]

Eq. 1

where \( \text{P}_{\text{net}} \) is net photosynthesis (µmol O₂ g dry wt⁻¹ hr⁻¹), \( \text{P}_{\text{gross,max}} \) is the maximum rate of gross photosynthesis at saturating irradiance (µmol O₂ g dry wt⁻¹ hr⁻¹), \( \alpha \) is the initial slope of the curve ([µmol O₂ g dry wt⁻¹]•[µmol photons m⁻² s⁻¹]⁻¹), \( I \) is irradiance (µmol photons m⁻² s⁻¹), and \( \text{R}_d \) is the respiration rate in the dark (µmol O₂ g dry wt⁻¹ hr⁻¹). \( \text{P}_{\text{gross,max}} \) was calculated posthoc from \( \text{P}_{\text{net,max}} \) (the maximum rate of net photosynthesis at a saturating irradiance [µmol O₂ g dry wt⁻¹ hr⁻¹]) and \( \text{R}_d \), where \( \text{P}_{\text{gross,max}} = \text{P}_{\text{net,max}} + \text{R}_d \). The saturation irradiance (\( I_k \)) was determined by \( \text{P}_{\text{gross,max}}/\alpha \). These parameters allowed us to further test the photosynthetic performance of *F. gardneri* conditioned with a range of temperatures.
Effect of seawater temperature on photosynthesis

We measured net photosynthesis of *F. gardneri* under saturating irradiance at 2°C intervals between 6°C and 22°C. Based on results of the preliminary PI curves (above), which showed no significant difference in saturating irradiance ($I_s$) among the three temperatures tested, I used an irradiance of 1400 µmol photons m$^{-2}$s$^{-1}$ for all $P_{\text{gross, max}}$ measurements as this level was reliably above minimum saturating irradiance for all temperatures. For the dark respiration measurements, all algal tissue was dark-adapted for at least 1 h. I measured 10 replicate apical tips of *F. gardneri* for each temperature evaluated.

Net Photosynthesis Model

Using parameters from the net photosynthesis measurements in conjunction with varying irradiance (I, 0–1500 µmol photons m$^{-2}$s$^{-1}$) and water temperature (6–22°C) combinations, I developed a simple model to predict net photosynthesis ($P_{\text{net}}$). This predictive model is based on best curve fits for $P_{\text{gross, max}}$ (second order polynomial curve fit) and $R_d$ (a linear equation); both parameters are dependent on seawater temperature. However, there were no differences in initial slope of the PI curve (α) between temperatures, therefore a fixed value was used for all conditions. The $P_{\text{net}}$ model, which is dependent only on water temperature and irradiance, is valid only for submersed conditions, and I assumed that $P_{\text{net}} \approx 0$ during aerial exposure, since the mean net photosynthesis for *F. gardneri* has been shown to be as much as two orders of magnitude lower in air than in water (Williams and Dethier 2005).

We then used this model to reconstruct $P_{\text{net}}$ based on environmental conditions recorded at FHL over two years (October 2010-October 2012)
Seawater temperature was measured in situ and underwater downwelling irradiance was estimated from surface irradiance and water depth following Beer’s Law:

\[ I_{d,uw}(z) = I_{air} e^{-K_d z} \]  

Eq. 2

where \( I_{d,uw} \) is the downwelling underwater irradiance (µmol photons m\(^{-2}\)s\(^{-1}\)) at depth \( z \) (in meters), \( I_{air} \) is the downwelling irradiance measured in air (µmol photons m\(^{-2}\)s\(^{-1}\)), \( K_d \) is the vertical light attenuation coefficient for summer (0.373 m\(^{-1}\), based on Dethier and Williams 2009). Tidal predictions were used to estimate water depth (\( z \)) at an intertidal elevation of +1m MLLW (Xtide, http://tbone.geol.sc.edu/tide). Thus, when the predicted tidal level was < +1 m MLLW, then the alga was assumed to be aerially exposed and \( P_{net} = 0 \).

To evaluate the impact an increase in seawater temperature would have on \( P_{net} \) over the tidal range of \( F. gardneri \), I calculated the yearly average \( P_{net} \) for FHL from hourly recorded data, and compared it to simulated increases in temperature +2°C and +4°C above recorded temperatures at intertidal elevations ranging from -1.0 m to +2.5 m MLLW, at 0.1 m intervals. Using only recorded temperature conditions at FHL, I then manipulated the timing of the tide to quantify yearly average \( P_{net} \), shifting the tidal timing at FHL by -6 hrs, -3 hrs, +3 hrs, and +6 hrs from ambient (+0 hrs difference). I then evaluated the difference in yearly average \( P_{net} \) at the five tidal adjustments using the tidal cycle at Tatoosh Island, WA. Tatoosh Island is located ~190 km west of FHL, typically exhibiting a 3 – 5 h difference in tidal timing ahead of FHL (Xtide). Preliminary results suggested that the greatest difference in yearly average \( P_{net} \) was between +0 m and 0.5 m MLLW intertidal elevation, therefore I used these two elevations to explore the effects of exposure and submersion time on yearly average \( P_{net} \).
In order to evaluate the likelihood that the southern latitudinal range limit of *Fucus gardneri* was set by seawater temperature conditions, I compared yearly average $P_{net}$ using temperatures recorded during 2010-2012 at FHL, as well as at Monterey, CA, the recorded southern limit of *F. gardneri* (Blanchette et al. 2008), and at San Diego, CA, considerably farther south than the current range limit. I also estimated $P_{net}$ at each of these sites using temperature increases of 2°C and 4°C above current conditions. Seawater temperature data for the three sites were obtained from NOAA Buoy Center (www.ndbc.noaa.gov). I recognize that this analysis is based only on seawater temperatures, and thus ignores all other environmental conditions likely to change over this range. The analysis also does not consider any potential physiological differences among populations. However, I made the assumption that if any differences do exist, populations farther south are likely to be even more thermally tolerant than those at FHL. This comparison thus provides a conservative estimate of yearly average $P_{net}$ for *F. gardneri* along the northeastern Pacific, and actual values could be higher at southern sites if algae were acclimatized or adapted to those conditions.

A linear regression was used to determine the relationship between algal respiration and temperature. A two-factor mixed model ANOVA was used to compare yearly average $P_{net}$ at FHL at current temperatures and increases of 2°C and 4°C with changes in tidal elevation. Sensitivity analyses was used to evaluate differences in yearly average $P_{net}$ between FHL and Tatoosh Island, and compare yearly average $P_{net}$ with response to temperature differences between latitudinal sites (Friday Harbor, WA, Monterey, CA, and San Diego, CA). All statistical tests were run with Systat 12.
RESULTS

Effect of seawater temperature on photosynthesis

Best curve fits were applied to the photosynthetic performance measurements of F. gardneri in response to changes in temperature in the dark (Rd) and at a saturating irradiance (P_{gross, max}) (Figure 2). A linear regression analysis showed a significant increase in respiration, Rd, with temperature ($r^2 = 0.798$, $P < 0.001$) from 34.70 ± 2.41 µmol CO$_2$ g dry wt$^{-1}$hr$^{-1}$ at 6°C up to 49.33 ± 3.17 µmol CO$_2$ g dry wt$^{-1}$hr$^{-1}$ at 22°C. A curvilinear regression (second order polynomial) curve was fit to the photosynthesis data to describe the effect of temperature on P_{gross, max}, and showed a maximum value of 16°C, followed by a decrease in P_{gross, max} from 18° to 22°C (Figure 2). Temperature had a small effect on the light-saturated, maximum rate of photosynthesis of submerged F. gardneri, with Q$_{10}$ values of 1.52 at 6–16°C, 1.59 at 8–18°C, and 1.15 at 10–20°C, demonstrating the greatest rate of change in photosynthesis associated to temperature change for the 8–18°C range, and quickly declining for the 10–20°C range.

Net Photosynthesis Model

The predictive model of $P_{net}$ for F. gardneri based on the measured seawater temperature and downwelling irradiance calculated from aerial measurements at FHL (shown as points overlaid on the contour plot, Figure 3), suggest that submerged F. gardneri at FHL never experienced temperatures above optimal levels. The two-factor mixed model ANOVA showed a significant difference in net photosynthesis for F. gardneri when evaluating the interaction of tidal elevation × temperature change ($F_{2,102}=3.939$, $P=0.022$) (Suppl. Figure 2). As expected,
simulations show that photosynthesis decreases with increased tidal elevation, especially > +1 m MLLW.

The $P_{\text{net}}$ model simulations for different adjustments to the timing of the tide for FHL showed the greatest increase in $P_{\text{net}}$ with shifts in tidal timing of -6 hrs and +6 hrs (Suppl. Figure 3). Using tide predictions for Tatoosh Island, only -6 hrs and -3 hrs tidal timing shift predicted an increase in yearly average $P_{\text{net}}$ for all intertidal elevations. However, a tidal timing shift of +6 hrs caused a decrease in $P_{\text{net}}$ at tidal elevations < +1 m MLLW, but an increase in $P_{\text{net}}$ at tidal elevations > +1 m MLLW (Suppl. Figure 3). The yearly average $P_{\text{net}}$ for shifts in tidal timings demonstrated the lowest $P_{\text{net}}$ at 0 hrs tidal timing for FHL at 0 m and 0.5 m tidal elevation, however for Tatoosh Island, yearly average $P_{\text{net}}$ was lowest at +3 hrs for both tidal elevations (Figure 4).

The model simulations evaluating yearly average $P_{\text{net}}$ for current recorded temperatures (+0°C), and increases of 2°C and 4°C for San Diego, CA, Monterey, CA, and Friday Harbor, WA revealed differences in sensitivity to temperature increases between the three locations, but more importantly show that the current southern limits is likely not set by thermal performance (Figure 5). Modeled $P_{\text{net}}$ for San Diego, CA temperatures showed positive yearly average $P_{\text{net}}$, even though this location is further south than the southern range limit for this species (Blanchette et al. 2008). However, the model predictions for this location suggest a decline in $P_{\text{net}}$ with increased seawater temperature, assuming that thermal sensitivity is comparable to algae from the northern site. In contrast, there is no difference in yearly average $P_{\text{net}}$ for $F. gardneri$ based on current and projected temperatures for Monterey, CA. And finally, yearly average $P_{\text{net}}$ at FHL is predicted to increase with increasing seawater temperature (Figure 5).
DISCUSSION

This study demonstrated that the photosynthetic response of *Fucus gardneri* is strongly dependent on surrounding seawater temperature at high levels of irradiance, and that at most sites an increase in seawater temperature will either have no effect on performance, or will slightly increase photosynthetic performance. Whereas previous studies have explored the plasticity of fucoids to wave action (Blanchette et al. 2000, Dethier and Williams 2009), desiccation (Harker et al. 1999, Dethier and Williams 2009, Gylle et al. 2009), and habitat type (Schonbeck and Norton 1978, 1980, Chapman 1995, Wernberg et al. 2011), this study is one of the first to characterize the net photosynthesis of *F. gardneri* under a range of irradiance and temperature combinations in submerged conditions (but see Alexandridis et al. 2012).

Our results show that 16–18°C is the optimal temperature for *F. gardneri* in submerged conditions at Friday Harbor, suggesting a strong link between the organism’s fitness and the thermal environment (Pörtner 2010) while undergoing maximum rates of gross photosynthesis. The initial positive effect of temperature (<16°C) is attributed to the role of temperature in carbon fixation during photosynthesis up to a thermal maximum (Davison 1991). This increase in photosynthetic activity to increasing temperature is quantified by a $Q_{10}$ value, which for *F. gardneri* has been shown to vary between 1.1 and 1.5 (Madsen and Maberly 1990, present study), for temperatures between 6° and 20°C. Reduction in gross photosynthesis at temperatures above 18°C can be attributed to several factors such as temperature sensitive enzymes of photophosphorylation and electron transport and plastoquinone diffusion (Davison 1991). Oxygen-dependent thermal tolerance could also explain the near linear increase in respiration with increasing temperature observed in submerged conditions (Zou et al. 2007, Allakhverdiev et
al. 2008). However, these results should be interpreted with caution because they were conducted on individuals from one location, there was a low power of comparison among the PI curves (n=3 per temperature), and because the net photosynthesis model is based on the algal tissue from the apical tip rather than the entire thalli. Moreover, experiments measuring temperature effects were conducted at saturating irradiance, and modeled at lower irradiance levels using a generic PI curve. Thus, explicit tests at lower irradiance levels are still needed. Additionally, I did not account for the effect of ultraviolet radiation (UVR) on Fucus photosynthetic activity over the range of temperatures evaluated; however, I suggest that in situ UVR may also affect macroalgal net photosynthesis, primarily during shallow submerged conditions and aerial exposure (Raven and Hurd 2012).

The optimum temperature of 16°C is ~2°C warmer than the highest recorded seawater temperature at FHL from 2010 to 2012. This suggests that F. gardneri may be able to withstand the projected average seawater temperature increase of 2.3°C by the year 2090 for the North Pacific region. Notably, however, this increase is based on changes in average conditions only and it is highly likely that temperatures in excess of the optimum will occur during rare, extreme events such as those recorded in other parts of the world. For example, in summer 2012 temperatures in the Gulf of Maine were up to 3°C higher than the 1982-2011 climatology (Mills et al. 2012). Somero (2010) suggested that with projected future climate change and organism acclimatization to increasing temperature, there will be ‘winners’ and ‘losers’ in the intertidal; the findings from this study suggest F. gardneri at FHL will likely increase net photosynthesis with increasing seawater temperatures. These simulations predict that F. gardneri at low tidal elevations (< +1 m MLLW) will experience an approximately 10% increase in yearly average photosynthesis with a 4°C increase in seawater temperature above ambient.
The comparison of the P_{net} models for *F. gardneri* based on tidal timing suggests that the timing of high tide can theoretically influence yearly net photosynthesis by as much as 25% (Figure 4). The tidal timing and submergence time can influence photosynthesis by determining algal submergence during midday irradiance exposure, though this is dependent on tidal elevation of *Fucus* algae. The tidal regimes of FHL and Tatoosh Island demonstrate that the timing of the tide, tidal range, and duration of exposure time between these two locations influences yearly average P_{net} of *F. gardneri*; however, these effects are likely to be compounded with differences in irradiance and water temperature between the two sites. The P_{net} model simulations showed FHL tidal timing manipulations caused the greatest change in net photosynthesis when shifting the tide -6 hrs and +6 hrs from current conditions (i.e., ambient), though Tatoosh Island demonstrated an increase in net photosynthesis when shifting the tide -6 hrs and -3 hrs from ambient. Because simulations for Tatoosh Island used the same environmental temperature and irradiance conditions measured at FHL, differences observed in the yearly average P_{net} are only attributable to differences in tidal timing and range for these two locations, and do not include the effects of differences in other environmental conditions (e.g., irradiance, water temperature).

The P_{net} model comparison for temperature data from San Diego, CA, Monterey, CA, and Friday Harbor, WA suggests that *F. gardneri* has a positive net photosynthesis at all three locations, though Monterey, CA has been identified as the approximate southern limit of this species. This suggests the southern limit for *F. gardneri* is not solely temperature dependent, and is potentially limited by other environmental conditions (i.e., spatial competition, foraging pressure), or by some combination of stressors. However, macroalgae at the more southern sites are expected to display a reduced photosynthetic response with increasing temperature.
conditions, whereas Friday Harbor shows a continued increase in photosynthesis with an increase of 2° and 4°C in seawater temperature.

Future applications of this $P_{\text{net}}$ model should incorporate aerial exposure and other abiotic conditions and biotic interactions that are driving $Fucus$ fitness in the intertidal zone. Duration of aerial exposure and environmental conditions during exposure can dictate algal success and net photosynthesis (Williams and Dethier 2005). In the simple $P_{\text{net}}$ model I suspended photosynthetic activity during aerial exposure and did not account for potential deleterious effects of desiccation, UVR, and heat stress during low tide events, especially during warm summer days. Other abiotic conditions that may influence photosynthesis along with temperature and irradiance are nutrient levels (Nygard and Dring 2008), salinity (Nygard and Dring 2008, Gylle et al. 2009), and CO$_2$ concentrations (Zou and Gao 2002). Future studies can explore the role these other environmental conditions may have on the $P_{\text{net}}$ of $F. gardneri$.

This study is an initial step at describing the net photosynthesis of $F. gardneri$ using a range of seawater temperatures, and therefore is a critical first step that can be expanded upon to explore how other environmental factors (e.g., salinity, grazing pressure, water motion, and aerial exposure) contribute to net photosynthesis in this intertidal alga.
REFERENCES


FIGURES

Figure 1. Frequency distribution of surface seawater temperatures from October 2010 to October 2012 at Friday Harbor Laboratories, Washington (http://depts.washington.edu/fhl/fhl_wx.html). The first peak in the frequency of temperature (7 – 8.5°C) is largely based on fall and winter-time measurements, whereas the second peak (9.5 – 10.5°C) is primarily comprised of spring and summer time measurements. The inset figure is of time-series data from which the frequency distribution was calculated, with hourly seawater temperature measurements ranging 6° to 16°C (y-axis) from October 2010 to October 2012 (x-axis).
Figure 1.
Figure 2. Maximum gross photosynthesis ($P_{\text{gross, max}}$, closed circles) and dark respiration ($R_d$, open circles) for apical tips of *Fucus gardneri* in seawater. Symbols are means ± SE of n=10 replicates per temperature. A linear regression analysis showed there was a significant increase ($r^2 = 0.798$, $P < 0.001$) in $R_d$ (µmol CO$_2$ g dry wt$^{-1}$hr$^{-1}$) increasing with temperature, from 6 to 22°C. For $P_{\text{gross, max}}$ (µmol O$_2$ g dry wt$^{-1}$hr$^{-1}$), the photosynthetic output increases with temperature up to 18°C, after which $P_{\text{gross, max}}$ shows a decline at 20 and 22°C.
Figure 3. Contour plot of the predictive model of $P_{\text{net}}$ for *Fucus gardneri* ($\mu$mol O$_2$ g dry wt$^{-1}$hr$^{-1}$) for a range of irradiance (0 – 1500 $\mu$mol photons m$^{-2}$s$^{-1}$) and seawater temperature (6 – 22°C) combinations. The overlaid points are environmental data based on temperature and underwater irradiance measurements from October 2010 – October 2012 at Friday Harbor Laboratories, WA.
Figure 3.
Figure 4. The $P_{\text{net}}$ model simulations using ambient temperature conditions, evaluating the difference in tidal timing for 0 m tidal elevation (A) and 0.5 m tide tidal elevation (B) for Friday Harbor Laboratories (black bars) and Tatoosh Island (white bars). The yearly average $P_{\text{net}}$ for the five tidal timing shifts (-6 hrs to +6 hrs) showed the lowest $P_{\text{net}}$ at 0 hrs tidal timing for FHL, but was lowest at +3 hrs for Tatoosh Island at 0 m and 0.5 m tidal elevation of the alga.
Figure 4.
Figure 5. *Fucus gardneri* yearly average $P_{net}$ model predictions for current recorded temperatures (+0°C), and increases of 2°C and 4°C for Friday Harbor, WA (insert A), Monterey, CA (insert B), and San Diego, CA (insert C). The dashed line (near Monterey, CA) is the currently recorded southern limit of *F. gardneri* (Blanchette et al. 2008). These data show yearly average $P_{net}$ is predicted to increase with increasing seawater temperature for Friday Harbor, WA, no difference in yearly average $P_{net}$ for Monterey, CA, and a decline in $P_{net}$ with increased seawater temperature in San Diego, CA. However, San Diego, CA is modeled to have positive net photosynthesis, though this location is further south than the southern limit of *Fucus* algae in the northeastern Pacific.
Figure 5.
Suppl. Figure 1. Photosynthesis vs. Irradiance curves for non-reproductive apical tips of *Fucus gardneri* in seawater (n=3 replicates per temperature). The blue circles represent the O2 production measurements at the ambient 10°C, black squares represent the high temperature conditions (14°C), and the red triangles are the extreme condition (18°C). The curve fits are based on the $P_{\text{net}}$ model prediction (Eq. 1). The single-factor ANOVA tests revealed no significant differences in $P_{\text{net,max}}$ ($F_{2,6}=0.868$, $P=0.467$), $R_d$ ($F_{2,6}=1.387$, $P=0.318$), or $\alpha$ ($F_{2,6}=2.342$, $P=0.177$) for all three temperatures.
Suppl. Figure 2. $P_{\text{net}}$ model simulations based on ambient temperature (+0°C), and +2°C and +4°C above ambient over the natural tidal range of *Fucus gardneri* (-1m to +2.5m MLLW). The magnitude of the greatest difference in net photosynthesis is between temperatures at the lower tidal elevations.
Suppl. Figure 3. $P_{\text{net}}$ model simulations based on ambient temperature conditions over the natural tidal range of *Fucus gardneri* (-1m to +2.5m MLLW). A. Yearly average $P_{\text{net}}$ of FHL with a shift of the tidal timing -6hrs, -3 hrs, ambient (0 hrs), +3 hrs, and +6 hrs from ambient. B. Yearly average $P_{\text{net}}$ of Tatoosh Island with varying tidal timing. The largest magnitude and change in net photosynthesis increases for FHL occurred when shifting tidal timing -6 hrs or +6 hrs, but for Tatoosh Island the largest magnitude and change in net photosynthesis occurred when shifting the tidal timing -6 hrs and -3 hrs.
CHAPTER II

The Nutrient Effect: Quantifying how nutrient enrichment influences the ecophysiology of a primary producer\(^1\)

\(^1\)In review: Colvard, N.B. and B. Helmuth. Ecological Applications.
ABSTRACT

Urbanization of coastlines is leading to increased introduction of nutrients from the terrestrial environment to nearshore habitats. While such nutrient influxes can be detrimental to the organisms in these coastal environments due to increased eutrophication and subsequent reduced oxygen, they could also have positive effects (i.e., increased food availability) on species that are nitrogen-limited and would benefit from increased nitrogen levels. The potential benefits from nutrient enrichment in an environment may counteracting some of the negative impacts of increasing temperatures. Characterizing the physiological response of organisms to simultaneous changes in multiple stressors such as these is an important first step in predicting how global climate change may lead to changes at more local levels. Here, I evaluated how nutrient enrichment (i.e., nitrogen) affected photosynthesis and growth in Fucus vesiculosus, a foundational macroalgal species in the North Atlantic rocky intertidal zone. Specifically, I quantified net photosynthesis in ambient and nutrient-enriched tissues over a range of temperature conditions to characterize thermal performance. Respiration was unaffected by nutrient treatment; however, there was a significant increase in photosynthetic oxygen production for nutrient-enriched tissue compared to ambient at elevated (≥18°C) temperatures. Nutrient-enriched algal blades also showed a significant increase in tissue growth compared to individuals grown under ambient conditions. This study contributes to a growing body of literature showing the complexity of responses to changes in multiple drivers, and highlights the importance of studying the impacts of global climate change within the context of more local environmental conditions.
INTRODUCTION

A major scientific challenge in the coming century is to forecast how the effects of a rapidly changing environment will influence how organisms interact with their surroundings, particularly in systems that are already environmentally stressful. The marine intertidal environment serves as one of these systems that already experiences large thermal fluctuations over short temporal periods (Scavia et al. 2002, Helmuth et al. 2014, Pachauri et al. 2014); and the strength and frequency of those stressors is likely to increase over the coming decades (Sagarin et al 1999, Harley et al. 2006). Recent changes in seawater temperature and other sublethal climatic and non-climatic drivers have been shown to have a substantial effect on organism phenology (Edwards and Richardson 2004, Harley et al. 2006, Mills et al. 2013), native species ranges (Barry et al. 1995, Parmesan and Yohe 2003, Hawkins et al. 2009, Mills et al. 2013), and the expansion of invasive species (Carlton 2000, Stachowicz et al. 2002). However, less attention has focused on local adaptation of native species to environmental change on small spatial scales (Untersee and Pechenik 2007, Somero 2010, Hoffman and Sgrò 2011), as well as the potential for large environmental heterogeneity within these proximal systems and the impact that has on endemic species (Freestone and Inouye 2006, Helmuth et al. 2006, Stein et al. 2014).

Macroalgae provide unique habitat services through structural support (i.e., serving as a refuge) and nutritional support (i.e., food resource) for many species within rocky intertidal ecosystems. *Fucus vesiculosus* algae is one of the most prevalent and ubiquitous macroalgal species within the intertidal in the northwestern and northeastern Atlantic, thus providing multiple ecosystem services over a large spatial range (Lubchenco 1987, Pearson et al. 2000, Nygård and Ekelund 2006). Multiple studies have therefore evaluated the thermal performance
of *F. vesiculosus* in context of anticipated environmental conditions with climate change (Pearson et al. 2000, Nygård and Dring 2008, Graiff et al. 2015), in order to test phenotypic acclimation of physiological performance traits (e.g., growth, photosynthesis). *F. vesiculosus* has demonstrated increased growth and photosynthesis under warming conditions, suggesting this species has a high tolerance for thermal stress. However, there is a paucity of studies that have evaluated the effect of high temperature stress in conjunction with nutrient enrichment in intertidal macroalgae.

Anthropogenic introduction of nitrogen through agriculture, fossil fuel combustion, and other human activities (i.e., land clearing and draining wetlands) has dramatically altered the global cycle of nitrogen (Vitousek et al. 1997). Human activities have nearly doubled the rate at which nitrogen enters terrestrial systems and has greatly increased the nitrogen subsequently transferred to estuarine and coastal ocean systems through rivers and watersheds (Nixon 1995, Howarth 2008). The increased development and urbanization of coastal communities has further exacerbated the problem by increasing surface water run-off (Nixon 1995, Valiela et al. 1997, Nixon and Buckley 2002, Howarth 2008). Coastal ecosystems are quite sensitive to external perturbations, and under extreme nutrient addition to an environment eutrophication and anoxia has occurred (Odum 1985, Rapport et al. 1985, Schindler 1987, Galloway et al. 2004, Phoenix et al. 2012).

However, rocky intertidal and open-ocean environments are well below moderate nitrogen levels found in estuarine, wetland, and river-outlet systems (de Jonge et al. 2002, Howarth and Marino 2006, Bricker et al. 2008). When evaluating nutrient enrichment in the rocky intertidal, nitrogen levels should be framed within the context of this system being nutrient-limited, and small increases in available nitrogen can have a large impact on the
organisms in these environments that are typically exposed to very low nitrogen levels. Therefore, increased nitrogen input from sewage and fertilizer runoff from coastal development (Nixon 1995, Valiela et al. 1997, Howarth 2008) can cause large-scale microalgal blooms (Nixon and Buckley 2002, Bricker et al. 2008), which influence water oxygen levels and potentially influence other macrophyte, invertebrate, and fish species in the affected habitats (Heugens et al. 2001, Thornber et al. 2008, Orth et al. 2006, Altier and Gedan 2014).

In contrast, a growing body of literature has pointed to the positive influence that the nutritional state can have on the physiological sensitivity of plants and animals to changes in the environment (Galloway et al. 2004, 2008). For example, several studies have shown that increased food availability can increase tolerance to otherwise stressful temperatures in marine invertebrates (Schneider et al. 2010). However, considerably less is known about effects of nutrient enrichment on marine macroalgae undergoing thermal stress. Macrophytes are typically thought to be nitrogen limited (Galloway et al. 2004, Elser et al. 2007, Harpole et al. 2011), suggesting that some increase in available nitrogen could be beneficial (Vitousek et al. 1997, Galloway et al. 2004, 2008). Previous research has demonstrated that increases in available nitrogen can lead to an increase in plant productivity and biomass accumulation over short temporal scales (Blanchette et al. 2000, Stachowicz 2001, Bracken 2004, Elser et al. 2007, Harpole et al. 2011). In terrestrial environments, nitrogen supply can directly affect photosynthetic production and leaf litter decomposition rates (Hunt et al. 1988, Berg and Tamm 1991, Boxman et al. 1995). In marine and coastal ecosystems, nitrogen can be seasonally limiting at high latitudes and chronically limiting at lower latitudes (Sarmiento et al. 1993, Herbert 1999), thus influencing organism production and success in these respective...
environments. Environmental nutrient levels also increase growth, tissue nutrient levels, and protective chemical production that deters herbivory (Van Alstyne and Pelletreau 2000).

In coastal marine systems, the distribution of environmental nitrogen limitation differs both geographically and temporally (Lapointe 1987, Fujita et al. 1989, Wootton et al. 1996, Van Alstyne and Pelletreau 2000). Though phosphorus and iron serve important roles as nutrients available for algal productivity, nitrogen has been shown to be most influential in northern temperate coastal waters (Van Alstyne and Pelletreau 2000, Bracken 2004, Perini and Bracken 2014, Bracken et al. 2015). Fast-growing macrophytes are more dependent on environmental nutrient levels compared to large, slow-growing plants, suggesting that maximum growth rates are associated with high sensitivity to nutrient stress (Pedersen 1995). The carbon/nutrient balance hypothesis (CNBH) predicts that when nutrient levels are abundant macroalgae will allocate carbon to tissue growth, and when nutrient levels are deplete the plant will allocate carbon to anti-herbivore defenses (i.e., carbon-based phlorotannins in Fucus) (Cronin and Hay 1996b, Van Alstyne and Pelletreau 2000). Plant growth is often more limited by low nutrient availability than from photosynthesis (Chapin 1980); therefore, it is important to consider environmental nutrient levels when quantifying growth and photosynthetic production over time.

It remains unclear how increased nitrogen delivery will affect coastal ecosystems undergoing temperature increases resulting from global climate change. Macroalgae are responsible for a considerable amount of the photosynthetic productivity in coastal marine habitats (Nixon et al. 1986, Binzer and Sand-Jensen 2002, Binzer et al. 2006, Dethier and Williams 2009). Although tidal height, desiccation, and high light stress have all been shown to negatively affect photosynthetic production during extreme exposure conditions (Underwood and Jernakoff 1984, Harley 2003, Williams and Dethier 2005, Lamote et al. 2012), temperature
is positively correlated with photosynthesis and growth in macrophytes until a temperature optimum is reached (Smith and Berry 1986, Matta and Chapman 1995, Colvard et al. 2014). However, an additional increase in temperature, though small, beyond this optimal thermal state can result in a dramatic decline in organism performance. Therefore, understanding the synergistic effects of changes in temperature and nutrients will better our understanding of how foundational species within intertidal communities will respond to predicted environmental conditions in the coming decades.

Here I evaluate how nutrient enrichment (i.e., nitrogen) and temperature affect photosynthesis and growth in *Fucus vesiculosus*, an intertidal macroalga that has served as a model study organism in North Atlantic rocky intertidal systems (Lubchenco 1983, Serrão et al. 1999, Pearson et al. 2000, Nygård and Ekelund 2006). I quantified the photosynthetic production of *F. vesiculosus* over a range of irradiance and seawater temperature combinations representative of projected temperatures under climate change scenarios (Pachauri et al. 2014), and under both ambient and nutrient-enriched conditions. This species serves as a model organism since it disproportionately affects the basal productivity of the North Atlantic rocky intertidal (Dijkstra et al. 2012).

METHODS

*Study Organism and Location*

I examined the thermal performance (photosynthesis and growth) of *Fucus vesiculosus* individuals in response to ambient and nutrient-enriched states. All algal tissue used in these experiments was collected from non-reproductively active individuals collected in July 2014
from the mid-intertidal region (~1.0 m above Mean Lower Low Water, MLLW) of rocky shores near the Northeastern University Marine Science Center in Nahant, MA (42.42°N, 70.90°W). All individuals were placed in a flow-through seawater system for 24 hours prior to the start of each experimental trial. Only whole individuals with little to no tissue damage were used, and all epiphytic algae and grazing invertebrates were removed prior to the start of the experiment. The experiment was conducted in July and August 2014 for 36 d.

Experimental Design

This study consisted of two treatment levels – ambient and nutrient-enriched, each with ten replicate experimental mesocosms (7.5 L) consisting of four *F. vesiculosus* individuals per mesocosm. A mixed semi-diurnal tidal cycle was simulated in the experimental mesocosms, mimicking the tidal elevation at which the algae were collected. Each mesocosm had an independent water supply and contained a nutrient dispenser constructed from a perforated PVC cap with a 3% agar solution (Perini and Bracken 2014). The nutrient-enriched treatment contained a 2.0 M solution of sodium nitrate (NaNO₃) added to the nutrient dispensing agar solution, which was confirmed through preliminary tests to be the concentration needed to produce the desired level of elevated nitrogen in seawater. The ambient (control) treatment did not contain any added NaNO₃ (i.e., nutrients). To ensure proper nutrient levels in the mesocosms for the duration of the study the nutrient dispensers were replaced weekly, and weekly seawater samples (15 mL) were collected from each mesocosm in order to quantify seawater nitrogen levels. To determine nutrient uptake throughout the duration of the experimental trials (36 d) ~3 cm of tissue was clipped weekly from a fifth, sacrificial *F. vesiculosus* individual per mesocosm. At the end of the experiments, all *F. vesiculosus* individuals were dried at 70°C in a drying oven.
(Thermo Scientific HERAtherm Oven) for at least 48 hrs, and dry tissue weight recorded in order to normalize all photosynthesis measurements. The dried algal tissue was then ground to a fine powder using a Retch Mixer Mill MM400, in order to quantify the percent nitrogen levels in the tissue using a Thermo Scientific Flash EA 1112 NC soil analyzer. Nitrogen levels in the collected seawater were analyzed using a Unisense NO$_x$ Biosensor, which provided a quantitative value of total nitrate and nitrite (µM NO$_x$), hereafter referred to as nitrogen. Nitrogen levels in the algal tissue and in the seawater collected from the experimental mesocosms at the end of the study were statistically analyzed using two-tailed t-tests, comparing ambient and nutrient-enriched conditions.

**Tissue Growth**

Growth was calculated from changes in algal tissue wet weight and projected surface area. Tissue wet weight was recorded using a Mettler Toledo MS 105DU scale (0.0001 g sensitivity) at the start and end of the experiment, with algal tissue dabbed dry to remove surface seawater. Photographs were taken of all individuals at the start and end of the experiment, and projected surface area was calculated using ImageJ software (v. 1.37). Two-tailed t-tests were used to analyze growth differences between treatments, evaluating changes in wet weight and surface area.

**Photosynthesis versus Irradiance**

An Ocean Optics Neofox FOSPOR-R oxygen sensor was used to quantify oxygen production as a function of irradiance (PE curves) for *F. vesiculosus* algae, one individual per experimental mesocosm. Each PE curve consisted of a 20 min exposure in the dark (0 µmol
photons m\(^{-2}\)s\(^{-1}\)), and a 5 min exposure to each consecutive irradiance level (0, 15, 50, 75, 125, 230, 450, 820, and 1440 \(\mu\)mol photons m\(^{-2}\)s\(^{-1}\)). An external light source (300-W quartz-halogen lamp projector) and neutral density filters were used to create the nine irradiance conditions. All respiration and photosynthesis measurements were conducted in a 1 L closed system chamber that was continually mixed. A linear regression of the rate of change of oxygen inside the chamber was calculated once it became stable while in the dark (0 \(\mu\)mol photons m\(^{-2}\)s\(^{-1}\)) and for each consecutive irradiance level. PE curves were conducted at three different temperature conditions: 10°C, 14°C, and 18°C, and for each nutrient condition 5 individuals were haphazardly selected. These three temperatures are representative of low, medium, and high seawater temperatures during the summer season for this location, verified from a temperature logger located at \(~1.0\) m MLLW tidal elevation near the Marine Science Center, Nahant, MA (Suppl. Figure 1). All temperature manipulations for the closed photosynthetic chambers were provided by a recirculating water bath (VWR 1147P).

Best curve fits were applied to all points for each PE curve as described in Colvard et al. (2014) using the following photosynthesis equation (Jassby and Platt 1976):

\[
P_{\text{net}} = P_{\text{gross,max}} \times \tanh \left( \alpha \times \frac{I}{P_{\text{gross,max}}} \right) - R_d \quad (\text{Eq. 1})
\]

where \(P_{\text{net}}\) is the net photosynthesis (\(\mu\)mol O\(_2\) g dry wt\(^{-1}\)h\(^{-1}\)), \(P_{\text{gross,max}}\) is the maximum rate of gross photosynthesis at a saturating irradiance (\(\mu\)mol O\(_2\) g dry wt\(^{-1}\)h\(^{-1}\)), \(R_d\) is the respiration rate in the dark (\(\mu\)mol O\(_2\) g dry wt\(^{-1}\)h\(^{-1}\)), \(I\) is irradiance (\(\mu\)mol photons m\(^{-2}\)h\(^{-1}\)), and \(\alpha\) is the initial slope of the PE curves (\([\mu\)mol O\(_2\) g dry wt\(^{-1}\]) \times [\mu\)mol photons m\(^{-2}\)h\(^{-1}\)]. \(P_{\text{gross,max}}\) and \(R_d\) were measured through laboratory experiments, where \(P_{\text{gross,max}}\) was calculated posthoc using the equation:

\[
P_{\text{gross,max}} = P_{\text{net,max}} + R_d, \quad (\text{Eq. 2})
\]
where $P_{\text{net,max}}$ is the maximum rate of net photosynthesis at a saturating irradiance. Based on preliminary results and from the findings in Colvard et al. (2014) for *F. gardneri*, $P_{\text{gross,max}}$ and $R_d$ for *Fucus* spp. are temperature-dependent; therefore, different irradiance and temperature combinations resulted in different calculated $P_{\text{net}}$ values. The $P_{\text{net}}$ equation provided by Jassby and Platt (1976) does not account for photoinhibition ($\beta$), and based on previous photosynthetic measurements of *F. vesiculosus*, photoinhibition was not observed at high, ecologically relevant irradiances (N. Colvard unpublished data) and was therefore not included in the calculation. An ANCOVA was used to analyze the effect of treatment (i.e., nutrient), temperature (covariate), and the treatment $\times$ temperature interaction for the PE curves. For purposes of statistical analysis, light was log+1 transformed to meet assumptions of normality and homoscedasticity, confirmed by plot of residuals.

*Photosynthetic Thermal Performance*

Photosynthetic thermal performance curves were conducted using an Ocean Optics Neofox FOSPOR-R oxygen sensor inserted into a 1L closed chamber system, where temperature was controlled by a recirculating water bath. All *F. vesiculosus* individuals (one individual per experimental mesocosm) were dark-adapted inside a closed water bath at the desired temperature for at least 30 min prior to any measurements. Dark respiration ($R_d$) measurements were conducted by shading the chamber and water bath, and recording the reduction in percent oxygen ($\%O_2$) for 20 min. The rate of $\%O_2$ change inside the chamber was calculated once the rate stabilized, after which an external light source (300-W quartz-halogen lamp projector) was turned on to induce photosynthesis. Maximum net photosynthesis ($P_{\text{net,max}}$) was recorded following 20 min exposure at a saturating irradiance (1000 $\mu$mol photons m$^{-2}$s$^{-1}$), determined
from the PE curves. The rate of %O₂ change for P_{net,max} was calculated once the rate stabilized from exposure to light. P_{gross,max} was calculated as P_{net,max} + R_d. R_d and P_{net,max} were measured using an adaptive mesh refinement approach for temperatures between 6°C and 30°C. In an adaptive mesh refinement approach (Berger and Oliger 1984) the refined regions (i.e., temperatures) consist of finer spacing as the organism performance approaches an optimum output (i.e., P_{max}); therefore, providing a more descriptive determination of the thermal optimum performance for *F. vesiculosus*, rather than a course approximation of the optimum. Irradiance for P_{net,max} measurements was provided by an external halogen lamp light source.

Best curve fits were applied to the photosynthetic performance measurements for *F. vesiculosus* as a function of seawater temperature in the dark (R_d) and at a saturating irradiance (P_{gross,max}). R_d was determined by fitting a linear regression curve to the respiration (i.e., consumption of O₂) measurements with increasing seawater temperature. P_{gross,max} was determined by fitting the photosynthetic performance with a curvilinear, third-degree polynomial. ANCOVAs were used to analyze the effect of treatment (i.e., nutrient), temperature (covariate), and the treatment × temperature interaction for R_d and P_{gross,max} data.

**Chlorophyll Fluorescence Measurements**

A Pulse Amplitude Modulation chlorophyll fluorometer (Jr. PAM, Walz, Effeltrich, Germany) was used to measure light induction curves at the conclusion of the nutrient enrichment experiment (day 36). Ten individuals were haphazardly selected, one from each of the mesocosm treatments (nutrients/no nutrients), and were dark adapted for at least 30 min prior to measurements. All trials were conducted at 18°C in 35 µm filtered seawater. Light induction curves were used to describe characteristic changes in chl-α fluorescence yield upon illumination.
of dark-adapted photosynthetic tissue. Light induction curves were performed on two regions of *Fucus vesiculosus* tissue: the apical tip and the thallus wing tissue along the side of the algal blade. The apical tip has been shown to be the primary growing region of *Fucus* algae (King and Schramm 1976, Bonsdorff and Nelson 1996), and the wing or thallus tissue comprises a large portion of the photosynthetically active tissue of *F. vesiculosus* (Hemmi et al. 2005).

Characterizing these two regions of the *F. vesiculosus* tissue helped elucidate small-scale differences in photosynthetic activity within a single individual, whereas the Neofox oxygen sensor setup quantified photosynthetic production for whole individuals. From the light induction curves, maximum quantum yield of PSII (\(\phi_{PSII}\)) and non-photochemical quenching (NPQ) values were analyzed. \(\phi_{PSII}\) can be used as a proxy for the photosynthetic capacity of the alga, whereas NPQ is closely related to the dissipation of excess light energy into heat in order to avoid photodamage. Two-tailed *t*-tests were used to analyze \(\phi_{PSII}\) and NPQ between nutrient treatments for both apical tip and thallus wing tissue. ANOVA tests was used to evaluate the interaction of treatment and tissue region for both \(\phi_{PSII}\) and NPQ data.

*Net Photosynthesis Model*

Using ecologically relevant irradiance (\(I, 0 – 1500 \mu\text{mol photons m}^{-2}\text{s}^{-1}\)) and seawater temperature (0 – 30°C) combinations in conjunction with the equation to calculate \(P_{\text{net}}\) (Equation 1), I developed simple models to predict net photosynthesis (\(P_{\text{net}}\)) for ambient and nutrient-enriched conditions. These predictive models are based on the best curve fits for \(P_{\text{gross,max}}\) (a curvilinear, third degree polynomial fit) and \(R_d\) (a linear fit) for both treatments. \(P_{\text{gross,max}}, R_d\), and \(\alpha\) (initial slope of PE curves) were temperature dependent for ambient and nutrient-enriched
conditions. Since the $P_{\text{net}}$ model is only valid when *F. vesiculosus* is submerged, during periods of aerial exposure at low tide $P_{\text{net}}$ was assumed to be 0 (Colvard et al. 2014).

Reconstructing the $P_{\text{net}}$ model to account for environmental conditions recorded near Nahant, MA from May 2013 to December 2014 provided an estimate of *F. vesiculosus* performance in the field. Seawater temperature data were obtained from an offshore NOAA buoy (NDBC Buoy: 44013) and compared to an *in situ* HOBO pendant logger located at +1 m MLLW in Nahant, MA. Aerial irradiance was recorded from a HOBO weather station located at the Northeastern University Marine Science Center, Nahant, MA. Underwater irradiance was calculated from aerial irradiance and water depth following Beer’s Law:

$$I_{d,uw}(z) = I_{\text{air}} \times e^{(-K_d^*z)}$$

$I_{d,uw}$ is the downwelling underwater irradiance ($\mu$mol photons m$^{-2}$ s$^{-1}$) at depth $z$ (m), $I_{\text{air}}$ is the downwelling irradiance measured in air ($\mu$mol photons m$^{-2}$ s$^{-1}$), and $K_d$ is the vertical light attenuation coefficient for Nahant, MA (0.480 m$^{-1}$, based on MWRA Water Quality measurements, [www.mwra.com/harbor/html/wq_data.htm](http://www.mwra.com/harbor/html/wq_data.htm)). Water depth ($z$) was estimated from tidal predictions at an intertidal elevation of +1 m MLLW (Xtide, [tbone.geol.sc.edu/tide](http://tbone.geol.sc.edu/tide)). Therefore, when the tidal height was <$+1$ m MLLW, the modeled alga was assumed to be aerially exposed and $P_{\text{net}} = 0$.

We calculated the yearly average $P_{\text{net}}$ for *F. vesiculosus* located at Nahant, MA from hourly recorded environmental data, and compared those results to a simulated increase in seawater temperature +3°C above recorded temperatures. Note that this approach does not account for projected changes in variance and thus is only a rough estimator of future changes, particularly considering that increases of this magnitude have already been reported in the Gulf of Maine (Mills et al. 2013). These evaluations were done over a tidal range of $-1.0$ m to $+2.0$ m
RESULTS

Seawater collected from the experimental mesocosms demonstrated elevated nitrogen levels in the nutrient-enriched treatment (20.95 ± 6.88 µM NO₃, mean±SE) mesocosms for the duration of the experiment (36 d). Analysis of seawater collected from the ambient (control) experimental mesocosms showed no change in nitrogen levels for the duration of the experiment (2.41 ± 0.33 µM NO₃, mean±SE) (Table 1). The results from a two-tailed t-test showed these water nitrogen levels were significantly different from one another (t = 2.01, P < 0.001).

Following the 36-d incubation period, the measured nitrogen levels in Fucus vesiculosus tissue were elevated in the nutrient-enriched treatment (1.741 ± 0.025 %N) compared to the tissue incubated under ambient conditions (1.178 ± 0.012 %N) (Table 1). Results from a two-tailed t-test showed these tissue nitrogen levels were significantly different from one another (t = 20.44, P < 0.001).

Tissue Growth

A two-tailed t-test showed significant growth differences in algal wet weight between ambient and nutrient-enriched tissues following the 36-d duration of incubation (t = 4.941, P < 0.001) (Table 1). Nutrient-enriched tissue had elevated tissue growth rate (0.021 ± 0.001 g d⁻¹) compared to ambient (0.016 ± 0.001 g d⁻¹). There was also a significant difference in the change
in normalized surface area growth between ambient (0.669 ± 0.43 cm$^2$) and nutrient-enriched tissues (0.873 ± 0.061 cm$^2$) ($t = 2.742, P = 0.008$) (Table 1).

**Photosynthesis versus Irradiance**

The ANCOVA showed significant effects of temperature ($F_{1,228} = 26.998, P < 0.001$), light ($F_{1,228} = 953.895, P < 0.001$), treatment × temperature ($F_{1,228} = 10.834, P < 0.001$), and temperature × light ($F_{1,228} = 18.776, P < 0.001$). The effect of treatment (nutrients) showed a trend towards significance ($F_{1,228} = 3.516, P = 0.062$), and all other interactions were non-significant. Nutrient-enriched *Fucus vesiculosus* tissue had higher photosynthesis levels than ambient tissue, but only at the highest temperature (18°C) recorded for the photosynthesis versus irradiance curves (PE curves) (Figure 1). There was no difference in the initial slope ($\alpha$) of the PE curves for all nutrient and temperature combinations. The half-saturation constant ($I_K$) for the PE curves showed similar values for ambient (44.355 μmol m$^{-2}$s$^{-1}$) and nutrient-enriched (42.438 μmol m$^{-2}$s$^{-1}$) treatments at 10°C, and for ambient (57.728 μmol m$^{-2}$s$^{-1}$) and nutrient-enriched (58.524 μmol m$^{-2}$s$^{-1}$) treatments at 14°C. The $I_K$ for the 18°C measurements had the greatest difference between ambient (56.922 μmol m$^{-2}$s$^{-1}$) and nutrient-enriched (78.252 μmol m$^{-2}$s$^{-1}$) treatments. None of the PE curves demonstrated photosynthetic inhibition ($\beta$) at high light levels, and therefore $\beta$ was not included in the photosynthetic analyses.

**Photosynthesis Thermal Performance**

Measurements of *F. vesiculosus* respiration in the dark ($R_d$) showed a near-linear response in oxygen consumption (presented as CO$_2$ production) as temperature increased from 6°C to 30°C (Figure 2). The ANCOVA demonstrated a significant effect of temperature ($F_{1,85} =$
212.848, P < 0.001), however, there was no effect of the nutrient treatment (F_{1,85} = 2.189, P = 0.143) nor an effect of the treatment × temperature interaction (F_{1,85} = 0.398, P = 0.530).

We fit curvilinear, third degree polynomial curves to the maximum gross photosynthesis (P_{gross,max}) data for nutrient-enriched and ambient individuals. The ANCOVA showed a significant effect of treatment (nutrients) (F_{1,75} = 30.195, P < 0.001), and temperature (F_{1,75} = 33.032, P < 0.001), and treatment × temperature interaction (F_{1,75} = 5.157, P = 0.026). Maximum photosynthetic production occurred at 26°C, followed by a decrease in P_{gross,max} at 28°C and 30°C for both treatment conditions (Figure 3). There was no difference between nutrient treatments in P_{gross,max} at low temperatures (6°C – 14°C); however, for ≥18°C P_{gross,max} measurements were greater for nutrient-enriched tissue compared to ambient (Figure 3).

Although the treatment × temperature interaction is weak, the position of the treatment lines is consistent across the levels of the covariate (temperature).

**Chlorophyll Fluorescence Measurements**

The measurements from the PAM fluorometer revealed the maximum quantum yield of PSII (\(\phi_{\text{PSII}}\)) for the apical tip tissue was greater for the nutrient-enriched treatment (0.739 ± 0.012) compared to the ambient condition (0.712 ± 0.007) (\(t = 3.003, P = 0.007\)). This difference was also observed for the thallus wing tissue, with increased \(\phi_{\text{PSII}}\) for nutrient-enriched tissue (0.703 ± 0.005) compared to ambient (0.675 ± 0.006) (\(t = 2.420, P = 0.026\)). There was no significant difference in \(\phi_{\text{PSII}}\) when evaluating the nutrient treatment × tissue region interaction (F_{1,36} = 0.726, P = 0.400) (Table 1).

Non-photochemical quenching (NPQ) was significantly different between ambient and nutrient-enriched tissue for both the apical tip tissue and the thallus wing tissue (Table 1). The
measurements of the apical tip region showed a significantly elevated NPQ for the ambient condition (1.635 ± 0.331) over nutrient-enriched tissue (0.810 ± 0.140) \((t = 2.295, P = 0.034)\). The thallus wing tissue also showed a significantly elevated NPQ for the ambient (2.071 ± 0.253) over nutrient-enriched tissue (1.136 ± 0.077) \((t = 3.737, P = 0.002)\). There was no significant difference in NPQ when comparing the nutrient treatment \(\times\) tissue region interaction \((F_{1,36} = 0.066, P = 0.799)\).

Net Photosynthesis Model

Based on temperature and irradiance values recorded near Nahant, MA, the \(P_{\text{net}}\) models suggested that \textit{F. vesiculosus} rarely experience environmental conditions needed to reach optimal production at any tidal elevation (Figure 4). Comparisons of average \(P_{\text{net}}\) for current (+0°C) recorded temperatures and an increase of 3°C with ambient nutrient conditions and elevated nutrient levels showed notable differences, with the highest yearly average \(P_{\text{net}}\) occurring in nutrient-enriched tissues at elevated temperatures (Figure 4).

DISCUSSION

The findings from this study show that \textit{F. vesiculosus} incubated in a nutrient-enriched treatment had higher oxygen production at warmer seawater temperatures, elevated maximum quantum yield of PSII (a proxy for photosynthetic capacity), a decrease in non-photochemical quenching (a mechanism to ameliorate light stress), and an increase in tissue growth compared to algae incubated under ambient conditions. These results support previous research that demonstrated elevated nitrogen levels led to increased photosynthetic performance and survival

The observed increase in *F. vesiculosus* growth in the nutrient-enriched treatment compared to the ambient condition suggests this foundational macroalgal species will experience increased productivity and tissue growth under moderate increases in nitrogen in the Southern Gulf of Maine. Nitrogen levels in *F. vesiculosus* tissue have been shown to exhibit a seasonal periodicity, with higher %N levels in the winter months, due to storm mixing and increased terrestrial runoff, and lower %N levels in the summer months when the stored nitrogen is more readily used during increased photosynthesis and growth activity (Townsend 1991, Perini and Bracken 2014). Tissue N levels from the nutrient-enriched treatment in the current study (1.741 ± 0.025) are representative of levels recorded in tissue sampled in the field at Nahant during May (1.996 ± 0.056) and October (1.951 ± 0.041), compared to lower levels measured in July and August (1.499 ± 0.087) when this experiment was conducted (N. Colvard, unpublished data). The elevated levels used in this experiment are thus representative of those in the field, but at levels that typically occur only under cooler temperatures. Increased temperature and irradiance levels in July and August allow for greater growth and photosynthetic production of the nitrogen-enriched algal tissue than ambient conditions. The increased growth in the nitrogen-enriched treatment is likely the result of a shift in carbon allocation, described by the carbon nitrogen balance hypothesis (CNBH) (Cronin and Hay 1996b, Van Alstyne and Pelletreau 2000)).

Contextually, the seawater nitrogen levels recorded for the nutrient-enriched conditions (20.95 ± 6.88 µM NO₃, mean±SE) are representative of elevated nitrogen levels for the rocky intertidal of New England (Perini 2013), which could be driven by environmental conditions
such as winter storm mixing, terrestrial run-off, or the added influx from combined sewer overflows (Lapointe 1987, Nixon 1995, Wootton et al. 1996, Howarth 2008). Keep in mind this study only exposed the *Fucus* algae to these elevated conditions for one month and demonstrated significant increases in tissue nitrogen and subsequent tissue growth. However, it is not clear what impact these elevated seawater nitrogen levels would have on *Fucus* algae over a longer duration, how much nitrogen would accumulate in the tissue, and how these changes may influence the ecology of this primary producer. Additionally, these nitrogen values are well below moderate nitrogen levels for river, wetland, and estuarine environments (de Jonge et al. 2002, Howarth and Marino 2006, Bricker et al. 2008). From 1993 to 2015 the Boston Harbor nitrogen levels have ranged from 0 to 69 µM NO₂ + NO₃, whereas the Mystic River nitrogen levels have ranged from 0.01 to 290 µM NOₓ and the Charles River nitrogen levels have ranged from 0.02 to 202 µM NOₓ (based on MWRA Water Quality measurements, www.mwra.com/harbor/html/wq_data.htm). Though the nitrogen levels used in this study are not representative of eutrophic conditions, in the traditional sense (Bricker et al. 2008), these levels do provide an initial indication of how *Fucus* algae will respond to elevated levels during the warmest time period of the year.

There was no observed difference between nutrient treatments for *F. vesiculosus* respiration, suggesting there is no added cost or stress on dark respiration with added nitrogen. Therefore, future seawater temperature increases will likely have a greater impact on *F. vesiculosus* respiration than will nutrient enrichment. However, photosynthetic oxygen production was greater for the nutrient-enriched tissue compared to tissue grown under ambient conditions, specifically for seawater temperatures between 18°C and 30°C. The results showed that 26°C is the thermal optimum for *F. vesiculosus* while submerged for both the ambient and
nutrient-enriched treatments. The initial positive effect of temperature (<26°C) on oxygen production is based on the rate of carbon fixation during photosynthesis (Davison 1991), with an increased rate of fixation as seawater temperature increased. The decline in oxygen production at temperatures above 26°C is likely attributed to temperature sensitive enzymes of photophosphorylation and electron transport and plastoquinone diffusion (Davison 1991). Therefore, stressful thermal conditions negatively affect enzymatic activity and proper transport of energy within the photosynthetic cellular structures.

Seawater temperatures for Nahant, MA recorded from May 2013 to May 2015 logged a maximum of 26.0°C - the optimal temperature for photosynthetic production - only four times (Suppl. Figure 1), suggesting that this species has not yet reaching it thermal optimum for this location (Stuart-Smith et al. 2015). Average seawater temperatures in the Gulf of Maine are projected to increase an average of 3°C by the end of the century (Pachauri et al. 2014), which may allow for increases in Fucus vesiculosus productivity. Since F. vesiculosus serves as a foundation species in this habitat (Dijkstra et al. 2012), this anticipated success would likely influence the success of other rocky intertidal organisms that directly or indirectly depend on F. vesiculosus for their own survival. However, it must be noted that these projected increases in average temperature of 3 – 3.5°C do not account for higher variance. For example, a summer heat wave in 2012 produced sea surface temperatures in the Gulf of Maine that were up to 3°C warmer than the average from the reference period of 1982 – 2011 (Mills et al. 2013), thus demonstrating that interannual variability in weather can exceed predicted changes in long-term average conditions. These rare, extreme events can potentially have deleterious effects on some intertidal organisms whose physiology is driven by local conditions and not broad scale climate shifts, per se. For Fucus algae, thermal anomalies such as this can potentially result in greater
photosynthetic production during emersed periods, though it is not know what effect it would have during aerial exposed immersion periods. Further research is needed to explore how small-scale oscillatory thermal variation affects foundational primary producers, as these species serve to influence (either directly or indirectly) several higher-level trophic organisms within their community.


This study showed that nutrient enrichment had a synergistic, positive effect on oxygen production for *Fucus vesiculosus* (Rhee and Gotham 1981, Staehr and Sand-Jensen 2006, Rosa et al. 2013) experiencing higher temperature conditions. These findings underscore the potential
resilience of this basal producer in the Southern Gulf of Maine to future environmental changes. Depending on how the basal photosynthetic producer in the community responds to increases in temperature and nitrogen - based on current environmental conditions - will affect interspecific interactions with other organisms within the assemblage and their success in an ever-changing environment. Further research is required to identifying how acute and chronic stressors on foundation species will scale to impacting the ecosystem community that is reliant on the success of these habitat-forming organisms (Harley et al. 2006, Helmuth 2009, Harley et al. 2012).
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Table 1. Summary of the experimental results and statistics for ambient (control) and nutrient-enriched (treatment) conditions for the mesocosm containers and *Fucus vesiculosus* tissue. The measured nitrogen in the experimental mesocosm containers is the average (±SE) µM Nitrogen in seawater samples collected throughout the duration of the experiment. The measured %N in *F. vesiculosus* is the average (±SE) from the experimental tissue following the enrichment period and corresponding photosynthesis and growth measurements. The relative growth rate (g•d⁻¹) and normalized surface area growth (cm²) measurements of *F. vesiculosus* are the average (±SE) for the two treatment conditions. The Quantum Yield of PSII (ϕPSII) and Non-photochemical Quenching (NPQ) results are the average (±SE) of the apical tip and thallus wing region of *F. vesiculosus* (n=10) from ambient and nutrient-enriched conditions. Statistical results for ϕPSII and NPQ are apical tip and thallus wing region, respectively.
Figure 1. Photosynthesis vs. Irradiance curves for A. ambient (control) and B. nutrient-enriched (treatment) individuals at 10°C, 14°C, and 18°C. The ANCOVA showed there was a significant effect of temperature ($F_{1,228} = 26.998$, $P < 0.001$), light ($F_{1,228} = 953.895$, $P < 0.001$), treatment $\times$ temperature interaction ($F_{1,228} = 10.834$, $P < 0.001$), and temperature $\times$ light interaction ($F_{1,228} = 18.776$, $P < 0.001$). The effect of treatment (nutrients) showed a trend towards significance ($F_{1,228} = 3.516$, $P = 0.062$).
Figure 1.
Figure 2. Dark respiration (Rₐ) measurements of ambient (white circle, solid regression line) and nutrient-enriched (black circle, hashed regression line) *F. vesiculosus* tissue were conducted using an adaptive mesh approach from 6°C to 30°C. The ANCOVA showed a significant effect of temperature ($F_{1,85} = 212.848, P < 0.001$), however, there was no effect of treatment (i.e., nutrient) ($F_{1,85} = 2.189, P = 0.143$) nor an effect of the treatment × temperature interaction ($F_{1,85} = 0.398, P = 0.530$).
Figure 2.
Figure 3. Maximum gross photosynthesis ($P_{\text{gross, max}}$) of ambient (white circle, solid curve line) and nutrient-enriched (black circle, hashed curve line) $F. \ vesiculosus$ with curvilinear, third degree polynomial curve fits were measured using an adaptive mesh approach from 6°C to 30°C in order to determine the thermal optimum. The ANCOVA showed a significant effect of treatment (nutrients) ($F_{1,75} = 30.195, P < 0.001$), and temperature ($F_{1,75} = 33.032, P < 0.001$), and treatment $\times$ temperature interaction ($F_{1,75} = 5.157, P = 0.026$).
Figure 3.

O₂ Production (μmol O₂ g dry wt.⁻¹ hr⁻¹)

Temperature (°C)
Figure 4. The yearly average $P_{\text{net}}$ modeled over a tidal elevation range of -1.0 m to 2.5 m at current, ambient seawater temperature (+0°C, black lines) and the projected increase in seawater temperature by 2090 in the North Atlantic (+3°C, red lines). The solid lines indicate ambient nutrient levels and the dashed lines indicate the nutrient-enriched conditions. There are notable increases in yearly average $P_{\text{net}}$ with elevated seawater temperature and nutrient enrichment.
Figure 4.

![Graph showing the yearly average net O$_2$ production (µmol O$_2$ g dry wt/hr) as a function of tidal elevation of algae (m) for two temperatures: +0°C and +3°C. The graph illustrates the decrease in net O$_2$ production with increasing tidal elevation for both temperatures.]
Suppl. Figure 1. A histogram of the seawater temperature recorded from May 2013 to May 2015 at Pump House Beach, Nahant, MA, near the Northeastern Marine Science Center. All temperature counts are when the logger was completely submerged, >2 m tidal height.
CHAPTER III

Evaluating herbivore response to algal population structure and environmental nutrient conditions

ABSTRACT

Ecosystems are experiencing dramatic changes in environmental conditions brought on by both climate change and urban development. Nowhere have these stressors been more apparent than coastal environments, which are subject to the perturbations from increased urbanization, terrestrial runoff from land development and agricultural practices, and coastal sewage discharge. Intertidal organisms already experience large fluctuations from daily tidal periodicity; however, in the coming century, the added stressors from climate change and other human disturbances are projected to exceed the physiological thresholds of many of these species. Although studies have often emphasized the negative aspects of nutrient enrichment resulting from increased run-off, others have suggested potential benefits of increased nutrients to enhancing resilience against other physiological stressors, such as increased temperature. It is therefore unclear under what sets of conditions increased coastal nutrients may be beneficial to primary producers such as macroalgae. To quantify potential spatial variation in environmental nutrient levels, and how this might drive variation in algal productivity, this study evaluated seawater and *Fucus vesiculosus* tissue samples collected from three different locations (sites) within the greater Boston area over the course of one year. Additionally, I quantified *Fucus* tissue growth during the predominant growing period (i.e., summer months) in order to establish within- and between-site differences in tissue production, potentially associated with environmental nutrient (i.e., nitrogen) levels. Lastly, the ubiquitous and prevalent gastropod mesograzer *Littorina littorea* was used to quantify whether prior exposure history influenced grazing pressure on different source populations of *Fucus* algae. The findings from this study suggest there were differences in chlorophyll *a* and nitrogen levels in the collected seawater, and
differences in percent nitrogen in the *Fucus* tissue, when evaluated on a range of spatial and temporal scales. Analysis of the seawater nitrogen and tissue nitrogen showed there were significant lag times in the cross correlation analyses for each site, suggesting an interrelationship between seawater nitrogen driving tissue nitrogen levels. There was also a significant difference in growth rates (maximum length) for *Fucus* between sites, though no significant difference when evaluating changes in projected surface area. Finally, there was no significant difference when evaluating the total tissue consumed for snail and *Fucus* populations, and the snail × *Fucus* interaction. However, there was a significant difference in percent-grazed agar between snail populations, though no difference between *Fucus* populations and the snail × *Fucus* interaction. The findings from this study provide a novel approach to evaluating how nitrogen levels in the environment differ depending on location, that can influence primary producer growth, as well as the consumptive effects of a grazer.

**INTRODUCTION**

Anthropogenic alterations to the environment are of particular concern when they impact structuring species and primary producers within the ecosystem (de Jonge et al. 2002, Crain et al. 2008). Changes in environmental conditions can cause direct and indirect effects on the organisms that inhabit the affected ecosystem (Aber et al. 2001, Blaustein et al. 2010, Altieri and Gedan 2014); in some systems these alterations have led to a rapid decline in species abundance and diversity (Bellwood et al. 2004, Orth et al. 2006, Jackson 2008), and increased the success of biological invasions in others (Lodge 1993, Vitousek 1986). Additionally, nutrient enrichment is dramatically altering the nitrogen cycle of coastal systems, which directly affects organism
productivity and success (Howarth and Marino 2006, Nygård and Dring 2008, Dolbeth et al. 2011, Ågren et al. 2012). Urban development in coastal communities in recent decades has led to a significant increase in environmental nitrogen of estuarine and marine systems (de Jonge et al. 2002, Howarth and Marino 2006, Galloway et al. 2008), markedly affecting the species composition and abundance in these habitats (Smith and Schundler 2009). There is often high spatial and temporal variation in nutrient supply due to multiple environmental factors, both natural and human-mediated, thus making it difficult to specifically predict nutrient levels within the heterogeneous rocky intertidal system. The rate and frequency of change in nutrient cycling in coastal marine systems can be very rapid, causing nutrient limited macroalgae to experience large variation in available nutrients on small temporal scales (Elser et al. 2007, Perini and Bracken 2014). An increase in environmental nutrients has been shown to increase tissue growth (Elser et al. 2007, Ågren et al. 2012), alter plant structure and function (Chapman and Craigie 1977, Elser et al. 2007, Ågren et al. 2012), and change the photosynthetic capacity of primary producers (Mooney and Gulmon 1982, Nygård and Dring 2008, Colvard and Helmuth in review). Though the focus on nutrient enrichment in an environment has concentrated on the negative effects of eutrophication (Nixon 1995, Herbert 1999, Howarth and Marino 2006, Dolbeth et al. 2011), recent studies have emphasized that lower levels of nutrient loading can be beneficial to some organisms when their environments are nutrient limited, which would otherwise limit growth (Nygård and Dring 2008, Ågren et al. 2012).

The carbon/nutrient balance hypothesis (CNBH) predicts that the concentration levels of nutrients in an environment help regulate a plant’s allocation of resources to growth and defense (Bryant et al. 1983, Yates and Peckol 1993, Van Alstyne and Pelletreau 2000). Plants and algae are predicted to allocate carbon to growth in nutrient-rich environments when they are not light-
or carbon-limited; and when nutrients are limited they are predicted to allocate carbon towards carbon-based defenses (Van Alstyne and Pelletreau 2000). This suggests there may be seasonal differences in carbon-based growth and defense, depending on available nutrient levels in the environment. In temperate rocky intertidal communities, nitrogen has been shown to be the most influential nutrient (Van Alstyne and Pelletreau 2000), limiting algal tissue growth during warm summer months and sustaining tissue development during cold winter months when photosynthetic activity is reduced (Lapointe 1987, Fujita et al. 1989, Wootton et al. 1996, Van Alstyne and Pelletreau 2000). It is well understood what processes contribute to nutrient enrichment and the direct effects they have on individual organisms, but there is increasing attention placed on describing how these inputs affect ecological interactions and community dynamics (Conley et al. 1993, de Jonge et al. 2002, Smith and Schindler 2009).

Small-scale variability in nutrient levels in the environment affect species interactions and in particular herbivore consumption (Cronin and Hay 1996a,b, Cruz-Rivera and Hay 2000, Hemmi and Jormalainen 2002, Jormalainen and Ramsay 2009). Herbivores can serve a large role as mediators within an ecosystem by facilitating bottom-up and top-down effects that can control the populations within a community (Nielsen 2001, Bracken and Stachowicz 2007, Schmitz 2008, Bracken et al. 2014). Herbivores must balance between the threat of being eaten from higher-order predators and eating to survive, and therefore must adjust their behavior at times to maximize their fitness gains (Bracken 2004, Iacarella and Helmuth 2011). For herbivores in marine environments this balancing act can limit species zonation within a habitat (Hawkins and Hartnoll 1985), recruitment to new habitats (Stachowicz 2001), and growth and development rates (Steneck et al. 1991). Rocky intertidal herbivores must contend with the pressures of both abiotic (e.g., temperature, desiccation) and biotic (e.g., competition) stressors for aquatic and
terrestrial environments (Hawkins and Hartnoll 1985), with the duration and extent of exposure to those stressors dependent on tidal timing and season. For example, Underwood and Jernakoff (1984) demonstrated that macroalgal distribution and abundance within a community was largely dependent on the activity of grazers, and the distribution of grazers (namely molluscs) was directly associated to exposure conditions. The consumptive effects by marine mesograzers on the basal primary producers of the system are typically targeting specific tissue that is more palatable and/or more readily available (Bracken and Stachowicz 2007). The apical tip tissue and small blades are often targeted on macroalgae by herbivore grazers, since these tissues have less chemical defenses and are less morphologically developed (Taylor et al. 2002, Bracken and Stachowicz 2007). Since these tissues are noted as having high surface area to volume ratios and high growth rates, these regions are responsible for a large degree of nutrient uptake and photosynthetic activity compared to other thallus tissue. Therefore, consumption of these structures is disproportionately affecting macroalgal growth and development, nutrient uptake, and photosynthetic activity than consumption of other more developed tissues (Schaffelke et al. 1995, Van Alstyne 1989, Stewart and Carpenter 2003).

In the northwestern Atlantic, *Fucus vesiculosus* serves as a foundation species for rocky intertidal ecosystems (Lubchenco 1983). Since these habitats are expected to experience increased nutrient enrichment, and in more extreme cases eutrophication, from coastal development, nutrient levels in *Fucus vesiculosus* (hereafter referred to as *Fucus*) tissue are expected to increase (Bracken 2004). *Littorina littorea* snails are described as the dominant herbivore in this system, and the consumptive rates of these grazers on *Fucus* are dependent on carbon-based defense levels within the algal tissue (Van Alstyne 1988, Yates and Peckol 1993, Van Alstyne et al. 2009, Flöthe et al. 2014). Carbon-based defenses (i.e., phlorotannins) in algal
tissue are directly proportional to the tissue nitrogen levels, which are dependent on ambient environmental conditions (Van Alstyne and Pelletreau 2000, Hemmi et al. 2005). This suggests a wide range in carbon and nitrogen tissue levels based on habitat location. Studying the variability in nitrogen levels in algal tissue, as well as differences in herbivorous snail exposure history, provides insight into the ecological complexity of environmental conditions influencing the growth, grazing pressure, and ultimate success of *Fucus* algae (Van Alstyne et al. 2009).

Although some studies have addressed the negative aspects of nutrient enrichment attributed to anthropogenic alterations of the environment (Nixon 1995, Galloway et al. 2008, Cloern 1999, de Jonge et al. 2002), others studies have suggested potential benefits of increased environmental nutrients enhancing organism resilience against other physiological stressors (i.e., temperature) (Vitousek et al. 1997, Blanchette et al. 2000, Bracken 2004, Elser et al. 2007, Galloway et al. 2008, Harpole et al. 2011). It is unclear under what concentrations environmental nutrient (i.e., nitrogen) levels may be beneficial to primary producers such as macroalgae in a nutrient-limited environment, and how variable these nutrients are on a spatial and temporal scale. The present study aimed to described how environmental nitrogen levels may differ on a small spatial scale in the rocky intertidal of New England, and how those changes may influence macroalgal growth and grazing pressure from a ubiquitous and prevalent mesograzer. In order to describe environmental and ecological variability within the rocky intertidal, I: 1) compared nutrient levels in the seawater and *Fucus* tissue on a regional scale over the course of a year; 2) analyzed *Fucus* tissue growth at three different sites during the predominant growing period (i.e., summer months); and 3) described the differences in snail grazing pressure on *Fucus* based on prior exposure history. This research consisted of both field collections and laboratory mesocosm
manipulations to characterize the role of environmental conditions influencing the ecology of a rocky intertidal foundation species.

METHODS

Study Organism and Location

I used *Fucus vesiculosus* and *Littorina littorea* collected from the rocky intertidal habitats of Deer Island in Winthrop, MA, the East Point of Nahant, MA, and the opening to Marblehead Harbor (near Fort Sewell, Marblehead, MA) (Suppl. Figure 1). *Fucus vesiculosus* is a prevalent macroalgal species within the intertidal in the Northwestern and Northeastern Atlantic (Lubchenco 1983, Bäck et al. 1992, Bonsdorff and Nelson 1996, Lamote and Johnson 2008), found to be a dominant spatial competitor with other macrophytes in the rocky intertidal system (Lamote and Johnson 2008, Jormalainen and Ramsey 2009, Graiff et al. 2015). *Littorina littorea* snails serve as the dominant herbivore in the rocky intertidal of New England, ubiquitous in exposed and sheltered habitats and tolerant of a range of environmental conditions (i.e., thermal and salinity stresses) (Kemp and Bertness 1984, Watson and Norton 1985, Yates and Peckol 1993). Deer Island serves as the sewage treatment plant for the greater Boston area, controlled by the Massachusetts Water Resource Authority (MWRA), and was the southern most site in this study (~10 km south of Nahant, MA). The East Point of Nahant, MA is the location of the Northeastern University Marine Science Center, where the experimental portion of this study was conducted. The third location is the most northern site, near the opening of Marblehead Harbor (~10 km north of Nahant, MA). All three sites were further subdivided into four sub-
sites to quantify potential within and between site variation in seawater and *Fucus* tissue nitrogen levels.

**Seawater Sample Analysis**

To analyze chl *a* and nutrient levels in seawater, water samples (0.4 L, *n* = 4 samples sub-site<sup>−1</sup>, 4 sub-sites site<sup>−1</sup>) were collected from Deer Island, Nahant, and Marblehead, MA from October 2014 to September 2015. All seawater samples were filtered through 0.7 µm glass microfiber filters (Whatman GF/F), and chl *a* was extracted from the filtered particulate in 10 mL 90% acetone solution in 15 mL Falcon tubes for 24 hrs. Following the extraction period, the solution was thoroughly-mixed and then centrifuged at 3000 rpm for 5 min to remove large particulates from suspension. The acetone/chl *a* samples were analyzed in a dimly-lit room using an Ocean Optics USB4000+ spectrometer connected in series to a 10 mm cuvette system and Ocean Optics LS1 tungsten halogen light source. A mixed-effects model (lme4 model with Satterthwaite approximation for degrees of freedom) was used to statistically analyze chl *a* data, with site and month treated as fixed effects, and sub-site treated as a random effect. All chl *a* data were log transformed.

Nitrogen levels in the collected seawater were analyzed using a Unisense NO<sub>x</sub> Biosensor, which provided a quantitative value of total nitrate and nitrite (µM NO<sub>x</sub>), hereafter referred to collectively as nitrogen. All field-collected seawater was filtered on day of collection (as described previously), and water samples were then frozen at −20°C until nitrogen analysis was conducted. To quantify water nitrogen levels, all samples were rapidly thawed and held at a constant 25°C in a temperature-controlled water bath. For each measurement, the NO<sub>x</sub> biosensor was allowed to reach a steady state (~ 2 to 3 min) before each reading. All calculated nitrogen
levels were the average of 5 sequential readings from the NO\textsubscript{x} biosensor, in order to account for small variability in the signal. A mixed-effects model (lme4 model) was used to statistically analyze the water nitrogen data, with site and month treated as fixed effects, and sub-site treated as a random effect. All water nitrogen data were square root transformed.

Fucus vesiculosus Tissue Analysis

To quantify nitrogen levels within the algal tissue over the course of one year, Fucus was collected from the mid-intertidal region (~1.0 m above Mean Lower Low Water, MLLW) from the three field sites (n = 4 individuals sub-site\(^{-1}\), 4 sub-sites site\(^{-1}\)) to determine within site variability, and allow for across-site comparisons. These field collections were conducted once a month from October 2014 to September 2015 to quantify potential seasonal changes in nitrogen levels in Fucus tissue. All collected algae were rinsed in freshwater, cleaned of epiphytes, and dried at 70°C in a drying oven (Thermo Scientific HERAtherm) for at least 48 hrs. A Retch Mixer Mill MM400 was used to grind the dried algae to a fine powder, from which tissue nitrogen levels were quantified using a Thermo Scientific Flash EA 1112 NC soil analyzer, providing % Nitrogen and C:N ratios of the Fucus tissue. Mixed-effects models (lme4) were used to statistically analyze the tissue nitrogen data, with site and month treated as fixed effects, and sub-site treated as a random effect. The % Nitrogen data and the C:N data were log transformed.

Fucus vesiculosus Field Growth

To quantify tissue growth of Fucus in the field, 40 individuals site\(^{-1}\) (10 individuals sub-site\(^{-1}\)) were tagged using numbered metal tags epoxied to the intertidal rock surface at the base of
each algal individual. Tagging was done in May 2015, with all individuals selected haphazardly at ~ MLLW +0 m tidal elevation for each respective sub-site. Initial linear maximum length of each individual alga was measured immediately upon emersion during low tide while the alga was still hydrated. Each individual alga was photographed during this same immersion period. Approximately 80 d later, in August 2015 all algal individuals that remained were re-measured, the linear maximum length was recorded and photographs were taken of each alga (n = 9 site\(^{-1}\)) (Dethier et al. 2005, Lachnit et al. 2009). Using image analysis software (ImageJ) I was able to calculate projected surface area (resolution in mm\(^2\)) of the *Fucus* and determine the growth rate over time. These two methods of measuring tissue growth allowed me to quantify linear growth and area growth over the summer season in these localized habitats. To analyze differences in growth rates (length and area) between research sites I used ANOVAs with sub-sites nested within sites. The measured maximum length data were arc-sine transformed, and the projected surface area data was square root transformed.

*Cross Correlation Function Analysis*

In order to evaluate the potential relationship between the seawater nitrogen time series data and the *Fucus* tissue nitrogen time series data I used a cross correlation function analysis, specifically comparing the two data sets at each respective site – Deer Island, Nahant, and Marblehead. This analysis established the seawater nitrogen as the x-variable and *Fucus* tissue nitrogen as the y-variable; therefore, a negative output would indicate *Fucus* tissue nitrogen lags seawater nitrogen, and a positive output would indicate the *Fucus* tissue nitrogen leads seawater nitrogen over time. This statistical comparison provided a quantitative analysis of how seawater
nitrogen may be a driver for nitrogen in *Fucus* tissue, and potentially providing insight into how changes in environmental conditions will impact macroalgal nutrient levels.

**Snail Grazing**

To quantify grazing pressure on algal tissue from littorine snails, *Fucus* was first collected from the three field sites in June 2015, dried at 70°C for 48 hours, and ground to a fine powder, as described previously. Using a 3% agar solution, dried *Fucus* tissue was mixed into solution and the algae agar was aliquotted (2 mL each) into 50 mm wide plastic petri dishes (n = 20 site⁻¹). *Littorina littorea* snails (n = 90 snails site⁻¹, 3 snails per grazing replicate) all of a similar size class (12 – 16 mm in length) were collected from the three sites. All snails were collected on the same day and starved for 48 hrs prior to the start of the experiment. All snails were weighed (air and buoyant weight) at the start of the experiment to confirm similar sizing for all algal and snail population combinations. With three different snail and *Fucus* populations, this design produced 9 different pairing combinations, allowing me to determine if grazing pressure was potentially driven by site-specific identity (e.g., Nahant snails grazing more on Nahant *Fucus*), or by snail source population (e.g., different grazing between snail populations). Prior to the start of the grazing experiment, *Fucus* tissue nutrient (C and N) levels were measured from the three sites (n = 4 samples sub-site⁻¹, 4 sub-sites per site) confirming no between site differences. After 72 hrs, final weights of grazed agar and control agar replicates were made to determine average tissue consumed. I evaluated grazing pressure by the snails of the algae agar by (a) total tissue consumption (i.e., weight lost from consumed agar) and by (b) percent agar grazed (i.e., image analysis of agar feeding cups). I used ANOVAs to analyze the total tissue
consumed and percent agar missing. The response variables (agar-tissue weight and proportion agar consumed) were both arc sine square root transformed.

All data processing and analyses were conducted using RStudio (Version 0.99.467, 2009-2015 RStudio, Inc.). All data that were transformed for analysis was done so that assumptions of normality were met, and all transformations were confirmed by Shapiro-Wilk normality tests.

RESULTS

Seawater Sample Analysis

The mixed-effects model analysis for chl $a$ ($\mu$g/L) levels showed there were significant differences for site ($P = 0.024$) and month ($P < 0.001$), but not for site $\times$ month ($P = 0.252$) over the one year analyzed (Figure 1A). The mixed-effects model analysis for the nitrogen levels in the collected seawater showed there were significant differences between between month ($P < 0.001$) and site $\times$ month ($P < 0.001$), but site showed a trend towards significant ($P = 0.080$). Tukey-Kramer HSD pairwise analysis comparing each site $\times$ month combination revealed that when evaluating within a given month Deer Island and Nahant were significantly different in October ($P = 0.025$). All other site $\times$ month comparisons within a given month were not significantly different. There was a strong seasonal effect on nitrogen levels in collected seawater, with higher levels in winter months ($5.940 \pm 0.401$ $\mu$M NO$_3$, December – February) compared to levels in late summer and fall months ($1.119 \pm 0.238$ $\mu$M NO$_3$, July – September) (Figure 1B).
Fucus vesiculosus *Tissue Analysis*

The mixed-effects model analysis for %N revealed significant differences for month (P < 0.001) and site × month (P = 0.010) over the one year analyzed, and a trend towards significance for site (P = 0.071) (Figure 1C). Tukey-Kramer HSD pairwise analysis comparing each site × month combination showed that when evaluating within a given month Deer Island and Marblehead showed a trend towards significance in June (P = 0.082). All other site × month comparisons within a given month were not significantly different. The %N calculated in *Fucus* tissue demonstrated a clear seasonal trend, with the highest tissue nitrogen levels in March for all three sites (3.698 ± 0.079 %N), and lowest recorded nitrogen levels in September for all three sites (1.371 ± 0.045 %N) (month, P < 0.001).

The mixed-effects model analysis for C:N ratio showed significant differences for month (P < 0.001) and site × month (P = 0.032) over the one year analyzed, though no significant difference between site (P = 0.144) (Suppl. Figure 2). Tukey-Kramer HSD pairwise analysis comparing each site × month combination showed that when evaluating within a given month there were no significantly different site combinations.

*Cross Correlation Function Analysis*

A cross correlation function (CCF) analysis was used to test the interrelationship between seawater nitrogen and *Fucus* tissue nitrogen levels. This statistical approach was helpful for identifying seawater nitrogen as useful predictors of algal tissue nitrogen. The CCF analysis comparing seawater nitrogen and algal tissue nitrogen was done on each site independently. For Deer Island (Figure 2A) the results showed the nitrogen levels in *Fucus* tissue significantly lagged 0, -1, and -2 months, with the peak at -2 months, behind seawater nitrogen levels. The
results for Nahant (Figure 2B) data demonstrated significant lags at 0, -1, and -2 months, with the peak lag at -1 month. These results indicate algal tissue nitrogen levels lagged 1 month behind seawater nitrogen levels at this location. The CCF analysis for Marblehead (Figure 2C) showed significant lags at 0, -1, -2, and -3 months, with the peak lag at -2 month. This signified that Marblehead algal tissue nitrogen lagged 2 months behind seawater nitrogen for this site. These results confirm for all three locations that seawater nitrogen levels are the leading condition, with algal tissue nitrogen levels lagging behind environmental conditions between one and three months (depending on location).

Fucus vesiculosus *Field Growth*

The findings from the ANOVA on maximum length growth rate in *Fucus* tissue, with sub-sites nested within site, showed there was a significant difference between sites (F<sub>2,15</sub> = 5.381, P = 0.017), though no significant difference between nested sub-sites (F<sub>2,15</sub> = 1.349, P = 0.292) (Figure 3A). Post-hoc Tukey-Kramer HSD tests determined a significant difference in maximum length growth rate of *Fucus* for Deer Island (0.011 ± 0.0013 cm•d<sup>-1</sup>, mean ± SE) compared to Marblehead (0.006 ± 0.0007 cm•d<sup>-1</sup>), though Nahant (0.007 ± 0.001 cm•d<sup>-1</sup>) *Fucus* growth was not significantly different from Deer Island or Marblehead growth.

For the projected surface area measurements, the results of the ANOVA showed there was no statistical difference between Deer Island (0.033 ± 0.01 cm<sup>2</sup>•d<sup>-1</sup>), Nahant (0.028 ± 0.003 cm<sup>2</sup>•d<sup>-1</sup>), and Marblehead (0.045 ± 0.014 cm<sup>2</sup>•d<sup>-1</sup>) sites (F<sub>2,15</sub> = 0.353, P = 0.708), and no significant difference between nested sub-sites (F<sub>2,15</sub> = 0.082, P = 0.922) (Figure 3B).
Snail Grazing

Evaluating the results of the two-way ANOVA for the total tissue consumption (i.e., change in weight (g) consumed of algae agar) of Fucus vesiculosus by snails showed there was no significant difference in grazed tissue between algal populations ($F_{2,168} = 1.729, P = 0.181$), snail populations ($F_{2,168} = 1.322, P = 0.269$), or the Fucus population × snail population interaction ($F_{4,168} = 0.087, P = 0.986$) (Figure 4A). Deer Island snails appeared to consume less Fucus tissue as the source population moved from south (Deer Island) to north (Marblehead); and there was no trend observed in the Nahant or Marblehead snail populations (Suppl. Table 1).

The image analysis of the algae agar provided a quantitative value of total grazing of the snails over the algae agar surface (Suppl. Table 2). The results from this two-way ANOVA demonstrated a significant difference in % grazed agar when comparing snail populations ($F_{2,171} = 4.973, P = 0.008$), but no significant difference between Fucus populations ($F_{2,171} = 0.323, P = 0.724$) or the Fucus population × snail population interaction ($F_{4,171} = 1.971, P = 0.101$) (Figure 4B). Tukey-Kramer HSD pairwise comparisons were applied post-hoc to compare snail populations, with Deer Island snail percent grazing pressure being significantly different from Nahant snails ($P = 0.010$) and Marblehead snails ($P = 0.041$). Nahant snails were not significant different in grazing pressure from Marblehead snails ($P = 0.873$).

DISCUSSION

This study demonstrated that nitrogen levels in the environment and in algal tissue differed between sites in relatively close proximity, which influenced algal tissue growth (maximum length), and found there are potential differences in grazing pressure based on the
population of a dominant mesograzer. New England rocky intertidal systems have been shown to be nutrient-limited, specifically nitrogen-limited (Howarth 1988, Howarth et al. 1996, Perini and Bracken 2014); therefore, the nutrient enrichment from terrestrial runoff or outflow from water treatment facilities will have a dramatic effect on primary producers (Nixon 1995, Howarth 2008). As human populations continue to grow in coastal cities, there will be an increase in land-use, habitat restructuring, and waste production, all of which will directly contribute to environmental nutrient levels (Nixon 1995, Valiela et al. 1997, Howarth 2008).

The measured seasonal fluctuation in environmental chl \(a\) and nitrogen levels in the seawater showed the variability these coastal organisms are exposed to over a year. The increased chl \(a\) levels in the seawater in late spring and summer are indicative of increased phytoplankton abundance, potentially causing greater competition for available light and nutrients for photosynthetic production with macroalgal species (Menge and Branch 2001, Menge et al. 1997, Harley et al. 2012). Chlorophyll \(a\) levels were measured in order to provide a possible metric for water quality (i.e., water clarity) levels at the respective study sites and serve as an explanation for how differences in environmental nitrogen levels may influence the abundance of microalgae (Bricker et al. 2008), which can be a light resource competitor with \textit{Fucus} algae. The statistical difference between the three study sites showed that chl \(a\) levels were typically greater at Deer Island compared to Nahant and Marblehead, suggesting that the Deer Island location had more phytoplankton in the water column. These findings support the visual observations of the three sites made during the course of this study, with Deer Island typically having much poorer water clarity than Nahant and Marblehead.

Seawater nitrogen levels are highest during winter months (December through February), primarily attributed to the cold water temperatures and the increase in water mixing from storm
activity (Townsend 1991, Townsend 1998, Perini 2013). This increase in seawater nitrogen eventually led to an increase in macroalgal tissue nitrogen levels, which peaked in March. As light and temperature conditions increased in late spring and summer the available nitrogen in the algal tissue dropped dramatically, attributed to increased tissue growth and photosynthetic activity. The observed difference in seawater nitrogen when evaluating the site × time interaction (P < 0.001) was largely attributed to Deer Island having higher nitrogen levels in winter (December and January) and late spring (April through June) compared to the seawater nitrogen readings from Nahant and Marblehead during those respective time periods.

Though this study demonstrated elevated seawater nitrogen levels at Deer Island, the location of the greater Boston water treatment facility, compared to Nahant and Marblehead, these levels are well below the moderate nitrogen levels recorded in estuarine and coastal wetland environments (de Jonge et al. 2002, Howarth and Marino 2006, Bricker et al. 2008). The nitrogen conditions recorded from these rocky intertidal habitats are not representative of eutrophic conditions in the traditional sense, but rather are meant to serve as a comparison for the large spatial and temporal variability in environmental nitrogen on a small local scale (10s of meters) along the New England coastline. In the coming century, nutrient concentrations in coastal communities will likely rise many-fold from terrestrial run-off including that from Combined Sewer Overflows (Nixon 1995, Howarth 2008), and as storm activity and frequency are projected to increase from climate change (Love et al. 2005, Perini 2013). It is therefore paramount to quantify the effect of small-scale changes in environmental nutrient levels in order to anticipate how organisms will respond.

The analysis of % nitrogen in Fucus tissue demonstrated similar seasonal patterns for all three study sites, with maximum nitrogen levels observed in March, indicative of the algal
species taking up the available nitrogen from the environment. With the increased seawater nitrogen levels during winter months, this provided an increase in nitrogen to macroalgal species that typically are nitrogen-limited in this region (Perini 2013). The results for the CCF analysis confirm tissue nitrogen significantly lagged seawater nitrogen (2 to 3 months) for all three sites, suggesting an interrelationship between seawater nitrogen driving tissue nitrogen levels. The lowest recorded % nitrogen in *Fucus* tissue was in September, which was likely attributed to the macroalgae using the stored tissue nitrogen collected from winter and spring months for photosynthetic activity and tissue growth during summer months, when light and temperature conditions are higher.

The analysis of *Fucus* growth in the field from May through September showed a significant difference between sites for the maximum length growth rate, primarily driven by the linear growth of *Fucus* at Deer Island compared to Nahant and Marblehead sites. This would suggest that the higher nitrogen levels in the environment at Deer Island from April through June contributed to higher tissue nitrogen levels, and therefore led to greater linear tissue growth at this site. Based on the CNBH described previously, with increased available nitrogen plants and algae will allocate carbon to growth, which support this study’s findings for *Fucus* growth at Deer Island compared to Nahant and Marblehead sites. However, this also suggest that *Fucus* in higher nitrogen environments (i.e., Deer Island) may be more susceptible to herbivores, since the algae are dedicating carbon to growth instead of defense from grazing. The differences in seawater nitrogen and algal tissue nitrogen did not differ as drastically between Nahant and Marblehead during the course of the field measurements.

The findings from the grazing experiment indicated there were differences in the percent grazed algae agar for the three *Littorina littorea* snail populations, but no differences when
evaluating the total tissue consumed. For the purposes of this experiment, the total tissue consumed was treated as a proxy for the amount of food consumed, whereas the percent grazed agar was treated as a proxy for the amount of surface area covered by the snails. This approach allowed me to determine if the snails stayed in a more limited area but were eating all the food in that space, or if the snails continually grazed over the whole surface of the available agar feeding cups but not completely consuming all algae agar in their path. The findings from the percent grazed agar showed there was a significant difference between snail populations \(P = 0.008\). This would suggest how mobile the snails were during grazing, with higher percent grazed agar values indicating more coverage than lower percent grazed values. This difference in percent grazed agar is most prominent on Marblehead *Fucus*, indicating the Deer Island snails grazed least on this algal population in comparison to the Nahant and Marblehead snails. There was no apparent trend in Nahant or Marblehead snails grazing on the three algal populations, indicating these two snail populations do not differ in the percentage of tissue they grazed over. However, it is not clear what is driving the difference in percent grazed agar between the three snail populations, and further research is needed to explore the potential mechanism influencing herbivore grazing pressure on intertidal macroalgae. There were no significant differences between total tissue consumed when comparing snail populations, *Fucus* populations, and the interaction.

The grazing pressure from herbivores can largely affect the success of primary producers within a habitat (Sousa et al. 1981, Hawkins and Hartnoll 1983, Hauxwell et al. 1998), and these pressures will change based on environmental conditions. Herbivores within the marine intertidal environment are subject to both aquatic and terrestrial environments (Lubchenco and Menge 1978, Hawkins and Hartnoll 1985, Menge 1992, Poore et al. 2012), so even small-scale changes
projected from climate change or anthropogenic disturbance from coastal development can have a dramatic influence on these coastal organisms. Since herbivores can serve as mediators by facilitating bottom-up and top-down control within a community (Nielsen 2001, Bracken and Stachowicz 2007, Schmitz 2008, Bracken et al. 2014) there will likely be drastic effects on species abundance and richness within habitats that are experiencing an increase in environmental nutrients.

As the human population continues to increase there will be increased pressure on natural ecosystems and the services they provide. The anticipated changes to the environment from increased anthropogenic pressure will be quite evident in coastal habitats. These coastal systems are subject to habitat alteration from increased urbanization and from changes in biogeochemical conditions (i.e., carbon and nitrogen cycles) (de Jonge et al. 2002, Howarth and Marino 2006, Galloway et al. 2008, Ågren et al. 2012). The changes that have already occurred from human population growth near coastal systems have led to an increase in biological invasions (Lodge 1993, Vitousek 1986) in some habitats, while others are experiencing a rapid decline in species abundance and diversity (Bellwood et al. 2004, Orth et al. 2006, Jackson 2008). The findings from this study have demonstrated environmental nutrient conditions have a direct effect on a macroalgal primary producer’s growth and the consumptive effects of a dominant herbivore on that primary producer. With the projected increase in coastal development in the coming century, and the subsequent nutrient enrichment in rocky intertidal communities, this study suggests Fucus macroalgae will have increased tissue growth that will directly affect habitat heterogeneity and species interactions in this system. The proliferation of Fucus algae with increased environmental nutrient levels can affect invertebrate species that use this primary producer as a food source or for structural protection from abiotic and biotic stressors. Increased Fucus growth
from increased environmental nutrients could also lead to exclusion of some spatial and resource competitor species, which would alter the diversity within the rocky intertidal. It is becoming increasingly evident that multiple environmental stressors and drivers are concurrently affecting the physiology and ecology of organisms in the natural world, and it is paramount to quantify the frequency and magnitude of these effects, and their potential interaction, in order to effectively forecast the future success or loss of species richness and community diversity (Crain et al. 2008).
REFERENCES


Bäck et al. 1992


Figure 1. These time series data show the monthly average (± SE) temporal variability in chlorophyll $a$ (A), seawater nitrogen (B), and % nitrogen in *Fucus vesiculosus* tissue (C) from October 2014 to September 2015. There were significant differences in chl $a$ ($\mu$g/L) for site ($P = 0.024$) and month ($P < 0.001$), but not for site $\times$ month ($P = 0.252$) over the one year analyzed. There were significant differences in seawater nitrogen levels ($\mu$M NO$_3$) for month ($P < 0.001$) and site $\times$ month ($P < 0.001$), but site was not significant ($P = 0.080$). The %N calculated in *Fucus* tissue demonstrated significant difference for month ($P < 0.001$) and site $\times$ month ($P = 0.010$) over the one year analyzed, and a trend towards significance for site ($P = 0.071$).
Figure 1.
Figure 2. Cross Correlation Function (CFF) analysis of seawater nitrogen and *Fucus vesiculosus* tissue nitrogen collected October 2014 to September 2015 from Deer Island (A), Nahant (B), and Marblehead (C). The filled circles represent significant correlations between the seawater and tissue nitrogen monthly matchups; open circles are non-significant monthly matchups.
Figure 2.
Figure 3. The ANOVA for maximum length growth rate (± SE, cm•d⁻¹) in *Fucus* tissue (A), with sub-sites nested within site, showed there was a significant difference between sites ($F_{2,15} = 5.381$, $P = 0.017$), though no significant difference between nested sub-sites ($F_{2,15} = 1.349$, $P = 0.292$).

The ANOVA for the projected surface area (± SE, cm²•d⁻¹) measurements (B) showed there was no statistical difference between sites ($F_{2,15} = 0.353$, $P = 0.708$), and no significant difference between nested sub-sites ($F_{2,15} = 0.082$, $P = 0.922$).
Figure 3.
Figure 4. Two-way ANOVA for the total tissue consumption (A) of *Fucus vesiculosus* by *Littorina* snails showed there was no significant difference in grazed tissue between algal populations ($F_{2,168} = 1.729, P = 0.181$), snail populations ($F_{2,168} = 1.322, P = 0.269$), or the *Fucus* population $\times$ snail population interaction ($F_{4,168} = 0.087, P = 0.986$). The % Grazed Agar (B) results demonstrated a significant difference in snail populations ($F_{2,171} = 4.973, P = 0.008$), but no significant difference between *Fucus* populations ($F_{2,171} = 0.323, P = 0.724$) or the *Fucus* population $\times$ snail population interaction ($F_{4,171} = 1.971, P = 0.101$). Presented data are mean ± SE.
Figure 4.
SUPPLEMENTAL TABLES

Suppl. Table 1. The average (±SE) tissue consumed (g) of Fucus vesiculosus in an algae agar mixture by Littorina littorea snails from three different populations (Deer Island, Nahant, and Marblehead). Graphical results are displayed in Figure 4.

<table>
<thead>
<tr>
<th>Tissue Consumed (g)</th>
<th>Fucus vesiculosus Population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Deer Island</td>
</tr>
<tr>
<td>Deer Island</td>
<td>0.029 ± 0.004</td>
</tr>
<tr>
<td>Nahant</td>
<td>0.031 ± 0.004</td>
</tr>
<tr>
<td>Marblehead</td>
<td>0.029 ± 0.003</td>
</tr>
</tbody>
</table>

Suppl. Table 2. The average (±SE) percent grazed Fucus vesiculosus in an algae agar mixture by Littorina littorea snails from three different populations (Deer Island, Nahant, and Marblehead). Graphical results are displayed in Figure 4.

<table>
<thead>
<tr>
<th>% Grazed Agar</th>
<th>Fucus vesiculosus Population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Deer Island</td>
</tr>
<tr>
<td>Deer Island</td>
<td>11.703 ± 2.765</td>
</tr>
</tbody>
</table>
Suppl. Figure 1. Map of the three field sites in the greater Boston area located ~10 km apart. Subsites for each site identified by black filled circles. Panel A – Marblehead, MA is the northern-most site. Panel B – Nahant, MA is the location of the Northeastern University Marine Science Center. Panel C – Deer Island, MA is the southern-most site and location of Massachusetts Water Resources Authority’s sewage treatment plant.
Suppl. Figure 2. C:N in *Fucus vesiculosus* tissue for October 2014 to September 2015. C:N ratios for all three sites showed significant differences for month (P < 0.001) and site × month (P = 0.032) over the one year analyzed, though no significant difference between site (P = 0.144).