THE ROLE OF SEASONALITY, SEAWEED TRAITS AND SEAWEED-HERBIVORE INTERACTIONS IN NUTRIENT CYCLING IN THE SOUTHERN GULF OF MAINE

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ABSTRACT OF THESIS

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ABSTRACT

Environmental fluctuations associated with both natural events and anthropogenic impacts can appreciably alter the functioning of ecosystems. For example, in the Gulf of Maine, there is substantial spatial and temporal variation in availability of nutrients that fuel primary production. Macroalgae are important primary producers in intertidal and shallow benthic habitats, and they play essential roles in absorbing nutrients (e.g., inorganic C, N, and P) and mediating their availability (e.g., as organic C, N, and P) to the communities that they support. The ability of macroalgae to take up nutrients depends on nutrient availability. However, sometimes a nutrient in short supply can restrict access to another abundant nutrient, resulting in co-limitation. Macroalgae can exhibit remarkable changes in tissue quality due to tidal elevation and seasonal nutrient variability. These changes in tissue quality may influence herbivore preference, consumption rates, and the flow of materials through marine systems. Here, I evaluated potential biotic and abiotic factors mediating rates of nutrient uptake and transfer in marine communities. I used an observational approach based on long-term, weekly sampling of ambient nutrient levels and macroalgal tissue quality at two tidal elevations and across taxa. I coupled the observational approach with laboratory experiments to investigate nutrient interactions such as co-limitation, and the impact on primary producers. Additionally, I conducted feeding assays to assess herbivore impacts on macroalgae from different positions on the shore and how these impacts vary with season. Observational data revealed strong seasonal patterns in ambient and macroalgal tissue nutrient levels and indicate that ambient nitrate levels may be restricting algal ability to access ambient phosphate. Laboratory results confirmed these observations of co-limitation, illustrating that increased N availability enhances P uptake efficiency in seaweeds. Results of feeding assays demonstrated varying net effects of herbivores on macroalgae, depending on tidal elevation and season via a combination of consumption and facilitation via nutrient recycling. These results highlight how both top-down and bottom-up processes are influenced by, and in turn contribute to, nutrient availability. My research illustrates how species interactions and abiotic conditions interact to mediate nutrient cycling in intertidal ecosystems.
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A wise man once told me, “Grad school is hard”. While I fully agree with him, the friendships I have made and experience I have gained has definitely made it all worthwhile.
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INTRODUCTION

Seasonal and environmental fluctuations

In the face of the variety of natural and anthropogenic impacts on ecological systems it is important to consider multiple factors to gain a realistic understanding of the complex interactions that support productive communities (Bracken et al. 2011). A variety of abiotic and biotic processes interact to determine community structure, species interactions and overall productivity in biological communities (Menge et al. 1997). Many of these processes are seasonal, such as changes in temperature, light, nutrient availability and community composition. Monitoring community-level responses to natural fluctuations, such as seasonal processes, can provide important insights for predicting responses to larger disturbances on a global scale. Traditionally, seasonal and multi-year studies have been relatively sparse, since this research can be logistically challenging due to the extended period of time and effort required. However, the need for these types of long-term monitoring efforts is becoming increasing clear due to the many threats facing natural communities.

Nutrient availability and constraints on primary producers:

Nitrogen and phosphorus are essential nutrients for all organisms due to their roles in the basic cellular machinery of life (Sterner and Elser 2002). These nutrients occur in a variety of forms and concentrations throughout ocean waters. Commonly occurring forms of nitrogen in seawater that are accessible to macroalgae include inorganic nitrate ($\text{NO}_3^-$), nitrite ($\text{NO}_2^-$), and ammonium ($\text{NH}_4^+$), collectively termed dissolved inorganic nitrogen (DIN). Major sources of DIN in coastal habitats are upwelling from the deep-ocean and terrestrial run-off (Parsons et al. 1984). Physiological studies show that ammonium is the most energetically efficient form of
DIN for macroalgae; it can be directly used to make new amino acids, whereas other forms must first be converted to ammonium upon assimilation (Lobban and Harrison 1994). Consequently, studies have found that many macroalgae preferentially absorb ammonium (Wheeler and Srivastava 1984, Brenchley et al. 1997) especially when facing energy limitation due to seasonal factors such as reduced access to light (Phillips and Hurd 2003). This indicates that local sources of ammonium such as invertebrate excretion might be important nitrogen sources for seaweeds during certain times of the year but not others.

Phosphorus also occurs in seawater in inorganic and organic forms, and macroalgae absorb the majority of required phosphorus as inorganic orthophosphate ions ($\text{PO}_4^{3-}$) (Lobban and Harrison 1994, Runcie et al. 2004). The majority of orthophosphate in ocean waters is derived from terrestrial sources such as weathering rock. Organic forms of phosphorus are readily available in coastal waters due to anthropogenic inputs and terrestrial runoff (Parsons et al. 1984). These can be used by macroalgae but require phosphatase enzymes to remove the organic portion before assimilation. Organic forms of phosphorus are therefore more energetically expensive (Lobban and Harrison 1994).

In the Gulf of Maine, nutrient supply to coastal ecosystems is influenced by a combination of biotic and abiotic factors. The temperate climate of the region combined with the bathymetry of the gulf produces pronounced seasonal variation in many natural processes. For example, towards the end of winter, coastal surface water becomes very cold and dense, causing it to sink to the ocean floor, driving deep water to the surface. This deep water is replete with sediment-derived nutrients. This annual process, known as seasonal overturn, creates an influx of nutrients in late winter and early spring, fueling increased primary production (Townsend 1998). Additionally, weather-related fluctuations such as increased wave action due to storms can alter
nutrient availability. This increased tidal mixing prevents water-column stratification, allowing nutrients that would normally be trapped at depth to be transported to the surface where they can fuel productivity (Townsend 1991). In a recent assessment of nutrient cycling in the Gulf of Maine, Townsend et al. (2010) suggested that climate change might be impacting the sources of water and nutrients entering the gulf. Specifically, Arctic melting may be increasing fresh water inputs into the gulf, replacing salty, slope water inputs, resulting in decreased nitrate and increased silicate concentrations in coastal waters. These large-scale environmental fluctuations highlight the need for local, long-term monitoring, to assess the impact of global change on biological processes.

These seasonal and anthropogenic changes in nutrient availability can impact the growth and functioning of primary producers (Elser et al. 2007), altering their essential roles in nutrient cycling at the base of food chains. The theory of ecological stoichiometry provides scientists with a basis to study the cycling of nutrients and impacts on biological processes. However, even before the development of this theory, scientists recognized the importance of nutrient availability in autotroph fitness. Early studies attempted to identify which nutrients were limiting autotroph growth by quantifying elemental ratios (C:N, N:P etc.) necessary to fuel growth and essential physiological functions (Chapman and Cragie 1977, Wheeler and North 1981).

Redfield et al. (1963) defined the C:N:P ratio of marine autotrophs as 106:16:1, with an N:P ratio <10 indicating N limitation and N:P ratio >30 indicating P limitation (Ryther and Dunstan 1971). However, an extensive survey of the C:N:P of marine macrophytes led Atkinson and Smith (1983) to conclude that while Redfield’s ratio may be appropriate for phytoplankton, marine macrophytes have an average C:N:P of 550:30:1. This indicates that overall, macroalgae tend to be P limited. Conversely, many studies have demonstrated that coastal ecosystems, and
therefore the growth of near-shore, benthic primary producers is often N limited (Lobban and Harrison 1994, Elser et al. 2007). Based on these findings, the role of P as a limiting nutrient in coastal systems was largely thought to be negligible due to ample P from terrestrial sources such as rock erosion and terrestrial runoff. However, Lapointe et al. (1992) illustrated that primary producer growth may be P limited in tropical ecosystems that lack terrestrial P sources.

Additionally, over the past several decades, water treatment efforts have focused on removing P from wastewater with fewer efforts to control N-inputs into natural systems. This may be driving N:P ratios in coastal waters towards P-limitation (Howarth and Marino 2006, Pedersen et al. 2010). Clearly, there is still much debate regarding the causes and consequences of nutrient limitation in marine systems and anthropogenic activities which alter the balance of these essential nutrients only further hinders the ability of scientists to assess their role in primary productivity.

Traditionally, the study of nutrient limitation of primary production was dominated by the idea that the nutrient in shortest supply will limit growth (Liebig’s Law of the Minimum), which asserts that only a single nutrient can be limiting at a given time. However more recently, in the face of changing nutrient regimes, it has become clear that understanding interactions between nutrients will be important in assessing impact of nutrient limitation on primary production (Saito et al. 2008, Harpole et al. 2011).

The incentive to study nutrient interactions has lead to emergence of the idea of nutrient co-limitation, in which two nutrients interact to limit primary productivity. Recently, several authors have provided varying definitions of what should and should not be considered co-limitation, depending on primary producer response to one or both limiting nutrients (Saito et al. 2008, Harpole et al. 2011, Agren et al. 2012). While these definitions allow us to categorize
observations of autotroph responses to nutrient enrichment, few authors have investigated mechanisms causing these responses. Agren et al. (2012) point out the likely existence of several different mechanisms, leading to the need for several definitions to fully encapsulate the complicated interactions between nutrients that limit primary productivity. Ultimately, these interactions between N and P equate to constraints on the N:P ratios of primary producers (Sterner and Elser 2002), and the extent to which these organisms are adapted to alter their uptake of essential nutrients in the face of limitation determines the extent to which their growth and functioning will be limited (Agren et al. 2012). This indicates that to better understand co-limitation, we need to identify mechanisms by which uptake of specific nutrients is enhanced or suppressed.

*Importance of seaweed*

Macroalgae are important primary producers in coastal marine systems and contribute to a significant percentage of total ocean primary productivity (Mann 1973). Additionally, large, canopy-forming macroalgae can serve as foundation species on rocky shores (Stachowicz 2001). In New England, brown algae such as *Ascophyllum nodosum* (L.) Le Jolis and *Fucus vesiculosus* L. fulfill this role, providing coexisting understory flora and fauna with habitat structure and relief from stressors such as desiccation and predation (Bertness et al. 1999).

Macroalgae are mediators of nutrient cycles in coastal ecosystems. They are able to absorb ambient nutrients such as nitrogen and phosphorus and make them available to herbivores at the next trophic level, providing an essential link in nutrient transfer throughout the community. A variety of seaweed traits and environmental factors can influence the unique role that each species or individual plays in nutrient cycling. For example, species with different
morphologies or from distantly related lineages might exhibit differences in nutrient uptake physiology, storage, and usage (Fujita et al. 1989, Wheeler and Bjornsater 1992, Phillips and Hurd 2003). Environmental factors influencing uptake rates include tidal elevation (Bracken et al. 2011), season (Hurd and Dring 1990), UV radiation, flow (Hurd et al. 1996), and other agents of physical stress. For example, several studies have illustrated that mild desiccation may enhance nitrate (Thomas et al. 1987) and phosphate (Hurd and Dring 1991) uptake for some macroalgal species.

Even within species, variable nutrient uptake rates can occur, depending on past nutritional history and internal nutrient reserves. For instance, several workers (Fujita 1985, Thomas and Harrison 1985) have illustrated that N starved seaweeds display increased rates of nitrate and ammonium uptake compared to individuals exposed to sufficient N preceding the incubations. Further, different forms of nutrients may interact to influence seaweed uptake abilities. D’Elia and DeBoer (1978) found that nitrate uptake was suppressed in the presence of ammonium for several seaweed species, however other studies have failed to detect this nutrient interaction (Thomas et al. 1987), indicating that some species can overcome this limitation.

Furthermore, nutrient availability influences the internal nutrient status of autotroph tissue, which may impact uptake rates. For instance, Fujita (1985) and Thomas and Harrison (1985) measured higher nitrate uptake rates in N-starved macroalgae compared to those growing under sufficient N conditions prior to uptake incubations. Similarly, Runcie (2004) measured enhanced phosphate uptake rates in p-starved Ulva lactuca and Catenella nipae, compared to P enriched individuals.

Seasonal fluctuations can influence macroalgal growth and physiological functions. For instance, many algae, especially in temperate waters, display patterns of growth that are closely
linked to nutrient availability (Chapman and Cragie 1977, Hanisak 1979, Gagné et al. 1982). However, in areas where solar radiation is significantly reduced in the winter, light has also been shown to limit algal growth, despite ample nutrients (Chapman and Lindley 1980). When light is limiting growth, seaweed are able to store excess nutrients in tissues for use later in the year, when light conditions are optimal but ambient nutrients become scarce (Chapman and Cragie 1977). This storage ability decouples growth from nutrient availability, making it difficult to assess algal nutrient limitation (Fujita et al. 1989). When internal nutrient reserves are depleted, algae may reduce growth in the summer when ambient nutrients are sparse and environmental conditions are stressful (Cubit 1984). These macroalgal traits can impact the roles of these primary producers in nutrient cycling.

**Tidal elevation**

Extensive research has shown that species distribution and abundance as well as important traits such as defense and foraging activity change across latitudinal gradients (Sousa et al. 1981, Gaines and Lubchenco 1982, Pennings and Paul 1992, Pennings et al. 2001, Moles et al. 2011). Similarly, the vertical gradient of physical stress in the intertidal zone drives ecological processes in this habitat. Specifically, macroalgae living at different tidal elevations experience different environmental stressors, such as increased desiccation stress high on the shore and increased herbivore pressure low on the shore (Hawkins and Hartnoll 1983). These macroalgae also experience different degrees of access to nutrients due to differences in the amount of time spent underwater, where they can take up nutrients. This environmental gradient is associated with variation in the physiological functions of macroalgae, which include reproduction, growth
and photosynthetic rates (Williams and Dethier 2005) and nutrient cycling dynamics (Bracken et al. 2011).

Tide-height gradients are associated with changes in nutrient uptake rate and storage capacity. For instance, Phillips and Hurd (2003) found that seaweed species living high on the shore were not N limited, despite significantly reduced immersion times compared to low shore species. Uptake experiments revealed higher NO$_3^-$ and NH$_4^+$ uptake rates in high shore species, an attribute that allowed them to obtain sufficient nutrients despite limited access.

While many studies have compared traits of different species living high versus low on the shore, few studies have examined intraspecific adaptations in seaweeds whose range spans the environmental gradient from low to high intertidal zone. Among these few, Thomas et al. (1987) examined nutrient dynamics and performed reciprocal transplants of a red intertidal alga. Results indicated that low-shore individuals increased their uptake rates when transplanted to the high intertidal. However, this increased uptake was not enough to compensate for reduced immersion time, and seaweed tissue N declined in low-to-high transplants. These studies illustrate the important role that tidal elevation plays in creating an environmental stress gradient that shapes ecological processes in this community.

*Herbivores*

Ecologists have historically debated the role of top-down (i.e. predation and herbivory) versus bottom up (i.e. resource supply) forces in shaping natural communities (Hairston et al. 1960, Hunter and Price 1992). Recently, it has been recognized that both top-down and bottom-up processes play essential roles in ecological communities, and studies have highlighted the importance of understanding the interactions between consumers and resources in mediating
species interactions, community structure, and ecosystem functioning (Menge et al. 1997, Hauxwell et al. 1998, Menge 2000, Nielsen 2001). One example of these types of interactions is when organisms with top-down roles (predators and herbivores) modify bottom-up processes such as nutrient supply. For example, in intertidal systems, herbivores and other nearby organisms can provide a source of recycled nutrients by excreting ammonium (Bracken et al. 2007), facilitating macroalgae growth. In fact, one study demonstrated that regenerated nutrients supplied up to 79% nitrogen required for algal growth (Taylor and Rees 1998). Similarly, another study showed that these autochthonous nutrient inputs from invertebrate excretions may be equally or even more important to primary production than allochthonous nutrient inputs from physical processes (Aquilino et al. 2009).

Several factors can influence the role of recycled nutrients in overall nutrient supply to coastal ecosystems. Local hydrodynamic factors play a role in determining the residence time of nutrients once they are excreted (Taylor and Rees 1998), therefore recycling may contribute more to nutrient supply in wave-protected areas or during times of year when wave action is reduced. Furthermore, previous studies indicate that ammonium excretion rates of gastropods may change seasonally (Navarro and Torrijos 1994), and depending on environmental conditions or food sources, potentially impacting nutrient recycling.

While modifying bottom-up processes, herbivores also play important top-down roles in intertidal communities. Classic ecological experiments have illustrated the essential role of molluscan herbivores in controlling algal biomass on temperate rocky shores (Lubchenco 1978). In their recent global review of marine herbivory, Poore et al. (2012) estimated that intertidal communities are impacted more by herbivores than any other ocean ecosystem. This intense herbivory can be influenced by a number of factors. For instance, some studies have found that
rates of herbivory increase with increasing temperature (Barker and Chapman 1990, Hillebrand et al. 2009). This idea is supported by metabolic theory, which predicts increased metabolism in organisms at higher temperatures (Allen et al. 2005). Adding complexity, Poore et al. (2012) found that temperature interacts with season to determine the impact of marine herbivores. They found that in the winter and spring, increasing temperatures were actually associated with decreased impact of herbivory, and that in the summer and fall there was no relationship between herbivory and temperature.

Environmental factors such as temperature and season may also impact the density of herbivores in an area. Barker and Chapman (1990) illustrated that in New England, densities of herbivorous gastropods vary throughout the year, with the highest densities in the summer. Nutrient availability can also change the impact of herbivory in marine habitats, and many studies have demonstrated a positive relationship between ambient nutrient levels, and/or seaweed tissue nutrient levels and rates of herbivory (Onuf et al. 1977). Stress plays an important role in shaping interactions and processes in intertidal communities, and several studies have attempted to quantify the impact of stress on herbivory. For instance, Renaud et al. (1990) found that the impact of desiccation stress on herbivory varied with seaweed species; desiccation increased herbivory of one species due stress-induced loss of defense compounds and decreased herbivory of another species due to decreased protein tissue levels in the seaweed.

Several studies have suggested that herbivore preference may be influenced by overall quality or carbon:nutrient ratio of autotroph tissue (Mattson 1980a, Hauxwell et al. 1998, Boyer et al. 2004, Thornber et al. 2008). Ecological stoichiometry examines how elemental ratios like these can impact processes from the cellular to the ecosystem or even biosphere level, including important impacts on autotroph-herbivore interactions (Elser et al. 2000, Sterner and Elser 2002).
Autotrophs may have extremely variable stoichiometry, due to their ability to store nutrients in excess of what is necessary for growth. Herbivores however do not have this storage ability and therefore need to maintain a relatively constant stoichiometry to ensure normal growth and functioning (Sterner and Elser 2002). This need to maintain nutrient ratios may lead to foraging decisions based on resource stoichiometry i.e. nutrient content of autotrophs could influence herbivore preference (Hemmi and Jormalainen 2002, Boyer et al. 2004, Schmitz 2008).

Studies have shown that starved or nutrient limited herbivores will eat more of a chemically defended resource if it has a low carbon:nutrient ratio (Cronin and Hay 1996). In this case, the metabolic needs of the herbivore override the negative consequences associated with the secondary metabolite (Cruz-Rivera and Hay 2003). In addition to this direct impact of variable autotroph stoichiometry on herbivore preference, carbon:nutrient ratios can also influence the type and/or quantity of secondary metabolites produced (Yates and Peckol 1993, Mata et al. 2012), or their effectiveness in deterring herbivores (Duffy and Paul 1992, Van Alstyne et al. 2009). This suite of factors adds complexity to the study of nutrient cycling and seaweed-herbivore interactions in intertidal communities.
OBJECTIVES

The research summarized above illustrates the abundance of biotic and abiotic processes that influence the cycling and flux of nutrients through natural systems. Anthropogenic activities are altering natural communities at alarming rates, including impacts on nutrient availability and the drivers of nutrient cycling. In the face of these disturbances, assessing the functioning of these ecosystems, especially via long-term studies that capture seasonal and inter-annual variation, is becoming increasingly important in order to fully understand our impact on these systems and build a bank of knowledge to inform conservation and management efforts.

The main objective of this study was to elucidate mechanisms contributing to nutrient availability in the southwestern Gulf of Maine, as well as document how seasonal changes in nutrient availability contribute to intertidal nutrient cycling. Observational data were collected to document nutrient availability to intertidal communities across large temporal scales, as well as the ability of macroalgae to access these nutrients and make them available to higher trophic levels. These observations were coupled with laboratory manipulations investigating interactions between limiting nutrients as well as assessing the seasonality of seaweed-herbivore interactions, including the importance of nutrient regeneration. Overall, the results contribute to our understanding of nutrient cycling in intertidal communities to allow for predictions of how these communities may respond to large-scale natural and anthropogenic fluctuations.
The specific objectives of this study were:

**Objective #1:** Document the impact seasonal fluctuations on nutrient supply to Canoe Beach, Nahant, MA and the response of macroalgae to this nutrient variation via long-term, weekly sampling.

**Objective #2:** Investigate the manner in which interactions between limiting nutrients influence macroalgal growth, tissue quality, and nutrient uptake dynamics via laboratory enrichment and uptake experiments.

**Objective #3:** Examine variation in the positive and negative impacts of herbivores on seaweeds, across tidal elevations and seasons.
MATERIALS AND METHODS

Study Site and Organisms:

All field collections took place at Canoe Beach (42.42, -70.91), a moderately protected, north-facing beach with long stretches of continuous rock. Canoe Beach is located on the eastern tip of Nahant, Massachusetts, a peninsula extending into the southwestern Gulf of Maine, just north of Boston Harbor. All sample analysis and experiments were performed at Northeastern University’s Marine Science Center, directly adjacent to the collection site.

On the rocky shores of the Gulf of Maine, *Fucus vesiculosus* is an abundant brown fucoid alga that dominates the mid-intertidal zone, extending patchily into the high and low zones. *Chondrus crispus* is a turf-forming, red alga that dominates the low intertidal and subtidal zone. Competition with low-zone macrophytes like *Chondrus crispus* has been shown to set the lower distributional limit of *Fucus vesiculosus* in the intertidal zone, while desiccation stress most likely sets the upper distributional limit (Lubchenco 1980). *Littorina obtusata* is an herbivorous snail found throughout the intertidal zone that actively grazes and resides on fucoid algae, including *Fucus vesiculosus* (Long et al. 2007).

Objective #1:

Ambient Nutrients:

In order to document ambient nutrient levels and their variation throughout the year, I collected weekly water samples (*n*=5) at Canoe Beach, Nahant, Massachusetts from January 2010 to December 2012. Water samples were not collected during February and March of 2011. Water samples were collected by hand, at the surface of the water, in plastic, acid washed, 15mL tubes. Water samples were immediately analyzed or frozen for storage until analysis. The
concentration of nitrate and phosphate in water samples was measured with a Quikchem 8500 Automated Ion Analyzer (Lachat Instruments, Loveland, CO).

To determine the role of environmental fluctuations in nutrient availability, local wave height data were obtained from GOMOOS (Gulf of Maine Ocean Observation System) buoys. Linear regressions relating ambient nutrients to wave height one day prior to water sample collection were used to assess the role of weather-related fluctuations in delivering nutrients to Canoe Beach.

Algal Tissue Nutrients:

In order to document algal tissue nutrient levels in relation to nutrient availability and seasonality, I collected algal samples from January to December in 2010 and 2012. Algal samples were collected weekly in 2010 and bi-weekly in 2012. In order to examine nutrient dynamics between individuals of the same species living at different elevations on the shore, samples of Fucus vesiculosus (hereafter Fucus) were haphazardly collected both in the high (n=5) and low (n=5) intertidal zone, at 1.7m and 0.2m above mean lower-low water, respectively. In order to examine nutrient dynamics between two different species with diverse lineages experiencing similar nutrient availability, samples of Chondrus crispus (=5) were collected in the low intertidal zone, where the alga is associated with Fucus. Chondrus crispus was collected in 2010 only. All algal samples were wrapped in aluminum foil, dried in an oven at 70°C until no further weight change could be detected, and then stored in an airtight cabinet with silica beads prior to nutrient analysis.

To analyze tissue carbon (C), nitrogen (N), and phosphorus (P) content, dried algae were ground to a fine powder using a mixer mill. Tissue N and C content was measured with the NC
Soil Analyzer Flash EA 1112 Series (ThermoFisher Scientific, Waltham, MA), an elemental analyzer that combusts the sample and measures the nitrogen and carbon gas released (Wheeler and North 1981). Tissue phosphorous content was measured with a magnesium sulfate digestion, using methods modified from Fourquarean and Zieman (1992) for total phosphorous determination. Extracted phosphorus in solution was measured with the Quikchem 8500 Automated Ion Analyzer.

Observations of ambient nutrient availability and algal tissue nutrients were compared using ANOVA. Ambient nitrate and phosphate levels were compared between years, and weeks nested within years (a random factor). Algal tissue % N, P and C were compared between years, weeks nested within years (a random factor) and between tide heights (a fixed factor).

**Objective #2:**

**Enrichment Experiment:**

In order to examine the interacting roles of ambient nitrogen and phosphorus in determining macroalgal tissue quality and growth, we conducted a laboratory enrichment experiment to simulate natural fluctuations of ambient nutrients. PVC agar nutrient dispensers inside 2-gallon buckets were used to create 4 treatments ($n=10$): +N, +P, +NP, and Control (no nutrient addition). All dispensers contained a 3% agar solution. In addition to agar, each nitrate dispenser contained a 0.75M solution of sodium nitrate ($\text{NaNO}_3$) and deionized water. Each phosphate dispenser contained a 0.35M solution of potassium phosphate ($\text{KH}_2\text{PO}_4$) and deionized water. Each +NP dispenser contained a mixture of both $\text{NaNO}_3$ and $\text{KH}_2\text{PO}_4$ in the same concentrations as in the individual +N and +P treatments. The dispensers were tested extensively, via water sampling, before the experiment began, and then weekly throughout the
experiment, to ensure desired levels of enrichment were achieved. In +N and +NP mesocosms, nitrate levels were maintained at an average ± S.E. of 15.17 ± 3.83 µmol L⁻¹. In +P and +NP mesocosms, phosphate levels were maintained at an average ± S.E. of 4.9 ± 1.9 µmol L⁻¹. Levels of nitrate in +P and control mesocosms and levels of phosphate in +N and control mesocosms did not differ significantly from ambient water (Figure 1). Enrichment concentrations were chosen based on maximum nutrient levels experienced by seaweeds in the field based on my own observational data.

![Graph showing nutrient levels](image)

**Figure 1:** Mean ± SE nitrate and phosphate levels (µmol L⁻¹) in mesocosms of different treatments and ambient seawater, calculated from samples collected weekly throughout enrichment experiment.

*Fucus vesiculosus* individuals with initial wet weights between 8-20g were collected from the mid-intertidal zone (1m above mean lower-low water) and 4 individuals (subsamples) were placed in each replicate bucket. These algal individuals were zip-tied to a corner of the square of lobster cage mesh, elevated one inch above the bottom of each bucket. Nutrient dispensers were zip-tied to the center of the square of lobster cage mesh, so each algal individual was at equal distance from the dispenser. Buckets were held in large, flowing seawater tanks (20
per tank), which filled and drained on a timer that mimicked the natural tide cycle. Each bucket had a single drainage hole below the lobster cage so that at low tide when the holding tank drained, the buckets also drained. Each bucket received constant flowing seawater from a piece of tubing that was attached to the lobster cage, creating circulation to dispense the nutrients at high tide and draining out the drainage holes at low tide.

Growth of seaweed was measured weekly throughout the experiment, by removing each algal individual from the bucket, weighing it, and calculating growth as a percentage of initial mass [\% Growth = (\Delta \text{Mass}/\text{Initial Mass}) \times 100\%]. Tissue N, P, and C content of each algal individual was measured before and after enrichment via methods described above. Growth and tissue nutrients of Fucus in the enrichment experiment were compared using 2-way ANOVA to examine impacts of N enrichment, P enrichment and the interaction of the two nutrients on response variables. Prior to comparisons, subsamples within replicate buckets were averaged. Using averages per replicate, % growth in each week, and initial and final % tissue N and P were compared between enrichment and control treatments.

Uptake Experiment:

The enrichment experiment was coupled with a laboratory nutrient uptake experiment, measuring the ability of Fucus to absorb nitrate and phosphate following enrichment. Algal nutrient uptake rates were measured in 8 1-L chambers following a design modified from Bracken et al. (2011). The experimental setup maintained artificial seawater (Instant Ocean, 35‰) at constant temperatures (14±0.3°C), while circulating water to create high flow velocities (18.1 ± 3.1 cm/s) and providing saturating light levels (>1000 µmol photon m⁻² s⁻¹), to mimic conditions experienced in the field. Four Fucus individuals from each treatment were randomly
selected from the enrichment experiment, and each individual was cut into 4, 2-6 g pieces (vegetative apical tissue only). After cutting, seaweeds were placed in a flowing seawater tank for at least 12 hours prior to uptake incubation to allow for recovery from cutting. Following this recovery period, the four pieces of seaweed from a single individual were placed in 4 individual chambers. The four chambers were spiked with NaNO$_3$ or KH$_2$PO$_4$ standard solutions to create a gradient of initial concentrations of nitrate (2, 15, 30, and 50 µmol·L$^{-1}$) or phosphate (1, 3, 5 and 10 µmol·L$^{-1}$). Water samples (6 ml) were taken from the chambers at time zero and then every 10 minutes for one hour. Nitrate and phosphate concentrations in water samples were measured with the Quikchem 8500 Automated Ion Analyzer. After uptake incubations, seaweeds were dried at 65°C until no further weight change was detected. Using the dry mass of each piece of algae, we calculated the biomass-specific uptake rate (µmol·h$^{-1}$·g$^{-1}$) of each algal individual as a function of the initial nitrate or phosphate concentration (µmol·L$^{-1}$) in each chamber. I then estimated Michaelis-Menten parameters for each algal individual [$V=(V_{\text{max}}*S)/(K_s + S)$] where $V$ (µmol·h$^{-1}$·g$^{-1}$) is the biomass-specific uptake rate, $V_{\text{max}}$ (µmol·h$^{-1}$·g$^{-1}$), is the maximum uptake rate at saturating concentration, $S$ (µmol·L$^{-1}$) is the substrate concentration, and $K_s$ (µmol·L$^{-1}$) is the substrate concentration at $V_{\text{max}}/2$. The ratio of $V_{\text{max}}$ over $K_s$ is equal to the initial slope of the relationship, which describes an individual’s uptake ability at low nutrient concentrations (Bracken et al. 2011), relevant to those experienced the GOM.

I used a generalized linear model with a log link and an inverse Gaussian distribution to evaluate differences in uptake rates between seaweed from different treatments in taking up NO$_3^-$ or PO$_4^{3-}$ [i.e. uptake as a function of nutrient (NO$_3^-$ or PO$_4^{3-}$), enrichment treatment (C, N or P), and nutrient X treatment].
I used AIC model selection to compare Michaelis-Menten model fit and linear model fit and found overarching support for the Michaelis-Menten model. Using Michaelis-Menten parameters for nitrate and phosphate uptake of *Fucus* from each enrichment treatment, we estimated uptake rates over the entire range of ambient concentrations tested (0-50 and 0-10 µmol·L⁻¹ for N and P respectively). I used these estimations to generate projected uptake curves, to compare uptake rates across this range of concentrations for seaweed from each enrichment treatment.

*Objective #3:*

*Feeding Assays:*

In order to determine the role of tidal elevation and seasonal fluctuations on herbivore preference, and macroalgae-herbivore interactions, paired choice versus no choice feeding assays were conducted, based on a modification of methods of Thornber et al. (2008) and Long et al. (2007). Assays were conducted in spring and summer of 2010, 2011 and 2012. The herbivorous snail *Littorina obtusasta* was given a choice of *Fucus* individuals collected in the high and low intertidal zone. Herbivores were collected haphazardly along the shore. All choice treatments (high versus low) were paired with no choice treatments (both high and both low). Snails were allowed to graze for 3 days. Algae and snails were weighed before and after the experiment to determine amount of grazing based on change in algal tissue mass. Nutrient (C, N and P) content of algae were measured before grazing to determine the role of algal tissue content in herbivore preference. Paired *t*-tests were used to assess difference in algal growth between high and low individuals, due to consumption, facilitation and the total impact of herbivores. One-sample *t*-tests were used to test for significant impact of herbivores on algal growth.
Feeding assays were designed with treatments that allow for measurement of both consumptive (e.g. grazing) and non-consumptive (e.g. facilitation) effects of herbivores on algae. Mesocosms were crafted from plastic containers, which were cut half, and plastic mesh was glued in the middle, to split the container into two equal sides. Split mesocosms had equal amounts of *Fucus* on each side, and snails on one side (the grazing treatment). Ammonium excretions of herbivores were able to diffuse across the mesh barrier, in order to account for autogenic growth of seaweed in the presence of herbivores (facilitation treatment). Consumption was calculated by subtracting growth of *Fucus* in the grazing treatment from growth in the facilitation treatment. Each of these mesocosms was paired with a control, no grazer, no facilitation mesocosm with *Fucus* on both sides and snails on neither side. This treatment allowed for quantification of the impact of herbivores on *Fucus* via excretion or other non-consumptive effects. Facilitation was calculated by subtracting the growth of *Fucus* in the control treatment from growth in the facilitation treatment.

*Herbivore Excretion and Abundance:*

I quantified seasonal variation in nutrient recycling by measuring the ammonium excretion rates of *Littorina obtusata* during spring and summer, in conjunction with feeding experiments. To accomplish this, freshly collected snails (25 grams) were placed in individual closed chambers (*n*=8), which circulate seawater and maintain it at a constant temperature to mimic natural conditions. Water samples were taken from each chamber at time zero and then every twenty minutes for two hours. Water samples were then for ammonium concentration on a spectrophotometer at 650nm (Solorzano 1969, Liddicoat et al. 1975). The change in concentration of ammonium over time was used to calculate an excretion rate in µmol NH₄⁺ per
gram snail mass per hour. Excretion rates were compared between spring and summer with a 1-way ANOVA.

Additionally, field surveys of *L. obtusata* abundance in the high and low intertidal zone were conducted at the same time as feeding assays and excretion experiments to document seasonal variation in natural herbivore density. Herbivore density was compared between tide heights and seasons with a 2-way-ANOVA. Excretion rates and field density data were used to estimate field excretion totals across tidal elevations and seasons.

All data were examined and transformed, when necessary, to meet the assumptions of each statistical test. In several cases, even after transformation, data did not conform to the ANOVA assumption of normally distributed residuals, however I decided to proceed with the analysis because ANOVA is robust to deviation from this assumption (Schmider et al. 2010). All analyses were performed in R Studio version 0.97.318.
RESULTS

Objective 1:

Ambient nutrients:

Monitoring of ambient nutrients revealed a seasonal trend in nitrate availability that was repeated during all three years of sampling (Figure 2a). In 2010, the highest nitrate levels were observed in March (average±SE: 7.99±2.43 μmol L\(^{-1}\)) and lowest in July (0.58±0.42 μmol L\(^{-1}\)) with an average value throughout the year of 2.42±0.23. Although sampling was not performed throughout 2011, of the obtained samples, highest nitrate levels were observed in January (9.32±2.18) and lowest in June, when nitrate levels were below the detectable limit. In 2012, the highest nitrate levels were observed in January (13.34±0.64 μmol L\(^{-1}\)) and nitrate levels were below the detectable limit during late May and early June, and throughout July, with an average value throughout the year of 2.54±0.39. Results of ANOVA indicate that nitrate levels varied significantly from week to week in 2010 and 2012 (p<0.001), however average nitrate availability did not vary between 2010 and 2012 (p=0.329).

Figure 2a:
Figure 2b: Weekly mean ± SE ambient nitrate (a) and phosphate (b) in µmol L⁻¹, measured from samples (n=5) collected from January 2010 to December 2012 at Canoe Beach. Samples were not collected during February and March 2011.

Ambient phosphate levels did not adhere to a seasonal pattern, as did nitrate levels (Figure 2b). In 2010, the highest phosphate levels were observed in July (0.96±0.15) and lowest in April (0.028±0.024), with an average throughout the year of 0.53±0.22 µmol L⁻¹. In 2011 and 2012 however, phosphate levels rose significantly, and became more variable than those observed in 2010. Specifically, there was a trend toward higher phosphate levels during late summer and fall during both 2011 and 2012. Average phosphate availability in 2012 was 1.13±0.38 µmol L⁻¹, more than double the average in 2010. Results of ANOVA indicate that phosphate levels varied significantly from week to week in 2010 and 2012 (p<0.001). Additionally, average phosphate levels varied significantly between 2010 and 2012 (p<0.001), contrary to average nitrate levels.
Figure 3: Linear regression between ambient nitrate and phosphate levels (µmol L\(^{-1}\)) for three years of sampling indicates a significant relationship between the two nutrients.

A linear regression relating ambient nitrate and phosphate for all sampling dates from 2010, 2011, and 2012, indicates a significant correlation between concentrations of the two nutrients (R\(^2\)=0.05, p=0.019, Figure 3). Additionally, the intercept of the regression is significantly different than zero (p<0.001) indicating that when ambient nitrate is completely depleted, ambient phosphate remains in the water column. In 2010 the highest N:P ratios were detected in April (35.81±37.37) and lowest in July (0.66±0.19), with an average throughout the year of 6.03±0.94 (Figure S a). In 2012 N:P ratios were less extreme, with highest levels detected in January (13.56±0.80) and lowest in June and July when the N:P ratio was zero due to nitrate concentrations below detectable levels. Average N:P throughout 2012 was 2.33±0.39, less than half that of 2010. Results of ANOVA indicate that N:P ratios varied significantly from week to week in 2010 and 2012 (p=0.0034). Additionally, average N:P ratios varied significantly between 2010 and 2012 (p<0.001).

In 2010, there was a positive relationship between wave height and ambient nitrate in the winter (R\(^2\)=0.31, p=0.093) and spring (R\(^2\)=0.52, p=0.008) months. However, wave height and
nitrogen availability were not related in summer (R²=0.03, p=0.56) or fall (R²=0.03, p=0.85) months (Figure 4a). In 2012, linear regressions revealed no significant relationships between wave height and ambient nitrate (p>0.05), however regression lines for winter and fall (Figure 4b) indicated a slight trend. These data indicate that waves are important in delivering nutrients to intertidal communities during some months of the year but not others and that this seasonal relationship varies from year to year as well.

**Figure 4a:**

![Graph showing the relationship between wave height and nitrate concentration.](image-url)
Figure 4b: Ambient nitrate (µmol L⁻¹) plotted versus mean wave height (m) on the day before water samples were collected in 2010 (a) and 2012 (b).

In 2010, linear regressions examining the relationship between wave height and phosphate availability revealed a positive relationship during the winter ($R^2=0.56$, $p=0.013$). However, no relationship was detected for the rest of the year ($R^2<0.03$, $p>0.1$, Figure S2). In 2012, wave height and ambient phosphate were positively related during the summer ($R^2=0.56$, $p=0.020$). However, no relationship was detected for other seasons of the year ($R^2<0.2$, $p>0.1$, Figure S6).

**Algal Tissue Nutrients:**

Documentation of algal tissue nutrients revealed that macroalgal tissue nitrogen content was tightly coupled to nitrogen availability in both 2010 and 2012 (Figures 5a and 6a). Algal tissue nitrogen mirrored nitrate availability during both years, with highest levels in March and February, and lowest in July and August in 2010 and 2012, respectively. This pattern persisted
regardless of position on the shore or algal species. In both 2010 and 2012, linear regressions between ambient nitrate and *Fucus* tissue nitrogen revealed a strong positive relationship (2010: $R^2=0.35$, $p<0.001$; 2012: $R^2=0.45$, $p<0.001$; Figure 7).

The highest tissue N levels were detected in *Fucus* in February and March of 2010, and in February of 2012 when tissue N levels reached 2.63% dry weight (DW) (Figure 5a). Lowest levels were detected in July of 2010 (0.66% DW) and August of 2012 (0.55% DW). *Fucus* tissue nitrogen varied between weeks ($p<0.001$), but not between years ($p=0.99$) or between tide heights ($p=0.58$). However, there was a significant interaction between week and tide height ($p<0.001$), indicating that difference in tissue N between tide heights depended on week. Therefore, individual weeks were tested for significant differences between tide heights. Significant differences in tissue nitrogen between *Fucus* individuals collected at high versus low tidal elevations were detected on 8 of the 46 total sampling dates in 2010 and on 7 of 22 total sampling dates in 2012 ($p<0.05$). These few differences were scattered throughout the year, giving no indication that these differences were related to variation in environmental conditions. When differences were detected, there was no consistent trend in whether high or low individuals had higher or lower nitrogen tissue content.

As with *Fucus*, *Chondrus crispus* tissue nitrogen varied throughout the year in 2010 ($p<0.001$). Despite identical access to nutrients, *Chondrus crispus* tissue contained an average of 88% more nitrogen than low zone *Fucus* ($p<0.001$). The highest tissue N levels were detected in *Chondrus crispus* in January and March of 2010 when tissue N levels reached 4.98% dry weight (DW). Lowest levels were detected in July of 2010 (1.16% DW).
Figure 5: Weekly water and algal tissue nitrogen (a) and phosphorus (b) in 2010. Points represent the mean ± SE % tissue N and P of seaweed and average µmol L⁻¹ nitrate and phosphate in water for samples collected each week (n=5).
Documentation of algal tissue phosphorus content revealed an interesting seasonal trend, despite the lack of a trend in phosphate availability in 2010 and 2012 (Figures 6a and 6b, respectively). Disregarding a dramatic spike in tissue P levels that lasted for two weeks of September of 2010, algal tissue phosphorus mirrored the seasonal trend of nitrate availability, with highest levels in March of both years, and lowest levels in June 2010 and August 2012. Linear regressions illustrate a positive relationship between ambient nitrate and Fucus tissue phosphorus in both 2010 ($R^2=0.22$, $p<0.001$) and 2012 ($R^2=0.33$, $p=0.005$). However, there was no correlation between ambient phosphate and Fucus tissue phosphorus during either year (2010: $R^2<0.001$, $p=0.969$; 2012: $R^2=0.003$, $p=0.953$; Figure 7).

The highest tissue P levels were detected in Fucus in September of 2010 when tissue P levels reached 0.33% dry weight (DW). Aside from these values, the highest Fucus tissue P levels in were detected in March during both years (0.27% DW 2010, 0.24% DW 2012). Lowest levels were detected in July of 2010 (0.097% DW) and August of 2012 (0.072% DW).

Fucus tissue phosphorus varied with week ($p<0.001$), year ($p=0.041$) and tidal elevation ($p=0.035$). Additionally, there was a significant interaction between week and tide height ($p=0.012$), indicating that the difference in tissue P between tide heights depended on week. Therefore, individual weeks were tested for significant differences between tide heights. Differences in tissue phosphorus between Fucus individuals collected at high versus low tidal elevations were detected on 9 of the 46 total sampling dates in 2010 and on 6 of 22 sampling dates in 2012 (ANOVA: $p<0.05$). In 2010, on 8 out of these 9 sampling dates, Fucus from the low zone had higher tissue phosphorus levels than high zone Fucus. In 2012, there was no consistent trend in whether high or low individuals had higher or lower phosphorus tissue content.
As with *Fucus, Chondrus crispus* tissue phosphorus varied throughout the year in 2010 (p<0.001). Despite identical access to nutrients, *Chondrus crispus* tissue contained an average of 60% more phosphorus than low zone *Fucus* (p<0.001). The highest tissue P levels were detected in *Chondrus crispus* in September of 2010 when tissue P levels reached 0.63% dry weight (DW). Aside from this value, highest *Chondrus crispus* tissue P levels in were detected in February (0.42% DW). Lowest levels were detected in July (0.092% DW), when *Chondrus crispus* P levels actually dropped below the lowest recorded *Fucus* P levels of 2010.

**Figure 6a:**
**Figure 6b:** Weekly water and algal tissue nitrogen (a) and phosphorus (b) in 2012. Points represent the mean ± SE % tissue N and P of seaweed and average μmol L⁻¹ nitrate and phosphate in water for samples collected each week (n=5).

*Fucus* tissue N:P ratio varied with week (p<0.001), year (p<0.001) and tidal elevation (p=0.036, Figure S1). Additionally, there was a significant interaction between week and tide height (p=0.005), indicating that difference in tissue N:P between tide heights depended on week. In 2010, *Fucus* tissue N:P ranged from 3.05±0.19 in September to 13.49±1.21 in May, with an average throughout the year of 9.07±0.043. In 2012, *Fucus* tissue N:P was higher throughout the year than in 2010, ranging from 7.50±0.41 in August to 14.16±3.31 in October, with an average of 10.39±0.31. As with *Fucus*, *Chondrus crispus* tissue N:P varied throughout the year in 2010 (p<0.001), with highest values observed in October (22.83±3.21) and lowest in September (3.61±0.17), and an average throughout the year of 10.82±0.10. While the average N:P values of low zone *Fucus* and *Chondrus crispus* differed by only 1.8 units, *Chondrus* N:P was more variable than that of *Fucus* throughout the year, resulting in overall significant
difference between the N:P ratios of the two seaweeds in 2010 (p<0.001).

_Fucus_ tissue C:N ratio varied with week (p<0.001), but not with year (p=0.85) or tidal elevation (p=0.14, Figure S3). However, there was a significant interaction between week and tide height (p<0.001), indicating that difference in tissue C:N between tide heights depended on week. In 2010, _Fucus_ tissue C:N ranged from 13.68±0.50 in March to 59.93±3.75 in July, with an average throughout the year of 28.48±0.17. Compared to high individuals, _Fucus_ from the low intertidal exhibited more extreme C:N ratios throughout the year, ranging from 14.55±0.71 in February to 75.80±4.21 in August, with an average throughout the year of 30.21±3.35. As with _Fucus_, _Chondrus crispus_ tissue C:N varied throughout the year in 2010 (p<0.001), with highest values observed in October (24.79±2.07) and lowest in September (6.24±0.32), and an average throughout the year of 12.45±0.082. The year-long average C:N of low zone _Fucus_ was more than double that of _Chondrus_ and _Fucus_ C:N spanned a much larger range, resulting in a significant difference between the C:N ratios of the two seaweeds in 2010 (p<0.001). Carbon content for both high and low _Fucus_ and _Chondrus crispus_ did not vary throughout the year in either 2010 or 2012 (p<0.001, Figure S4), indicating that differences in C:N ratio were driven by changes in tissue N.
**Figure 7:** Linear regressions illustrating relationships between ambient nutrients (µmol·L⁻¹) and *Fucus* tissue nutrients (%D.W.) for each sampling date in 2010 (black) and 2012 (grey).
A linear regression relating Fucus % tissue N and P for all sampling dates from 2010 and 2012, indicates a significant correlation between algal tissue levels of the two nutrients ($R^2=0.50$, $p<0.001$, Figure 8).

**Objective 2:**

*Enrichment Experiment:*

Results of 2-way ANOVA indicate that neither N ($p=0.199$) nor P ($p=0.133$) enrichment affected *Fucus* growth rates (Figure 9). Additionally there was no interactive impact of the two nutrients on algal growth ($p=0.353$). Seaweed grew an average of $0.007\pm0.001$ grams per day throughout the experiment.
Figure 9: Mean ± SE growth for each treatment measured weekly throughout the enrichment experiment.

Algal tissue nutrients increased in all enrichment and control treatments during the experiment. Tissue N levels increased 1.1 to 1.3 fold with N enrichment (Figure 10a). Tissue P levels increased by 1.4 fold of initial levels with P enrichment (Figure 10b).

Figure 10: Mean ± SE nutrient accumulation in *Fucus* tissues of N (a) and P (b) after 6 weeks of enrichment.
Accumulation of nutrient in *Fucus* tissues resulted in final tissue N levels were 30-48% higher in N enriched individuals compared to P enriched and control individuals (p<0.001, Figure 11a). Final tissue P levels were 38-58% higher in P enriched individuals compared to N enriched and control individuals (p<0.001, Figure 11b).

**Figure 11a:**

**Figure 11b:**

*Figure 11:* Mean ± SE initial and final *Fucus* tissue N (a) and P (b) for enrichment and control treatments.

**Uptake Experiment:**

Results of uptake incubations indicate that previous nutrient exposure impacted the ability of *Fucus* to absorb both nitrate and phosphate (Figure 12). Control seaweeds exhibited the highest nitrate uptake rates, with an average $V_{max}/K_s$ of 0.563 (Figure 13). P enriched seaweeds displayed the lowest nitrate uptake rates, with an average $V_{max}/K_s$ of 0.383. In phosphate uptake incubations, N enriched seaweeds exhibited the highest uptake rates, with an average $V_{max}/K_s$ of 0.834. Control seaweeds had the lowest phosphate uptake rates with an average $V_{max}/K_s$ of 0.430 (Figure 13). The generalized linear model revealed a significant nutrient x treatment interaction ($\chi^2 = 6.59, p = 0.037$), which highlights the fact that uptake in the treatments respond differently to the different nutrients. Subsequent comparisons of treatment means revealed that this pattern emerged because there were no significant differences between control and treatment means for
NO₃⁻ uptake (C vs. N: $\chi^2=0.31$, $p=0.578$; C vs. P: $\chi^2=2.82$, $p=0.093$), however PO₄³⁻ uptake was enhanced by N enrichment (C vs. N: $\chi^2=6.21$, $p=0.013$; C vs. P: $\chi^2=1.97$, $p=0.160$).

**Figure 12a:**
![Nitrate Uptake Graph](image)

**Figure 12b:**
![Phosphate Uptake Graph](image)

**Figure 12:** Nitrate and phosphate uptake rates for N enriched, P enriched and Control seaweeds. Points represent measured mean uptake rates from nitrate (a) and phosphate (b) uptake incubations. Lines represent projected uptake rates over the entire range of ambient concentrations tested, calculated via equations generated from measured uptake rates.
Figure 13: Mean nutrient uptake efficiency ($V_{\text{max}}/K_m$) of control versus enriched seaweeds at low nutrient concentrations generated via Michaelis-Menten models. Asterisk indicates significant difference in phosphorus uptake efficiency of N enriched seaweed as indicated by GLM results.

Objective 3:

**Feeding Assays:**

Results of feeding assays indicate that there was considerable spatial and temporal variation in seaweed-herbivore interactions in this system (Figure 14). For instance, in the spring of 2010, herbivores preferred to consume seaweed from the high zone, however in all subsequent assays there were no significant differences in consumption between high and low seaweeds. However in the summer of 2011, herbivores once again consumed more high than low seaweed, and this difference in consumption was marginally significant ($p=0.082$). In the spring of 2010, seaweeds exhibited positive growth in the presence of herbivore excretions, however in subsequent assays there was no significant positive growth via facilitation.
**Figure 14:** Feeding assay results in choice treatments

**Figure 14:** Results of feeding assays during spring and summer 2010, 2011 and 2012. Bars represent mean ± SE seaweed growth in grams per day for seaweed collected from the high (black) versus low (grey) intertidal, due to grazing, facilitation and the total impact of herbivores in choice treatments. Asterisks represent significant change in growth (one-sample t-test, p<0.05). P-values are results of paired t-tests between growth of high and low seaweed.
Figure 15a: Mean total consumption (g. day\(^{-1}\)) of *Fucus* by *L. obtusata* during spring versus summer feeding assays (a) and of *Fucus* collected in the high versus low zone (b) for 2010, 2011, and 2012 experiments.

Figure 16a: Mean total growth (g. day\(^{-1}\)) of *Fucus* via *L. obtusata* facilitation of *Fucus* collected in the high versus low zone (a) and during spring versus summer feeding assays (b) for 2010, 2011, and 2012 experiments.

Results of 3-way ANOVA indicate that consumption rates were not significantly different across tidal elevations, seasons or years (p>0.05). However there was a marginally significant
trend towards increased consumption in summer versus spring (p=0.12, Figure 15a). Similarly, there was a trend towards greater consumption of seaweed collected in the high versus low intertidal (Figure 15b). It is likely that the large amount of variation between assays, as evident in Figure 14, lead to the failure of ANOVA to detect significant differences in consumption, despite observed trends.

Seaweeds collected from the high zone grew 1.7x more due to herbivore excretion than seaweeds from the low zone (p=0.038, Figure 16b). This difference in growth between heights was dependent on season, and overall, seaweed grew 4x more in the spring than in the summer, due to herbivore excretions (Height x Season: p=0.015, Figure 16a). Additionally, growth of seaweed in facilitation treatments varied with year (p=0.051) and in 2010, seaweed in facilitation treatments grew 12-160x more than in 2011 and 2012. The total impact of herbivores on seaweed growth was not different across tidal elevations, seasons, or years (p>0.05). Overall, results in choice (Figure 14) treatments did not differ from results in no-choice (Figure S5) treatments.

*Herbivore Excretion and Abundance:*

Results of excretion experiments indicate that *Littorina obtusata* excreted over 5x more ammonium in spring than in summer (ANOVA: p<0.001), with an average excretion rate of 4.36 µmol NH₄⁺ g⁻¹ h⁻¹ in spring, and 0.72 µmol NH₄⁺ g⁻¹ h⁻¹ in summer (Figure 17).
Figure 17: Back-transformed mean ± 95% CIs μmol of ammonium excreted per gram of snail tissue mass per hour during spring and summer excretion experiments in 2012.

Herbivore abundance surveys revealed that *L. obtusata* were roughly 2x more abundant in the high compared to low intertidal, regardless of season (2-way ANOVA: height: p=0.0024, height*season: p>0.05, Figure 18). Similarly, within a single zone of the intertidal, herbivores were roughly 2x more abundant during the summer compared to the spring (2-way ANOVA: season: p=0.0035).

Figure 18: Mean ± SE herbivore density (no. m$^{-2}$) from surveys in the high and low intertidal during spring and summer 2012.

Results of field excretion calculations indicate that *L. obtusata* contributes an average of 4x
more ammonium to the intertidal during spring, compared to summer, regardless of tidal
elevation (2-way ANOVA: season: p<0.001, season*height: p>0.05). Regardless of season, these
herbivores contribute an average of 2x more ammonium in the high intertidal, compared to the
low (2-way ANOVA: height: p=0.006, Figure 19) based on their density measured in field
surveys.

**Figure 19:** Estimated average ± SE field excretion rates based on measured excretion rates during
different seasons, and measured herbivore density across tidal elevations and seasons.
DISCUSSION

Collectively, my measurements and experiments highlight the role of environmental fluctuations, across seasons, and years, in mediating biological processes on southern GOM rocky shores. Documentation of ambient nutrients indicate that coastal waters may be N-limited for much of the year and that limited N availability may be impacting seaweed’s ability to access ambient P via N-P co-limitation. However, the nutrient storage capacities of perennial, slow growing macroalgae may help them overcome this nutrient limitation, perhaps explaining why nutrient enrichment did not increase growth of these individuals. Instead, these large macrophytes may respond to ambient nutrient variation in more subtle ways, via the changes in tissue stoichiometry and nutrient uptake rates demonstrated here. The impacts of these environmental fluctuations span trophic levels, illustrated by changes in herbivory rates and herbivore-mediated nutrient recycling, across tidal elevations, seasons and years. Overall, the results presented here emphasize the importance of natural environmental fluctuations and seasonal variation in driving community-level processes in this system.

Ambient Nutrients:

Long-term sampling revealed pronounced seasonal variation in nutrient availability. For example, from winter to summer, ambient nutrient concentrations were reduced by nearly an order of magnitude during all three years. Nitrate availability exhibited a pattern that has been previously reported, offshore, for GOM waters (Fournier et al. 1977, Pastuszak et al. 1982, Petrie and Yeats 2000). Nitrate levels were relatively high in January and February 2010 likely due to wave action and lack of water-column stratification, which creates vertical movement of nutrients from the deep. In March, nitrate levels rose quickly and dramatically, due to seasonal
overturn, which creates an influx of deep, nitrate-rich water. These nutrients combined with increased temperature and light level of spring to fuel high levels of primary production of both seaweeds and phytoplankton. By June this increased primary production had depleted ambient nitrate at the surface, and water-column stratification trapped nitrate at depth for the remainder of the summer. As air temperatures cooled and fall storms began to once again create vertical mixing, sediment-derived nitrate was once again able to reach the surface and levels remain at elevated concentrations for the rest of the year.

In 2012, this pattern in nitrate availability roughly repeated itself, however the spring influx of nitrate due to seasonal overturn was not as pronounced and nitrate levels were sporadic throughout winter and spring. This may be due to higher winter temperatures in 2012. Average air temperatures from January to March were 1.8°C higher, and similarly average surface water temperatures were 1.7°C higher in 2012 than in 2010. Water temperatures may not have been cold enough to induce the sinking of cold, dense surface waters that leads to pronounced influx of nitrate due to seasonal overturn. While a distinct spike in nitrate availability did not occur in 2012, nitrate levels were elevated at times until April, when nitrate was depleted, likely due increased primary production associated with the spring bloom. For the remainder of 2012, nitrate levels followed a similar pattern as in 2010.

While ANOVA detected significant differences in phosphate levels throughout the year in 2010, phosphate availability did not adhere to a seasonal pattern. In fact, phosphate levels were relatively consistent throughout the year, with the exception of April and May, when phosphate levels dropped nearly an order of magnitude. This drop in phosphate levels may have been due to a depletion of phosphate from the water column via increased primary production associated with the spring phytoplankton bloom. This lack of a seasonal trend in phosphate availability has
been reported in previous studies (Petrie and Yeats 2000). These authors speculate that relatively constant phosphate levels throughout the year in the GOM could be due to the activity of phosphate recycling phytoplankton in surface waters (Harrison 1983).

In 2011 and 2012, phosphate levels were higher overall, and more variable throughout the year. An interesting trend of abnormally high (based on averages observed here and reported in the literature) phosphate levels in the latter half of the year repeated itself in both 2011 and 2012. Since weathering rocks supply the majority of available phosphate to coastal areas, I investigated the relationship between phosphate levels and average monthly rainfall (Figure S6) obtained from the Department of Conservation and Recreation’s rainfall-monitoring program. While there was no significant relationship between rainfall and phosphate levels in 2011 and 2012, rainfall averages were considerably higher in the latter half of 2011 and 2012, compared to 2010. This is likely due to hurricanes Irene in August 2011, and Sandy in October 2012, which brought large amounts of rain to the New England area during the time of elevated phosphate levels. A previous study of GOM nutrient levels found higher than average level of both phosphate and nitrate after storm events, due to terrestrial runoff (Love et al. 2005), emphasizing the important impact of all environmental fluctuations, whether terrestrial or marine in origin, on marine habitats.

To my knowledge, the present study is the first to measure near-shore ambient nutrient levels throughout the year in the GOM. While other studies have reported ambient nutrient concentrations in the GOM, most are oceanographic studies in which samples are collected offshore and at various depths, via research vessels (Durbin et al. 1995, Petrie and Yeats 2000, Townsend et al. 2001). Additionally, many of these studies collect samples at only one or few dates throughout the year. However, the few studies that have monitored surface nutrient levels
throughout the year have detected similar minimum (0-1 µmol N, 0.02-1 µmol P) and maximum (>10 µmol N, >2 µmol P) nutrient levels to this study. Additionally, as previously mentioned, studies have reported similar seasonal trends in nitrate availability and interestingly, a lack of seasonal trends in phosphate availability, as reported here (Petrie and Yeats 2000).

Throughout nearly the entire sampling period, seawater N:P ratios were considerably lower than the commonly accepted value of 16 (Redfield et al. 1963). Only in April 2010, when phosphate levels fell dramatically for several weeks, were seawater N:P ratios above 16. On most other sampling dates, seawater N:P rarely rose above 10. These levels are consistent with previous observations in New England (Lapointe et al. 1992) and suggest that coastal waters in this system are N-limited. The linear regression relating ambient nitrate further justifies the assumption that N is most limiting in this system. When nitrate is completely depleted from the water column, detectable levels of phosphate remain, indicated by the significant positive intercept of the relationship.

This study revealed strong seasonal and inter-annual variation in the impact of waves on nutrient availability. Wave height was positively correlated with ambient nitrate during winter and particularly spring of 2010, but this relationship disappeared in summer and fall and was not detected at all in 2012. Previous studies have established that waves have the potential to impact nutrient availability to near-shore communities. For example, Armstrong and LaFond (1966) illustrated that waves have the ability to vertically move the thermocline, which simultaneously moves nutrients, trapped in deep cold waters, to the surface. By moving the thermocline, and stirring up sediments, waves can make nutrients more available to primary producers at the surface. For example, via monthly sampling for one year, Martinez and Rico (2002) found a significant relationship between wave force and algal tissue nutrient content. In the present
study, the lack of a relationship between waves and nutrient availability during the warmer months of 2010 suggests that thermal stratification of the water column was strong enough to trap nutrients at depth, despite the fact that similar wave heights occurred throughout the year (compare colored points on Figure 4a). This suggests that in 2010, waves were the driver of nutrient availability during part of the year, but high temperatures producing a strong thermocline was a stronger force during others.

Storms can cause intense waves, and studies investigating the impacts of storms locally and worldwide, have found significant impacts on physical and biological processes (Trussell 1997), especially associated with increases in nutrient availability (Fanning et al. 1982, Gremare et al. 2003). The lack of a strong relationship between wave height and nutrient availability in 2012 may be due to the abnormally warm winter and the fact that there were half as many winter storms in 2012 (8) compared to 2010 (16), in New England, according to the NOAA Extreme Weather Database. Furthermore, because wave height data was obtained from a buoy located approximately 17 miles away from the water-sampling site, the ability to detect these relationships was less powerful than if wave height had been measured on site. The variability in the relationship between waves and nutrient availability in this system emphasizes the context-dependency of mechanisms driving natural processes, and highlights the need for long-term studies documenting this natural variation.

While much of the observed variation in nutrient availability was likely driven by natural, seasonal processes, anthropogenic impacts are also important to consider. All sampling was performed in a relatively impacted area; at a beach located only 9 miles from the dense urban center of Boston. Additionally, the GOM is home to many productive, hence recently overexploited, marine fisheries. Overfishing can alter ecosystems in many ways, in fact Pilskaln
et al. (1998) demonstrated that bottom trawling in the GOM may play an important role in resuspending sediment, changing nutrient availability in certain areas of the gulf, or at certain times of year. Interestingly, these authors report that January to April is the most active time for bottom trawling and that one of the most impacted areas is Wilkinson Basin, located in the southwestern GOM near Nahant. This indicates that, in addition to natural drivers like tidal-mixing, and storm events, anthropogenic factors are likely contributing to observed elevated nutrient levels during this time of year.

*Algal Tissue Nutrients:*

Throughout both years of sampling, algal tissue nitrogen content mirrored the seasonal pattern of nitrate availability, regardless of species or position on the shore, indicating that both species are able to absorb nitrate as it is available. Interestingly, throughout the year, there were few differences in % tissue N between *Fucus* individuals collected in the high versus low intertidal, despite the fact that *Fucus* in the low zone is submerged on average for over 30% more time than *Fucus* in the high zone, and therefore has more time access to ambient nutrients. The lack of difference in tissue content indicates that high *Fucus* individuals must have an adaptation to allow them to maintain tissue nutrient levels despite limited access to nutrients. Several studies have illustrated that high intertidal seaweeds may have greater nutrient uptake rates compared to low individuals (Thomas et al. 1987, Fischer et al. 2001, Phillips and Hurd 2003), including mechanisms such as desiccation enhanced uptake (Hurd and Dring 1991). Additionally, work by my lab group has demonstrated higher nitrate uptake rates in high compared to low intertidal *Fucus vesiculosus* individuals, as a potential adaptive mechanism for dealing with limited nutrient access (Benes and Bracken *in prep*).
*Fucus* tissue nitrogen levels fluctuated considerably throughout the year during both sampling years. For example, in the winter, % tissue N was 5x greater than summer minimum levels. Compared to other studies documenting seaweed tissue nutrient levels throughout the year, values observed in this study are similar, however slightly lower than most other studies. For instance, Pedersen and Borum (1996) reported a range of tissue N concentrations of 0.9-3.5% DW for *Fucus vesiculosus* in the Baltic Sea, and Asare and Harlin (1983) reported a similar range of 1-3.2% DW for *Fucus vesiculosus* in Rhode Island Sound. Reports of the seasonal tissue N ranges of other brown algae also match results in this study; Wheeler and Bjornsater (1992) reported a tissue N range of 0.86-2.2% DW for the intertidal fucoid alga *Pelvetiopsis limitata* in Oregon, and Delgado et al 1994 reported a range of 1.51-3.47% DW for the intertidal fucoid alga *Cystoseira mediterranea* in the Mediterranean Sea. Additionally, all of the aforementioned studies detected seasonal patterns in seaweed tissue nitrogen levels similar to the present study, with highest levels in winter and lowest levels in summer. The occurrence of these seasonal patterns in different species and diverse sampling locations around the world, including some locations with vastly different environmental conditions than the GOM (such as the Baltic Sea), indicates that this seasonal variation in tissue N levels may be a universal trait of fucoid algae in temperate coastal waters.

In both 2010 and 2012, algal tissue phosphorus varied according to a seasonal pattern that resembled that of ambient nitrate and algal tissue nitrogen. This trend emerged despite the lack of a seasonal trend in phosphate availability. These observations indicate that phosphate uptake and/or storage may be dependent on nitrate availability and/or seaweed tissue nitrogen levels. In his seasonal study of tissue nutrients for seven species of macroalgae, Komfeldt (1982) reported that tissue P levels did not track availability, indicating that P-uptake might be similarly
constrained in multiple algal species and locales.

In September 2010 there was a rapid rise in algal tissue P in which tissue P levels doubled from one week of sampling to the next, and stayed elevated for two weeks of sampling before returning to post-spike levels. Because of the abnormality of this observation, samples were processed 3x to ensure this observation was not due to an error in sample processing. However, since seaweeds across tidal elevations and taxa all exhibited similarly elevated P levels, this observation seems legitimate. During this time of elevated seaweed tissue P levels, ambient nitrate levels increased 5-fold from one sampling date to the next. This tight coupling of nitrate availability and algal P tissue levels further justifies the observation that N plays a role in P uptake and storage in this community. Similarly, Bjornsater and Wheeler (1990) demonstrated the impact of N-P co-limitation on tissue nutrients of green macrophytes in the *Ulva* genus. They found that tissue P declined in N-limited seaweed. However, there was no impact of P-limitation on seaweed tissue N. In the nitrate deplete waters of the North Atlantic this type of co-limitation can have severe consequences for primary producers, reducing their ability to access P, despite relatively high and constant availability of this essential nutrient.

Few studies of *Fucus vesiculosus* nutrient dynamics have been performed. Of the few studies, a large majority have been performed in the Baltic Sea (Pedersen and Borum 1996, Pedersen and Borum 1997, Nygård and Dring 2008, Pedersen et al. 2010). In the Baltic Sea, seaweeds are exposed to very different conditions than those in the present study, including estuarine salinity and constant submersion. Previous studies have shown that emersion stress (Hurd and Dring 1991) and changes in salinity (Munda and Kremer 1977) can impact algal nutrient dynamics, therefore these factors must be kept in mind when comparing *Fucus* in this study to *Fucus* in the Baltic Sea.
In his study of nutrient dynamics in the invasive green alga *Codium fragile* sps. *tomentosoides*, Hanisak (1979) defined ‘critical nutrient levels’ in seaweeds as the tissue nutrient content that just limits maximum growth, i.e. the threshold level required to sustain maximum growth. Since then numerous studies have measured critical N (N_c) levels (Fujita et al. 1989, Pedersen and Borum 1997), and to a lesser extent P levels (O'Brien 1987, Pedersen et al. 2010), for individual algal species.

Pedersen and Borum (1997) estimated N_c=1.71 % DW for *Fucus vesiculosus*. Fujita et al. (1989) estimated an N_c=1.5% DW for *Pelvetiopsis limitata*, an intertidal Fucoid alga found on temperate shores of the Pacific Northwest. Based on these values, in 2010, *Fucus* tissue N remained above the critical value throughout winter and into spring. Even after ambient nitrate levels fell in April, *Fucus* maintained high tissue N levels until the end of May. Similarly, in 2012, *Fucus* tissue levels were above the critical value from January to April and then again in November and December. While these high tissue nutrient levels indicate that *Fucus* could be growing at maximum rates for a large majority of the year, previous studies have shown that seaweed growth slows in the winter in temperate climates due to light limitation (Chapman and Lindley 1980). Therefore, during winter, N not used for growth accumulates in thallus storage pools to fuel growth during future times of low ambient nutrients. *Fucus* tissue N levels were below the critical value from May to November indicating that growth may have been N-limited during this time. However, throughout 2010 and 2012, *Fucus* tissue N levels fell below the subsistence level reported by Pedersen and Borum (1996) of 0.55% on just one sampling occasion during August 2012. This indicates that although growth may have been N-limited, tissue nutrient levels were never low enough that growth ceased entirely.

Pedersen and Borum (2010) estimated a critical P tissue level in *Fucus vesiculosus* of
PC = 0.12 %DW. During both years of sampling *Fucus* tissue P levels remained above this critical value from January to May and again from November to December. Tissue P levels fell below this critical value on many dates between June and October during both 2010 and 2012 indicating that *Fucus* growth may have been P limited during the summer and fall. Interestingly, tissue P levels fell below critical values during the same time of year as critical N values, further emphasizing the probable interaction between these two essential nutrients.

Throughout 2010, *Chondrus crispus* maintained tissue N and P levels that were on average 88% and 60% higher (respectively) than observed levels in low zone *Fucus*, with which *Chondrus crispus* co-occurs in the intertidal. Despite identical access to ambient nutrient levels, the red alga was able to absorb and store significantly more nutrients. Furthermore, *Chondrus crispus* N content tracked nitrate availability more closely than *Fucus*, as illustrated by spikes in *C. crispus* tissue N corresponding with spikes in ambient nitrate. Elevated nutrient content compared to *Fucus*, and rapid response to changes in nutrient availability is most likely due to the ability of red algae to store excess nutrients in specialized pigment molecules known as phycobiliproteins (Asare and Harlin 1983). *Chondrus crispus* tissues contain several different types of phycobiliproteins, however phycoerithrin in particular has been shown to be important in N storage (Lobban and Harrison 1994). Furthermore, Rhee and Briggs (1977) demonstrated that phycoerithrin content is reduced in *Chondrus crispus* due to high light intensity, experienced by non-shaded intertidal individuals in the summer. These authors explain that this pigment gives *Chondrus crispus* its red color, so phycoerithrin depleted individuals exhibit green thalli. In the present study, specimens collected during the summer of 2010, when tissue nutrients levels were at their lowest, appeared green, indicating low phycoerithrin levels. The ability of this algal species to bind considerably more nutrients in its tissue compared to other species may
contribute to its role in nutrient cycling in this community.

Due to essential roles of nitrogen and phosphorus in the growth and functioning of all biological organisms, N:P ratios are often used to assess nutrient limitation, especially in primary producers. In extensive surveys of marine primary producers, Atkinson and Smith (1983, 1992) estimated the C:N:P ratios of marine macrophytes as 55:30:1 and 800:49:1, respectively. Based on these ratios both seaweeds in the present study would be considered N-limited throughout the entire study, with N:P ratios that remained below 20, with the exception of several isolated dates, during both years of sampling. However, these large surveys consider many primary producers with distinct lineages and morphologies and from environments around the world, and therefore average values may be very different than values of individual species in specific locations. In fact, other studies that have assessed N:P ratios in one or several algal species have demonstrated that thick, branching, slow-growing macroalgae have considerably reduced N:P ratios compared to those of thin, fast-growing species with simple morphologies (Wheeler and Bjornsater 1992). Notably, throughout both years of sampling, seaweeds maintained N:P ratios well above ambient levels, illustrating the essential ability of these macrophytes to bind nutrients in their tissues in order to survive in the relatively nutrient deplete waters of the GOM.

*Enrichment Experiment:*

Experiments testing the relative importance of N and P in terrestrial (Vitousek et al. 2010), freshwater (Elser et al. 1990) and marine phytoplankton communities (Downing et al. 1999) are numerous. Conversely, studies evaluating co-limitation in marine macrophytes are less common, and most have been performed in tropical rather than temperate waters (Lapointe 1987, Fong et al. 1993). The attempt to experimentally validate field observations of N-limitation of P uptake
and assimilation by seaweed yielded mixed results. No difference in seaweed growth between enrichment treatments and controls was observed throughout the experiment. Nutrients accumulated in *Fucus* tissues, but these nutrients were not used for growth. Initial algal tissue N levels were 0.77% DW, less than half the N<sub>c</sub> value of 1.71% reported for *Fucus* by Pedersen and Borum (1996), while initial P levels of 0.12% DW, matched the P<sub>c</sub> value reported for this alga by the same authors in a later study (Pedersen and Borum 2010). This suggests that seaweed were not P limited prior to the experiment, and so the lack of a growth response to P enrichment is not surprising.

Several possible explanations may explain the lack of a growth response in the N and NP enrichment treatments. The first is simply that large, slow growing macrophytes such as *Fucus* are less sensitive to changes in nutrient availability, due to their slow growth rates and tissue storage capacity (Fujita et al. 1989, Wheeler and Bjornsater 1992). Several studies have illustrated that slow-growing species often show little to no growth response to nutrient enrichment, compared to that of faster-growing ephemeral species (Pedersen and Borum 1997, Pedersen et al. 2010). Another explanation for the lack of growth response is that seaweeds were allocating added nutrients to functions other than growth, such as reproduction or storage. Lehvo et al. (2001) observed a reduction in growth associated with the production of reproductive receptacles in the fall in *F. vesiculosus* in the Northern Baltic. The present experiment was performed in late summer, so seaweed may have been allocating nutrients towards reproduction rather than growth. Finally, seaweed might not have responded to enrichment because some other resource was limiting their growth. Seaweeds were living in an artificial environment. While experimental conditions were designed to imitate the natural habitat, a number of factors, such as light levels or water circulation/flow intensity could have deviated enough from
preferred conditions to limit growth. The lack of a growth response was likely due to a combination of these aforementioned factors.

The slightly reduced growth of *Fucus* in the N enrichment treatment was initially surprising. However, Harpole et al. (2011) noted that of the 641 experiments complied in their meta-analysis, 15% reported some negative response to enrichment, including reduced growth in enriched treatments compared to controls. The authors point out that negative impacts could be due to over-accumulation of the nutrient resulting in toxicity, or simply, that adding a single nutrient causes the other, non-added nutrient, to limit growth.

While no growth response was observed, enriched seaweeds took advantage of increased nutrient levels by absorbing and storing a significant amount of excess nutrients compared to controls. In fact, all enriched seaweeds were able to absorb added nutrients, regardless of the presence of other nutrients. In other words, the uptake and assimilation of N or P did not seem to depend on the other nutrient. This is likely because nutrients were added in such high concentrations that seaweed were able to overcome co-limiting interactions between N and P. In the oligotrophic waters of the GOM, seaweeds likely obtain all nutrients via active transport, in which they expend energy to move nutrients against the concentration gradient from the water column into their tissues (Lobban and Harrison 1994). This involves transport proteins, which is likely why N availability is linked to nutrient uptake. However, the high nutrient levels in the experimental mesocosms created an environment in which seaweeds did not need to perform active transport, and they could simply absorb nutrients via passive diffusion, which likely eliminated the effects of co-limitation.

While no limitation of phosphate accumulation by nitrate was detected in final seaweed tissue analyses, results of the uptake experiment indicate that N and P were indeed interacting to
influence overall seaweed nutrient dynamics. In nitrate uptake incubations, control *Fucus* exhibited the highest uptake efficiency \( \frac{V_{\text{max}}}{K_s} \), although these differences were not significant, likely due to low sample size. These results were expected, since these seaweeds had been N-starved, compared to enriched seaweeds, prior to uptake incubations. Several studies have shown that nutrient starved seaweed exhibit higher uptake rates, in an attempt to fill depleted nutrient stores (Fujita 1985, Thomas and Harrison 1985, Runcie et al. 2004). The lowest nitrate uptake rates were measured in P enriched seaweeds. It is possible that due to the accumulation of tissue phosphorus, these seaweeds had an ideal N:P ratio, and therefore did not need to take up additional nitrate (Figure S7).

Results of the generalized linear model indicate that N enriched seaweeds displayed the highest phosphate uptake efficiency. These results confirm the hypothesis based on field observations that nitrogen may be necessary for the uptake of phosphate. Unlike enhanced nitrate uptake in N-starved seaweed, phosphate uptake efficiency was not enhanced in P-starved individuals. It is probable that although these control seaweed had depleted P stores, they did not have sufficient N to facilitate P uptake.

The strong relationship between nitrogen availability and seaweed tissue phosphorus, throughout two years of sampling, in combination with experimental validation of N limitation of P uptake make a compelling case for N-P co-limitation in this system. Rhee (1974) detected a similar pattern of N limitation of P uptake in the freshwater phytoplankton *Scenedesmus* spp., however to my knowledge this pattern has not been previously demonstrated in marine macrophytes.

Saito et al. (2008) have asserted that N-P limitation usually falls under their Type I: Independent Nutrient Co-limitation definition, which occurs when both nutrients are in such
short supply that they are both limiting. However, throughout the present study P availability was relatively consistent, and ambient concentrations and N:P ratios were not indicative of P limitation (Downing 1997). Therefore the pattern observed is more consistent with what Saito and colleagues refer to as Type III: Biochemically Dependent Limitation, in which reduced availability of one nutrient limits autotroph ability to take up another, non-limiting nutrient. While the authors of this study discuss this type of limitation only regarding interactions with trace metals and other micronutrients, there seems to be no reason why this type of interaction might not occur between macronutrients such as N and P as well. Indeed, several authors have made a case for the intrinsic linkage of N and P in the cellular machinery of all biological organisms (Sterner and Elser 2002, Loladze and Elser 2011), leading to phenomena such as the highly conserved Redfield Ratio, and interactions between these essential nutrients such as those observed here. The cellular mechanisms behind this type of co-limitation have received little attention. However, in their 2002 review of plant responses to P-limitation, Rausch and Bucher report that P-starved autotrophs increase production of transport proteins (an N-dependent process) to increase access to P. Further, one of these transport proteins has been identified in the unicellular green alga Chlamydomonas (Wykoff et al. 1999). Additionally, Bari et al. (2006) show that the signaling pathway associated with plant responses to P-deficiency is rendered non-functional when N is limiting. These previous works clearly indicate that N plays a role the ability of primary producers to access P, and provides a mechanism for the biochemically-dependent co-limitation observed in the present study.

These results indicate that nutrient limitation of primary production is often more complex than can be described by a single rule such as Liebig’s Law of the Minimum, because interactions between nutrients may cause limitation by a nutrient despite its ample availability.
As anthropogenic activities continue to alter global biogeochemistry, understanding the mechanisms underlying interactions between limiting nutrients will be essential in order to determine impact of limitation on community and ecosystem-level nutrient cycling.

*Feeding experiments:*

Herbivores impact primary producer communities in a variety of ways. For instance, on rocky intertidal shores, herbivorous snails play an important role in controlling primary producer biomass (Lubchenco 1978), while also providing essential nutrients to these primary producers (Bracken 2004). Results of feeding experiments illustrate that due to a combination of consumption and facilitation, the overall impact of herbivores on *Fucus vesiculosus* in the Southern GOM varies across tidal elevations and seasons.

Combined results of feeding assays demonstrate that herbivores tended to prefer to consume high zone *Fucus*, compare to individuals collected in the low zone indicating differences in palatability between high and low algal individuals. While herbivore preference may change based on nutritional quality of the food source (Mattson 1980b, Boyer et al. 2004), few differences in *Fucus* tissue nutrients were detected between tidal elevations throughout two years of sampling, therefore this not a probable explanation of observed herbivore preference.

Many studies have illustrated changes in plant palatability across environmental gradients (Bolser and Hay 1996, Pennings et al. 2001, Scheidel et al. 2003), and several authors have suggested that these differences may arise due to trade-offs between defense and stress tolerance (Thornber et al. 2008). For instance high intertidal seaweeds experience considerably more desiccation stress due to long emersion times, than lower intertidal individuals; thus they may allocate more resources to dealing with this stress, than to avoiding herbivory (Renaud et al. 1990). Deal (1997) illustrated that palatability of *Fucus vesiculosus* increased with increasing
tidal elevation, due to increased environmental stress at higher tidal elevations. Furthermore, tissue toughness has been shown to play a role in seaweed palatability (Pennings and Paul 1992), and high-zone *F. vesiculosus* individuals have considerably thinner and less robust thalli than lower-zone intertidal counterparts, even when fully hydrated (V. Perini, personal observation), potentially making them more palatable to herbivores.

While much work has been done on seaweed palatability, only one other study has examined herbivore preference for macroalgae along an intertidal gradient and no general trends of changes in palatability across tidal elevations were detected (Thornber et al. 2008). However, herbivores preferred to eat food sources with which they did not co-occur on the shore. These authors suggest that while this result may be due to evolutionary arms race between plant-defense and herbivory, it may also have been an artifact of the chosen herbivores and their respective method of eating. This illustrates that environmental gradients might not always relate to changes in palatability and highlights the fact that a suite of other processes change across environmental gradients and might be influencing herbivore preference.

Interestingly, herbivore abundance surveys revealed that *L. obtusata* was more abundant at higher tidal elevations, regardless of season. This may imply that *L. obtusata* is more desiccation resistant than other intertidal species, and prefers the high intertidal due to the occurrence of fewer predators and competitors. Additionally, considering this herbivore’s preference for high-zone *Fucus*, it may prefer to dwell in the high intertidal due to the increased palatability of its preferred food source.

While consumption rates did not vary significantly with season, there was a trend of higher consumption rates in summer. Several studies have indicated that herbivory rates might be influenced by temperature and/or season (Hillebrand et al. 2009, Poore et al. 2012). For instance,
Barker and Chapman (1990) measured consumption rates of *L. obtusata* on *F. vesiculosus*, detecting higher rates at higher temperatures, similar to results of the present study. This phenomenon is supported by the ideas of metabolic theory, which imply that increasing temperatures would increase organismal metabolism, increasing energy requirements leading to increased consumption rates (Allen et al. 2005).

Seasonal variation in seaweed tissue nutrients might also be an explanation for differences in consumption rates across seasons. Experiments examining herbivore preference based on nutrient content of food source are numerous and many have detected herbivore preference for food sources with elevated nutrient content (Hauxwell et al. 1998, Boyer et al. 2004). These authors have argued that herbivores choose more nutritious food sources, because they gain more nutrients per unit of food eaten, conserving energy associated with foraging. Furthermore, it may also be argued that if a food source were less nutritious, herbivores would have to eat more of it to obtain the same amount of nutrients as eating less of a more nutrient rich food source. In the present study, seaweed tissue nutrients were nearly 2x higher in the spring than in the summer, therefore increased consumption rates in the summer may be necessary for herbivores to obtain sufficient levels of essential nutrients.

Several authors have detected that changes in tissue nutrient levels can influence secondary metabolites (Yates and Peckol 1993, Steinberg 1995). This suggests that perhaps it is not the altered nutrition of food sources that is influencing herbivore preference, but the induction or depression of defense compounds at different tissue nutrient levels. For instance, Bryant et al. (1983) explain that as nutrient content decreases in autotroph tissue, growth becomes limited, so carbon that cannot be used for growth is allocated to secondary metabolites. This implies that herbivores should be able to consume more autotroph tissue as tissue nutrient levels increase,
due to less secondary metabolites. However, pooled results of feeding assays indicate that the opposite was true for this study. Herbivores ate more in the summer when ambient and algal tissue nutrients were at their lowest levels. Therefore, increased metabolic rates of herbivores at higher temperatures and decreased tissue nutrient content of food source, both of which necessitate increased consumption, are likely explanations for the results observed in this study.

Seaweeds collected from the high intertidal displayed higher growth in the presence of herbivore excretions compared to their low intertidal counterparts, indicating they were better able to take advantage of this added resource. Considering that *L. obtusata* is more abundant in the high intertidal, seaweeds living at this tidal elevation may be more accustomed to exploiting this resource. High-zone *Fucus* has been shown to have higher nitrate uptake rates to compensate for the nutrient limitation associated with long emersion times (Benes and Bracken, *In prep*). If these individuals have similarly enhanced ammonium uptake rates, this may provide a mechanism by which seaweeds in the high-zone are better able to benefit from herbivore excretions. The observed changes in seaweed growth associated with herbivore excretions emphasize the importance of this autochthonous resource and its dependence on environmental factors.

Herbivore facilitation of seaweed growth was greater in the spring than the summer, corresponding with the higher excretion rates of *L. obtusata* measured during this season. Navarro and Torrijos (1994) detected seasonal variation in the excretion rate of a predatory marine gastropod and measured the highest ammonium excretion rates in the spring. These differences could be driven by lower nutrient availability in the summer. Given the reduced nutrient availability there is less to consume and less to recycle via excretions. Shifts in nutrient recycling rates impact growth of macroalgae and change nutrient transfer rates in the community,
potentially impacting organisms at higher trophic levels and the overall functioning of the ecosystem.

Field surveys of herbivore abundance help to put results of laboratory feeding and excretion experiments into context, to estimate the ammonium contribution of herbivores to nutrient supply for *Fucus* in the field. Due to the higher densities of herbivores in the high intertidal zone, seaweeds are supplied with nearly 2x as much ammonium per square meter than in the low intertidal, regardless of season. Since high seaweeds have less access to nutrients due to less emersion time, higher levels of ammonium excretions, due to a higher density of herbivores in this zone may help to prevent nutrient limitation in these seaweeds. This additional source of nutrients may contribute to the lack of difference in seaweed tissue N levels between high and low *Fucus* individuals observed in this study.

Due to higher excretion rates in the spring, seaweeds are supplied with roughly 2x as much ammonium per square meter in the spring, compared to summer, at both high and low tidal elevations. In the nutrient poor waters of the Gulf of Maine, autochthonous nutrient sources such as herbivore excretion may help supplement allochthonous nutrient supply to prevent nutrient limitation of primary productivity. Furthermore, ammonium is a more efficient source of N for seaweeds compared with nitrate. For instance, Wallentinus (1984) measured ammonium uptake rates in *F. vesiculosus* that were 3x higher than the nitrate uptake rates measured in this study. Additionally, Taylor et al. (1998) demonstrated that some seaweeds can absorb ammonium via passive diffusion, in contrast to energy intensive active transport which is the primary uptake mechanism by seaweeds for most inorganic nutrients (Lobban and Harrison 1994).

While the study site is a moderately wave-protected shore, hydrodynamic forces such as wave action may impact the ability of primary producers to access autochthonous nutrients.
excreted by herbivores. For instance, Probyn and Chapman (1983) suggest that waves may reduce the residence time of nutrients, carrying them away from the shore before seaweeds can absorb them. However, Taylor and Rees (1998) assert that since excreting invertebrates live in such close proximity to seaweeds (often right on the thallus), and because dense beds of seaweeds reduce water movement within the bed, these ammonium excretions are accessible and likely an important source of N for these primary producers. Indeed, Aquilino et al. (2009) demonstrated higher ammonium concentrations and abundance of seaweed above a mussel bed on a wave-swept shore, confirming that invertebrate excretions are important even when local hydrodynamic forces may suggest otherwise. In the nutrient-depleted waters of the GOM, herbivore excretions may be an important resource, providing primary producers with additional nutrients when ambient nutrients may be limiting. Thus seasonal variation in this resource could be an important driver of seasonal changes in primary production and nutrient cycling throughout this community.

Conclusion:

The research presented here further develops our understanding of the drivers of nutrient cycling in coastal communities, while also highlighting the context-dependency of natural processes. The large temporal scale of this study highlights that while seasonal processes in this system are relatively consistent from year to year, environmental fluctuations can lead to inter-annual variation and potential impacts on primary production and nutrient cycling. Seasonal trends in Fucus tissue quality were consistent across tidal elevations, indicating that this species has acclimated to the stress and nutrient limitation associated with high tidal elevation. Seasonal trends in algal tissue quality were also consistent across taxa, however the ability of Chondrus
*crispus* to bind considerably more nutrients in its tissues than *Fucus*, may indicate different roles of the two species in nutrient cycling. The combined observational and experimental approach of this study illustrates that while GOM waters are likely nutrient-limited, local species may be adapted to exploit high nutrient concentrations when they occur, to avoid nutrient limited growth during times of low ambient nutrients. Furthermore, observational data indicate and experimental data confirm that N availability is limiting seaweed’s ability to access ambient P in this system, likely due to biochemically-dependent co-limitation. The impacts of these seasonal fluctuations span trophic levels, leading to changes in the negative and positive impact of herbivores on seaweeds across tidal elevations and seasons. These changes are likely due to mechanisms such as increased seaweed palatability due to reduced defense compound production under stressful conditions in the high intertidal, and elevated ambient and seaweed tissue nutrient levels which lead to increased rates of nutrient recycling by herbivores in the spring. Response of multiple trophic levels to seasonal fluctuations emphasizes how these processes may impact community-wide nutrient cycling. My research has demonstrated the many natural and potentially anthropogenic impacts contributing to nutrient availability and cycling in this system. Results emphasize the necessity of considering the interactive effects of multiple processes, across long temporal scales in order to better understand, protect and conserve threatened natural systems.
APPENDIX A: Supplementary Figures

Figure S1a:

Figure S1b:

Figure S1: Seaweed tissue N:P ratios for 2010 (a) and 2012 (b)
Figure S2a:

![Graph showing relationship between ambient phosphate concentration and wave height for 2010.](image)

Figure S2b:

![Graph showing relationship between ambient phosphate concentration and wave height for 2012.](image)

Figure S2: Ambient phosphate (μmol L⁻¹) plotted versus mean wave height (m) on the day before water samples were collected in 2010 (a) and 2012 (b)
Figure S3a:

![Graph showing Seaweed Tissue C:N ratio for 2010](image)

Figure S3b:

![Graph showing Seaweed Tissue C:N ratio for 2012](image)

Figure S3: Seaweed tissue C:N ratio throughout 2010 (a) and 2012 (b).
Figure S4a: Seaweed tissue carbon (%DW) in 2010.

Figure S4b: Seaweed tissue carbon (%DW) in 2012.

Figure S4: Seaweed tissue carbon (%DW) in 2010 (a) and 2012 (b).
Figure S5: Results of feeding assays during spring and summer 2010, 2011 and 2012. Bars represent mean ± SE seaweed growth in grams per day for seaweed collected from the high (black) versus low (grey) intertidal, due to grazing, facilitation and the total impact of herbivores in no-choice treatments. Asterisks represent significant change in growth (one-sample t-test, p<0.05). P-values are results of paired t-tests between growth of high and low seaweed.
Figure S6: Linear regressions between monthly mean rainfall (inches) and ambient nitrate and phosphate ($\mu$mol L$^{-1}$) for 2010, 2011 and 2012.

Figure S7: Final Fucus tissue N:P ratios after 6 weeks of enrichment.
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