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## The nonconsumptive and consumptive effects of the invasive green crab (*Carcinus maenas*) on macroinfaunal diversity, abundance and ecosystem functioning in a New England salt marsh

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The nonconsumptive and consumptive effects of the invasive green crab (*Carcinus maenas*) on macroinfaunal diversity, abundance and ecosystem functioning in a New England salt marsh

A thesis presented

by

Genevieve Bernatchez

To

The Department of Biology

In partial fulfillment of the requirements for the degree of  
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In the field of

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ABSTRACT OF THESIS

Submitted in partial fulfillment of the requirements  
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## ABSTRACT

Traditional studies of trophic cascades have revealed how predators by limiting abundance of their prey, have positive indirect effects on basal resources. However, recent studies have shown that predators can also indirectly affect such resources without consuming their prey by way of anti-predator foraging adaptive behavior (nonconsumptive effects). For example, on rocky shores, waterborne risk cues from the predatory green crab (*Carcinus maenas*) cause strong trophic cascades by suppressing the grazing rate of the herbivorous snail, *Littorina littorea*, on algal resources. *L. littorea* is also an abundant herbivore in northern New England salt marshes but it is unknown whether *C. maenas* exerts a similar indirect effect in this system.

Through field experiments conducted in a salt marsh located at the Wells National Estuarine Research Reserve and Scarborough in southern Maine, I examined how nonconsumptive and consumptive effects of *C. maenas* on *L. littorea* indirectly influenced benthic macroinfaunal density and diversity, and how these interactions affected marsh primary production and nutrient availability. I hypothesize that predator-induced changes in *L. littorea* foraging behavior reduced the intensity of foraging competition among macroinfauna for a shared food source (epibenthic diatoms and algae), resulting in an increase in density of macroinfaunal organisms. *C. maenas* has become increasingly abundant in salt marshes, and it is critical that we examine how nonconsumptive and consumptive effects of this predator on prey populations influence salt marsh community dynamics. Our results suggest that both consumptive and nonconsumptive predator effects influenced macroinfaunal densities and composition. However, the top-down interactions had no influence on primary production nor on

nutrient availability. Furthermore, we need a greater understanding of how nonconsumptive predator effects may influence ecosystem level properties such as biodiversity, productivity, stability and energy flow.

**DEDICATION**

To my parents, Jean and Ginette Bernatchez,  
who always supported and believed in me

AND

To my husband, Roger Coleman,  
for your unconditional love

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## General Introduction

Salt marshes are intertidal communities dominated by non-woody, salt tolerant plants. They are characterized by striking zonations of highly productive, halophytic plants capable of dealing with harsh physical conditions (Bertness 1999). Salt marshes are biogenic communities, with *Spartina alterniflora* being the foundation species (Pennings & Bertness 2001). Their distribution is worldwide but here in the United States they are primarily found along the east coast and gulf coast but are also present on the west coast in lesser density (Bertness 1999).

On the north Atlantic coast of the United States, the establishment of a salt marsh generally beginning with the colonization of *S. alterniflora*. These plants can tolerate flooded soil, high salinity conditions, can easily spread vegetatively by rhizomes and rapidly colonize a new habitat. Once the plants start to establish themselves they decrease wave action and water flow, accelerating sedimentation, limiting erosion and allowing for other species to establish and thrive (Frey & Basan 1985). Overtime, sediment accumulation and binding lead to vertical growth of marshes, and to the development of high marsh habitats that are not flooded daily by tides. Salt marshes develop preferably in areas where there is slow moving water, where the sediment can accumulate and where marsh plants can colonize. This is one of the major reasons why we find fewer salt marshes on the west coast, where wave action is too strong and there is little accumulation of sediment. In general, the mouth of estuaries and protected shores are ideal location for marshes to grow.

The typical north Atlantic east coast low marsh zone is characterized by well drained soils, dominated by *S. alterniflora*'s tall form, which can reach 1-2 meters tall (Bertness 1999). Just behind the low marsh zone, landward, where the drainage is reduced and soils become anoxic, *S. alterniflora* is often less than 25cm tall and is referred to as short-form (Bertness 1999). The high

marshes which are not flooded daily are generally dominated by marsh hay *Spartina patens*, followed by *Juncus gerardii* (black needle rush). At the terrestrial border *Phragmites australis* and other varieties of terrestrial plants can also be found.

Salt marshes are critical habitats providing a variety of ecosystem services; including sheltering coastline from storms, preventing erosion, and acting as a buffer zone between land and ocean by filtering runoff and removing nutrients (Turner 1976; Bertness 1999; Pennings & Bertness 2001). Many commercially important species use marshes as nurseries (Bertness 1999), and many bird species use marshes for reproduction and feeding station during migration. They are important ecosystems, which are disappearing at an alarming rate (Dreyer & Niering 1995). In the New England area alone about 80% of the marshes have disappeared due to human activity (Bertness et al. 2002). Human manipulation has greatly affected salt marshes over time. The creations of dikes, dams, land development, introduction of new species, harvesting, nutrient pollution are among the impacts caused by human activities affecting the salt marshes (Portnoy & Giblin 1997; Mitsch & gosselink 2000; Bromberg Gedan et al 2009). Consequently, we are slowly losing one of the most productive and valuable ecosystems (Costanza et al 1997; Bertness 1999; Bromberg Gedan et al 2009). It is imperative that we protect and restore those habitats. However, in order to protect and manage salt marshes, we need to better understand their ecosystem functioning, ecology and species interactions.

Salt marshes have been studied for decades and resource availability (bottom-up forces) has been traditionally perceived as the principal factor controlling salt marsh primary productivity (Valiela & teal 1974; Mendelssohn 1979). Early studies showed that plant assemblages and growth were highly influenced by nutrients availability. Numerous fertilizer experiments have demonstrated a positive correlation between added nutrients and increased in

primary production, often leading to conclusions of nitrogen limitation (Valiela & Teal 1974; Gallagher 1975; Osgood & Zieman 1993). Salt marsh florae receiving increased amounts of nitrogen show increased rates of primary production, with a change in the abundance of certain species and an increase in the nitrogen content of the plants (Osgood & Zieman 1993; Siliman & Zieman 2001). In light of the fertilizer experiments other scientists have suggested that nitrogen limitation in the inner marsh is not a function of nitrogen availability per se, but a result of the inhibition of uptake kinetics, induced by environmental factors such as hypoxia, salinity and sulfide concentration (Bradley and Morris, 1990).

It is clear that bottom-up forces play an important role in shaping the plant community in salt marshes. However, in recent years, a growing number of studies have shown that nitrogen may not be the only limiting factor controlling salt marsh plant growth but the interactions among critical nutrients such as carbon, nitrogen and phosphorus coupled to other physical factors might play an equally important role in regulating salt marsh primary productivity (Bedford et al. 1999; Güsewell et al. 2003; Tessier & Raynal 2003; Crain 2007)

Other studies have also observed that where the supply of nitrogen is increased the number of herbivore grazers increases as well (Sala et al. 2008). Historically, consumers (Top-down forces) were thought to play a minimal role in shaping the salt marsh community dynamics. Nevertheless, recent studies have documented how top-down forces may play a significant role in salt marsh productivity (Silliman & Zieman 2001; Sala et al. 2008). For example, grazing consumers such as snow geese, snails and sesarmid crabs have been shown to decrease aboveground plant biomass and play a major role in regulating plant growth (Smith and Odum 1983; Silliman & Zieman 2001; Alteri et al. 2012).

The concept of trophic cascades has greatly improved our understanding of the implications of food web interactions to community structure in a variety of systems. Trophic cascades demonstrate how predators can affect their prey in two ways: by directly reducing their prey's density through consumptions or by altering their behavior, morphology, and physiology (nonconsumptive) (Hairston et al. 1960; Sih et al. 1985; Carpenter & Kitchell 1993; Menge et al. 1994; Boertje et al. 1996; Estes et al. 1998; Werner & Peacor 2003). Early studies have focused on how predators regulate prey densities and the cascading effects predators have on the abundance of the prey's resources. However predator not only affect prey by eating them, but can alter their behavior such as foraging effort, morphology to reduce their risk of mortality. Nonconsumptive effects can have important implications in community dynamics. A growing body of work suggests that nonconsumptive effects of predators on prey population can also drive strong trophic cascades. We now know that nonconsumptive effects play an important in shaping community structures in rocky shore habitats. Experiments conducted by Trussell (2002, 2004) showed that when green crab predatory cues are present herbivorous snails react to predatory cues by reducing their feeding, seeking refuge, and thus reducing their grazing pressure on basal resources such as fucoids algae, which therefore can thrive. Predatory effects are important top-down mechanisms driving trophic cascades. Other examples have also been found in both terrestrial and freshwater habitats but it is not known if these interactions observed in marine habitats will also be observed in salt marsh located on the northern coast of New England where the predatory green crab and the common periwinkle are also present.

The majority of the recent studies focusing on top-down forces in salt marsh community have been conducted in southern salt marshes on the Atlantic coast but these effects could differ in northern marsh communities due to differences in species composition (Bertness 1999). An

important grazer found to exert strong top-down forces in southern marshes, *Littoraria irrorata*, is notably absent in northern marshes. This grazer can ingest live tissue of *S. alterniflora*, creating scars, rendering the plant susceptible to fungal infections (Silliman & Newell 2003). *L. irrorata* are preyed upon by a variety of predators including blue crabs, which are also absent from northern marshes (Silliman & Bertness 2002). In contrast, one of the major herbivores found in northern salt marshes is the common periwinkle *Littorina littorea*. This herbivore does not appear to apply the same grazing pressure as *L. irrorata* on *S. alterniflora* blades but could potentially have the ability to eat the rhizomes (Bertness 1984). In northern marshes, *L. littorea* is exposed to predation pressure from *C. maenas*, an important predatory crab that feeds on a variety of prey including bivalves, gastropods and juvenile fish (Elner 1981). We know very little about the potential of top-down effects exerted by *C. maenas* and *L. littorea* in northern marshes in comparison to their counterparts in southern marshes.

A better understanding of how both top-down (predator effects) and bottom-up forces interact to influence ecological dynamics and growth of northern salt marshes is now needed. One of the major epibenthic predators in northern salt marshes is *C. maenas*, which can cause strong trophic cascades by suppressing the grazing rate of *L. littorea* on algal resources in rocky shores habitats. *L. littorea* is an important herbivore in northern New England salt marshes, where it grazes on epibenthic algae such as diatoms. Given that *C. maenas* has become increasingly common in marsh systems, it is critical that we examine how the consumptive and nonconsumptive effects of this predator on prey populations influence salt marsh community dynamics and ecosystem function.

The major objective of my research is to examine the nonconsumptive and consumptive interactions between *C. maenas* and *L. littorea* and their effects on the salt marsh community.

Recently, there has been a strong emphasis in ecology on the relationship between top-down, bottom-up effects and ecosystem functioning. The potential for predator effects may significantly change our view of how predators influence important ecosystem processes such as productivity, stability and energy flow.

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## **Chapter 1: How nonconsumptive and consumptive interactions between *Carcinus maenas* and *Littorina littorea* influenced both density and diversity of macroinfaunal species**

### **Introduction**

It has long been established that predators, by consuming prey, can have indirect effects on their prey's resources (Hairston et al. 1960, Carpenter et al. 1987; Boertje et al. 1996; Beckerman et al. 1997; Estes, et al. 1998; Rudgers et al. 2003). A predator's consumptive effects play an important role in shaping community structure and species interactions in many natural systems (Carpenter et al. 1987; Boertje et al. 1996; Beckerman et al. 1997; Rudgers et al. 2003), but similar effects may also emerge without predators consuming prey (Lima 1998; Peacor & Werner 2001; Peckarsky et al. 2008). In response to predation risk, prey can change their behavior, morphology, and life history in order to avoid predation. Trophic cascades can occur when nonconsumptive predator effects cause prey to reduce foraging activity or shift to alternative, safer habitats or resources. Nonconsumptive predator effects on community structure and species interactions can emerge more quickly than consumptive effects because they affect more prey simultaneously in comparison to consumptive effects where predators only impact one prey at a time (Werner 1991; Schmitz et al. 1997; Trussell et al. 2002; Trussell et al. 2004). Nonconsumptive predator effects can also indirectly affect competition among prey species and among prey resources (Schmitz 1998; Peacor & Werner 2001). For example, predator-induced reductions in prey foraging can reduce the intensity of intra- and inter-specific competition for a shared food source (Peacor & Werner 2001)

The majority of nonconsumptive and consumptive studies have been conducted in terrestrial, freshwater and rocky shore habitats. The relative importance of these effects,

however, is not well understood in other systems. We have a very poor understanding of their influence in other habitats such as salt marshes.

Classic research in salt marshes focused on the effects of nutrient availability (bottom-up effects) on community structure and dynamics (Valiela & Teal 1974; Gallagher 1975; Mendelssohn 1979; Osgood & Zieman, 1993), but recently ecologists have documented how top-down forces may also play an important (Posey et al. 1995; Silliman & Zieman 2001; Silliman & Bertness 2002; Bertness et al. 2001; Ho & Pennings 2008). For example, in salt marshes in the southeastern United States, grazing pressure from the periwinkle *Littoraria irrorata* can have a strong influence primary production by *Spartina alterniflora* (Silliman & Zieman 2001). Little is known, however, about how top-down effects operate in Northern marshes where the species composition differs from Southern marshes (Bertness 1999).

We need a better understanding of how top-down forces in Northern salt marshes may influence ecological dynamics. Along the rocky shores habitats of north-Atlantic coast of the United States, the invasive predatory green crab (*Carcinus maenas*) causes strong trophic cascades by suppressing the grazing rate of the common periwinkle (*Littorina littorea*) on algal resources (Trussell et al. 2002; Trussell et al. 2004). Both *C. maenas* and *L. littorea* are abundant species found in northern New England salt marshes (Bertness 1985). Given that *C. maenas* has become increasingly common in Northern marshes, it is critical that we study how nonconsumptive and consumptive effects of *C. maenas* on prey populations may possibly influence its prey's competitors such as the benthic macroinfaunal community. To examine how the nonconsumptive and consumptive effects between *C. maenas* and *L. littorea* influenced both density and diversity of macroinfaunal species, I manipulated both predatory cues and snail density in two separate field experiments conducted during the summer of 2006 and 2007 in a

southern Maine salt marsh. During these experiments I also investigated the phenotypic and behavioral responses of *L. littorea* in presence of predatory crab risk cues. I show that both nonconsumptive and consumptive effects affect the macroinfaunal community, however, differences in nonconsumptive interaction strength among macroinfaunal functional feeding groups highlight the potential importance of functional diversity in mediating the effects of predators on community.

## **Materials and methods**

### Study site

All experiments were conducted at the Wells National Estuarine Research Reserve, located in Southern Maine (43°20'03.17"N, 70°32'33.41"W), U.S.A. The Wells reserve consists of over sixteen hundred acres of protected estuarine waters, shoreline, adjacent uplands and marshes.

Experiments were conducted on the mud flat adjacent to the low marsh zone, dominated by tall form *Spartina alterniflora*, on the southern (downstream) portion of the Little River. This habitat supports a variety of epibenthic species including the predatory green crab *Carcinus maenas* and the herbivorous snail *Littorina littorea*, as well as a diverse macroinfaunal community consisting of various species of bivalves, crustaceans and polychaetes.

### 2006 Experimental design and setup

The initial experiment examined how nonconsumptive and consumptive interactions between *C. maenas* and *L. littorea* influenced both density and diversity of macroinfaunal species. I randomly applied two treatments (nonconsumptive and consumptive effects) in a fully

factorial design with six replicates per experimental combination for a total of 48 replicates. The nonconsumptive treatment consisted of two treatment levels where either two crabs or no crab was added to each enclosure. In the nonconsumptive treatment, to prevent crabs from preying on snails, their claws were bound shut using duct tape and cable ties. The consumptive treatment consisted of four treatment levels where 0, 1, 2 or 4 snails I remove manually every four days from appropriate replicates in order to simulate consumptive predatory effects without the confounding effect of crab risk cues. I initially stocked each enclosure with 25 snails, which is representative of the mean snail density ( $\pm$  SE) ( $25.05 \pm 2.82$ ) sampled in  $0.25\text{m}^2$  quadrats at this site (unpublished data) and culled them at four day intervals resulting in removal of 0%, 25%, 50% and 100% over the duration of the experiment. Enclosures were constructed of  $\frac{1}{4}$  inch diameter PVC piping ( $0.5 \times 0.5 \times 0.25\text{m}$ ) and covered by galvanized mesh (0.5mm mesh diameter). Enclosures were anchored in the mudflat adjacent to the *S. alterniflora* zone and extended 10 cm down into the sediment to minimize crab emigration. The experiment was started on July 6<sup>th</sup>, 2006 and lasted 25 days.

### Macroinfaunal sampling

At the end of the experiment I collected 5 sediment cores from each enclosure using a syringe of 4cm in diameter and 3cm long. Sediment cores were brought back to the lab, sieved using a USA standard test sieve (0.5mm mesh diameter) and all organisms collected were preserved in 70% ethanol. Using a dissecting microscope, organisms were counted and identified to the lowest possible taxonomic level. Density (#/ core) and diversity (Shannon Weiner index) of macroinfaunal organisms were calculated for each core. To evaluate any effect on functional

diversity, organisms were further sorted into appropriate functional feeding groups: surface feeders, interstitial feeders, and filter feeders (Table 1).

#### Phenotypic plasticity in shell thickness and tissue mass

To compare variation in shell morphology of *L. littorea* in response to the presence and absence of predatory risk cues, 5 snails per enclosure were tagged and followed for growth in shell length, shell thickness and soft tissue mass following the methods of Trussell (1996, 2000). Briefly, snails were initially weighed while submerged in seawater using a Mettler Toledo Model AB104-S electronic balance ( $\pm 0.01$ grams), providing a reliable estimate of the shell mass. Snails were then air-dried and an absorbent tissue was used to remove excess water from the shell opening before weighing in air to obtain snail dry weight. Subtracting wet weight from dry weight yielded tissue mass. Shell length was measured with digital calipers ( $\pm 0.01$  mm) as the maximum dimension of the shell parallel to the aperture. Shell thickness was measured at the lip of the shell next to the body whorl and at the lip opposite. Immediately following the experiment, snails were again measured using these methods. Growth in shell and tissue mass was determined by subtracting initial values from final values.

#### 2007 Experimental design and setup

The second experiment was designed to differentiate effects of *C. maenas* bioturbation and predatory risk cues on the macroinfaunal community. For this experiment, I used larger enclosures (0.5 X 1 X 1m); the surface area covered by these new enclosures ( $0.5\text{m}^2$ ) was twice the size of enclosures used in the previous experiment, allowing me to anchor them in the mudflat/*S. alterniflora* interface; half of each enclosure enclosed mudflat while the other half

enclosed *S. alterniflora*. By anchoring the enclosures at the mudflat/*S. alterniflora* interface I crabs and snails were allowed access to both preferred habitats.

I randomly applied two treatments (nonconsumptive and consumptive effects) in a fully factorial design with eight replicates per experimental combination for a total of 48 replicates. The nonconsumptive treatment consisted of three levels where either two caged crabs, two bioturbator crabs, or no crabs were added to each enclosure. Similar to the previous experiment, bioturbator crabs were allowed to roam freely in the enclosures with their claws bound shut. Caged crabs were individually contained in small galvanized cages (15cm X 15cm) anchored within the enclosure, preventing them from physically disturbing the sediment through walking. To simulate consumptive predatory effects without the confounding effect of crab risk cues, I manually removed snails from appropriate replicates. Enclosures were initially stocked with 50 snails and each day, I removed 0 or 1 snail, resulting in 0% and 100% respective reduction in snail population by the end of the experiment. The experiment was started on July 12<sup>th</sup>, 2007 and lasted 50 days.

#### Snail behavior

To detect changes that nonconsumptive and consumptive treatments have on snail behavior, during the 2007 experiment, I counted the total number of snails found on the mudflat and on *S. alterniflora* leaves on a weekly basis. The height at which snails were found on the leaves was also recorded.

## Statistical analysis

### Macroinfaunal sampling

For the experiments conducted both in 2006 and 2007, macroinfaunal density and diversity were analyzed using a two-way nested ANOVA that considered nonconsumptive and consumptive treatments as fixed effects. Because sediment cores samples were not independent, I considered ‘enclosure’ to be nested within nonconsumptive and consumptive treatments in my analysis. Differences among functional feeding groups were analyzed with a multivariate analysis of variance (MANOVA), which used functional feeding groups as the repeated measure. To satisfy the MANOVA homogeneity of variances, data were log-transformed. All analyses were performed on JMP software (version 5.0.1a).

### Phenotypic plasticity

A two-way analysis of covariance (ANCOVA) with enclosures (random) nested within each nonconsumptive and consumptive effect (fixed) was conducted to test for differences in shell thickness and tissue mass. Initial shell length and tissue mass were used as covariates for shell thickness and tissue mass analyses respectively.

### Snail behavior

The snail density and location were analyzed with repeated-measures ANOVA that considered predatory risk cues treatment as a fixed effect and sampling time as a random, repeated effect.

## Results

### 2006 experiment- macroinfaunal sampling

The experiment examined how nonconsumptive and consumptive interactions between *C. maenas* and *L. littorea* affected the abundance and diversity of macroinfaunal species. Both nonconsumptive (ANOVA,  $F_{1,40} = 14.6162$   $P = 0.0005$ ) and consumptive (ANOVA,  $F_{3,40} = 4.4417$   $P = 0.0087$ ) effects positively influenced macroinfaunal density. Macroinfauna density in the presence of predatory risk cues was 50% greater compare to the macroinfauna density in the absence of crabs. The results indicate that the positive direct effect on macroinfaunal density produced by the most intense snail removal (100%) was comparable to the positive nonconsumptive effect produced by predator risk cues. This indicates that the nonconsumptive indirect effect on macroinfauna was similar to the direct effects on macroinfauna of the most intense simulated predation scheme.

The analysis of macroinfaunal diversity, using the Shannon-Weiner index, within nonconsumptive and consumptive treatments also yielded highly significant results. In the presence of predatory risk cues, macroinfaunal diversity remained high regardless of the consumptive treatment (ANOVA,  $F_{1,40} = 6.0767$   $P = 0.0181$ ). The consumptive treatment also positively influenced macroinfaunal diversity (ANOVA,  $F_{3,40} = 2.9288$   $P = 0.0452$ ) indicating that consumptive and nonconsumptive effects are equally important mechanisms impacting diversity in this system (Figure 1).

With respect to macroinfaunal functional feeding groups, MANOVA revealed that there was a significant interaction between consumptive and nonconsumptive effects on macroinfaunal density in all three functional feeding groups (Univar unadj epsilon,  $F = 2.2368$ ,  $P = 0.0391$ ) (Figure 2). Each functional feeding group responded differently to the crab and snail

combinations; for the deposit surface feeders at the no snail removal treatment, when crabs are present there is a greater density compare to when no crabs are present. For the interstitial feeders there is a crab effect when no snail are remove but that effect disappears has the snail density decreases. For the filter feeders there is no nonconsumptive effect or no consumptive effect. Results suggest that the indirect effects of crab risk cues increased with the snail removal and both treatments led to changes in functional feeding group densities but these effects were particularly strong for surface deposit feeders.

#### 2006 experiment- phenotypic plasticity in shell thickness and tissue mass

Analysis of covariance revealed that snails exposed to predatory crab risk cues developed significantly thicker shells (ANCOVA,  $F_{1,40} = 295.3112$ ,  $P < .0001$ ) than those snails contained in cages without crabs present (Figure 3). Significant reductions in tissue mass was also induced by the presence of predatory crab risk cues (ANCOVA,  $F_{1,40} = 52.1496$ ,  $P < .0001$ ). Snails exposed to crab cues had 20% less tissue mass on average than snails from the no crab treatment (Figure 4).

#### 2007 experiment- macroinfaunal sampling

The second experiment, conducted in 2007, was designed to differentiate the direct effect of *C. maenas* bioturbation from the indirect effect of *C. maenas* predatory risk cues on the macroinfaunal community. Both nonconsumptive (ANOVA,  $F_{2,40} = 3.2964$ ,  $P = 0.0472$ ) and consumptive (ANOVA,  $F_{1,40} = 6.7821$ ,  $P = 0.0128$ ) effects positively influenced macroinfaunal density. Macroinfauna density in the presence of predatory risk cues was 37% greater compare to the macroinfauna density in the absence of crabs and 14% greater compare to the density in the

presence of bioturbator crabs. My results also suggest that caged crabs had a stronger positive effect than did bioturbator crabs, indicating that direct bioturbation effects counteracts positive indirect effects of crab risk cues (Figure 5).

Macroinfaunal diversity was positively influenced by consumptive effects (ANOVA,  $F_{1,40} = 1.9546$ ,  $P = 0.0398$ ). Functional feeding groups responded similarly to both the consumptive (Univar unadj epsilon,  $F = 5.8878$ ,  $P < 0.0001$ ) and nonconsumptive (Univar unadj epsilon,  $F = 11.8013$ ,  $P = 0.0001$ ) treatments. However nonconsumptive effects were particularly strong for the surface deposit feeders (Figure 6).

#### 2007 experiment - snail behavior

Analysis of snail behavior revealed that in presence of crab risk cues a higher density of snails were found on *S. alterniflora* leaves away from the mudflat (Repeated measures analysis,  $F_{2,21} = 6.9180$ ,  $P = 0.0049$ ) (Figure 7). Snails responded to predatory risk cues by seeking shelters on higher *S. alterniflora* leaves, avoiding the mudflat area when crabs were present.

## Discussion

The 2006 experiment data suggest that both nonconsumptive and consumptive effects contribute to the net predatory effect of *C. maenas* on the macroinfaunal species. Predatory crab cue effects were comparable to the effects of the most intense snail removal (consumptive effects) on the macroinfaunal species. When crab cues were present, the density and diversity of macroinfaunal community were greater indicating a positive indirect effect.

The positive indirect effect produced by the most intense snail removal (100%) was comparable to the positive nonconsumptive indirect effect on macroinfaunal abundance. I

hypothesize that predator-induced changes in snail foraging behavior, in response to the predatory risk cues, reduced the intensity of competition between snails and macroinfaunal for a diatoms and periphyton (Relyea 2000; Peacor & Werner 2000; Mowles et al. 2011) (Figure 8). These positive nonconsumptive effects were particularly strong for the surface deposit feeders, as predicted by my hypothesis. Surface deposit feeders are a group of organisms including species as *Corophium sp.*, *Polydora sp.* and *Streblospio benedicti* (Table 1); grazers who actively forage on diatoms and algae growing at the surface of the sediment (Smith et al. 1996; Fauchald & Jumars 1979) potentially directly competing with the efficient herbivorous grazer *L. littorea*. Previous experiments have demonstrated that when exposed to crab predatory risk cues, *L. littorea* showed a greater behavioral response, resulting in a lower level of foraging (Trussell et al. 2002; Trussell et al. 2004). This reduction in foraging allows *L. littorea*'s competitors' access to shared resources, indicating that nonconsumptive effect can influence species interactions and has a strong impact on trophic interactions. In my experiment, I observed that the functional feeding group that responded most to both nonconsumptive and consumptive effects were the surface deposit feeders. The interstitial deposit and filter feeders did not react as strongly to the treatments, further supporting my hypothesis that interstitial and filter feeders do not share a common food source with *L. littorea* and are not in competition with one another. Therefore a reduction in the snail's feeding activity would not be directly beneficial to them. The differences among nonconsumptive strengths among functional feeding groups highlight the potential importance of functional diversity in mediating the effects of predators on community dynamics. Additionally, my field experiment showed a phenotypic response of *L. Littorea* to predatory crab cues. Experiments conducted in laboratory settings have demonstrated how snails held in presence of crab risk cues exhibit an increase of shell thickness and a reduction in tissue mass

due to a reduction in feeding while in presence of predators cues (Trussell 1996, 2000). My field experiment was able to demonstrate similar results; on average, the snails held in cages with crabs had a 20% reduction in tissue mass. I also observed that snails would seek shelter by climbing higher on the cages walls to potentially avoid predation, therefore reducing the time spent on the sediment foraging for food, allowing the other organisms access to that food supply.

Not only did the macroinfaunal density respond positively to both nonconsumptive and consumptive effects but also the diversity. In recent years, scientists have emphasized how biodiversity is important to ecosystem functioning (Cardinale et al. 2002; Griffin et al. 2008; Stachowicz et al. 2008). Cardinale (2002) showed that by increasing both richness and evenness of suspension-feeding aquatic insect in stream; the topographical complexity of the benthic floor was also increased allowing greater feeding success for other species. This change in topography also resulted in a deceleration of the flow allowing other suspension feeders better access to food particles. Other studies have also shown links between the number of species in an ecosystem and its functions and services (Hooper et al. 2002, 2005; Worm et al. 2006). With increased biodiversity, ecosystems have been shown to be more resistant to invasion and more resilient to diseases, especially among plants (Tillman). More diverse ecosystems also offer more prey species for predators and often attracted a greater diversity of predators. My experiments revealed that the presence of crabs had a positive effect on diversity; when crabs were present I sampled a greater number of macroinfaunal species. My results highlight the importance of trophic interactions positively influencing functional diversity suggesting that top-down interactions may play an important role in shaping the community structure and furthermore impacting the ecosystem functioning.

In 2007, I conducted another experiment similar to the previous one, with some

differences; I used larger and taller enclosures anchored in the mudflat/*S. alterniflora* interface. By positioning the enclosures on the interface crabs and snails were allowed access to both preferred habitats. At low tide crabs tend to seek shelter from desiccation in the *S. alterniflora* tall form and often snails were seen grazing on the mudflat (personal observation). By having the enclosures on the interface the organisms were allowed to behave more naturally. One of the objectives of this experiment was to differentiate the direct impact of the crabs' bioturbation from the indirect effect of their predatory risk cues; to see if the crabs' bioturbation could directly benefit the macroinfaunal community by introducing more nutrients, oxygen and water into the sediment (Bertness 1985). Ecosystem engineers, such as crabs, can have important indirect effects on benthic communities by altering structural complexity and sediment characteristics (Bertness 1985) and may be important in shaping the community structure.

The results from this experiment were similar to the previous one; I observed that when crabs are present there is an overall increase in macroinfaunal density, but a post-hoc analysis revealed no significant difference between the crab cues and the bioturbator crabs indicating that there is no direct positive effect from the crabs' physical bioturbation. In essence, the predatory crab cues are driving these interactions. As the crab bioturbation treatment did not exhibit any direct positive effect on the macroinfaunal density. If the bioturbation would have been beneficial to the macroinfaunal organisms one would have expected to see a positive response from both the interstitial and surface deposit feeders, by spreading more nutrients throughout the sediment via bioturbation. However in this experiment only the surface deposit feeders significantly increased in density. Both experiments highlight how nonconsumptive cues are important in shaping and changing community functioning. When crabs are present, the snails reduce their feeding, allowing access to food to the other organisms present in the system. In

response to predatory crab cues, snails exhibited an anti-predator behavior avoidance. Early on in the experiment the majority of the snails were found climbing higher on the *S. alterniflora* leaves, avoiding the mudflat where the crabs were found. These responses are consistent with those of many experiments conducted in the recent years (Trussell et al. 2002; Mowles et al. 2011), which clearly documented an avoidance response of *L. Littorea* in presence of *C. maenas* cues. Furthermore, the results of both experiments demonstrate that the behavioral responses of one species can influence interspecific interactions favoring other species, with a change in foraging competition in the presence of predatory cues.

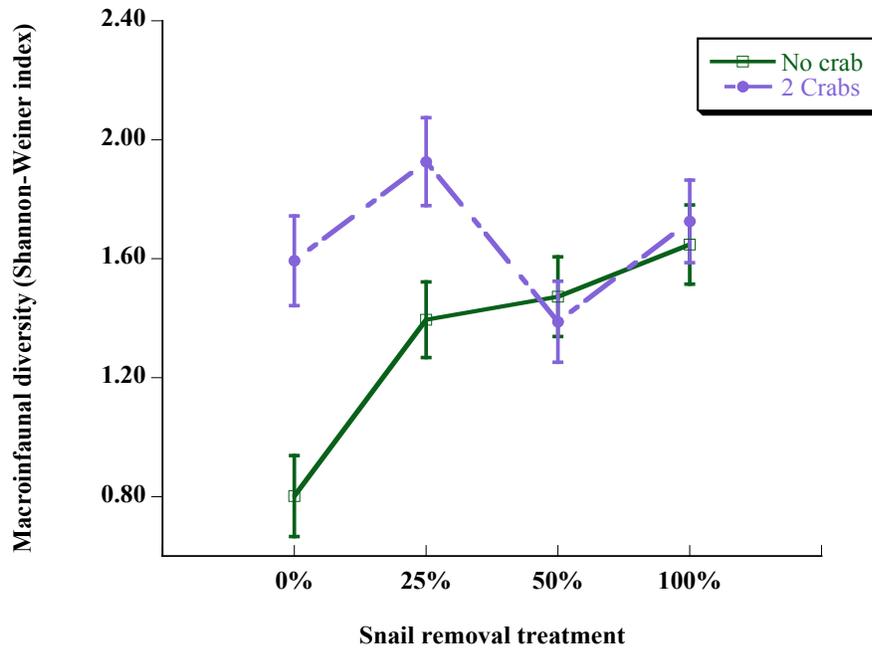
With increasing knowledge of the importance of the nonconsumptive effects of predators on trophic interactions and system functioning it is becoming clear that these effects have significant impacts in various systems including salt marshes. My experiment demonstrates how an interaction modification in lower trophic level competitors was affected differently in presence of predatory cues. These experiments highlight the importance of better understanding how top-down interactions play an important role in shaping the salt marsh communities and how these interactions might influence the bottom-up processes equally important in these systems.

## Tables

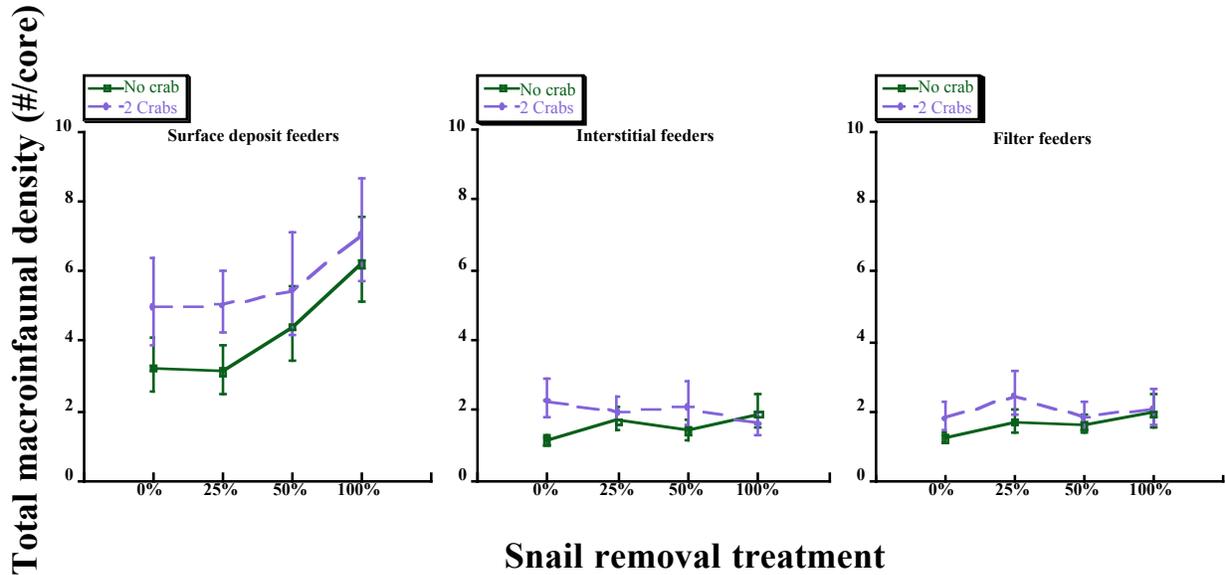
**Table 1.** Functional feeding groups of dominant taxa found in core samples

Species	Phyla	Functional feeding groups
<i>Corophium spp.</i>	Arthropoda	Surface Deposit Feeders
<i>Leitoscoloplos fragilis</i>	Polychaeta	Surface Deposit Feeders
<i>Marenzelleria viridis</i>	Polychaeta	Surface Deposit Feeders
<i>Polydora spp.</i>	Polychaeta	Surface Deposit Feeders
<i>Pygospio elegans</i>	Polychaeta	Surface Deposit Feeders
<i>Streblospio benedicti</i>	Polychaeta	Surface Deposit Feeders
<i>Neanthes spp.</i>	Polychaeta	Interstitial deposit feeders
<i>Eteone heteropoda</i>	Polychaeta	Interstitial deposit feeders
<i>Tubificidae</i>	Polychaeta	Interstitial deposit feeders
<i>Mya arenaria</i>	Mollusca	Filter feeders
<i>Mytilus edulis</i>	Mollusca	Filter feeders

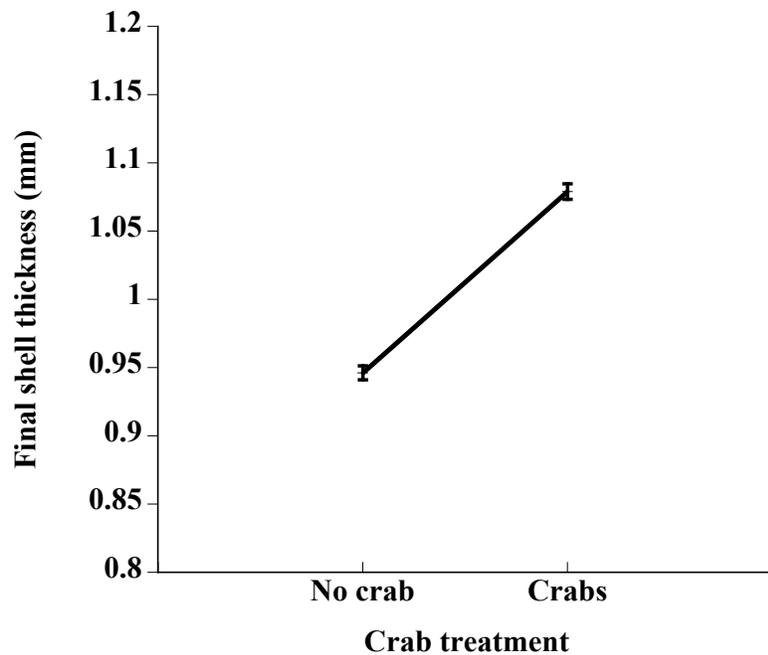
## Figures



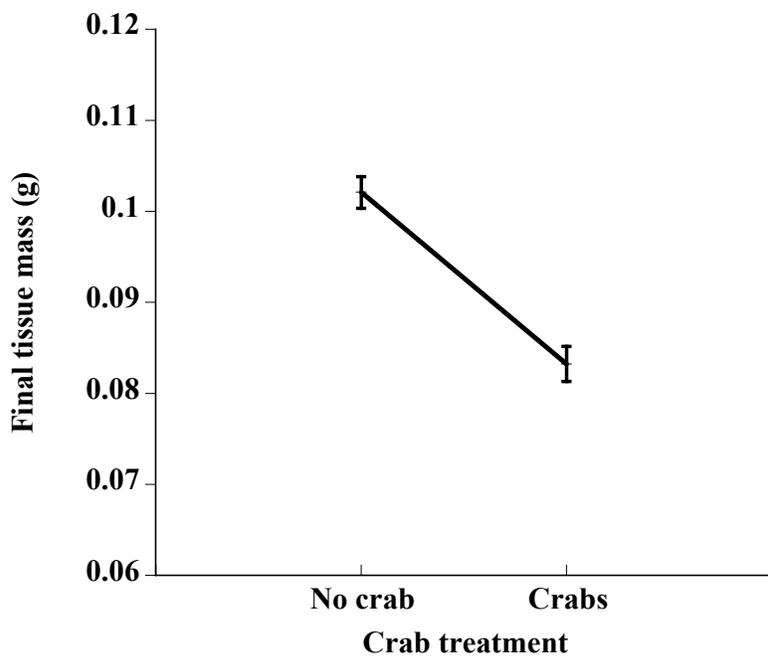
**Figure 1.** Macroinfaunal Shannon-Weiner diversity (+/- standard error) in the presence and absence of crabs and at different levels of simulated predation (0%, 25%, 50%, 100%)



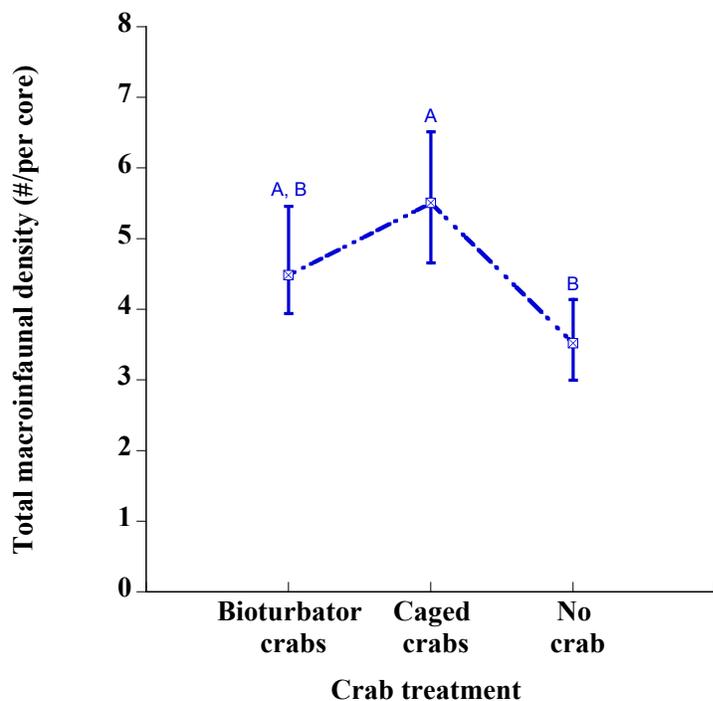
**Figure 2.** Macroinfaunal density of three functional feeding groups (deposit feeders, interstitial feeders and filter feeders) in the presence and absence of crab risk cues (2 crabs or 0 crabs) and at different levels of simulated predation (0%, 25%, 50% or 100%). Analyses performed on log<sub>10</sub> transformed data; reverse-transformed means (+/- 95% confidence intervals) are presented for clarity.



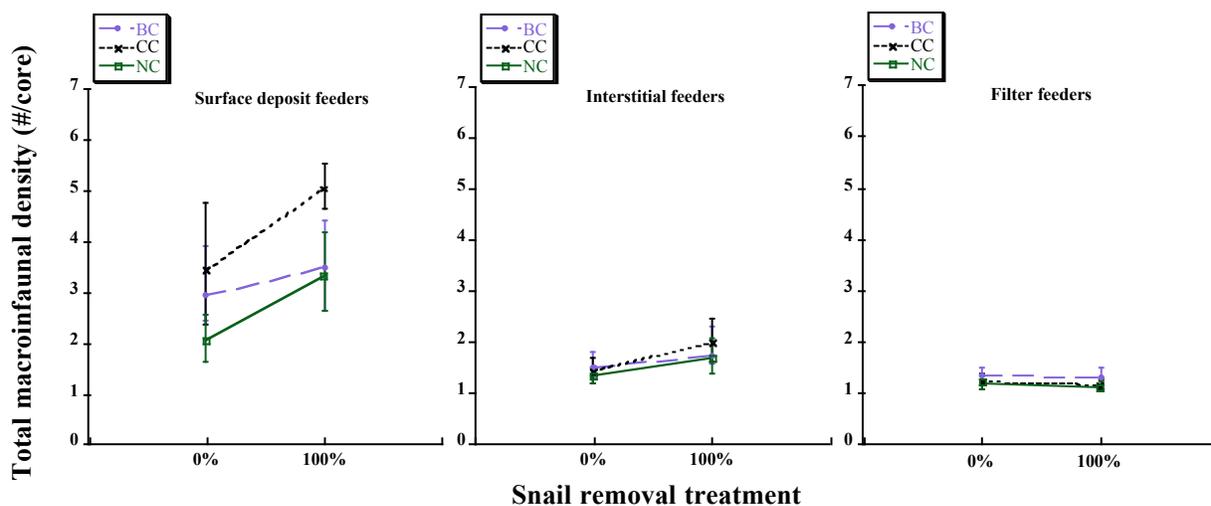
**Figure 3.** Final shell thickness (+/- standard error) for snails in the presence and absence of crabs.



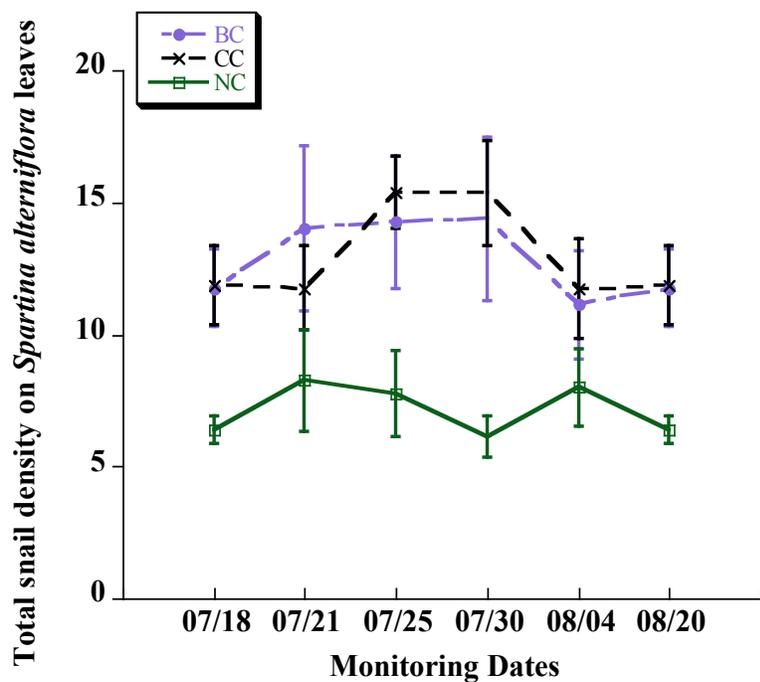
**Figure 4.** Final tissue mass (+/- standard error) for snails in the presence and absence of crabs.



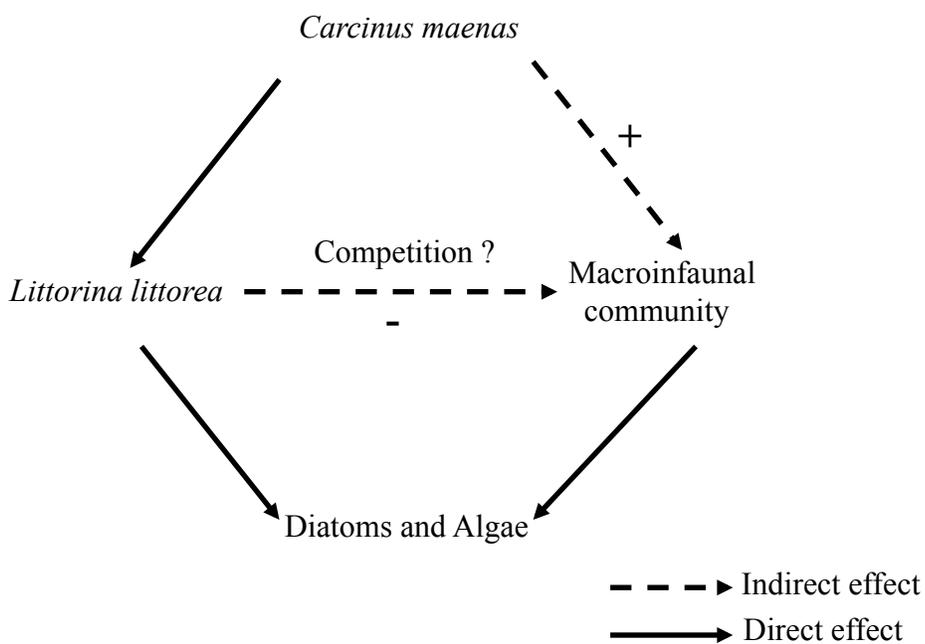
**Figure 5.** Total macroinfaunal density in the presence of bioturbator crabs, of caged crabs and of no crabs. Analysis was performed on  $\log_{10}$  transformed data. Reverse-transformed means ( $\pm$  95% confidence intervals) are presented for clarity.



**Figure 6.** Macroinfaunal density of three functional feeding groups (deposit feeders, interstitial feeders and filter feeders) in presence of bioturbator crabs (BC), of caged crabs (CC) of no crabs (NC) and at different levels of simulated predation (0% or 100%). Analyses performed on  $\log_{10}$  transformed data; reverse-transformed means ( $\pm$  95% confidence intervals) are presented for clarity.



**Figure 7.** Snail density found on *S. alterniflora* leaves in presence of bioturbator crabs (BC), of caged crabs (CC) and of no crabs (NC) at different monitoring dates throughout the experiment



**Figure 8.** Species interactions detected in the Wells salt marsh

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## **Chapter 2: The effects of *Carcinus maenas*, *Littorina littorea* and macroinfaunal community interactions on nutrients availability and *Spartina alterniflora* growth**

### **Introduction**

The majority of research conducted in salt marsh has focused on how physical and chemical parameters can influence *Spartina alterniflora* growth, the foundation species in salt marsh community (Valiela & Teal 1974; Mandelsohn 1979; Morris 1982; Bradley & Morris 1990). In early studies, bottom-up forces were thought to be the driven force controlling the primary productivity of salt marshes (Valiela & Teal 1978; Mandelsohn 1979; Morris 1982; Bradley & Morris 1990). Studies have showed that *S. alterniflora* nutrient uptake and production are highly influenced by increased nutrient and oxygen availability (Odum & Snalley 1959; Teal 1962; Valiela and Teal 1974; Gallagher 1975; Mandelsohn 1979; Howes et al. 1981). Conversely salinity and hydrogen sulfide can have a negative impact on *S. alterniflora* growth (Morris 1982; Osgood & Zieman 1993; Bradley and Morris 1990). Overall, primary production in nearshore marine systems is thought to be nitrogen limited and salt marshes are no exceptions (Valiela and Teal 1974; Osgood and Zieman 1998). Nitrogen limitation on *S. alterniflora* growth has been documented in experiments conducted in Massachusetts by Valiela and Teal (1974). Their experiments demonstrated that the application of fertilizer, which included nitrogen, has had a positive growth effect on *S. alterniflora* short form whereas phosphorus addition did not have a positive effect. Overall, primary production in nearshore marine systems is thought to be nitrogen limited and salt marshes are no exceptions (Valiela and Teal 1974; Osgood and Zieman 1998). The early studies conducted on nutrients limitation have identified nitrogen as the limiting factor in both marine and terrestrial habitats and phosphorus as the limiting factor in freshwater

habitats (Likens 1972). A phosphorus increase in lakes has been shown to lead to eutrophication and proliferation of blue-green algae (Likens 1972; Paerl 2009). However, in recent years there has been an increase debate on which primary nutrients (P or N) are limiting in the different ecosystems and how their interactions can impact the primary productivity (Elser et al 2007). An increasing body of work suggests that nitrogen might not be the only factor limiting primary production but phosphorus might equally play an important role in regulating plant growth in marine habitats (Paerl 2009). Several studies have looked at the importance of nitrogen to phosphorus ratio in plant tissue as indicator of ambient levels of nutrient and nutrient limitation (Bedford et al. 1999; Güsewell et al. 2003; Tessier & Raynal 2003; Crain 2007) The studies have shown that N:P ratios can be effective predicators of nutrient limitation, on average a N:P > 16 would indicate a phosphorus limitation, where a N:P < 14 would indicate a nitrogen limitation, and a N:P ration between 14 and 16 could indicate that either N or P can be limiting or there is a co-limitation (Koerselman & Meuleman 1996; Tessier & Raynal 2003). Studies conducted on salt marsh plant tissue and sediment surfaces consistently indicated a nitrogen limitation with ratios under the threshold of 14 (Verhoeven et al. 1996; Bedford et al. 1999). Some studies have found that differences in *S. alterniflora* tissue element composition can reflect the availability of nutrients and maybe use as an indicator of deficiency experienced by plants (Güsewell et al. 2003).

Recently, however, ecologists have documented how strong top-down forces can play an important role in salt marsh production (Posey et al. 1995; Bertness et al. 2002; Silliman & Zieman 2001; Silliman & Bertness 2002; Ho & Pennings 2008). Historically, top-down forces were thought to play a minor role in salt marsh community dynamic. Consumer control (top-down forces) has shown to play a critical role in regulating species interactions and shaping

community in terrestrial, freshwater and rocky shore habitats (Carpenter & Kitchell 1993; Boertje et al. 1996; Beckerman et al. 1997; Rudgers et al. 2003). By consuming prey, predators can have indirect effects on their prey's resources (Hairston et al. 1960, Carpenter et al. 1987; Boertje et al. 1996; Beckerman et al. 1997; Estes, et al. 1998, Rudgers et al. 2003). Furthermore, through nonconsumptive effects, predators can also have similar indirect effects in shaping community structures (Werner 1991; Schmitz et al. 1997; Trussell et al. 2002; Trussell et al. 2004). Predatory effects (nonconsumptive and consumptive) are important top-down mechanisms controlling ecological interactions in various ecosystems.

The first studies focusing on top-down forces in salt marsh community were conducted primarily in southern salt marshes on the Atlantic coast, where the species composition differ greatly from the northern salt marshes (Bertness 1999). An important grazer found to exert strong top-down forces in southern marshes, *Littoraria irrorata*, is notably absent in northern marshes. This grazer can ingest live tissue of *S. alterniflora*, creating scars, rendering the plant susceptible to fungal infections (Silliman & Newell 2003). In contrast, one of the major herbivore found in northern salt marshes is the common periwinkle *Littorina littorea*, this herbivore do not appear to apply the same grazing pressure as *L. irrorata* do on *S. alterniflora* blades but could potentially have the ability to eat the plants' rhizomes (Bertness 1984). In northern marshes, *L. littorea* is exposed to the predation pressure from *C. maenas*, an important predatory crab that feeds on a variety of prey including bivalves, gastropods and juvenile fish (Elner 1981). We know very little about the potential of top-down effects exerted by *C. maenas* and *L. littorea* in northern marshes in comparison to their counterparts in southern marshes.

We need a better understanding of how both top-down (predator effects) and bottom-up forces interact to influence ecological dynamics and primary productivity of northern salt

marshes. Both *C. maenas* and *L. littorea*, are abundant species found in northern New England salt marshes and it is critical that we study how their interactions may influence nutrients availability and the resulting effects on *S. alterniflora* growth. To examine how the interactions among *C. maenas*, *L. littorea* and macroinfaunal species on nutrient availability affected *S. alterniflora* growth I manipulated predatory risk cues, snail density and fertilizer application in two separate field experiments conducted during the summer of 2008 and 2010 at two separate locations. The first experiment was conducted in 2008 at the Wells National Estuarine Research Reserve in southern Maine; the second experiment was conducted in 2010 at the Scarborough River marsh, also in southern Maine. The experiments' objectives are: (1) to understand how the trophic interactions observed between *C. maenas*, *L. littorea* and the macroinfaunal community could potentially impact the growth of *S. alterniflora*, and how (2) the strength of the trophic interactions may vary with increased bottom-up influence via increased nutrient availability.

## **Materials and methods**

### **Study sites**

The first experiment was conducted, during the 2008 summer months, at the Wells National Estuarine Research Reserve, located in Southern Maine (43°20'03.17"N, 70°32'33.41"W), U.S.A. The Wells reserve consists of over sixteen hundred acres of protected estuarine waters, shoreline, adjacent uplands and marshes. The experiment was carried out in the mudflat/*S. alterniflora* interface on the southern (downstream) portion of the Little River.

The second experiment was conducted during the summer of 2010 in the Scarborough River marsh, adjacent to the Eastern Trail (43°33'53.21"N, 70°21'53.35"W), U.S.A. The site is located in the Scarborough Marsh Wildlife Management Area and is considered a protected area.

The experiment was carried out in the mudflat/*S. alterniflora* interface in the inlet North of the Eastern Trail.

These two sites were chosen due to their differences in ambient ammonium concentrations. Porewater samples collected at the Wells National Estuarine Research Reserve location showed high concentrations of ammonium. Conversely, ammonium concentration levels sampled at Scarborough River marsh were much lower (Table 1).

#### Experimental design and setup

For both experiments, I examined how the interactions among *C. maenas*, *L. littorea* and macroinfaunal species on nutrient availability affected *S. alterniflora* growth. I used a similar design for each experiment; I randomly applied three treatments (predatory risk cues, presence of snail and fertilizer addition) in a fully factorial design with five replicates per experimental combination for a total of 40 replicates. The predatory risk cues treatment consisted of two treatment levels where either two crabs or no crabs were added to each enclosure. In the predatory risk cues treatment, to prevent crabs from preying on snails, their claws were bound shut using a combination of duct tape and cable ties. The snail treatment was randomly assigned to the enclosures by adding 0 or 50 snails. For the fertilizer treatment, I used the Scott turf builder lawn fertilizer (29% N, 3% P, and 4% K). I drilled 16 holes in the sides of 50-mL plastic centrifuge tubes; I anchored 4 centrifuge tubes per enclosure under the surface of the sediment. For the enclosures with added fertilizer, each centrifuge tube contained 20.4g of fertilizer wrapped in nylon mesh to promote a slow release. The centrifuge tubes were evenly distributed and change biweekly for the duration of the experiment.

Enclosures were constructed of ¼ inch diameter PVC piping (1 X 1 X 1m) and covered by galvanized mesh (0.5mm mesh diameter). For these experiments, the enclosures were anchored on the mudflat/*S. alterniflora* border, thus allowing the crabs and snails to have access to both preferred habitats. The 2008 experiment, conducted in Wells, started on July 15<sup>th</sup> and lasted 57 days. The 2010 experiment, in Scarborough, started on July 13<sup>th</sup> and lasted 40 days.

### Nutrients sampling

In each enclosure, one sipper was anchored to collect porewater samples for nutrient analysis. The sippers consisted of white plastic bottle (4oz) with 2 rows of holes drilled near the top of the bottle. The holes were covered with 50micron mesh. The sippers were attached to a PVC pole and anchored just underneath the sediment surface. Porewater samples were collected at the end of the experiment and analyzed for ammonium ( $\mu\text{mol/L}$ ) and phosphorus ( $\mu\text{mol/L}$ ) concentrations.

Using a syringe, porewater samples were siphoned out and filtered in the field using a Whatman GF/F filters. For the ammonium analysis, I used the salicylate method #8155 on a HACH DR/2400 spectrophotometer and for the phosphorus analysis I used the LaChat Quickem 8500 methods for phosphate analysis.

### *Spartina alterniflora* growth and nutrient concentrations

To monitor the *S. alterniflora* growth rate, I tagged and measured 5 plants in each enclosure. At the end of the experiment, the tagged plants were then collected, measured, dried, and weighed. Tissue samples, from 3 plants were grinded using a Retsch oscillating Mill

MM400. The samples were stored in microtubes and kept in the drying oven at 60°C until further analysis.

For both experiments, *S. alterniflora* carbon and nitrogen tissue contents were determined for each sample by using a gas chromatographic elemental analyzer (Flash EA 1112 series NC soil analyzer) that uses flash combustion in an oxygen atmosphere to determine the concentrations of carbon and nitrogen. To determine the *S. alterniflora* leaves phosphorus content, I processed the samples using the method described by Fourqurean et al. (1992) using a magnesium sulfate extraction. Following the extraction the samples were analyzed using the LaChat Quickem 8500 methods for phosphate analysis.

#### Snail behavior

To detect changes that predatory risk cues treatments have on snail behavior, during both experiments, I counted the total number of snails found on the mudflat and on *S. alterniflora* leaves on a weekly basis.

#### Statistical analyses

##### Nutrient sampling

For both experiments conducted in 2008 and 2010, porewater ammonium and phosphorus concentrations were analyzed using a three-way nested ANOVA that considered predatory risk cues, snail and fertilizer treatments as fixed effects. To satisfy ANOVA assumptions the data were  $\log_{10}$  transformed. All analyses were performed on JMP software (version 5.0.1a).

### *S. alterniflora* growth and nutrient concentrations

*S. alterniflora* final height and weight were analyzed using a three-way nested ANCOVA that considered predatory risk cues, snail and fertilizer treatments as fixed effects. Initial height was used as covariate for the final height analysis. Because plants from each enclosure are not independent, I considered “enclosure” to be the experimental unit and nested it within predator effect, snail effect and fertilizer effect in my analysis to avoid pseudoreplication.

Carbon, nitrogen and phosphorus tissue concentrations were analyzed using a three-way nested ANOVA that considered predatory risk cues, snail and fertilizer treatments as fixed effects. Because the plants samples from each enclosure are not independent, I considered “enclosure” to be the experimental unit and nested it within predator effect, snail effect and fertilizer effect in my analysis to avoid pseudoreplication.

To analyze the carbon, nitrogen and phosphorus tissue concentrations between the two experiments (Wells-2008 and Sacrborough-2010) I used a two-way ANOVA that considered fertilizer treatment and location as fixed effects.

*S. alterniflora* final heights difference between Wells and Scarborough was analyzed using a two-way nested ANCOVA that considered location and fertilizer treatment as fixed effects. Initial height was used as covariate for the final height analysis. Because plants from each enclosure are not independent, I considered “enclosure” to be the experimental unit and nested it within location and fertilizer effect in my analysis to avoid pseudoreplication.

### Snail behavior

Snail behavior was analyzed with repeated-measures ANOVA that considered predatory risk cues treatment as fixed effect and sampling time as a random, repeated effect.

## Results

### 2008 experiment- Nutrients

The first experiment was conducted at the mouth of the little river at the Wells National Estuarine Research Reserve in the summer months of 2008. I examined how the effects of *C. maenas*, *L. littorea* and macroinfaunal community interactions on nutrient availability affected *S. alterniflora* growth. I first analyzed the effects of the added fertilizer treatment, to confirm that enclosures with added fertilizer were enriched. The ammonium concentrations sampled were positively influenced by the addition of fertilizer (ANOVA,  $F_{1,39} = 47.2146$ ,  $p < .0001$ ); both predatory risk cues and snail treatments had no effect on the ammonium levels. The fertilizer treatment increased the level of ammonium, on average 16 times more, in fertilized enclosures (Figure 1.) The fertilizer treatment (ANOVA,  $F_{1,39} = 16.2002$ ,  $p = 0.0003$ ) also positively influenced the porewater phosphorus concentrations (Figure 2.). The fertilizer was effective at adding nutrients in the system.

### 2008 experiment- *S. alterniflora* growth and nutrient concentrations

The three-way nested ANCOVA results indicated that *S. alterniflora* aboveground growth did not differ significantly among the three treatments (predatory risk cues, snail and fertilizer). The addition of fertilizer did not enhance *S. alterniflora* growth; the increased nutrient availability had no positive effect (Table 1.). The analysis of nitrogen tissue concentrations revealed that percentage of nitrogen in tissue samples was positively influenced by the addition of fertilizer (ANOVA,  $F_{1,39} = 19.9672$ ,  $p < .0001$ ), tissue samples from added fertilizer enclosures had on average 29% more nitrogen than tissues collected from unfertilized enclosures. Both predatory risk cues and snail treatments had no effect on the presence of

nitrogen in *S. alterniflora* tissue samples. The average percentages of nitrogen in plants from fertilized enclosures was 2.19 (+/- 0.07)% whereas the unfertilized plants had on average 1.69 (+/- 0.07)%. The N:P ratio for 2008 was not significantly different between the fertilize and unfertilized treatment (ANOVA,  $F_{1,116} = 0.1819$ ,  $p = 0.6726$ ) and the average N:P ratio for the fertilized enclosures was 15.16 (+/- 0.89), where as the unfertilized N:P ratio was 14.78 (+/- 1.10).

#### 2008 experiments- Snail behavior

Throughout the experiment, I also monitored snail behavior in each enclosure. The MANOVA results indicated that a greater number of snails were found on the *S. alterniflora* plants in presence of crab risk cues (Repeated measures analysis,  $F_{1,16} = 5.4964$ ,  $P = 0.0323$ ). Snails responded to the crab risk cues by seeking shelter on high grounds by climbing on the *S. alterniflora* leaves. The snail climbing did not seem to impair the plants' growth; I did not find any evidence of snail grazing directly on the *S. alterniflora* leaves. After careful examination of leaves under dissection scopes I did not find any presence of scarring tissue.

#### 2010 experiment- Nutrients

The second experiment was conducted at the Scarborough River marsh, adjacent to the Eastern Trail during the summer months of 2010. This experiment was identical to the one conducted in 2008 at the Wells reserve. I examined how the effects of *C. maenas*, *L. littorea* and macroinfaunal community interactions on nutrients availability affected *S. alterniflora* growth. I first analyzed the nutrient concentrations between fertilized and unfertilized treatment, to confirm that enclosures, with added fertilizer, were enriched. The amount of ammonium was 261

times greater in fertilized enclosures than in the unfertilized (ANOVA  $F_{1,36} = 12.4033$ ,  $P = 0.0014$ ); both predatory risk cues and snail treatments again had no effect on the ammonium levels. Conversely, the levels of phosphate were not increased by the addition of fertilizer in this system.

#### 2010 experiment- *S. alterniflora* growth and nutrient concentrations

The three-way nested ANCOVA result indicates that the *S. alterniflora* aboveground growth significantly responded to the fertilizer treatment. Both the predatory risk cues and snails treatments had no effect on *S. alterniflora* aboveground growth. The addition of fertilizer did enhance *S. alterniflora* growth; the increase nutrient availability had a positive effects (ANCOVA,  $F_{1,193} = 19.7321$ ,  $p = 0.0001$ ), the fertilized plants were on averages 10 cm taller than the unfertilized plants. The percentage of nitrogen in tissue samples were positively influenced by the addition of fertilizer (ANOVA,  $F_{1,39} = 23.7568$ ,  $p < .0001$ ); both predatory risk cues and snail treatments had no effect on the presence of nitrogen in *S. alterniflora* tissue samples. The average percentages of nitrogen in plants from fertilized enclosures was 2.92 (+/- 0.06)% whereas the unfertilized plants had on average 2.51 (+/- 0.06)%. The N:P ratio for 2010 was not significantly different between the fertilize and unfertilized treatment (ANOVA,  $F_{1,116} = 0.1277$ ,  $p = 0.7232$ ) and the average N:P ratio for the fertilized enclosures was 14.27 (+/- 0.54), where as the unfertilized N:P ratio was 14.58 (+/- 0.78).

#### 2010 experiments- Snail behavior

Throughout the experiment, I monitored snail behavior in each enclosure. The MANOVA results indicated that a greater number of snails were found on the *S. alterniflora*

plants in presence of crab risk cues (Repeated measures analysis,  $F_{1,16} = 2.0753$ ,  $P < .0001$ ). Snails responded to the crab risk cues by seeking shelter on high grounds by climbing on the *S. alterniflora* leaves.

#### 2008 versus 2010 experiment

A two-way ANOVA showed that there was a significant difference in nutrients concentrations between the two locations (Table 3.). There was 5 times more ammonium available in the system at the Wells Reserve marsh compare to the Scarborough marsh. The phosphorus concentrations in the fertilized treatment were similar between the two marshes. However, the overall ambient levels of phosphorus found at the Wells Reserve were significantly lower than the ones sampled at Scarborough (Figure 4.). Phosphorus could be a limiting factor at the Wells reserve. The location and fertilizer treatment did not have an effect on the N/P ratios analyzed (Table 4.). All the ratios seem to fall between 14 and 16, indicating a possible N and P co-limitation or a phosphorus limitation or a nitrogen limitation (Koerselman & Meuleman 1996; Tessier & Raynal 2003). A two-way nested ANCOVA showed that there was a significant difference in *S. alterniflora* final height between the two locations (Table 5). The analysis also revealed that there was a significant interaction between location and fertilizer effects on the *S. alterniflora* final height (Figure 5). The analysis for *S. alterniflora* final height data showed that there was a significant difference in *S. alterniflora* final height between the two locations; the plants in the unfertilized enclosures at the Wells reserve were on average 20 cm taller than the plants found at Scarborough (Table 6.).

## Discussion

The results suggest that top-down interactions do not have any effect on the bottom-up processes in northern salt marshes. My experiments revealed that the trophic interactions (top-down effects) between *C. maenas*, *L. littorea* and the macroinfaunal community did not have any impact on the primary productivity of salt marshes. Both predatory crab risk cues and presence of snails did not have a direct effect on the *S. alterniflora* aboveground growth. Recent studies conducted in southern marshes have clearly demonstrated that top-down forces can be important driving forces shaping the community dynamic in salt marshes (Silliman & Zieman 2001). Experiments conducted by Silliman (2001, 2002) clearly demonstrated that plant biomass can be controlled by grazers and their predators; when snails were exposed to predatory risk cues their grazing pressure was greatly reduced and plant biomass increased. However, in salt marshes found in southern Maine, the species composition is different and I did not observe the same interactions. There is no evidence of grazers actively foraging on *S. alterniflora*. In presence of the predatory crab (*C. maenas*), *L. littorea* seek shelter by climbing higher on the *S. alterniflora* but do not injure or forage while on the plant. Contrary to my observations, Bertness (1984) suggested that the removal of *L. littorea* at the leading edge of *S. alterniflora* resulted in an expansion of the plant. In his study, conducted on Mount Hope Bay in Rhode Island in the early 1980's, he found that *L. littorea* actively grazed on *S. alterniflora* rhizomes and restricted its growth (Bertness 1984). However, in my experiment conducted in southern Maine, I did not find any evidence of *L. littorea* grazing on *S. alterniflora*; snails tend to forage on the diatoms/algae mat growing at the surface of the sediment adjacent to the *S. alterniflora* zone (Personal observation). All the analyses indicate that the top-down forces at these locations do not play an important role in controlling the primary production of the marsh. Both crabs and

snails tended to use the marsh as a hiding place during low tide, seeking shelter from potential avian predators and physical stress such as desiccations (personal observations). The top-down interactions observed in this system indicated that the crab risk cues had a positive indirect effect on the macroinfaunal community; when crab risk cues were present, I observed a greater macroinfaunal density and diversity (Chapter 1). I originally hypothesize that the observed increase density of macroinfaunal, in presence or crab risk cues, would lead to a greater nutrient availability positively influencing *S. alterniflora* growth. Macroinfaunal organisms are important bioturbators; by moving through the sediment they increase the water exchange allowing oxygen and other nutrients into the sediment (Aller 1982; Kirstensen 1985). However, the increased macroinfaunal density did not directly benefit the primary production. The top-down interactions observed in this did not enhance the bottom-up processes.

Nutrients availability seems to play an important role in controlling the primary productivity of southern Maine salt marshes. The Wells (2008) experiment results indicate that the ammonium concentrations sampled in fertilized enclosures were 16 times greater than the concentrations found in the unfertilized enclosures. However, the plants did not respond to the fertilizer; aboveground plant growth was not significantly different between fertilize and unfertilized treatment. Previous experiments looking at the effects of added fertilizer in marshes have shown that plants' growth respond positively to added nutrients, especially nitrogen (Valiela & Teal 1974; Valiela et al. 1978; Mendelssohn 1979; Morris 1982). Historically, nitrogen has been characterized has a limiting factor in coastal waters (Boesh et al. 2001; Conley 2000; Fisher et al. 1988; Howarth & Marino 2006). In the system, the application of nitrogen did not stimulate plant growth. The enclosures used in the experiment were anchored on the mudflat/*S. alterniflora* tall-form border and were located near the mouth of the river less than

100 yards from the Atlantic Ocean. The enclosures were flooded twice daily by the tides and exposed to a constant influx of ocean water. The ambient nutrient concentrations, at this location, were already elevated (Personal observation). Water analyses conducted by the Wells Estuarine Research Reserve laboratory also revealed high levels of nutrients sampled in the marsh near the ocean intake. The plants in the enclosures were already exposed to high levels of nitrogen, adding more nutrients did not enhance their growth. Smart and Barko (1980) conducted a green house experiment, where *S. alterniflora* was cultured in sediments under conditions of nitrogen and phosphorus limitation to establish the critical tissue concentrations of these elements needed for the plants to grow. They established that the critical concentration of nitrogen needed for plants to grow is  $0.73 \pm 0.7 \%$  and the critical concentration of phosphorus needed was  $0.044 \pm 0.005 \%$ ; the critical concentration will only be exceeded when the availability of the nutrients exceeds the requirements of the plants. In the experiment, the average nitrogen concentrations in tissue samples was  $2.19 (\pm 0.07)\%$  for the fertilized plants, whereas the unfertilized plants had on average  $1.69 (\pm 0.07)\%$ . Nitrogen concentrations analyzed in plants from the experiment were all above the critical concentration of nitrogen needed to grow indicating ample nutrient availability in the system. For most plants nitrogen demands usually increase as plant size increases, in order to add new tissue plants need more nutrients (Smart and Barko 1980). All the plants in this study showed positive growth throughout the field season, even if the fertilizer treatment had no direct effect. The nitrogen tissue concentrations results indicate that the nitrogen was available to be allocated towards growth. The plants had the necessary nutrients for growth and neither nitrogen nor phosphorus did appear to be a limiting factor at the Wells Reserve. These results indicate that nitrogen limitation alone may not be the only factor influencing the primary productivity. Other factors could have an impact on the plant

growth; Valiela et al. (1974) said that nitrogen-limited *Spartina* shows increased growth following a substantial rainfall. A number of studies have shown that high salinity can impair plant growth (Ranwell et al. 1964; Mendelssohn 1979). Increased water exchange at the edge of the marsh may decrease the salinity stress by flushing the extra salts from the sediments and by providing additional nitrogen supplies.

Subsequently, I conducted a similar experiment at another location in 2010. The Scarborough River marsh was chosen primarily because the ambient nutrient concentrations were much lower than at Wells. Allowing me to also investigate if the top-down interactions would have an effect on the bottom-up processes in a low nutrients system. The nitrogen concentration in porewater samples at Scarborough was much lower ( $10.24 \pm 2.29$  ( $\mu\text{mol/L}$ )) compare to Wells ( $53.05 \pm 35.32$  ( $\mu\text{mol/L}$ )), indicating a potential N limiting factor. The results from this experiment indicate that the top-down forces had no significant effects on the primary productivity, similarly to what I had previously observed at the Wells Reserve. The trophic interactions observed between *C. maenas*, *L. littorea* and the macroinfaunal community had no impact on both the *S. alterniflora* growth and nutrient availability. The increased bioturbation caused by the macroinfaunal community did not enhance the *S. alterniflora* growth. However, the application of fertilizer did have a positive impact on *S. alterniflora* aboveground growth. The application of fertilizer resulted in a significant increase in plant growth, furthermore supporting my initial hypothesis that Scarborough marsh was nitrogen limited. The final plant heights sampled at Scarborough, in the fertilizer treatment, were similar to the Wells reserve plants. However, the average percentages of nitrogen in plants from fertilized enclosures was  $2.92 (\pm 0.06)\%$  and the unfertilized plants had on average  $2.51 (\pm 0.06)\%$ , which indicates that all plants had adequate amount of nutrients for growth even the plants in the unfertilized

enclosures. These results indicate that nitrogen limitation alone may not be the only factor influencing the primary productivity.

The N:P ratios calculated for both experiments showed that all the values were between 14 and 16 which indicates that neither N or P can be limiting or they can be co-limiting (Koerselman & Meuleman 1996). N:P ratios have recently been used as indicators of nutrient availability in several systems and wetlands were found to be P limited or co-limited by P and N, whereas marshes have been found to have lower N:P ratios and considered N limited (Bedford et al. 1999). The Wells reserve plants did not respond to added fertilizer and the ambient levels of nutrients were generally high, conversely the plants in Scarborough marsh positively responded to the nutrient application and appeared to be nitrogen limited.

The analysis for *S. alterniflora* final height data showed that there was a significant difference in *S. alterniflora* final height between the two locations; the plants tagged and measured at the Wells reserve were taller than the plants at Scarborough. However, when fertilized plants at Scarborough grew to the heights observed at the Wells reserve supporting, furthermore supporting the idea that Scarborough is nitrogen limited.

With the increasing appreciation of the significance of the interactions of top-down forces and bottom-up processes on ecosystems functioning it is becoming clear that these effects have important community impacts in various systems including salt marshes. However, my experiments seem to indicate that in northern Atlantic salt marshes bottom-up processes are the dominant factors controlling the primary productivity. My experiments demonstrated how primary productivity was affected by the addition of fertilizer in marshes with low levels of ambient nutrients. These experiments highlight the importance of better understanding how

nutrients such as nitrogen and phosphorus still play an important role in shaping northern salt marsh communities.

## Tables

Table 1. Summary of ambient levels of ammonium ( $\mu\text{mol/L}$ ) and phosphate ( $\mu\text{mol/L}$ ) sampled at two locations: the Wells National Estuarine Research Reserve and Scarborough River marsh both located in southern Maine.

Location	Ammonium ( $\mu\text{mol/L}$ )	Phosphate ( $\mu\text{mol/L}$ )
Wells National Estuarine Research Reserve	53.05 +/- 35.32	2.14 +/- 1.26
Scarborough River marsh	10.24 +/- 2.29	4.11 +/- 0.71

Table 2. Summary of three-way ANCOVAs on the effect of crab risk cues (crab, no-crab), snail (present, absent) and fertilizer (fertilize, unfertilized) on *S. alterniflora* growth at Scarborough in 2010.

Source	df	SS	F	P
Crab	1	306.952	0.7452	0.3948
Snail	1	0.18523	0.0005	0.9831
Fertilizer	1	8121.92	19.7321	0.0001
Crab X Snail	1	8.53379	0.0207	0.8865
Crab X Fertilizer	1	550.171	1.3480	0.2546
Snail X Fertilizer	1	846.006	2.0540	0.1620
Crab X Snail X Fertilizer	1	185.527	0.4511	0.5069
Rep [Crab X Snail X Fert.] & random	31	12491.8	2.9119	<.0001
Initial height (cm)	1	4716.67	34.0841	<.0001
Crab X Snail X Fert. X Ini. height	1	274.356	1.9826	0.1611

Table 3. Summary of two-way ANOVAs on the effect of fertilizer (fertilize, unfertilized) on ammonium and phosphorus concentrations sampled at two different locations (Wells (2008), Scarborough (2010)).

Source	df	SS	F	P
<i>Log Ammonium (<math>\mu\text{mol/L}</math>)</i>				
Location	1	9.0023	33.5636	<.0001
Fertilizer	1	15.31	57.07	<.0001
Location X Fertilizer	1	2.004	7.47	0.0078
<i>Log Phosphorus (<math>\mu\text{mol/L}</math>)</i>				
Location	1	0.360	2.952	0.0898
Fertilizer	1	1.287	10.5434	0.0017
Location X Fertilizer	1	0.48970	4.013	0.0487

Table 4. Summary of two-way nested ANOVAs on the effect of fertilizer (fertilize, unfertilized) on N/P ratios analyzed at two different locations (Wells (2008), Scarborough (2010)).

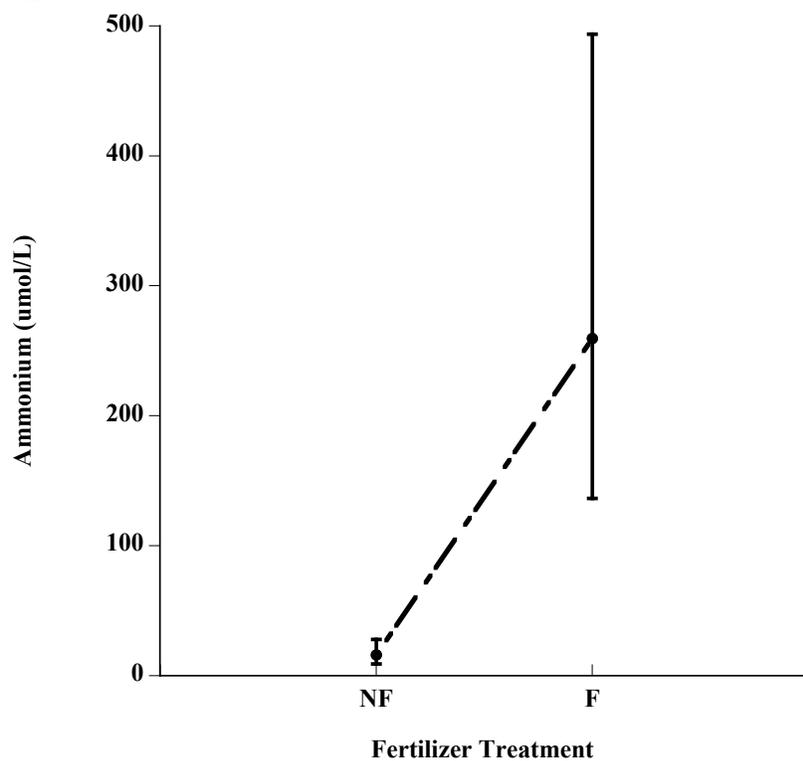
Source	df	SS	F	P
Location	1	25.2106	0.3821	0.5451
Fertilizer	1	1.43746	0.0218	0.8845
Location X Fertilizer	1	11.3096	0.1714	0.6843
Replicate [Location X Fertilizer] & Random	16	1057.02	1.6447	0.0596

Table 5. Summary of two-way nested ANCOVAs on the effect of fertilizer (fertilize, unfertilized) on *S. alterniflora* final height analyzed at two different locations (Wells (2008), Scarborough (2010)).

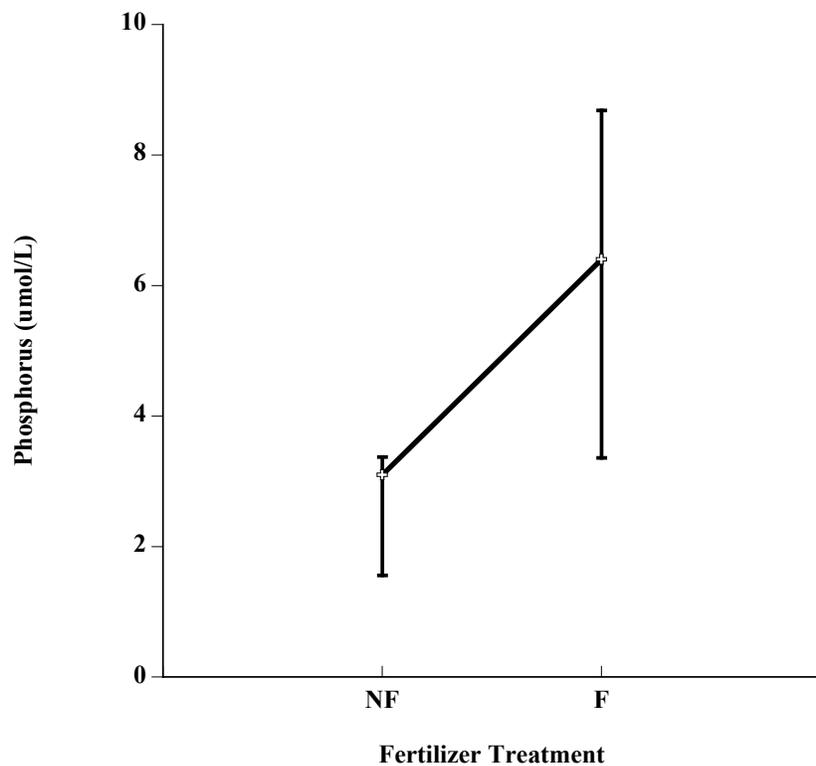
Source	df	SS	F	P
Location	1	12829.5	46.3657	<.0001
Fertilizer	1	2605.54	9.4164	0.0053
Location X Fertilizer	1	3713.3	11.9839	0.0034
Replicate [Location X Fertilizer] & Random	16	4904.27	1.5886	0.0692
Initial height	1	32032.4	166.0161	<.0001
Initial height X Location X Fertilizer	1	196.292	1.0173	0.3138

Table 6. *S. alterniflora* final average heights measured in presence and absence of fertilizer at two locations: the Wells National Estuarine Research Reserve (2008) and Scarborough River marsh (2010) both located in southern Maine.

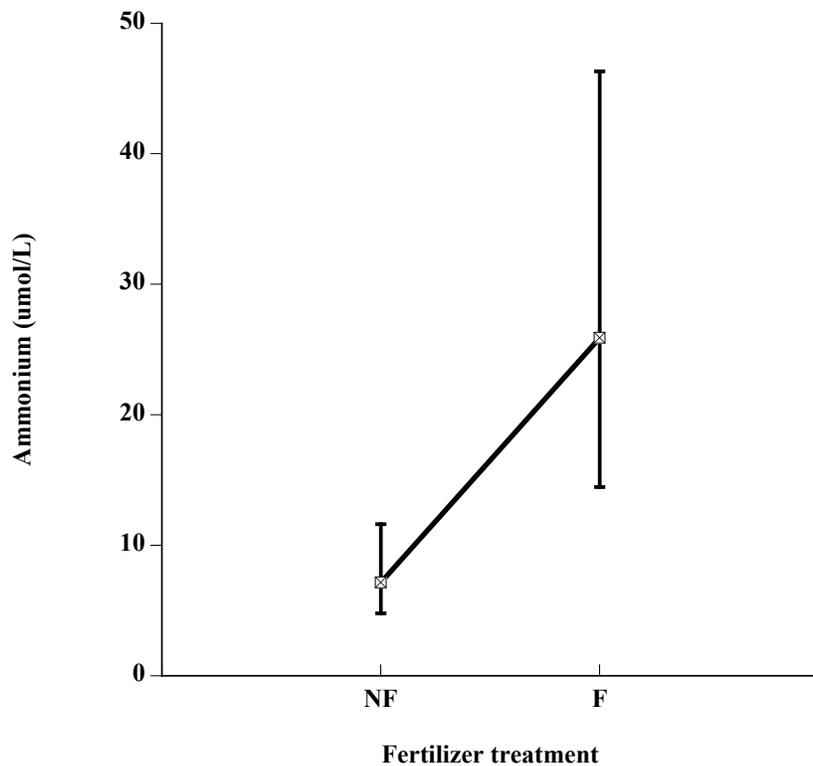
Fertilizer Treatment	Wells	Scarborough
Fertilize	106.38 +/- 2.02	98.93 +/- 1.86
Unfertilized	106.45 +/- 1.93	86.19 +/- 3.47

**Figures**

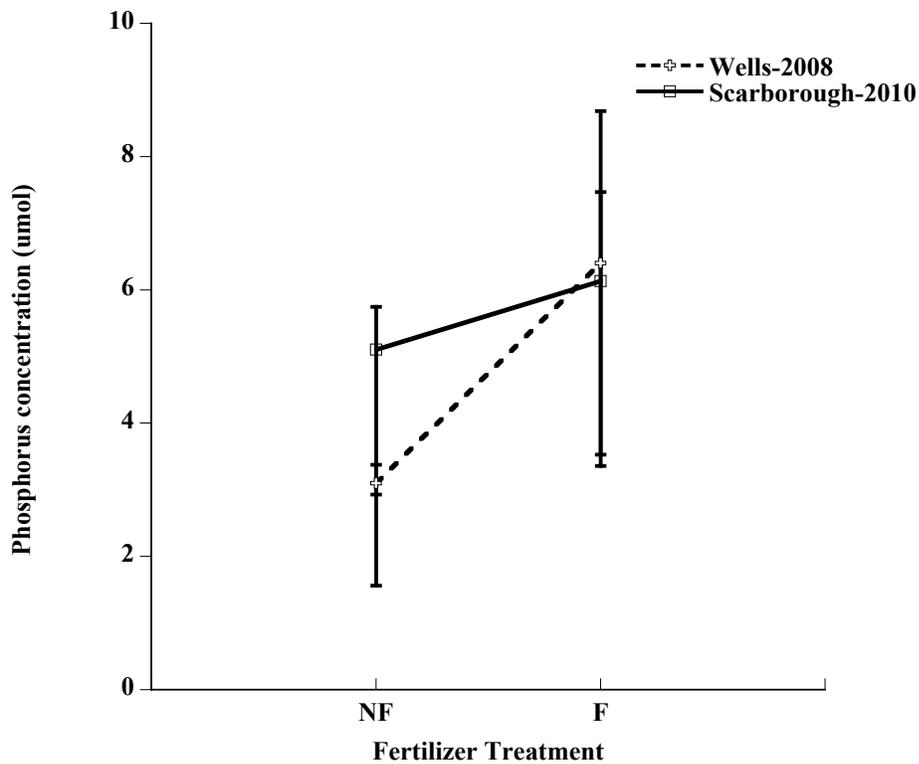
**Figure 1.** Porewater ammonium ( $\mu\text{mol/L}$ ) concentrations for the fertilized (F) and unfertilized (NF) treatment sampled at the Wells Reserve (2008). Analyses performed on  $\log_{10}$  transformed data; reverse-transformed means ( $\pm$  95% confidence intervals) are presented for clarity.



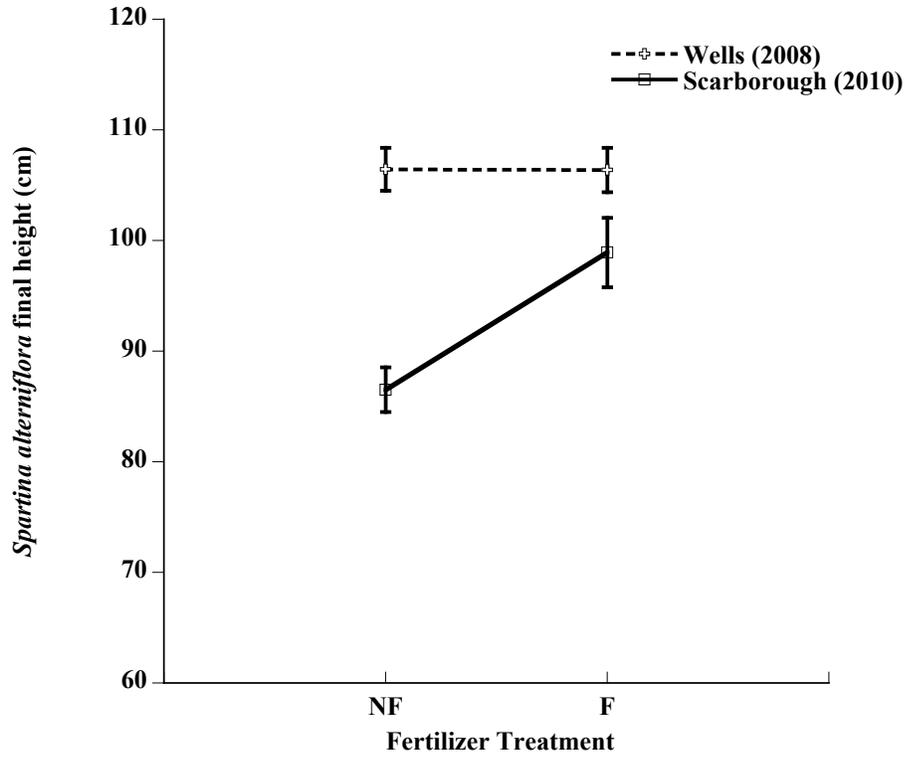
**Figure 2.** Porewater phosphorus ( $\mu\text{mol/L}$ ) concentrations for the fertilized (F) and unfertilized (NF) treatment sampled at the Wells Reserve (2008). Analyses performed on  $\log_{10}$  transformed data; reverse-transformed means ( $\pm$  95% confidence intervals) are presented for clarity.



**Figure 3.** Porewater ammonium ( $\mu\text{mol/L}$ ) concentrations for the fertilized (F) and unfertilized (NF) treatment sampled at Scarborough (2010). Analyses performed on  $\log_{10}$  transformed data; reverse-transformed means ( $\pm$  95% confidence intervals) are presented for clarity.



**Figure 4.** Porewater phosphate ( $\mu\text{mol/L}$ ) concentrations for the fertilized (F) and unfertilized (NF) treatment at two different locations (Wells (2008), Scarborough (2010)). Analyses performed on  $\log_{10}$  transformed data; reverse-transformed means ( $\pm$  95% confidence intervals) are presented for clarity.



**Figure 5.** *S. alterniflora* final height (cm) for the fertilized (F) and unfertilized (NF) treatment at two different locations: Wells (2008) and Scarborough (2010).

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