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Basal Food Web Dynamics in a Natural Eelgrass (Zostera marina) Community: Cage-Free Field Experimentation

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Basal Food Web Dynamics in a Natural Eelgrass (*Zostera marina*) Community: Cage-free Field Experimentation

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APPROVAL SHEET

This thesis is submitted in partial fulfillment of

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ABSTRACT

The relative strength of bottom-up and top-down processes operating within food webs is a fundamental determinant of community structure and function. In marine systems, inconspicuous but often highly abundant invertebrate herbivores (mesograzers) are implicated as strong consumers of primary production and important prey for higher-order consumers. Because of their small size, however, mesograzer abundance is not easily manipulated in the field, which limits our ability to adequately assess their grazing impacts. Seagrass systems present a pressing need for the study of food web dynamics because anthropogenic nutrient and sediment inputs decrease the amount of light reaching seagrass leaves, which limits the depth distribution of seagrasses via reduced photosynthesis to respiration ratios. Mesograzers benefit seagrass through their consumption of epiphytic algae and thus may mitigate the loss of seagrass beds due to nutrient enrichment. I test the relative impacts of nutrient enrichment and crustacean mesograzer abundance on epiphytes in a natural seagrass bed without using cages. My work presents the first cage-free tests of crustacean mesograzing impacts in natural seagrass communities. I successfully decreased crustacean abundance for extended periods of time in multiple experiments using a degradable chemical deterrent. Crustacean mesograzer reduction led to concomitant increases in epiphytic algal biomass, while nutrients increased epiphytes only in the absence of mesograzers. My results validate early work from mesocosm and field cage studies designed to test grazing impacts of mesograzers and support the hypothesis that mesograzers indirectly benefit seagrass through a positive indirect interaction.

Basal food web dynamics in a Chesapeake Bay seagrass community: field experimentation

INTRODUCTION

A fundamental question in ecology is how bottom-up and top-down processes acting within food webs determine the structure and function of ecological systems. Bottom-up processes are mediated by the availability of abiotic and biotic resources accessible for trophic transfer to organisms of increasing trophic level (Strong 1992). Top-down processes are the combined direct and indirect effects of consumption that permeate food webs (Hairston et al. 1960, Menge 1995). Bottom-up and top-down forces are both influential and act simultaneously (Hunter and Price 1992, Power 1992). A key remaining problem is to determine the relative strength of bottom-up and top-down processes in different systems and to predict how perturbations in these processes change community structure and function (Hunter and Price 1992, Hughes 1994, Jackson et al. 2001, Worm et al. 2002, Borer et al. 2005, 2006, Worm and Lotze 2006, Gruner et al. 2008).

Herbivores, as primary consumers, occupy a central position in food webs. They link primary producers and predators, providing a conduit through which bottom-up and top-down effects are conveyed. Experimental evidence suggests that nutrient availability affects plant biomass across many systems, but that bottom-up effects attenuate rapidly in many systems, often not affecting herbivores (Brett and Goldman 1997, Borer et al. 2006). Top-down effects of predation, on the other hand, more often set up trophic cascades in which consumption of herbivores results in increased plant biomass. Trophic cascades tend to be strongest in aquatic systems, particularly the marine benthos, which is dominated by invertebrate herbivores (Shurin et al. 2002, Borer et al. 2005, 2006, Gruner et al. 2008).

The implication of invertebrate herbivores in strong trophic cascades confirms these herbivores have strong density-dependent top-down effects on producers (Paine and Vadas 1969, Cyr and Pace 1993, Borer et al. 2005). One group of invertebrates that likely has strong top-down effects in natural systems but remains poorly understood ecologically is the mesograzers. As defined by Brawley (1992), mesograzers are "invertebrate grazers…larger than the average copepod but smaller than *c.* 2.5 cm" (p. 235) and include herbivorous arthropods, molluscs, and polychaetes. Mesograzers often utilize macrophytes (vascular plants and macroalgae), many of which they consume, for habitat and refuge from predators (Jernakoff 1996, Duffy and Hay 2000, Taylor and Brown 2006). Mesograzers can have large impacts on marine ecosystems through consumption of primary production, trophic transfer to near shore predators, and biogeochemical cycling (Kikuchi 1974, Edgar and Aoki 1993, Edgar and Shaw 1995,

Taylor and Rees 1998, Heck et al. 2000, 2006, Bracken et al. 2007). Mesograzers are highly abundant primary consumers in many marine systems and have been implicated in a number of trophic cascades (Brawley 1992, Duffy and Hay 2000, Duffy et al. 2005, Davenport and Anderson 2007).

Of the food webs featuring mesograzers, those founded on seagrasses are among the most ecologically and economically valuable. A better understanding of seagrass food web dynamics is urgently needed because of the ecological significance and rapid global decline of seagrass systems (Orth et al. 2006). Seagrasses are widespread marine vascular plants, occurring in coastal areas of all the continents except Antarctica (den Hartog and Kuo 2006). They are foundation species (*sensu* Dayton 1975, Bruno and Bertness 2001) in that they create relatively stable three-dimensional structure, which provides habitat for dense algal and animal populations (Williams and Heck 2001). They also provide many ecosystem services to humans (Constanza et al. 1997), including shoreline protection and nursery grounds for commercially important species (Kikuchi 1974, Gillanders 2006, Koch et al. 2006).

Seagrass systems are highly productive, yet herbivores directly consume relatively little seagrass production (Cebrián 1999). Rather, mesograzers consume primarily epiphytic algae and seagrass detritus (Brawley 1992, Jernakoff et al. 1996). In temperate systems, where direct grazing is weaker than in tropical systems and currently weaker than in the past (Madsen 1998, Domning 2001), mesograzers are the dominant herbivores (Valentine and Duffy 2006). A simplified view of the base of a temperate seagrass food web consists of two primary producer groups, macrophytes and epiphytic algae, and an assemblage of mesograzers (Figure 1). Seagrass provides structural habitat for epiphytes and mesograzers, epiphytes negatively affect seagrass through exploitative competition, while mesograzers indirectly benefit seagrass through their consumption of epiphytes (Jernakoff et al 1996, Valentine and Duffy 2006).

While the evidence suggesting that bottom-up effects attenuate rapidly in food webs is compelling, it is compiled from the results of many small-scale and short-term experiments (Borer et al. 2006). Based on observational studies, however, bottom-up effects are known to propagate up in marine food webs and influence fisheries yields (Aebischer et al. 1990, Nixon and Buskey 2002, Ware and Thomson 2005, Oczkowski et al. 2009). Even if bottom-up attenuation is a general trend across systems, the effects of nutrient supply to marine systems can have repercussions for the structure of established communities. This is true of coastal vegetated systems, where anthropogenic nutrient inputs favor bloom-forming algae (Valiela et al. 1997, Vitousek et al. 1997, Tefwik et al. 2005, but see Heck and Valentine 2007). Seagrass ecosystems are a case in point, where nutrient and sediment inputs threaten the persistence of entire communities (Short and Wyllie-Echeverria 1996). These inputs indirectly (via algal blooms) or directly (via turbidity) limit the amount of light that reaches seagrass leaf surfaces and ultimately decrease the depth distribution of seagrasses. Top-down control of algae by invertebrate

herbivores may help mitigate the loss of these systems in the face of global coastal change (Valentine and Duffy 2006, Andersson et al. 2009).

There is growing evidence that mesograzers have strong grazing impacts in seagrass and macroalgal systems, but our evidence is hampered by methodological constraints. Due to their small size and mobility, the grazing impacts of mesograzers, particularly crustaceans, are difficult to study in natural settings using traditional methods such as caging. Observational field studies that survey mesograzer communities describe patterns of plant and animal distributions in time and space (Marsh 1973, Nelson et al 1982, Edgar 1990b, Douglass et al. 2010), but they do not address the roles of mesograzers directly.

A variety of venues have been used in which to experimentally study mesograzers including laboratories, outdoor mesocosms, and in field cage exclosures/enclosures. It is unclear whether any of these venues accurately reflects field conditions, and thus I may lack the ability to definitely assess the role of mesograzers in natural settings.

A number of field studies suggest that mesograzer populations are limited by predation (Nelson 1979, Heck and Orth 1980, Stoner 1980, Orth et al. 1984, Duffy and Hay 1991, Taylor and Brown 2006). In a field cage experiment, Edgar and Aoki (1993) found that mesograzer production and biomass within enclosures reached similar levels irrespective of treatment (faunal composition and time of deployment). They suggested that mesograzer production in the field is limited chiefly by the amount of available food resources, which counters the proposition that bottom-up effects attenuate in marine systems (Borer et al. 2006) and contrasts with observational field studies that invoke predators as the main governor of mesograzer population sizes. Furthermore, greater quantity and quality of epiphytes in nutrient-induced blooms can support higher densities of mesograzers, which can increase top-down control and channels excess algal productivity up the food web (Moksnes et al. 2008, Spivak et al 2009b). Because mesograzers both influence plants and are influenced by them, important feedbacks may exist that determine dynamics of plant-herbivore interactions in time and space.

Laboratory experiments have been used to investigate mesograzer behavior, feeding preferences, grazing rates, and reproduction, but they often fail to address the contributions of mesograzers to natural communities (Duffy and Hay 1991, references in Brawley 1992). Using data from a laboratory experiment, Andersson et al. (2009) developed a model to predict the density of a common amphipod mesograzer (*Gammarus locusta*) required to control bloom-forming macroalgae (*Ulva* spp.) off the Swedish coast. They predicted that *G. locusta* was able to control macroalgal blooms at high densities, but that predation would limit grazer control. Changes in higher trophic levels, therefore, may also influence the balance between seagrasses and epiphytes through top-down effects on the mesograzer community (see Heck and Valentine 2006).

Mesocosm experiments have been widely employed because they approximate field conditions and allow for controlled manipulations of organisms not possible in field (e.g. Duffy and Hay 2000). Such studies clearly demonstrate the ability of mesograzers to control the growth of epiphytes on seagrasses, the role of biodiversity at various levels (e.g. mesograzer species richness, food-chain length), and important ecosystem functional responses to changes in biotic and abiotic conditions (Duffy and Hay 1991, Duffy et al 2003, 2005, France and Duffy 2006, Douglass et al 2008, Blake and Duffy 2010). The ability of mesocosms to recreate nature, however, is disputed (see Carpenter 1996, 1999, Drenner and Mazumder 1999, Skelly 2002, 2005, Chalcraft et al. 2005), and thus the validity of results from mesocosm experiments can come into question. While mesocosms allow the investigator to relatively rapidly assess effects of mesograzers at carrying capacity over multiple generations (Duffy and Harvilicz 2001), mesocosms may inflate carrying capacities observed in nature, increase food availability, and alter predator-prey interactions. The closed nature of mesocosms prevents dispersal of mesograzers and potentially results in unnaturally high densities of mesograzers (but see France and Duffy 2006). The walls of the mesocosms increase the surface area of the system and provide additional structural habit for floral and faunal settlement (reviewed in Englund and Cooper 2003). Predation effects may also be inflated due to the inability of prey to escape. More importantly, mesocosms fundamentally alter spatial dynamics of predator-prey interactions by placing highly motile predators into relatively small closed systems, which limits the experimenter's ability to adequately assess predation impacts. Finally, mesocosm studies tell us about potential effects (e.g. of diet, grazing rate, population growth) rather than realized effects in natural communities. Mesocosms are useful for conducting controlled experiments, but artifacts must be taken into account and care must be taken when extrapolating results to natural systems.

Field cage studies have addressed many of the same questions as mesocosm studies, namely the influence of bottom-up and top-down forces on the role of mesograzing in vegetated communities through the exclusion or enclosure of predators or mesograzers (predators – Nelson 1979, references in Brawley 1992, Heck et al. 2000, Heck et al. 2006, Davenport and Anderson 2007; mesograzers – Douglass et al 2007, Moksnes et al. 2008, Andersson et al. 2009, Spivak et al. 2009a). These studies indicate that nutrients and mesograzers can have measurable effects on communities, and they emphasize the importance of predators, omnivores, and sessile animals in more complex experiments.

Like mesocosms, cage experiments also suffer from artifacts (see Virnstein 1978, Hall et al. 1990). Although most experimenters account for cage effects on water flow and light regime using cage controls (open cages or partial cages), these controls continue to modify the environment (Miller and Gaylord 2007) and the behavior of animals (Steele 1996, Connell 1996). Cages (including cage controls) can attract organisms that would not have the same representation in treatments with no cages, including algae, mesograzers, and predators (Steele 1996, Douglass et al 2007, Moksnes et al. 2008). Cage experiments designed specifically to exclude or enclose mesograzers require the use

of very small mesh sizes, which easily become compromised in field conditions and likely compound the problems associated with water flow and light regime disruption (Virnstein 1978).

A novel method for reducing mesograzers *in situ*

Laboratory, mesocosm, and field cage studies have greatly improved our understanding of the consumptive role of mesograzers in model food webs. Much of this work, unfortunately, lacks definitive confirmation in the field. In the current study, I attempt to overcome the constraints of closed-system methods and bridge an important knowledge gap by experimentally testing the role of seagrass mesograzers in the field without using cages for the first time. Furthermore, I address the need to better understand the top-down and bottom-up forces shaping seagrass ecosystems by simultaneously manipulating mesograzer density and nutrient input in factorially-designed field experiments.

Poore et al. (2009) developed the first successful attempt to manipulate mesograzer abundance in the field without the use of cages. They employed a novel technique in which the insecticide carbaryl (1-naphthyl n-methylcarbamate) was incorporated into a plaster matrix, which slowly releases the insecticide in water and allows long-term exclusion of mesograzers in the field. While Poore et al. (2009) effectively reduced mesograzer densities in the field, mesograzer reduction did not result in responses of primary producers. Adapting the mesograzer reduction technique of Poore et al. (2009), I experimentally investigate the roles of mesograzers and nutrient inputs in determining the growth and abundance of primary producers in a natural seagrass system. My goals are 1) to evaluate the ability of mesograzers to influence algal epiphytes in the field, 2) to evaluate the interaction between grazers and epiphytes under different nutrient conditions, 3) to assess the relative importance of bottom-up and top-down forces in determining epiphyte accumulation, and 4) to identify if competitive interactions between epiphytes and eelgrass can be demonstrated in the field.

I first hypothesize that mesograzer reduction increases epiphyte accumulation in the field. I next hypothesize that nutrient fertilization increases mesograzer density through its effects on algal productivity and quality, yielding no net effect of nutrient enrichment on epiphytes under ambient grazing pressure (see Spivak et al. 2009). When fertilization and mesograzer reduction are combined, however, I expect a synergistic effect in which epiphyte accumulation is greater than that induced by mesograzer reduction alone. I finally hypothesize that grazing increases seagrass performance (e.g. vegetative growth) through a positive indirect interaction.

METHODS

Carbaryl is a reversible acetylcholinesterase inhibitor. Acetylcholinesterase (AChE) is a key enzyme that breaks down acetylcholine (ACh), an important neurotransmitter in animals, including invertebrates and humans. Carbaryl prevents AChE from breaking down ACh, which then builds up in the nervous system. This can cause continuous muscle contraction, possibly leading to uncontrolled movement, paralysis, convulsion, and death.

Carbaryl was designed to combat terrestrial arthropods and remains widely used in home and garden market sectors of the United States (EPA 2011). Carbaryl is also very effective against aquatic arthropods, which explains its long-term use in controlling burrowing shrimp, pests to intertidal oyster aquaculture in the U.S. Pacific Northwest (Dumbauld et al. 2006). The continued use of carbaryl is this system, however, speaks to the ability of the target organisms to recover from chemical disturbance, and other studies confirm that the use of carbaryl in water has limited effects on non-target taxa (Weis and Weis 1974, Duffy and Hay 2000, Dumbauld et al 2001). Carbaryl is toxic to fishes, but toxic concentrations are an order of magnitude higher than those toxic to crustaceans (Gunasekara et al. 2008). Mammals are at even lower risk from carbaryl because the compound is rapidly broken down internally and then excreted (Tomlin 2000). Repeated exposure at high levels can adversely affect humans, although most problems are associated with people who mix the chemical for large-scale agricultural purposes (chemical sprayers) and those who improperly store and handle carbaryl at home (Gunasekara et al. 2008).

Poore et al. (2009) developed a novel method for controlling mesograzer density in the field using a chemical deterrent rather than cages. I conducted 4 experiments using the grazer reduction methods developed by Poore et al. (2009). In all 4 experiments, wettable carbaryl powder (80% carbaryl by weight) was mixed with cold water and subsequently incorporated into dental plaster. I poured the wet mixture into 100 ml molds and inserted a loop of stainless steel wire into each mold to allow attachment to other objects. Plaster blocks were allowed to harden overnight after which time they were removed from molds and dried in a 60°C drying oven for 3 days. Two experiments tested the effectiveness of the method and two experiments factorially manipulated grazer reduction and nutrient fertilization to investigate top-down and bottom-up processes in a natural seagrass bed. These experiments represent the first field manipulations of seagrass mesograzers that do not employ cages.

TESTING THE EFFICACY OF GRAZER REDUCTION IN THE FIELD

In order to test my hypotheses concerning the role of mesograzers in natural seagrass beds, I first conducted two pilot experiments designed to determine the effective range of the grazer reduction method developed by Poore et al. (2009). In both experiments, I manipulated chemical deterrent and measured epifaunal biomass over space and time of exposure to experimental treatments. In both experiments I used artificial substrates (plastic kitchen scrubbers, hereafter scrubbers) to attract and collect epifauna. Scrubbers were made of bunches of plastic mesh that provided small crevices for small epifauna to colonize. Epifauna that recruited to scrubbers were collected and weighed to provide an estimate of epifaunal biomass (grams wet weight).

Experiment 1 – Concentration, Distance, and Time Experiment

I designed a field experiment to determine the effective distance of grazer reduction by carbaryl and to test the effective lifetime of a single block. I tested the effects of two concentrations of carbaryl on epifaunal biomass at four distances from a source and over two durations of treatment exposure. I conducted the experiment from 12 May 2009 to 20 May 2009 in the lower York River on a stretch of shallow, sandy bottom habitat near the Virginia Institute of Marine Science (Virginia, USA, 37° 15' N, 76° 30' W).

Experimental plots were created using 8 scrubbers attached to construction fencing in two parallel rows (Figure 2). Each row contained four scrubbers at 10, 30, 60, and 100 cm from a single point to which a plaster block could be attached. I deployed experimental plots directly on the sediment surface at equal depth (~ 1 m at high tide). The orientation of each plot was randomly assigned to one of eight 45 degree angles relative to north and treatments were randomly assigned to alongshore locations. Based on Poore et al. (2009), I separated plots from one another by 2 meters.

I tested the effectiveness of two grazer reduction treatments, high (10 % carbaryl by dry weight unmixed plaster) and low (3.3% by dry weight). In the high reduction treatment, 5 blocks were made with 55.5 g carbaryl, 222 ml water, and 555 g dental plaster. Low reduction blocks were made with 18.5 g carbaryl (one-third the dry-weight concentration of the high treatment) and the same amount of water and plaster. I included two control treatments; one used a plaster block without carbaryl and one used no block. Control blocks were made the same way as carbaryl blocks, but without the addition of carbaryl. Five replicates of each of 4 treatments resulted in 20 total experimental plots (Table 1).

I sampled mesograzers by removing one row of scrubbers from each plot 3 days after deployment and the other row 8 days following deployment. Scrubbers were removed from the array by removing each scrubber with a 250 μm filter bag and twisting them off of the construction fencing. The order of removal within a row was randomized

to minimize the effect of disturbance to adjacent scrubbers. All scrubbers were bagged and frozen at -20°C before processing for total epifaunal biomass described above. In addition to collecting biomass data, I identified and enumerated epifauna from a subset of samples.

Data Analysis:

I tested for differences in faunal biomass due to reduction treatments, distances, and time using a partially-nested linear model in which reduction treatment was analyzed between plots and distance and time were analyzed within plots. The model was constructed in R (R Development Core 2011) using the function 'aov', which allows error strata specification for designs with no missing values. Epifaunal biomass was modeled after square root transformation in order to improve normality and homogeneity of variance. Distance was treated as a covariate, and I treated time simply as a two-level factor because I only sampled scrubbers at two dates. All interactions were included in the model. *A priori* contrasts were used to test for differences between grazer reduction and control treatments, between low and high reduction treatments, and between block and no-block control treatments.

Experiment 2 – Distance and Direction Experiment

I conducted a second experiment designed to establish the spatial extent of grazer reduction influence and to inform the spatial design of other field experiments presented below. I tested the effect of a single concentration of chemical deterrent on epifaunal biomass at two distances and four directions from a deterrent source in a natural seagrass bed. I used the high reduction concentration from experiment 1 above and a single control that used a plaster block without deterrent. The two distances I used were 30 cm and 2 m, corresponding to the effective distance of grazer reduction and the distance between experimental plots presented in Poore et al. (2009). I conducted the experiment in a seagrass bed located in the Lower York River (Virginia, USA, 37° 15' N, 76° 25' W). At the time of study, this seagrass bed was a mostly monospecific, subtidal bed of eelgrass (*Zostera marina*), which featured large alongshore areas of equal depth that could accommodate the experiment.

I constructed experimental plots with two 4 m lengths of rope that were arranged perpendicularly and attached at their centers (Figure 3). Scrubbers were attached at the target distances from the center of the array in each of the four directions, resulting in 8 scrubbers per plot. Each treatment (grazer reduction and control) was replicated 4 times. Each plot was installed on the sediment surface and ropes were aligned with the cardinal directions. I arranged plots in a single row of equal depth alongshore and I spaced plots 2 meters from one another.

Data analysis:

I sampled all scrubbers after 7 days of exposure to treatments and I handled samples using the methods described in the previous experiment. Currently, 40 of the 64 total samples have been sorted. I fit linear models in R to run 2-way factorial ANOVA that tested for effects of treatment, distance, and their interaction on epifaunal biomass. Both treatment and distance were treated as two-level factors. Once data collection is completed, I will re-run the analysis with direction as a third factor.

FIELD TESTS OF BOTTOM-UP AND TOP-DOWN FORCES IN A NATURAL SEAGRASS BED

I conducted two experimental tests of bottom-up and top-down processes acting on the base of a natural eelgrass food web. I tested the effects of basal resources and primary consumption, respectively, by experimentally manipulating nutrients and grazers in the field.

Both experiments were conducted at the eelgrass bed used in the Distance and Direction Pilot above, each in a different season (fall and summer). Both experiments utilized the same plot design, which consisted of 3 PVC poles sunk into the sediment and arranged in a triangular array with equal sides of 30 cm (Figure 4). Poles were used to mark sites and to provide attachment points for experimental additions of fertilizer and plaster blocks. I chose a triangular plot shape to maximize grazer reduction under variable water flow conditions. I used the slow-release fertilizer Osmocote™ (N:P = 3:1) to elevate water-column nutrients. In both experiments, 300 grams of fertilizer were placed into perforated PVC tubes following previous work in the same system (Spivak et al. 2009). I also obtained water temperature data from the Virginia Institute of Marine Science's real time data buoy located at 37° 14' N, 76° 25' W, which collects water temperature every 15 minutes. I calculated minimum, maximum, and mean daily water temperature to compare biological and physical trends during each experiment.

Experiment 3 - Fall Nutrient and Grazer Manipulation

For the first experimental manipulation of nutrients and grazers, I used the high grazer reduction concentration from two experiments described above. Fertilization and grazer reduction were factorially manipulated resulting in 4 treatments (control, fertilization, grazer reduction, and fertilization + grazer reduction). The control treatment received a plaster block without chemical deterrent and an empty nutrient diffuser. Each treatment was replicated 10 times.

Experimental plots described above were installed at approximately equal depth in two rows parallel to shore. Each row was separated by 5 meters and individual plots within a row were separated by 2 meters. Each row was considered a block and 5 replicates of each treatment were allocated to each block. Within blocks, treatments were randomly assigned to experimental plots. I ran the experiment for 25 days beginning on 20 October 2008. Nutrient diffusers and plaster blocks were replaced weekly.

In order to quantify effects of fertilization and grazer reduction, I sampled mesograzers and micro-algal epiphytes prior to and during the experiment. I collected preliminary samples 3 days before addition of experimental treatments in between every other pair of experimental plots (i.e. 20 samples collected). During the experiment, I sampled mesograzers at days 4, 11, and 25. Epiphytes were sampled at days 11 and 25. All samples collected during the experiment were taken from the periphery of experimental plots in order to minimize the destructive effects of each sampling technique described below. Furthermore, at intermediate sampling dates (days 4 and 11) I only collected samples from half of the experimental plots chosen randomly within blocks. On each intermediate sampling date, I randomly chose one of three plot sides from which to collect samples and I did not sample from the same side twice. Upon breakdown at day 25, the center of the triangle formed by every experimental plot was sampled for mesograzers and epiphytes.

I sampled mesograzers using an epibenthic grab sampler modified from Virnstein and Howard (1987). This sampler allowed me to collect above-ground seagrass biomass and associated organisms from an area of bottom measuring 20 x 25 cm. All samples were frozen at -20°C before processing. Plant material (e.g. seagrass, macroalgae) was separated, dried at 60°C, and subsequently weighed. It was then combusted at 450°C and reweighed to obtain ash-free dry mass (AFDM). Mesograzers were identified and enumerated to determine mesograzer abundance. Mesograzer abundance was standardized to plant AFDM, resulting in habitat-specific density (hereafter, mesograzer density).

I estimated micro-algal epiphyte biomass using chlorophyll *a* as a proxy. I sampled micro-algal epiphytes by first collecting a single eelgrass shoot from each sampling location. Epiphytes were then scraped off of each shoot and rinsed onto Whatman™ GFF filters using saline water (20 ppt). Eelgrass shoots were retained for surface area measurement. Algal pigments were extracted in 20 ml 90% acetone at -20°C for 24 hours. Extracts were filtered through a 0.45 µm PTFE membrane filter (Millipore™), and absorbance of extracts was measured at 480, 510, 630, 647, and 750 nm using a Shimadzu UV-1650PC spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, MD). I calculated chlorophyll *a* biomass using absorbance values (Lorenzen 1967) and normalized biomass to eelgrass leaf area.

I also sampled water-column nutrients to verify the effectiveness of fertilization treatments. Samples were collected four days after treatment additions by collecting 25 ml of water from the center of the triangle formed by a subset of experimental plots. Water was filtered through a 0.45 µm PTFE membrane filter (Millipore), chilled, then frozen at -20°C until analyzed for NH_4^+, (NO_2^- + NO_3^-), and PO_4^{-3} concentrations using a Lachat QuikChem FIA+, 8000 series autoanalyzer (Hach Company, Loveland, CO). Nutrient analysis did not reveal statistically significant differences between fertilization and control treatments for any nutrient species.

Data analysis:

I tested for effects of fertilization, grazer reduction, and time on mesograzer density and micro-algal epiphytes using 3-way ANOVA. Fertilization and grazer reduction were two-level factors. Linear models were fit in R and marginal sums of squares were used for F-tests of model terms. Preliminary data were not used in the linear models. I only sorted a subset of mesograzer samples from 2 experimental treatments (control and grazer reduction). Because I sampled at unequally spaced time points and because I did not sample every plot on each sampling date, I treated time as a three-level factor with polynomial contrasts to look for trends over time. I analyzed crustacean mesograzer and gastropod mesograzer densities separately because they were predicted to respond differently to chemical reduction (Duffy and Hay 2000).

Experiment 4 – Summer Nutrient and Grazer Manipulation

I conducted a second field manipulation of nutrient and mesograzer at the lower York River eelgrass bed. I used the same experimental plots described above but modified the design to incorporate a procedural control that did not use plaster blocks or nutrient diffusers for a total of five treatments: no-block control, block control, nutrient fertilization, grazer reduction, fertilization + reduction. For grazer reduction treatments, I used one-half of the high reduction concentration used in the preceding experiments, i.e. 5% carbaryl by dry weight unmixed plaster. I ran the experiment in the summer for 38 days starting 18 June 2009. Nutrient diffusers and plaster blocks were replaced weekly.

Mesograzers and epiphytic algae were sampled using the methods described above for the Fall Nutrient and Grazer Manipulation. I conducted a preliminary sampling 3 days prior to treatment addition as described above. Mesograzers and epiphytic algae were sampled from the periphery of every experimental plot at 10, 24, and 38 days after treatment addition. Samples taken at each date were all taken from the same position relative to the triangle formed by experimental units. Each side of the triangle was sampled once. Small predators were sampled on the same days as mesograzers and nutrients.

I quantified two metrics of eelgrass performance, shoot growth and shoot density. Shoot growth was measured using a hole-punching technique (Zieman 1974). Individual shoots were marked in the field and all leaves exposed above the sheath were pierced using a syringe needle. Upon returning a week later, shoots were harvested and new growth was identified as the leaf material between the hole-punch on each leaf and the hole-punch on the oldest, non-growing leaf. The newly grown portions of leaves were removed, dried at 60°C, and weighed to determine dry mass (g DM wk^{-1}), a conservative measure of above-ground eelgrass growth. Because a response in eelgrass growth was expected to lag behind increases in epiphyte fouling due to treatment effects, I began marking shoots after treatments were applied. I marked one shoot from the periphery of half of the experimental plots on 25 Jun 2009. I marked one shoot from the periphery of

every experimental plot on subsequent dates: 2 July 2009, 9 July 2009, 16 July 2009, and 23 July 2009. I quantified shoot density as the number of eelgrass shoots present within experimental plots. I counted shoot density once prior to the experiment and again at the end of the experiment. I calculated proportional change (PC) in shoot density (SD) using the equation, $PC = \frac{SD_{final} - SD_{initial}}{SD_{initial}}$. Numbers are interpreted as the percent change in shoot density from the beginning to the end of the experiment.

In order to prevent resource allocation via rhizomatic tissue from areas of vegetative growth outside of the influence of experimental treatments or between plots, I severed the rhizosphere surrounding a 1 m^2 quadrat that was placed over each plot. I severed the rhizosphere weekly beginning on the first day I marked shoots for growth.

Data Analysis:

1. *Univariate Analysis*

Differences among treatments for mesograzers, micro-algal epiphytes, and eelgrass growth were analyzed using repeated measures ANOVA, in which fertilization and grazer reduction were each two-level factors and within-plot correlations through time were accounted for by fitting models with different correlation structures. Models were fit in R using generalized least squares with the function 'gls' in the package 'nlme' and I selected models using Akaike's Information Criterion (Pinheiro and Bates 2000). Marginal sums of squares were used in F-tests of model terms. I analyzed shoot density using two-way ANOVA to test for the additive and interactive effects of fertilization and grazer reduction.

2. *Structural Equation Modeling*

In addition to univariate methods, I employed structural equation modeling (Grace 2006) to analyze relationships between mesograzers, algae, and eelgrass during the experiment. I hypothesized that 1) macrophytes, composed of eelgrass and two red macroalgae (*Gracilaria* spp. and *Agardhiella* spp.), positively affect mesograzers and epiphytes through habitat provision and 2) mesograzers negatively affect epiphytic algae (Figure 5). My hypotheses directly influenced model construction.

I present a single exploratory model that incorporates data from multiple sampling dates and I use it to investigate direct and indirect effects of treatments and macrophytes on mesograzers and epiphytes and direct effects of mesograzers on epiphytes. I constructed models in AMOS version 18 (SPSS), I transformed data when appropriate to improve normality and linearize bivariate relationships, and I used maximum likelihood to calculate estimates. Unstandardized path coefficients were used for interpretation because of the inclusion of a categorical predictor (Deterrent), which does not have a standard deviation and thus cannot be standardized. Unstandardized path coefficients can

be interpreted as the unit change in the response due to 1 unit change in the predictor (Grace and Bollen 2005).

I constrained relationships (i.e. possible pathways) in the models based on my knowledge of the system and model results. When paths were not significantly different from zero, they were removed from the model. Typically, structural equation modeling uses the covariance matrix of the data and creates an estimated covariance matrix from the constructed network of pathways. Thus, I was able to identify areas of high residual covariance between two variables, which I used to decide whether or not an additional pathway between those variables made sense biologically and should be included in the model. To assess model fit, I used a χ^2 test of the hypothesis that observed and estimated covariance matrices were equal (Grace 2006).

Fate of Carbaryl in Marine Environments

In order to safely and effectively apply chemical deterrent in the field, I first investigated the toxicity and fate of carbaryl in the marine environment. I provide background to my source of chemical deterrence, carbaryl, and develop a simple model to predict how carbaryl enters and degrades in the environment within the context of my trophic manipulations.

Carbaryl has very low persistence in the environment so its effects also tend to be short-lived. Carbaryl is broken down in the environment in four main ways: hydrolysis, photolysis, microbial degradation, and metabolism in higher organisms (see Gunasekara et al. 2008 for degradation pathways). When carbaryl first enters the water, hydrolysis and photolysis are likely to be the first sources of carbaryl degradation. Armbrust and Crosby (1991) found that in filtered seawater without light, hydrolytic degradation resulted in a half-life of carbaryl of 24 hours. In the presence of light, the half-life was reduced to 5 hours. Carbaryl's major degradation product, 1-naphthol, was undetectable in the presence of light after 2 hours, although it was stable to hydrolysis.

In more realistic systems with sediments and diverse assemblages of organisms, the environmental fate will be altered. Carbaryl sorbs to soils fairly well (K_{oc} = 290 μg/g, Phillips and Bode 2004), which slows dispersal and potentially degradation. Carbaryl can be degraded in soils through microbial activity, and Venkateswarlu et al. (1980) found that this process occurs more rapidly in anoxic compared to aerated soils. While soil studies are limited to terrestrial systems, they are likely analogous in marine sediments. Degradation of carbaryl in higher organisms is known to occur in organisms ranging from plants, terrestrial oligochaetes, and mammals (Stenersen 1992, Tomlin 2000, Tomlin 2003). Carpenter (1986) found no effect of carbaryl on algal biomass or productivity in a tropical backreef system.

Carbaryl Degradation Model

In order to better understand the fate of carbaryl entering a natural system, I created a simple model of carbaryl loading and degradation in a model site using an experimental design proposed by the authors and degradation data from Armburst & Crosby (1993). Based on preliminary data indicating that most of a plaster block will dissolve after one week exposure to water, I assumed that 75% of plaster block mass entered the environment. Each plaster block I made contained roughly 12 grams of carbaryl. With an experiment containing a large number of blocks (I assumed 60 blocks), ~ 75 g of carbaryl enter the area each day (assumed to occur instantly at the beginning of the day), which is degraded by hydrolysis and photolysis. To provide enough space to accommodate the largest field experiments described above (Experiments 3 and 4), I assumed an area 216 m^2. With an assumed depth of 1 m, I started with a concentration of roughly 0.35 mg carbaryl/l in the system at the start of the day. I assumed a closed system of filtered seawater (i.e. no input or output of water and no biological degradation). I modeled degradation of the daily load using half lives of carbaryl in dark and light filtered seawater; 24 and 5 hours, respectively (Armbrust & Crosby 1993) following pseudo-first order kinetics in the light switching to the dark (Figure 6) and in the dark switching to the light (not shown) over a 24 hour period. I assumed 14 hours of sunlight at latitude 37°N on day of year 180. The final carbaryl concentration was below 0.05 mg/l in each case (light-to-dark, $[\text{carbaryl}]_{\text{final}} = 0.043$ mg/l; dark-to-light, $[\text{carbaryl}]_{\text{final}} = 0.039$ mg/l).

I did not included advection from the system due to currents and tides to maintain conservative concentration estimates. I also did not model sorption to sediments because carbaryl first contacts water in all of my field experiments described below and too little is known about aquatic sediment dynamics to propose a defensible model. In nature, my site would be a shallow, sunlit aquatic habitat and carbaryl would likely dilute and degrade rapidly in the environment. I do not expected any plant or animal populations to experience long-term effects following short-term (on the order of one month) use of carbaryl to reduce mesograzer abundance in the field.

RESULTS

Experiment 1 – Concentration, Distance, and Time Experiment

The gammarid amphipod, *Gammarus mucronatus*, dominated the assemblage of epifauna and was strongly affected by grazer reduction treatments (Table 1). This species occurs widely in the lower Chesapeake Bay, including in seagrass beds (Douglass et al. 2010).

Grazer reduction treatments were very effective at reducing faunal biomass at short range Figure 7a-b). Faunal biomass increased with distance in grazer-reduction treatments but did not differ systematically in control treatments (Table 2, treatment × distance, $P = 0.001$). A significant treatment by time interaction ($P = 0.004$) was due to differing responses in control treatments between the two sampling days (grazer-reduction treatments followed similar trends at both sampling days). *A priori* contrasts revealed highly significant differences between grazer-reduction and control plots ($P < 0.001$) and no significant differences between low and high reduction plots or between block and no-block controls.

The effectiveness of grazer reduction depended on the concentration of chemical deterrent, but for both tested concentrations the effective range appears to be less than one meter, with greatest reductions occurring at 10 and 30 cm from the deterrent source.

Experiment 2 – Distance and Direction Experiment

When samples were pooled across directions, I saw a clear reduction of faunal biomass in grazer reduction plots at 30 cm from blocks (Figure 7c-d, Table 3). I found no evidence of biomass reduction at 2 m in grazer reduction plots and no biomass differences with distance in control plots, a pattern which produces a significant reduction by distance interaction in a 2-way ANOVA (Table 3, $P < 0.001$). I did not have a large enough sample size to test for differences with direction from a plaster block, but visual inspection suggests that direction had no effect on faunal biomass. Arranging plots at least 2 meters apart for experiments involving grazer reduction at my site should prevent spill-over of grazer reduction into other plots.

Experiment 3 – Fall Nutrient and Grazer Manipulation

Mesograzer density declined strongly during the experiment. For crustaceans, this decline was exacerbated in grazer reduction treatments (Figures 8a, 9, Table 4). Gastropods decreased but were unaffected by reduction treatment (Table 5), and isopods maintained low densities throughout the experiment.

Micro-algal epiphytes increased through time in all treatments, possibly in response to decreased mesograzer density (Figure 8b). By day 24, when mesograzer density was near zero, I detected a positive fertilization effect on epiphyte biomass (Table 6, Fertilization × Sampling Date, $P = 0.011$). Epiphyte biomass was higher in reduction treatments at day 11, but this effect was not sustained in the absence of mesograzers.

Experiment 4 – Summer Nutrient and Grazer Manipulation

MESOGRAZERS

Over the course of the 6 week experiment, grazer reduction decreased field density of crustacean mesograzers by an average of 74% (Figure 10). Gammarid amphipods, caprellid amphipods, and isopods were reduced on average by 84%, 46%, and 70%, respectively. A single repeated measures ANOVA was run on crustacean mesograzer density because densities of zero were common within crustacean taxonomic groups, which would complicate linear modeling, and because patterns were similar across groups (Table 8, Figure 10). A significant deterrent by time interaction ($P = 0.001$) explains the changing relationship between densities in grazer-reduction and control plots through time. At day 38 I observed a smaller difference in densities between reduction and control plots, which is largely due to increased density of caprellid amphipods in reduction plots (Figure 10b).

Gastropod mesograzers showed no decrease in density due to chemical deterrence (Figure 10d, Table 9). Rather, gastropods increased, albeit non-significantly, in reduction plots at days 24 and 38. At day 24, mean gastropod density in reduction plots exceeded that in control plots by 38%. Fertilization did not significantly change mesograzer densities. A significant effect of sampling date ($P < 0.001$) describes a quadratic trend through time, where density increased at day 24 and subsequently decreased at day 38.

Because gammarid amphipods were the most speciose group of mesograzers and the most sensitive to grazer reduction, I investigated the effects of chemical deterrent on gammarid species richness (Figure 11). When pooled across sampling dates, median gammarid richness was 1 and 4 species in grazer reduction and control treatments, respectively (Mann-Whitney $U = 3164.5$, $P < 0.001$). The most consistently detected gammarid species was *Ampithoe longimana* (110 out of 120 total experimental samples, 40 out of 48 total reduction samples). Samples from reduction plots in which *A. longimana* was not detected contained at most one other gammarid species.

All small mobile epifauna sampled before and during the experiment using the epibenthic grab sampler are listed in Table 7 along with mean abundance per treatment. Mobile epipfaunal assemblages were dominated by gastropod and crustacean mesograzers. Non-grazing epifauna include predatory worms of the genus *Nereis*, predatory gastropods (*Acteocina canaliculata, Odostomia bisuturalis, Trophora nigra*), and the suspension feeder *Crepidula convexa*. Non-metric multidimensional scaling revealed a clear shift in assemblages (Figure 12). Assemblages in grazer reduction treatments were dominated by *Bittium varium*, *Nereis*. sp., and nudibranchs while assemblages in control treatments were characterized by crustaceans.

ALGAL EPIPHYTES

Reductions in crustacean mesograzer density resulted in concomitant blooms of micro-epiphytes (Figure 13a). Chemical deterrence led to a 200% increase in mean micro-epiphyte biomass averaged across sampling dates. A significant deterrent by time interaction (Table 9, $P < 0.001$) reveal changes in microalgal responses through time. Microalgal biomass was 447% higher in reduction treatments at day 24. By day 38, micro-epiphyte biomass in reduction plots was only 47.5% higher than in control plots. Fertilization did not significantly affect micro-epiphyte biomass over the entire experiment, but a marginally insignificant increase in micro-epiphytes was observed at week 2 in fertilization treatments.

Micro-epiphyte biomass displayed a strongly nonlinear, negative relationship with crustacean mesograzer density (Figure 14). The highest biomass of micro-epiphytes occurred in plots with no or very few crustacean mesograzers. As peracarid density increased, micro-epiphyte biomass decreased sharply and approached zero following exponential decline. The use of chemical deterrence generated a gradient in grazing pressure. Suppression of grazing pressure greatly increased the scope for micro-epiphyte growth, especially at week 4.

ZOSTERA PERFORMANCE/GROWTH

Eelgrass growth significantly declined through time and this decline was not due to treatment effects (Table 10, Figure 13b). The trend in eelgrass growth rate was inversely related to that of water temperature, a possible reflection of the thermal stress and summer senescence experienced by *Zostera marina* populations in Chesapeake Bay (Moore and Jarvis 2009).

Eelgrass shoot density within experimental plots was on average lower at the end of the experiment than during the preliminary sampling (Figure 13c), which is consistent with a negative effects of high temperature. However, a two-way ANOVA on the proportional change in shoot density revealed a significantly greater reduction in shoot density within reduction plots than control plots (Table 11), suggesting either direct

effects of chemical deterrent on eelgrass or indirect effects mediated by mesograzers and epiphytes.

In addition to measurements specifically taken for eelgrass performance, I obtained data on eelgrass performance from mesograzer samples (above-ground biomass) and micro-algal epiphyte samples (shoot surface area). The declines in above-ground biomass and shoot surface area mirror the trends I saw for eelgrass growth and shoot density (Figure 15). All eelgrass performance metrics declined through the course of the experiment.

Structural Equation Modeling

The final structural equation model fit well (Figure 16, $\chi^2_{28} = 25.42, P = 0.605$). I chose to analyze two sampling dates, days 10 and 24, because neither had missing data for any variable and because I observed weaker relationships at the end of the experiment potentially due to eelgrass dieback mentioned above (Figures 13a, 14). Path coefficients are listed in Table 13. Consistent with univariate analyses, I found strong effects of grazer reduction treatment on crustacean mesograzers, especially gammarid amphipods. In the univariate analyses, I standardized mesograzer abundance to macrophyte biomass. With structural equation modeling, I show that both groups of macrophytes (eelgrass and macroalgae) increase mesograzer abundance. Isopods and gastropods were not included in the final model because they highly reduced model fit and many paths to and from these taxa were non-significant.

Amphipods strongly reduced epiphyte biomass. Both gammarid amphipods and caprellid amphipods significantly reduced epiphyte biomass on day 10, but gammarids became the sole mediator of epiphyte biomass on day 24. On day 24, however, epiphyte biomass depended upon the biomass observed at day 10 (the model included both a direct path and correlated errors, Figure 16). The relationship between epiphyte biomass on different days reveals the accumulation of epiphytes over time. Macrophytes increased epiphytes at day 10, but this effect disappeared at day 24, making epiphyte biomass at day 10 the strongest predictor of epiphytes at day 24. Mesograzers at day 24 showed no dependence on mesograzers at day 10. However, there was a significant positive relationship between mesograzers at day 10 and macroalgae at day 24.

Due to the small number of samples relative to the number of estimated parameters in the model (16 estimated paths, 40 samples), I re-calculated path coefficients using Bayesian estimation to determine whether existing paths were significant (Lee and Song 2004). I deemed paths non-significant if 95% credible intervals around regression weights (path coefficients) included zero. One path (Gammarids24 → Epiphytes24) was nearly non-significant (mean = -0.111, lower = -0.222, upper = -0.001), but all other paths were clearly significant based on Bayesian estimation.

DISCUSSION

Mesograzers are abundant primary consumers in coastal marine food webs, but their grazing impacts remain poorly understood. Most previous studies investigating the consumptive role of mesograzers were conducted in laboratory or mesocosm settings, while the few studies addressing this topic in the field have used cages. Poore et al (2009) represents the only study to-date that has experimentally addressed the role of mesograzers in the field without cages. My study documents the first field experimental test of crustacean mesograzer impacts on primary production in a natural seagrass community that sidesteps the cage artifacts that have hampered previous research. The method of slow-release chemical deterrence reduced crustacean mesograzer density through time and only at close range. Unlike Poore et al. (2009), I found that crustacean mesograzers depressed micro-algal epiphytes. On day 24 of the Summer Nutrient and Grazer Manipulation, micro-algal epiphyte biomass was 447% higher in grazer reduction treatments relative to controls (Figure 13a). Although epiphyte biomass was highest at day 24, it was more dependent upon day 10 epiphyte biomass than on mesograzer density at day 24 based on my structural equation model (Table 12, Figure 16). This result confirms the cumulative effects of grazer reduction over time on seagrass epiphytes.

Non-linearity in the relationship between crustacean mesograzers and micro-algal epiphytes (Figure 14) suggests that grazing pressure saturates at low crustacean density. Consistent reduction in crustacean mesograzers over time greatly increased the scope for micro-algal biomass accumulation, such that by day 24 the relationship appears to be driven by a binary effect of treatment (reduction *vs.* control) rather than crustacean density *per se*. The variance in crustacean mesograzer density in control treatments at day 24 (Figure 14b black symbols) belies what was likely consistent grazing pressures through time.

Nutrient fertilization was largely unsuccessful at influencing micro-algal epiphyte biomass, except when mesograzer densities were near zero (Figures 8, 9). My finding that nutrient enrichment increased epiphyte biomass only in the absence of grazers is consistent with previous work in seagrass systems (Neckles et al. 1993, Williams and Ruckelshaus 1993, Heck et al. 2000, reviewed in Heck and Valentine 2006) and suggests strong top-down effects of grazers and efficient trophic transfer of primary production (Spivak et al. 2009b). During the Summer Nutrient and Grazer Manipulation, I did not observe significant nutrient effects on epiphytes possibly due to consistently high densities of the gastropod mesograzer, *Bittium varium*. However, the effects of gastropod mesograzers on micro-algal epiphytes remain unclear. I observed the highest gastropod

density and highest micro-algal biomass on the same day (day 24), which promotes two potential hypotheses. First, gastropods have low *per capita* effects on eelgrass epiphytes. Alternatively, gastropods may benefit epiphytes through nitrogen-rich excretions (Bracken and Nielsen 2004, Bracken et al. 2011). These hypotheses are not mutually exclusive and deserve further investigation.

Although grazer reduction treatments were effective at reducing the density of all crustacean mesograzers, the consistent presence of the gammarid amphipod, *Ampithoe longimana*, in grazer reduction plots during the Summer Nutrient and Grazer Manipulation potentially relates to their ability to withstand chemical deterrents naturally found in algae (Duffy and Hay 1991, 1994, Cronin and Hay 1996, Cruz-Rivera and Hay 2000, 2001, 2003, Sotka et al. 2009). Grazer reduction affected isopods less than amphipods. Gastropods were unaffected by grazer reduction as in previous work (Duffy and Hay 2000), but the shift in community composition induced by grazer reduction (Figure 10) elicited striking differences in epiphyte biomass and demonstrates carbaryl's taxonomic specificity.

Addressing my final hypothesis that mesograzers indirectly benefit seagrass through their consumption of epiphytic algae, I found that grazer reduction treatments exacerbated the decline in eelgrass shoot density during the experiment, consistent with earlier hypotheses (van Montfrans et al. 1984, Valentine and Duffy 2006). More work is needed to determine direct effects of carbaryl on eelgrass because if eelgrass takes up carbaryl into its tissues, it would have to use energy to degrade the compound (Xu 2000), which potentially confounds my results.

The differences I observed between fall and summer experiments are likely a result of seasonal patterns in Chesapeake Bay seagrass beds. During the Fall Nutrient and Grazer Manipulation, the loss of grazers and concomitant increase in epiphyte biomass was likely due to natural declines in mesograzer density (Marsh 1973, Douglass et al. 2010). Mesograzer populations are highly variable during fall months, and it appears that I observed a seasonal transition between top-down and bottom-up controlled states.

The weakening of patterns at the end of the Summer Nutrient and Grazer Manipulation is likely a consequence of annual summer dieback that occurs in Chesapeake Bay eelgrass beds. This dieback is largely driven by temperature-induced stress (Moore and Jarvis 2008). Above-ground eelgrass biomass (g AFDM) at a nearby site in the York River declined by 50% during the experiment (Duffy *unpublished data*), reflecting regional decline in eelgrass performance during my experiment.

My results largely complement previous studies of plant-herbivore interactions in seagrass communities, both in mesocosms and field experiments (Hughes et al. 2004, Heck and Valentine 2006). While mesocosm and field cage studies have experimental artifacts, they reveal patterns that reflect natural dynamics. Experiments from the same system as the current study (lab – Duffy and Harvilicz 2001, mesocosm – Duffy et al.

2003, 2005, Blake and Duffy 2010, field – Douglass et al. 2007) all reveal strong negative effects of mesograzers on seagrass epiphytes. With the novel technique developed by Poore et al. (2009), I confirm many of the findings from previous studies and I provide the best experimental test to date of the role of crustacean mesograzers in eelgrass communities.

Mesograzers can have strong top-down impacts on primary producer communities through their consumption of algae. Amphipods were both the most readily affected by chemical deterrence and showed the strongest relationships with seagrass epiphytes, suggesting that they are the most important consumers of primary production in Chesapeake Bay eelgrass communities. Because they are ubiquitous in coastal systems and are important prey for higher-order consumers, herbivorous amphipods likely play an important role in coastal food webs globally (Edgar and Shaw 1995, Jernakoff et al. 1996, Taylor 1998, Valentine and Duffy 2006).

My findings provide tentative support for the hypothesis that mesograzers and seagrasses exist in a mutualistic relationship (Valentine and Duffy 2006). Mesograzer reduction with chemical deterrent significantly accelerated the decline of eelgrass during the summer experiment, probably by allowing overgrowth by the large accumulations of epiphytic algae documented in the deterrent treatment. In their role as foundation species (*sensu* Dayton 1972, Bruno and Bertness 2001), seagrasses support rich and productive communities of epiphytes and epifauna. Seagrass productivity creates habitats utilized by higher-order consumers, many of which are economically important. Thus, my results highlight the potential importance of small, inconspicuous invertebrates in maintaining the structure and functioning of an important ecosystem type, which has implications for the management and conservation of both seagrasses and animals harvested for human consumption.

LITERATURE CITED

Aebischer, NJ, JC Coulson, JM Colebrook. 1990. Parallel long-term trends across four marine trophic levels and weather. *Nature* 347: 753-755.

Andersson, S, M Presson, P Moksnes, S Baden. 2009. The role of the amphipod *Gammarus locusta* as a grazer on macroalgae in Swedish seagrass meadows. *Marine Biology* 156: 969-981.

Armbrust, KL, DG Crosby. 1991. Fate of carbaryl, 1-naphthol, and atrazine in seawater. *Pacific Science* 45: 314-320.

Arbuckle, JL. 2009. Amos (Version 18.0) [Computer Program]. Chicago: SPSS.

Blake, RE, JE Duffy. 2010. Grazer diversity affects resistance to multiple stressors in an experimental seagrass ecosystem. *Oikos* 119: 1625-1635.

Borer, ET, BS Halpern, EW Seabloom. 2006. Asymmetry in community regulation: effects of predators and productivity. *Ecology* 87: 2813-2820.

Borer, ET, EW Seabloom, JB Shurin et al. 2005. What determines the strength of a trophic cascade? *Ecology* 86: 528-537.

Bracken, MES, CA Gonzalez-Dorantes, JJ Stachowicz. 2007. Whole-community mutualism: associated invertebrates facilitate a dominant habitat-forming seaweed. *Ecology* 88: 2211-2219.

Bracken, MES, E Jones, SL Williams. 2011. Herbivore, tidal elevation, and species richness simultaneously mediate nitrate uptake by seaweed assemblages. *Ecology* 92: 1083-1093.

Bracken, MES, KJ Nielsen. 2004. Diversity of intertidal macroalgae increases with nitrogen loading by invertebrates. *Ecology* 85: 2828-2836.

Brawley, SH. 1992. Mesoherbivores, p. 235-263, *In*: DM John, SJ Hawkins and JH Price (eds.), *Plant-animal interactions in the marine benthos*. Systematics Association Special Volume 46. Clarendon Press, Oxford, UK.

Brett, MT, CR Goldman. 1997. Consumer versus resource control in freshwater pelagic food webs. *Science* 275: 384-386.

Bruno, JF, MD Bertness. 2001. Habitat modification and facilitation in benthic marine communities, p. 131-158, *In*: MD Bertness, ME Hay and SD Gaines (eds.), *Marine Community Ecology*. Sinauer Associates, Sunderland, Massachusetts.

Carpenter, RC. 1986. Partitioning herbivory and its effects on coral reef algal communities. *Ecological Monographs* 56: 345-363.

Carpenter, SR. 1996. Microcosm experiments have limited relevance for community and ecosystem ecology. *Ecology* 77: 677-680.

Carpenter, SR. 1999. Microcosm experiments have limited relevance for community and ecosystem ecology: reply. *Ecology* 80: 1085-1088.

Cebrián, J. 1999. Patterns in the fate of production in plant communities. *The American Naturalist* 154: 449-468.

Chalcraft, DR, CA Binckley, WJ Resetarits, Jr. 2005. Experimental venue and estimation of interaction strength: comment. *Ecology* 86: 1061-1067.

Connell, SD. Exclusion of predatory fish on a coral reef: the anticipation, pre-emption and evaluation of some caging artefacts. *Journal of Experimental Marine Biology and Ecology* 213: 181-198.

Costanza, R, R dArge, R deGroot. et al. 1997. The value of the world's ecosystem services and natural capital. *Nature* 387: 253-260.

Cox, C. 1993. The problems with Sevin (carbaryl). *Journal of Pesticide Reform* 13: 31-36.

Cruz-Rivera E, ME Hay. 2000. Can quantity replace quality? Food choice, compensatory feeding, and fitness of marine mesograzers. *Ecology* 81: 201-219.

Cruz-Rivera E, ME Hay. 2001. Macroalgal traits and the feeding and fitness of an herbivorous amphipod: the roles of selectivity, mixing, and compensation. *Marine Ecology Progress Series* 218: 249-266.

Cruz-Rivera E, ME Hay. 2003. Prey nutritional quality interacts with chemical defenses to affect consumer feeding and fitness. *Ecological Monographs* 73: 483-506.

Cyr, H, ML Pace. 1993. Magnitude and patterns of herbivory in aquatic and terrestrial ecosystems. *Nature* 361: 148-150.

Davenport, AC, TW Anderson. 2007. Positive indirect effects of reef fishes on kelp performance: the importance of mesograzers. *Ecology* 88: 1548-1561.

Dayton, PK. 1975. Experimental evaluation of ecological dominance in a rocky intertidal algal community. *Ecological Monographs* 45: 137-159.

den Hartog, C, J Kuo. 2006. Taxonomy and biogeography of seagrasses, p. 1-23, *In*: AWD Larkum, RJ Orth, CM Duarte (eds.), *Seagrasses: Biology, Ecology and Conservation*. Springer, Dordrecht.

Domning, DP. 2001. Sirenians, seagrasses, and Cenozoic ecological change in the Caribbean. *Paleogeography, Paleoclimatology, Paleoecology* 166: 27-50.

Douglass, JG, JE Duffy, JF Bruno. 2008. Herbivore and predator diversity interactively affect ecosystem properties in an experimental marine community. *Ecology Letters* 11: 598-608.

Douglass, JG, JE Duffy, AC Spivak, JP Richardson. 2007. Nutrient versus consumer control of community structure in a Chesapeake Bay eelgrass habitat. *Marine Ecology Progress Series* 348: 71-83.

Douglass, JG, KE France, JP Richardson, JE Duffy. 2010. Seasonal and interannual change in a Chesapeake Bay eelgrass community: insights into biotic and abiotic control of community structure. *Limnology and Oceanography*, accepted 16 Feb 2010.

Drenner, RW, A Mazumder. 1999. Microcosm experiments have limited relevance for community and ecosystem ecology: comment. *Ecology* 80:1081-1085.

Duffy, JE, AM Harvilicz. 2001. Species-specific impacts of grazing amphipods in an eelgrass-bed community. *Marine Ecology Progress Series* 223: 201-211.

Duffy, JE, ME Hay. 1991. Food and shelter as determinants of food choice by an herbivorous marine amphipod. *Ecology* 72: 1286-1298.

Duffy, JE, ME Hay. 1994. Herbivore resistance to seaweed chemical defenses – the role of mobility and predation risk. *Ecology* 75: 1304-1319.

Duffy, JE, ME Hay. 2000. Strong impacts of grazing amphipods on the organization of a benthic community. *Ecological Monographs* 70: 237-263.

Duffy, JE, ME Hay. 2001. The ecology and evolution of marine consumer-prey interactions, p. 131-157, *In*: MD Bertness, ME Hay and SD Gaines (eds.), *Marine Community Ecology*. Sinauer Associates, Sunderland, Massachusetts.

Duffy, JE, JP Richardson, EA Canuel. 2003. Grazer diversity effects on ecosystem functioning in seagrass beds. *Ecology Letters* 6: 637-645.

Duffy, JE, JP Richardson, KE France. 2005. Ecosystem consequences of diversity depend on food chain length in estuarine vegetation. *Ecology Letters* 8: 301-309.

Dumbauld, BR, KM Brooks, MH Posey. 2001. Response of an estuarine benthic community to application of the pesticide carbaryl and cultivation of Pacific oysters (*Crassostrea gigas*) in Willapa Bay, Washington. *Marine Pollution Bulletin* 42: 826-844.

Dumbauld, BR, S Booth, D Cheney et al. 2006. An integrated pest management program for burrowing shrimp control in oyster aquaculture. *Aquaculture* 261: 976-992.

Edgar, GJ. 1990a. The use of size structure of benthic macrofaunal communities to estimate faunal biomass and secondary production. *Journal of Experimental Marine Biology and Ecology* 137: 195-214.

Edgar, GJ. 1990b. Population regulation, population dynamics and competition amongst mobile epifauna associated with seagrass. *Journal of Experimental Marine Biology and Ecology* 144: 205-234.

Edgar, GJ and C Shaw. 1995. The production and trophic ecology of shallow-water fish assemblages in southern Australia. III. General relationships between sediments, seagrasses, invertebrates and fishes. *Journal of Experimental Marine Biology and Ecology* 194: 107-131.

Edgar, GJ and M Aoki. 1993. Resource limitation and fish predation: their importance to mobile epifauna associated with Japanese *Sargassum*. *Oecologia* 95: 122-133.

Gillanders, BM. 2006. Seagrasses, fish, and fisheries, p. 503-536, *In*: AWD Larkum, RJ Orth and CM Duarte (eds.), *Seagrasses: Biology, Ecology and Conservation*. Springer, Dordrecht.

Gilliom, RJ. 2007. Pesticides in U.S. streams and groundwater. *Environmental Science & Technology* 41: 3407-3413.

Grace, JB. 2006. *Structural Equation Modeling and Natural Systems*. Cambridge University Press, Cambridge, UK.

Grace, JB, KA Bollen. 2005. Interpreting the results from multiple regression and structural equation models. *Bulletin of the Ecological Society of America* 86: 283-295.

Gruner, DS, JE Smith, EW Seabloom et al. 2008. A cross-system synthesis of consumer and nutrient control on producer biomass. *Ecology Letters* 11: 740-755.

Gunasekara, AS, AL Rubin, KS Gob et al. 2008. Environmental fate and toxicology of carbaryl. *Reviews of Environmental Contamination and Toxicology* 196: 95-121.

Haiston, NG, FE Smith, LB Slobodkin. 1960. Community structure, population control, and competition. *The American Naturalist* 94: 421-425.

Hall, SJ, D Raffaelli, WR Turrell. 1990. Predator-caging experiments in marine systems: a reexamination of their value. *The American Naturalist* 136: 657-672.

Harrison, PG. 1982. Control of microbial growth and of amphipod grazing by water-soluble compounds from leaves of *Zostera marina*. *Marine Biology* 67: 225-230.

Hauxwell, J, J Cebrián, C Furlong, I Valiela. 2001. Macroalgal canopies contribute to eelgrass (*Zostera marina*) decline in temperate estuarine ecosystems. *Ecology* 82: 1007-1022.

Hay, ME, W Fenical. 1988. Marine plant-herbivore interactions: the ecology of chemical defense. *Annual Review of Ecology and Systematics* 19: 111-145.

Hay, ME, T Colburn,D Downing. 1983. Spatial and temporal patterns in herbivory on a Caribbean fringing reef: the effects on plant distribution. *Oecologia* 58: 299-308.

Heck, KL, RJ Orth. 1980. Seagrass habitats: The roles of habitat complexity, competition and predation in structuring associated fish and motile macroinvertebrate assemblages, p. 449–464, *In*: VS Kennedy (Ed.), *Estuarine perspectives*. Academic Press, New York.

Heck, KL, JR Pennock, JF Valentine et al. 2000. Effects of nutrient enrichment and small predator density on seagrass ecosystems: an experimental assessment. *Limnology and Oceanography* 45: 1041-1057.

Heck, KL, JF Valentine. 2006. Plant-herbivore interactions in seagrass meadows. *Journal of Experimental Marine Biology and Ecology* 330: 420-436.

Heck, KL, JF Valentine. 2007. The primacy of top-down effects in shallow benthic ecosystems. *Estuaries and Coasts* 30: 371-381.

Heck, KL, JF Valentine, JR Pennock et al. 2006. Effects of nutrient enrichment and grazing on shoalgrass *Halodule wrightii* and its epiphytes: results of a field experiment. *Marine Ecology Progress Series* 326: 145-156.

Hughes, AR, KJ Bando, LF Rodriguez, SL Williams. 2004. Relative effects of grazers and nutrients on seagrasses: a meta-analytic approach. *Marine Ecology Progress Series* 282: 87-99.

Hughes, TP. 2004. Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reef. *Science* 265: 1547-1551.

Hunter, MD, PW Price. 1992. Playing chutes and ladders: heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. *Ecology* 73: 723-732.

Jackson, JBC, MX Kirby, WH Berger et al. 2001. Historical overfishing and the recent collapse of coastal ecosystems. *Science*: 293: 629-638.

Jernakoff, P, A Brearsley, J Nielsen. 1996. Factors affecting grazer-epiphyte interactions in temperate seagrass meadows, p. 109-162, *In*: AD Ansell, RN Gibson and M Barnes (eds.), Oceanography and Marine Biology: an annual review, Vol. 34. UCL Press.

Kangas, P, H Autio, G Hallfors et al. 1982. A general model of the decline of *Fucus vesiculosus* at Tvarminne, south coast of Finland in 1977-81. *Acta Botanica Fennica* 118: 1-27.

Kikuchi, T. 1974. Japanese contributions on consumer ecology in eelgrass (*Zostera marina* L.) beds, with special reference to trophic relationships and resources in inshore fisheries. *Aquaculture* 4: 145-160.

Koch, EW, JD Ackerman, J Verduin, M van Keulan. 2006. Fluid dynamics in seagrass ecology – from molecules to ecosystems, p193-225, *In*: AWD Larkum, RJ Orth and CM Duarte (eds.), *Seagrasses: Biology, Ecology and Conservation.* Springer, Dordrecht.

Lee, SY, XY Song. 2004. Evaluation of the Bayesian and maximum likelihood approaches in analyzing structural equation models with small sample sizes. *Multivariate Behavioral Research* 39: 653-686.

Lorenzen, CJ. 1967. Determination of chlorophyll and phaeo-pigments: spectrophotometric equations. *Limnology and Oceanography* 12: 343-346.

Lubchenco, J. 1978. Plant species diversity in a marine intertidal community: importance of herbivore food preference and algal competitive abilities. *The American Naturalist* 112: 23-39.

Madsen, J. 1998. Experimental refuges for migratory waterfowl in Danish wetlands. I. Baseline assessment of the disturbance effects of recreational activites. *Journal of Applied Ecology* 35: 386-397.

Marba, N, M Holmer, E Gacia, C Barrón. 2006. Seagrass beds and coastal biogeochemistry, p. 135-157, *In*: AWD Larkum, RJ Orth and CM Duarte (eds.), *Seagrasses: Biology, Ecology and Conservation*. Springer, Dordrecht.

Marsh, GA. 1973. The *Zostera* epifaunal community in the York River, Virginia. *Chesapeake Science* 14: 87-97.

Menge, BA. 1995. Indirect effects in marine rocky intertidal interaction webs: patterns and importance. *Ecological Monographs* 65: 21-74.

Miller, LP, B Gaylord. 2007. Barriers to flow: the effect of experimental cage structures on water velocities in high-energy subtidal and intertidal environments. *Journal of Experimental Marine Biology and Ecology* 344: 215-228.

Miller, MW, ME Hay. 1996. Coral-seaweed-grazer-nutrient interactions on temperate reefs. *Ecological Monographs* 66: 323-344.

Moksnes, P, M Gullström, K Tryman, S Baden. 2008. Trophic cascades in a temperate seagrass community. *Oikos* 117: 763-777.

Moore, KA. 2004. Influence of seagrasses on water quality in shallow regions of lower Chesapeake Bay. *Journal of Coastal Research* 45: 162-178.

Moore, KA, JC Jarvis. 2008. Environmental factors affecting recent summertime eelgrass diebacks in the lower Chesapeake Bay: implications for long-term persistence. *Journal of Coastal Research* 55: 135-147.

Neckles, HA, RL Wetzel, RJ Orth. 1993. Relative effects of nutrient enrichment and grazing on epiphyte-macrophyte (*Zostera marina* L.) dynamics. *Oecologia* 93: 285-295.

Nelson, WG. 1979. An analysis of structural patterns in an eelgrass (*Zostera marina* L.) amphipod community. *Journal of Experimental Marine Biology and Ecology* 39: 231-264.

Nixon, SW, BA Buckley. 2002. "A strikingly-rich zone" – nutrient enrichment and secondary production in coastal marine ecosystems. *Estuaries* 25: 782-796.

Oczkowski, AJ, SW Nixon, SL Granger et al. 2009. Anthropogenic enhancement of Egypt's Mediterranean fishery. *Proceedings of the National Academy of Science* 106: 1364-1367.

Orth, RJ, J van Montfrans. 1984. Epiphtye-seagrass relationships with an emphasis on the role of micrograzing: a review. *Aquatic Botany* 18: 43-69.

Orth, RJ, KL Heck, J van Montfrans. 1984. Faunal communities in seagrass beds: a review of the influence on plant structure and prey characteristics on predator-prey relationships. *Estuaries* 7: 339-350.

Orth, RJ, TJB Carruthers, WC Dennison et al. 2006. A global crisis for seagrass ecosystems. *BioScience* 56: 987-996.

Paine, RT, RL Vadas. 1969. The effects of grazing by sea urchins, *Strongylocentrotus* spp., on benthic algal populations. *Limnology and Oceanography* 14: 710-719.

Pinheiro, JC, DM Bates. 2000. *Mixed-effects models in S and S-Plus*. Springer Verlag, New York.

Polis, GA, DR Strong. 1996. Food web complexity and community dynamics. *The American Naturalist* 147: 813-846.

Poore, AGB, AH Campbell, PD Steinberg. 2009. Natural densities of mesograzers fail to limit growth of macroalgae or their epiphytes in a temperate algal bed. *Journal of Ecology* 97: 164-175.

Power, ME. 1992. Top-down and bottom-up forces in food webs: do plants have primacy? *Ecology* 73: 733-746.

R Development Core Team. 2011. R version 2.11.1. R Project for Statistical Computing, Vienna, Austria. <www.r-project.org>

Robertson, AI, KH Mann. 1980. The role of isopods and amphipods in the initial fragmentation of eelgrass detritus in Nova Scotia, Canada. *Marine Biology* 59: 63-69.

Roth, M, RH Richards, C Sommerville. 1993. Current practices in the chemotherapeutic control of sea lice infestations in aquaculture – a review. *Journal of Fish Diseases* 16: 1-26.

Sand-Jensen, K. 1977. Effect of epiphytes on eelgrass photosynthesis. *Aquatic Botany* 3: 55-63.

Short, FT, S Wyllie-Echeverria. 1996. Natural and human-induced disturbance of seagrasses. *Environmental Conservation* 23: 17-27.

Shurin, JB, DS Gruner, H Hillebrand. 2006. All wet or dried up? Real differences between aquatic and terrestrial food webs. *Proceedings of the Royal Society B* 273: 1-9.

Shurin, JB, ET Borer, EW Seabloom et al. 2002. A cross-ecosystem comparison of the strength of trophic cascades. *Ecology Letters* 5: 785-791.

Skelly, DK. 2002. Experimental venue and estimation of interaction strength. *Ecology* 83: 2097-2101.

Skelly, DK. 2005. Experimental venue and estimation of interaction strength: reply. *Ecology* 86: 1068-1071.

Sotka, EE, A McCarty, EA Monroe et al. 2009. Benthic herbivores are not deterred by brevetoxins produced by the red tide dinoflagellate *Karenia brevis*. *Journal of Chemical Ecology* 35: 851-859.

Spivak, AC, EA Canuel, JE Duffy et al. 2009a. Epifaunal community composition and nutrient addition alter sediment organic matter composition in a natural eelgrass *Zostera marina* bed: a field experiment. *Marine Ecology Progress Series* 376: 55-67.

Spivak, AC, EA Canuel, JE Duffy et al. 2009b. Nutrient enrichment and food web composition affect ecosystem metabolism in an experimental seagrass habitat.

Steele, MA. 1996. Effects of predators on reef fishes: separating cage artifacts from effects of predation. *Journal of Experimental Marine Biology and Ecology* 198: 249-267.

Steneck, RS, MN Dethier. 1994. A functional group approach to the structure of algal-dominated communities. *Oikos* 476-498.

Stenerson, J. 1992. Uptake and metabolism of xenobiotics by earthworms, p. 129-138. *In*: PW Greigh-Smith, H Becker, PJ Edwards and F Heimbach (eds.), *Ecotoxicology of Earthworms*. Intercepts Ltd. Andover, UK.

Stoner, AW. 1980. The role of seagrass biomass in the organization of benthic macrofaunal assemblages. *Bulletin of Marine Science* 30: 537-551.

Strong, DR 1992. Are trophic cascades all wet? Differentiation and donor-control in speciose ecosystems. *Ecology* 73: 747-754.

Taylor, RB. 1998. Density, biomass and productivity of animals in four subtidal rocky reef habitats: the importance of small mobile invertebrates. *Marine Ecology Progress Series* 172: 37-51.

Taylor, RB, TAV Rees. 1998. Excretory products of mobile epifauna as a nitrogen source for seaweeds. *Limnology and Oceanography* 43: 600-606.

Taylor, RB, PJ Brown. 2006. Herbivory in the gammarid amphipod *Aora typical*: relationships between consumption rates, performance and abundance across ten seaweed species. *Marine Biology* 149: 455-463.

Tegner, MJ, PK Dayton. 1987. El Niño effects on Southern California kelp forest communities. *Advances in Ecological Research* 17: 243-279.

Tomlin, CDS. 2000. *The Pesticide Manual*. 12th Ed. British Crop Protection Council. Surrey, UK.

Tomlin, CDS. 2003. *The Pesticide Manual*. 13th Ed. British Crop Protection Council. Surrey, UK.

Valentine JF, JE Duffy. 2006. The central role of grazing in seagrass ecology, p 463-501, *In*: AWD Larkum, RJ Orth and CM Duarte (eds.), *Seagrasses: Biology, Ecology and Conservation*. Springer, Dordrecht.

Valiela, I, J McClelland, J Hauxwell et al. 1997. Macroalgal blooms in shallow estuaries: controls and ecophysiological and ecosystem consequences. *Limnology and Oceanography* 42: 1105-1118.

van Montfrans, J, RJ Orth and SA VAY. 1092. Preliminary studies of grazing by *Bittium varium* on eelgrass production. *Aquatic Botany* 14: 75-89.

van Montfrans, J, RL Wetzel, RJ Orth. 1984. Epiphyte-grazer relationships in seagrass meadows: consequences for seagrass growth and production. *Estuaries* 7: 289-309.

Venkateswarlu, K, K Chendrayan, N Sethunathan. 1980. Persistence and biodegradation of carbaryl in soils. *Journal of Environmental Science & Health B* 15: 421-429.

Virnstein, RW 1978. Predator caging experiments in soft sediments: caution advised, p. 261–273, *In*: ML Wiley (ed.), *Estuarine Interactions*. Academic Press, New York.

Virnstein, RW, RK Howard. 1987. Motile epifauna of marine macrophytes in the Indian River Lagoon, Florida. I. Comparisons among three species of seagrasses from adjacent beds. *Bulletin of Marine Science* 41: 1-12.

Vitousek, PM, JD Aber, RW Howarth et al. 1997. Technical report: human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications* 7: 737-750.

Ware, DM, RE Thomson. 2005. Bottom-up ecosystem trophic dynamics determine fish production in the Northeast Pacific. *Science* 308: 1280-1284.

Weis, P, JS Weis. 1974. Schooling behavior of *Menidia menidia* in the presence of insectide Sevin (carbaryl). *Marine Biology* 28: 261-263.

Williams, SL, KL Heck. 2001. Seagrass community ecology, p. 317–337, *In*: MD Bertness, ME Hay and SD Gaines (eds.), *Marine Community Ecology*. Sinauer Associates, Sunderland, Massachusetts.

Williams, SL, MH Ruckleshaus. 1993. Effects of nitrogen availability and herbivory on eelgrass (*Zostera marina*) and epiphytes. *Ecology* 74: 904-918.

Worm, B, HK Lotze, H Hillebrand, U Sommer. 2002. Consumer versus resource control of species diversity and ecosystem functioning. *Nature* 417: 848-851.

Worm, B, HK Lotze, U Sommer. 2000. Coastal food web structure, carbon storage, and nitrogen retention regulated by consumer pressure and nutrient loading. *Limnology and Oceanography* 45: 339-349.

Xu, S. 2000. Environmental fate of carbaryl. Department of Pesticide Regulation: Sacramento, CA. < http://www.cdpr.ca.gov/docs/emon/pubs/fatememo/carbaryl.pdf>

Table 1. Ten most common taxa (mean abundance rounded to nearest whole number) recovered during Concentration, Distance, and Time Experiment. Data are grouped by distance and treatment, pooled across two sampling dates, and rounded to the nearest whole number.

	High Reduction				*Low Reduction*				*Block Control*				*No-Block Control*				Species
Species	**10 cm**	**30 cm**	**60 cm**	**100 cm**	**10 cm**	**30 cm**	**60 cm**	**100 cm**	**10 cm**	**30 cm**	**60 cm**	**100 cm**	**10 cm**	**30 cm**	**60 cm**	**100 cm**	**Average**
Gammarus mucronatus	1	4	92	152	9	64	96	148	98	129	88	186	155	76	146	106	104
Pluesymtes glaber	1	3	8	12	5	5	12	9	6	5	10	3	12	-	7	3	7
Paracaprella tenuis	3	-	8	2	-	3	2	4	3	-	-	5	2	-	3	9	4
Caprella penantis	2	3	5	3	6	4	3	6	2	1	1	2	2	-	1	3	3
Balanus improvisus	3	5	3	-	3	2	1	-	-	1	-	1	1	1	-	4	2
Corophium spp.	1	-	2	1	-	1	2	2	6	1	4	3	1	1	1	2	2
Panopeus herbstii	-	-	1	-	-	-	1	-	-	-	1	1	2	-	-	1	1
Idotea baltica	1	1	-	-	1	1	1	1	1	1	1	-	1	-	2	1	1
Callinectes sapidus	-	-	1	-	-	1	-	-	-	1	-	-	-	-	-	1	1
calanoid copepods	1	-	-	-	-	-	1	1	1	1	-	-	-	-	-	-	1
Treatment Average	2	3	25	37	5	19	21	38	22	27	24	37	31	26	48	30	27

Table 2. Results of Concentration, Distance, and Time Experiment. ANOVA table for linear model describing the effects of deterrent treatment, distance from source, and time of exposure on faunal biomass recovered from artificial substrates. Square root transformation of biomass was employed to improve normality and homogeneity of variance. *A priori* contrasts are listed below treatment.

Source	df	SS	*F*	*P*
Between Plots				
Treatment	3	1534.9	8.66	**0.001**
controls vs. grazer reduction	1	1387.0	23.49	**<0.001**
high deterrent vs. low deterrent	1	24.5	0.41	0.529
no-block vs. block control	1	123.5	2.09	0.168
Residual	16	944.9		
Within Plots				
Distance	1	999.3	54.91	**<0.001**
Time	1	24.5	1.34	0.249
Treatment × Distance	3	310.1	5.68	**0.001**
Treatment × Time	3	248.4	4.55	**0.005**
Distance × Time	1	24.0	1.32	0.253
Treatment × Distance × Time	3	22.7	0.42	0.742
Residual	128	2329.5		

Table 3. Results from Distance and Direction Experiment. ANOVA table for linear model describing the effects of grazer reduction treatment and distance from source on faunal biomass (natural log transformation) recovered from artificial substrates. F-tests were calculated using type III sums of squares.

Source	df	SS	*F*	*P*
Grazer Reduction	1	0.493	17.65	**1.7×10^{-4}**
Distance	1	0.901	32.31	**1.8×10^{-6}**
Reduction × Distance	1	0.617	22.10	**3.7×10^{-5}**
Residual	36	1.005		

Table 4. Results of Fall Nutrient and Grazer Manipulation: Crustacean Mesograzers. ANOVA table for linear model describing the effects of grazer reduction and sampling date on natural log-transformed crustacean mesograzer density. F-tests were calculated using type III sums of squares.

Source	df	SS	*F*	*P*
Reduction	1	16.44	42.84	**<0.001**
Sampling Date	2	10.57	13.77	**<0.001**
Reduction × Sampling Date	2	1.90	2.48	0.108
Residual	21	8.06		

Table 5. Results of Fall Nutrient and Grazer Manipulation: Gastropod Mesograzers. ANOVA table for linear model describing the effects of grazer reduction and sampling date on natural log-transformed gastropod mesograzer density. F-tests were calculated using type III sums of squares.

Source	df	SS	*F*	*P*
Reduction	1	0.31	0.33	0.574
Sampling Date	2	0.23	0.12	0.887
Reduction × Sampling Date	2	2.40	1.27	0.301
Residual	21	19.84		

Table 6. Results of Fall Nutrient and Grazer Manipulation: Micro-algal Epiphytes. ANOVA table for linear model describing the effects of chemical reduction, nutrient fertilization, and sampling date on natural log-transformed micro-algal epiphyte biomass. F-tests were calculated using type III sums of squares.

Source	df	SS	*F*	*P*
Reduction	1	0.44	3.97	0.052
Fertilization	1	0.34	3.10	0.084
Sampling Date	1	8.65	78.24	**<0.001**
Reduction × Fertilization	1	0.10	0.86	0.358
Reduction × Sampling Date	1	0.41	3.72	0.059
Fertilization × Sampling Date	1	0.77	7.00	**0.011**
Reduction × Fertilization × Sampling Date	1	0.09	0.81	0.371
Residual	51	5.64		

Table 7. Mobile epifauna (mean ± standard deviation) sampled during Summer Nutrient and Grazer Manipulation. Data during the experiment are pooled across dates. Abbreviations in parentheses are for use with Figure 12.

	Preliminary	*Experimental*					
Species		**X**	**C**	**N**	**P**	**NP**	**Species Mean**
Bittium varium (Bitt)	6.5 ± 3.8	49.1 ± 30.7	46.8 ± 24.1	36.1 ± 21.1	39.1 ± 19.6	48.4 ± 26.9	43.9 ± 25.0
Dulichiella appendiculata (Dulich)	1.0	34.6 ± 58.5	69.6 ± 92.7	23.3 ± 34.6	-	5.0	40.5 ± 65.1
Erichsonella attenuata (Erich)	21.0 ± 8.8	43.6 ± 45.1	40.0 ± 53.0	27.4 ± 26.5	8.3 ± 11.3	7.9 ± 7.9	25.9 ± 36.9
Cymadusa compta (Cyma)	31.1 ± 15.5	20.0 ± 26.1	24.7 ± 31.0	11.3 ± 17.6	1.8 ± 1.0	1.0	16.3 ± 24.3
Nereis sp.	6.1 ± 3.5	13.6 ± 13.1	13.4 ± 14.6	13.8 ± 12.5	18.4 ± 17.6	20.3 ± 15.1	15.9 ± 14.7
Elasmopus levis (Elas)	1.9 ± 1.1	11.3 ± 13.4	12.5 ± 17.5	11.3 ± 14.9	1.5 ± 0.7	1.0	10.9 ± 14.5
Ampithoe longimana (Al)	37.8 ± 23.4	14.6 ± 15.5	9.5 ± 8.7	8.5 ± 9.0	2.7 ± 1.9	3.1 ± 1.8	8.0 ± 10.1
Corophium spp. *(C* spp.)	13.1 ± 9.0	9.3 ± 11.0	7.6 ± 15.9	4.2 ± 4.0	1.0	1.0	7.0 ± 11.6
Gammarus mucronatus (Gamm)	13.6 ± 5.4	7.6 ± 7.2	6.4 ± 8.3	7.5 ± 17.1	2.0 ± 1.2	1.5 ± 1.2	6.3 ± 10.5
Caprella penantis (Cp)	12.3 ± 19.4	7.0 ± 6.6	7.6 ± 8.3	6.5 ± 8.0	1.7 ± 1.0	1.5 ± 1.3	5.5 ± 6.8
Idotea balthica	1.0	12.0 ± 15.6	1.0	-	1.0	1.0	4.7 ± 9.0
Hourstonius tortugae (Ht)		2.3 ± 1.5	5.0 ± 4.6	2.0 ± 1.4	1.5 ± 0.7	8.0 ± 11.4	4.4 ± 6.2
Nudibranch 2	4.5 ± 4.6	4.2 ± 3.0	3.4 ± 3.2	1.9 ± 1.0	4.2 ± 2.7	7.1 ± 6.7	4.3 ± 4.2
Paracaprella tenuis (Pt)	1.3 ± 0.5	2.5 ± 3.4	5.9 ± 5.9	1.9 ± 1.3	3.3 ± 2.3	5.3 ± 4.9	3.7 ± 4.1
Melita nitida		-	1.0	6.0	-	-	3.5 ± 3.5
Nudibranch 1	1.9 ± 1.0	3.5 ± 3.2	2.6 ± 3.1	3.6 ± 2.9	2.0 ± 1.7	3.4 ± 2.8	3.1 ± 2.8
Ampithoe valida	2.0 ± 1.4	1.0 ± 0.0	5.0	-	-	-	3.0 ± 2.8
Crepidula convexa (Cc)	2.5 ± 2.1	4.4 ± 5.7	3.5 ± 3.8	1.7 ± 0.9	3.1 ± 2.1	2.0 ± 1.6	2.9 ± 3.4
Acteocina canaliculata (Ac)		2.9 ± 2.9	1.0	1.3 ± 0.6	1.3 ± 0.5	2.5 ± 2.1	2.1 ± 2.0
Odostomia bisuturalis (Ob)		3.0	1.0	-	-	2.0	1.7 ± 0.8
Planarian	3.2 ± 2.4	1.3 ± 0.5	1.0	2.0 ± 1.4	1.8 ± 0.8	1.5 ± 1.0	1.6 ± 0.8
Triphora		-	2.0	-	-	1.3 ± 0.5	1.4 ± 0.6
Edotea triloba (Edot)		1.7 ± 0.8	1.0	1.3 ± 0.5	1.0	1.0	1.3 ± 0.6
Nudibranch 3	1.3 ± 0.6	-	1.0	1.0	1.0	1.3 ± 0.6	1.2 ± 0.4
Treatment Mean	12.7 ± 15.4	16.7 ± 26.9	17.2 ± 31.4	12.5 ± 18.6	10.4 ± 16.3	11.7 ± 19.0	14.1 ± 24.0

Table 8. Results of Summer Nutrient and Grazer Manipulation: Crustacean Mesograzers. ANOVA table for linear model describing the effects of chemical reduction, nutrient fertilization, and sampling date on natural log-transformed crustacean mesograzer density. Model run using generalized least squares procedure, which does not provide sums of squares. Model terms were tested using Wald tests. F- and P-values were calculated using type III sums of squares.

Source	df	*F*	*P*
Reduction	1	114.28	**<0.001**
Fertilization	1	0.02	0.902
Sampling Date	2	4.66	**0.012**
Reduction × Fertilization	1	0.13	0.720
Reduction × Sampling Date	2	7.29	**0.001**
Fertilization × Sampling Date	2	1.49	0.230
Reduction × Fertilization × Sampling Date	2	0.76	0.470
Residual	84		

Table 9. Results of Summer Nutrient and Grazer Manipulation: Gastropod Mesograzers. ANOVA table for linear model describing the effects of chemical reduction, nutrient fertilization, and sampling date on square root-transformed gastropod mesograzer density. Model run using generalized least squares procedure, which does not provide sums of squares. Model terms were tested using Wald tests. F- and P-values were calculated using type III sums of squares.

Source	df	*F*	*P*
Reduction	1	1.67	0.200
Fertilization	1	0.07	0.793
Sampling Date	2	20.20	**<.0001**
Reduction × Fertilization	1	2.13	0.148
Reduction × Sampling Date	2	2.45	0.093
Fertilization × Sampling Date	2	1.07	0.349
Reduction × Fertilization × Sampling Date	2	0.29	0.747
Residual	84		

Table 10. Results of Summer Nutrient and Grazer Manipulation: Micro-algal Epiphytes. ANOVA table for linear model describing the effects of chemical reduction, nutrient fertilization, and sampling date on natural log-transformed micro-algal epiphyte biomass. Model run using generalized least squares procedure, which does not provide sums of squares. Model terms were tested using Wald tests. F- and P-values were calculated using type III sums of squares.

Source	df	*F*	*P*
Reduction	1	50.30	**<0.001**
Fertilization	1	0.87	0.354
Sampling Date	2	1.76	0.178
Reduction × Fertilization	1	0.17	0.677
Reduction × Sampling Date	2	10.52	**<0.001**
Fertilization × Sampling Date	2	1.08	0.344
Reduction × Fertilization × Sampling Date	2	1.36	0.263
Residual	82		

Table 11. Results of Summer Nutrient and Grazer Manipulation: Eelgrass Growth. ANOVA table for linear model describing the effects of chemical reduction and nutrient fertilization on Box-Cox-transformed eelgrass growth. Model run using generalized least squares procedure, which does not provide sums of squares. Model terms were tested using Wald tests. F- and P-values were calculated using type III sums of squares.

Source	df	*F*	*P*
Reduction	1	0.02	0.899
Fertilization	1	0.00	0.995
Sampling Date	3	32.98	**<0.001**
Reduction × Fertilization	1	0.63	0.432
Reduction × Sampling Date	3	0.70	0.555
Fertilization × Sampling Date	3	0.27	0.845
Reduction × Fertilization × Sampling Date	3	2.32	0.083
Residual	65		

Table 12. Results of Summer Nutrient and Grazer Manipulation: Eelgrass Shoot Density. ANOVA table for linear model describing the effects of chemical reduction and nutrient fertilization on the proportional change in eelgrass shoot density (number of shoots per plot). Box-Cox power transformation was applied to improve normality and homogeneity of variance. F-tests were calculated using type III sums of squares.

Source	df	SS	*F*	*P*
Reduction	1	1.81	6.68	**0.015**
Fertilization	1	0.34	1.24	0.275
Reduction × Fertilization	1	0.03	0.10	0.751
Residual	28	7.59		

Table 13. Structural Equation Model Path Coefficients. All coefficients are unstandardized, so interpretation is on a unit basis. Maximum likelihood and Bayesian estimates are tested for significant differences from zero. All data except Deterrent are natural log-transformed to increase linearity of bivariate relationships. Numerals at the end of variable names denote day of sampling. The dotted line separates day 10 responses from day 24 responses. Lower and Upper bounds in Bayesian estimation are 95% credible intervals.

	Maximum Likelihood			*Bayesian Estimation*			
Path	Estimate	S.E.	*P*	Mean	S.D.	95% Lower	95% Upper
Deterrent → Gammarids2	-2.10	0.20	<0.001	-2.10	0.23	-2.54	-1.65
Deterrent → Caprellids2	-0.78	0.24	<0.001	-0.79	0.26	-1.32	-0.27
Seagrass2 → Gammarids2	0.87	0.37	0.020	0.87	0.43	0.01	1.71
Seagrass2 → Epiphytes2	0.41	0.09	<0.001	0.41	0.11	0.20	0.62
Macroalgae2 → Gammarids2	1.09	0.17	<0.001	1.09	0.20	0.70	1.48
Macroalgae2 → Caprellids2	0.77	0.21	<0.001	0.77	0.22	0.34	1.21
Macroalgae2 → Epiphytes2	0.28	0.05	<0.001	0.28	0.06	0.17	0.39
Gammarids2 → Epiphytes2	-0.17	0.02	<0.001	-0.16	0.03	-0.22	-0.11
Caprellids2 → Epiphytes2	-0.11	0.03	<0.001	-0.11	0.04	-0.18	-0.04
Deterrent → Gammarids4	-1.55	0.27	<0.001	-1.56	0.30	-2.13	-0.98
Gammarids2 → Macroalgae4	0.17	0.05	0.001	0.18	0.06	0.07	0.29
Seagrass2 → Seagrass4	0.43	0.13	0.001	0.44	0.15	0.15	0.72
Epiphytes2 → Epiphytes4	1.16	0.29	<0.001	1.14	0.33	0.53	1.86
Seagrass4 → Gammarids4	1.39	0.50	0.006	1.40	0.55	0.31	2.49
Macroalgae4 → Gammarids4	1.11	0.26	<0.001	1.11	0.29	0.55	1.68
Gammarids4 → Epiphytes4	-0.11	0.05	0.031	-0.11	0.06	-0.22	0.00

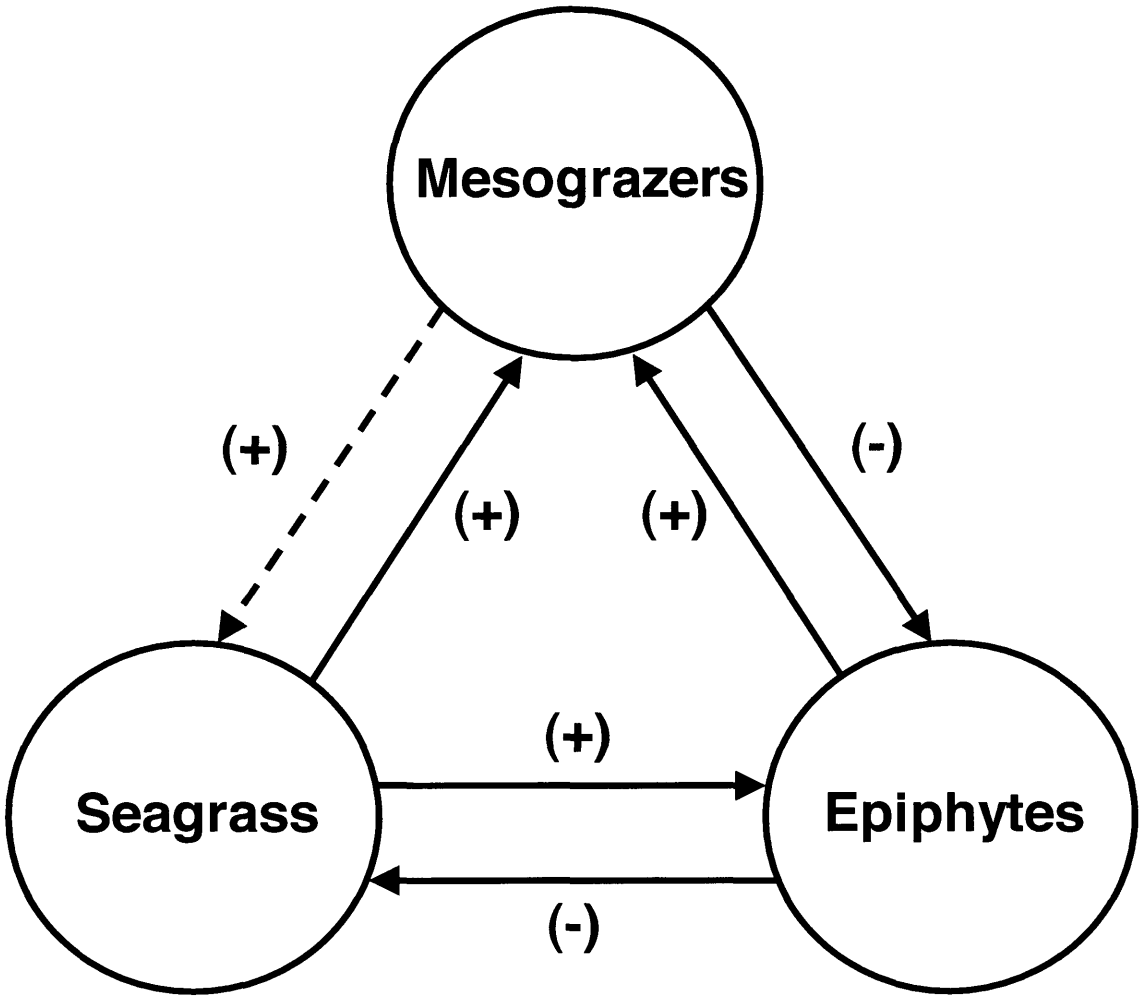


Figure 1. Conceptual diagram of the base of temperate seagrass food webs. Arrows leading from seagrass represent positive foundation species effects. The positive indirect interaction between mesograzers and seagrass (dashed arrow) is the result of consumption of epiphytes by mesograzers and the subsequent reduction in competition between seagrass and epiphytes.

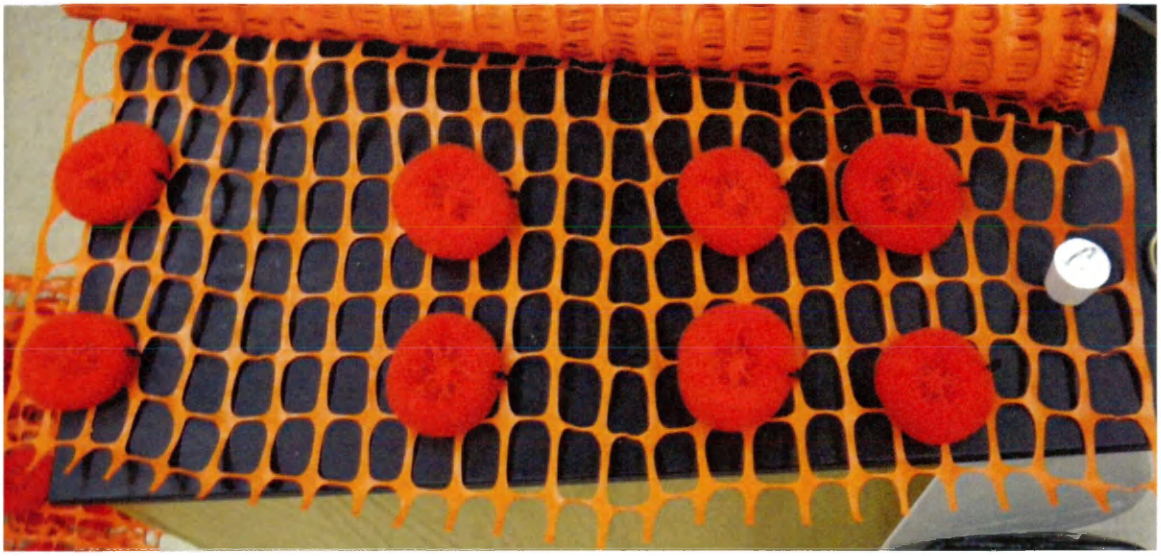


Figure 2. Experimental plot used in Concentration, Distance, and Time Experiment.

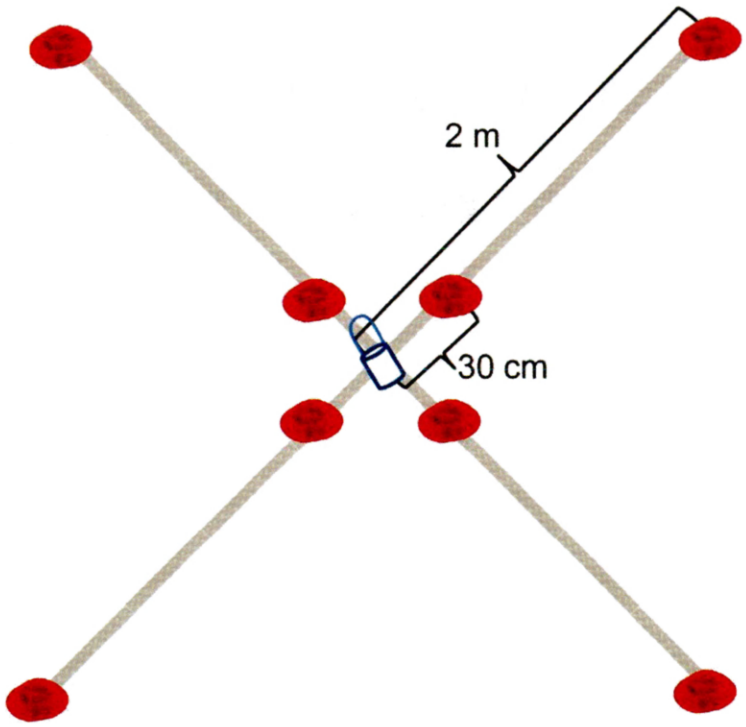


Figure 3. Experimental plot used in Distance and Direction Experiment. Red objects are artificial scrubber substrates. A plaster block is located in the center of the plot. Figure is not drawn to scale.

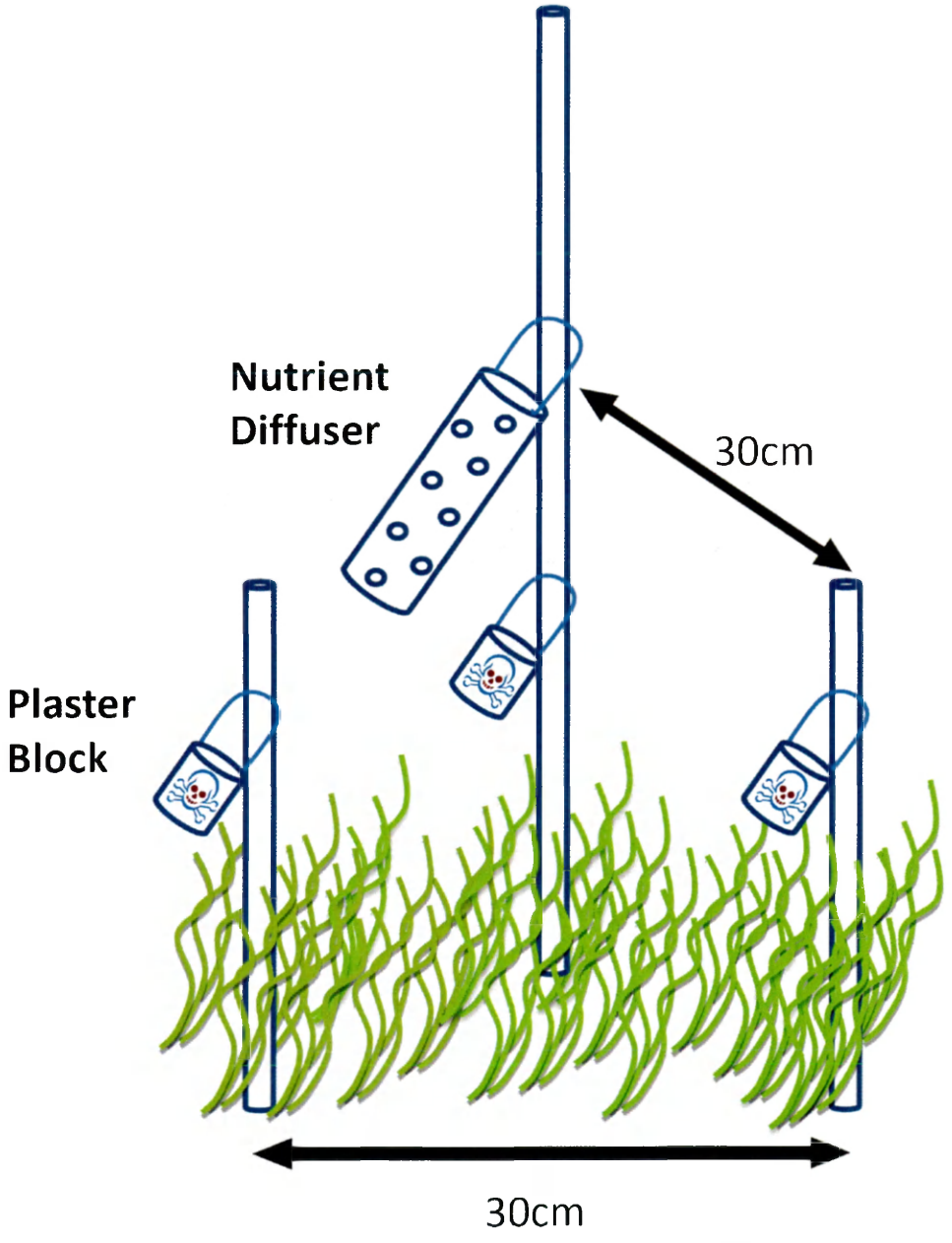


Figure 4. Experimental plot used in Nutrient and Grazer Manipulations (Experiments 3 and 4).

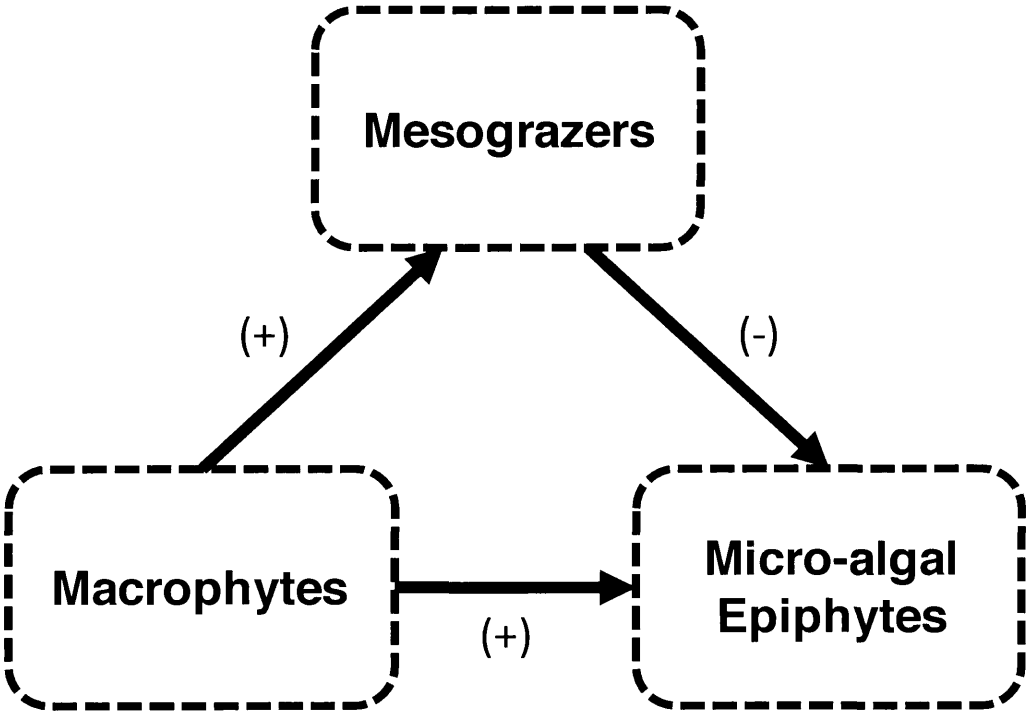


Figure 5. "Meta-model" of structural equation model used in analysis of Summer Nutrient and Grazer Manipulation.

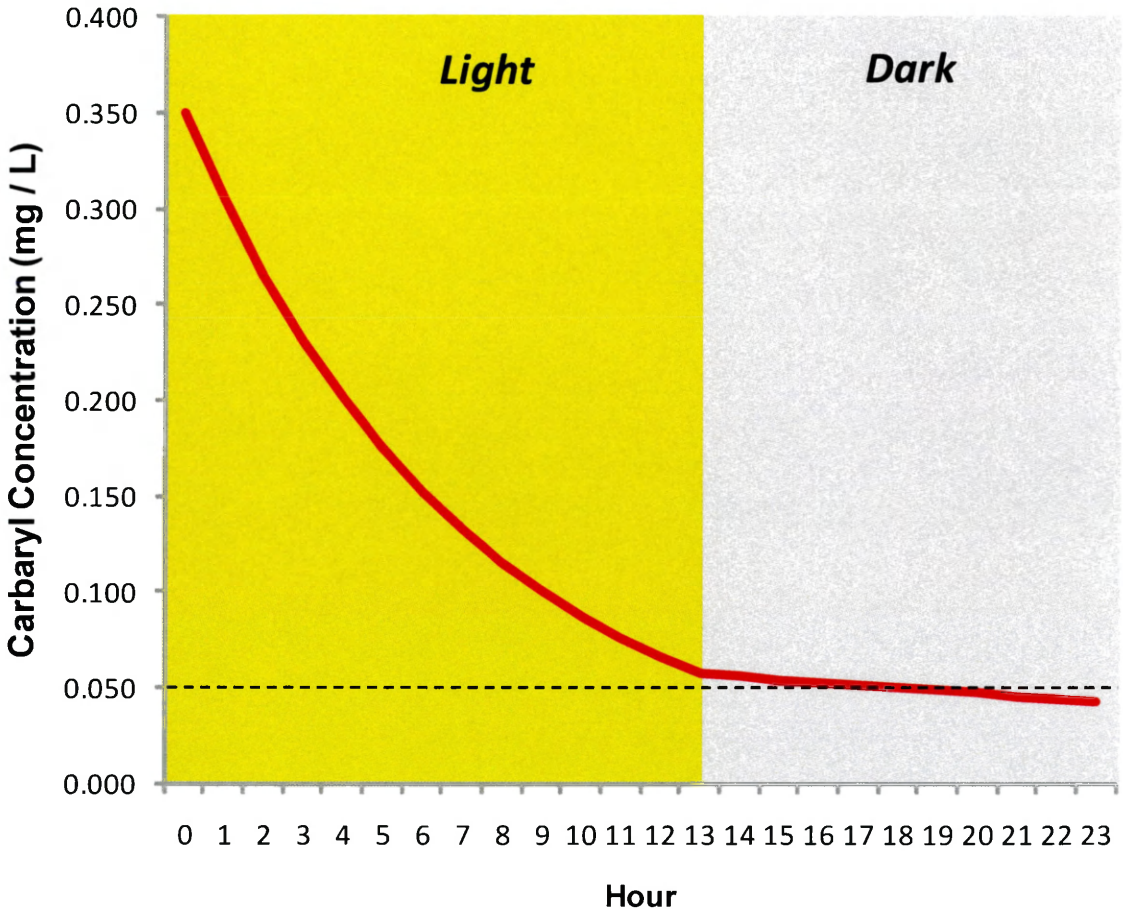
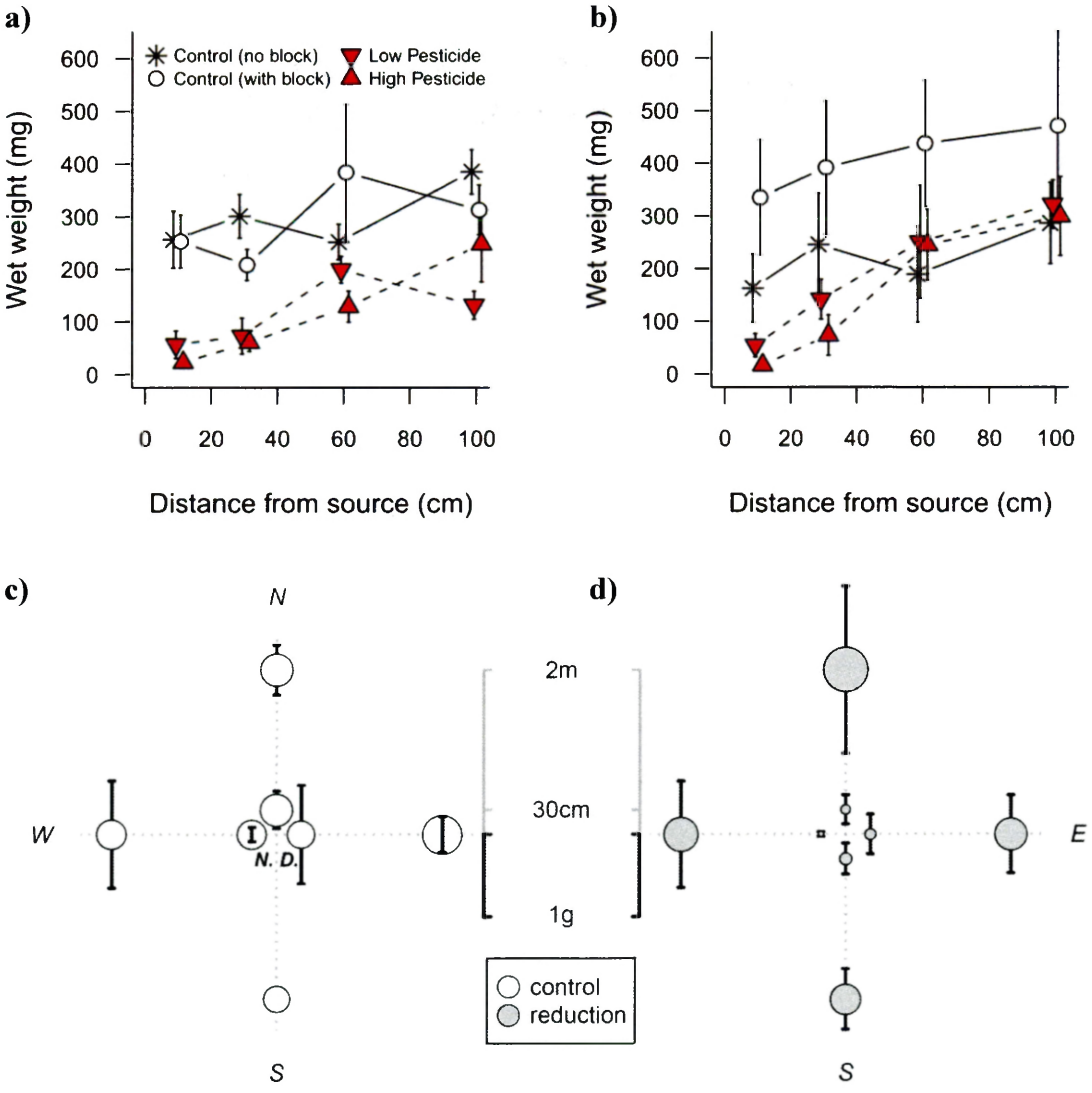


Figure 6. Modeled degradation of carbaryl via hydrolysis and photolysis over a 24-hour period. 14 hours of sunlight at assumed for a latitude of 37° N at day of year 180. Degradation modeled using first order kinetics and $H_{1/2}$ = 5 in light and $H_{1/2}$ = 24 in dark.

Figure 7. Results from experiments testing the efficacy of grazer removal using carbaryl in the field. (a-b): Faunal wet biomass (mean ± 1 SE) recovered during Distance and Direction Pilot after (a) 3 days and (b) 8 days exposure to experimental treatments. Symbols represent different treatments. Solid and dashed lines are used to distinguish deterred and control samples, respectively. (c-d): Bubble plot of faunal biomass (grams wet weight) from Distance and Direction Pilot. Symbol size is proportional to mean wet weight per treatment at each distance and direction (symbol size in legend represents 1 gram). Error bars represent ± 1 SD and bar length is depicted using y-axis (i.e. 1 meter = 1 gram). Control data (c) are shown with white symbols and grazer-reduction data (d) are shown with gray symbols. Points arranged on plots at orthogonal distances from an origin, corresponding to distances and directions from center of field plots. Cardinal directions are abbreviated in italics. N.D. = no data.


a)
b)
Control (no block)
Control (with block)
Low Pesticide
High Pesticide
Wet weight (mg)
Distance from source (cm)
c)
d)
N
W
E
S
N. D.
2m
30cm
1g
control
reduction

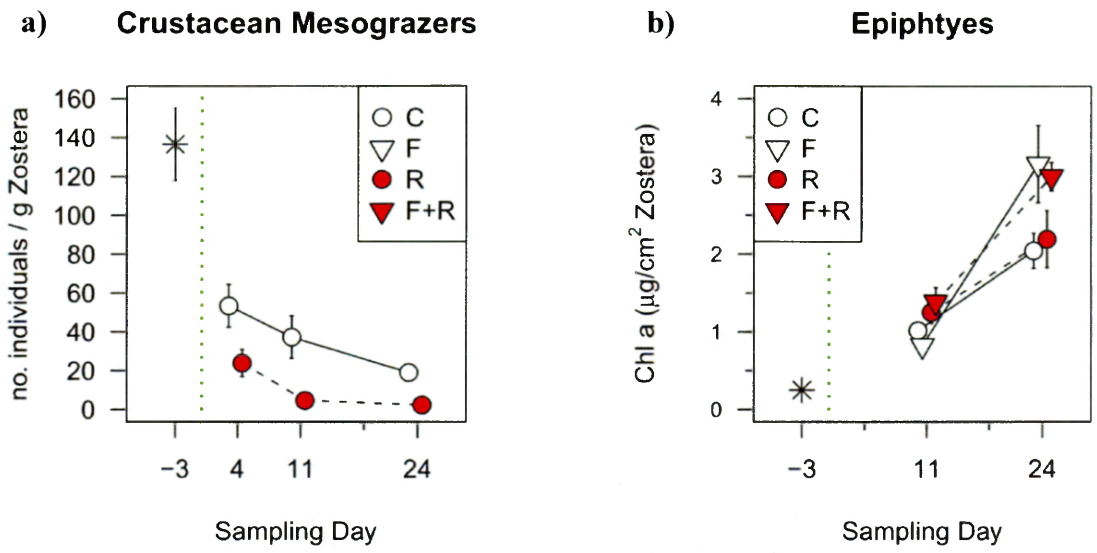


Figure 8. Time series of (a) crustacean mesograzer density and (b) micro-algal epiphyte biomass (mean ± 1 SE) during Fall Nutrient and Grazer Manipulation. Vertical dotted line denotes the beginning of experiment on 20 October 2008. Treatments: no-block control (X), block control (C), nutrient fertilization (F), grazer reduction (R), fertilization + reduction (F+R).

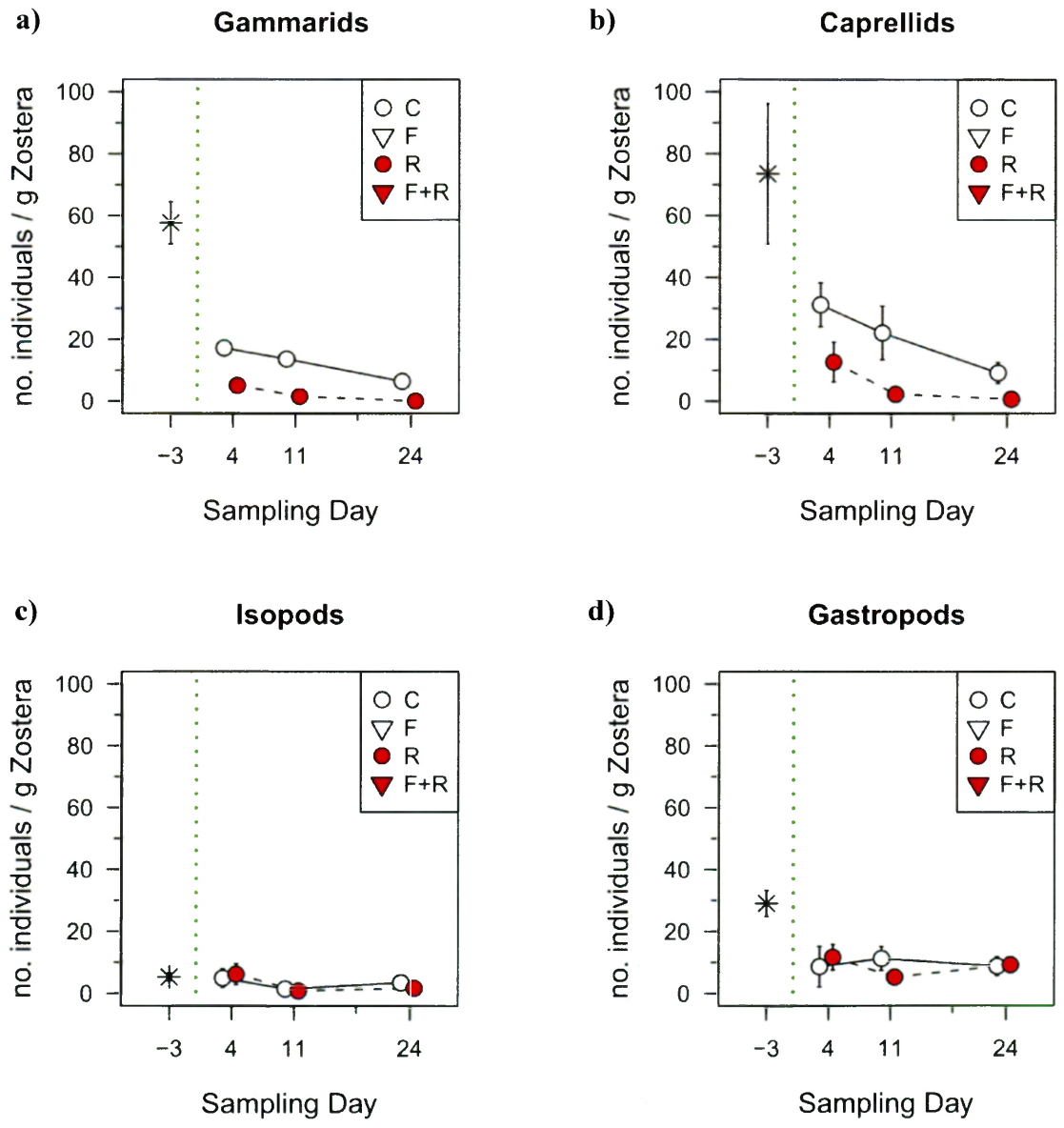


Figure 9. Density of mesograzer taxa (mean ± 1 SE) during Fall Nutrient and Grazer Manipulation. Vertical dotted lines denotes the beginning of experiment on 20 October 2008. Treatments: no-block control (X), block control (C), nutrient fertilization (F), grazer reduction (R), fertilization + reduction (F+R).

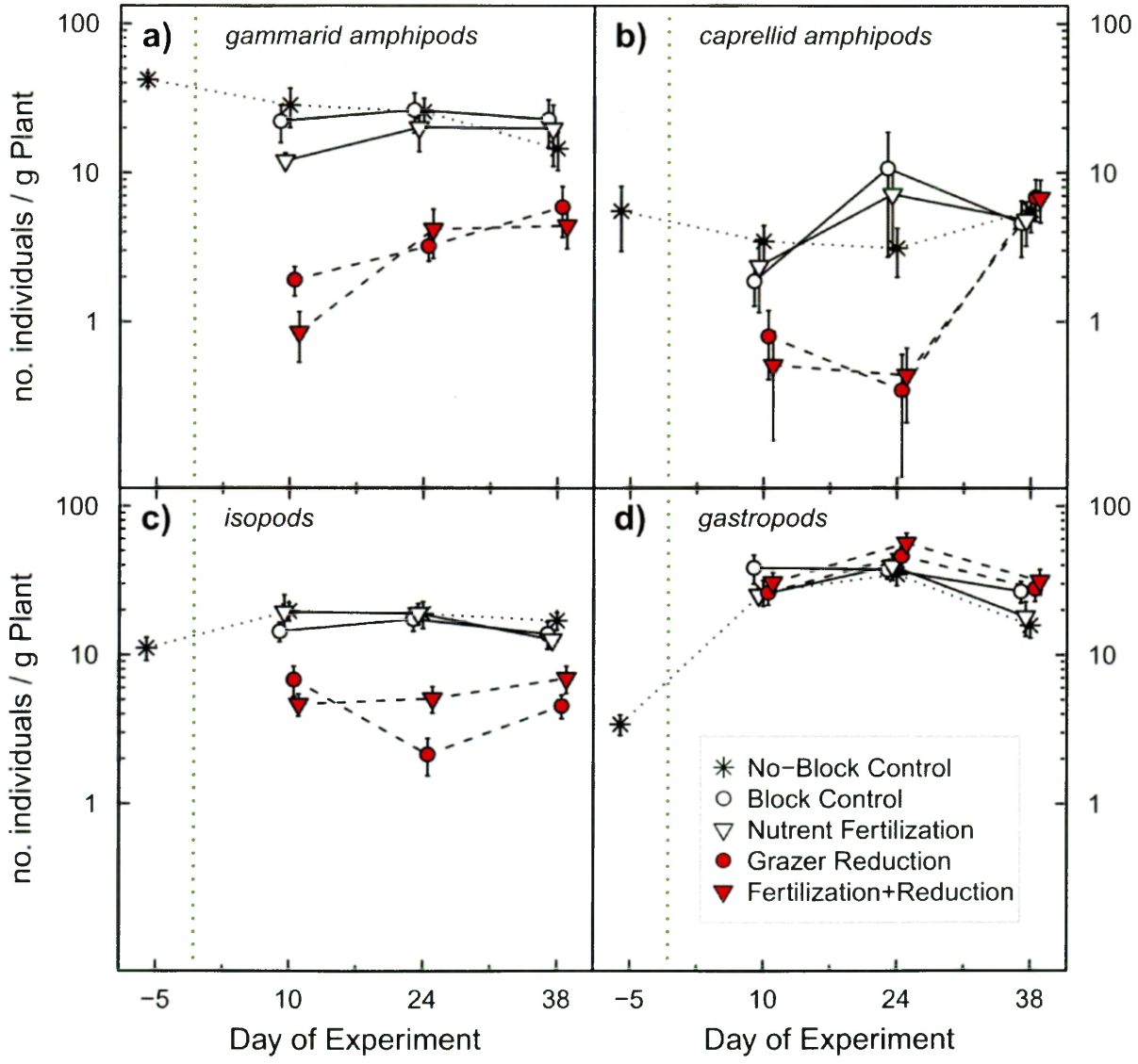


Figure 10. Density of mesograzer taxa (mean ± 1 SE) during Summer Nutrient and Grazer Manipulation. Vertical dotted line denotes the beginning of experiment on 20 June 2009. No-block treatment was not used in repeated measures ANOVA.

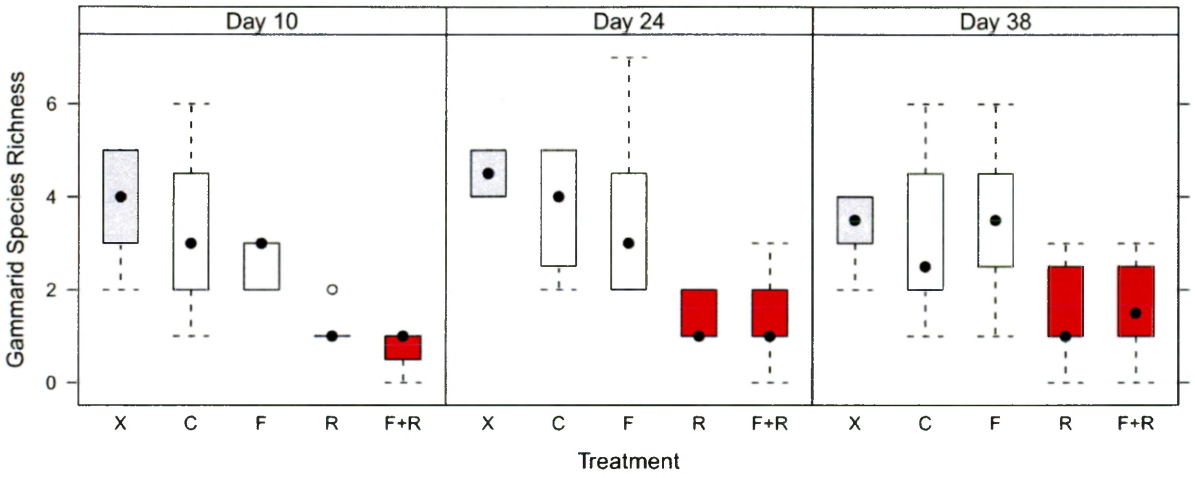


Figure 11. Gammarid species richness grouped by treatment and sampling date during Summer Nutrient & Grazer Manipulation. Treatments: no-block control (X), block control (C), nutrient fertilization (F), grazer reduction (R), fertilization + reduction (F+R). Boxes are 25% and 75% quartiles, the black circle in the middle of the box is the median, and whiskers extend to the extreme data points that are no more than 1.5 times the interquartile range from the box. Data exceeding 1.5 times the interquartile range are shown as unfilled circles.

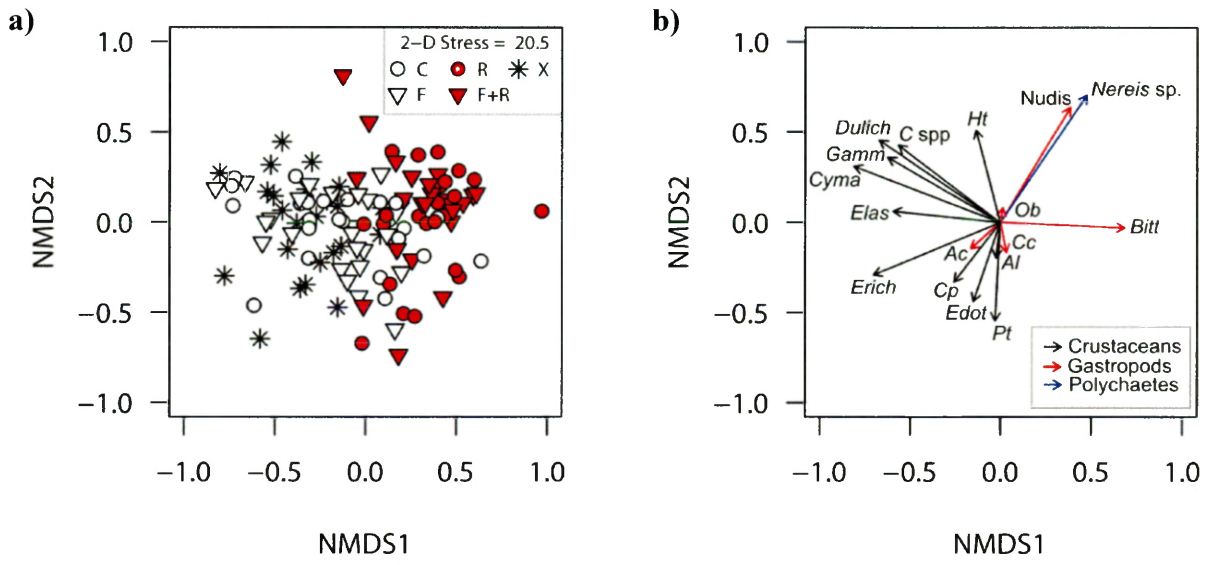


Figure 12. Non-metric multidimensional scaling of epifaunal assemblages recovered during Summer Nutrient and Grazer Manipulation. a) Data points are shown by treatment. b) vectors corresponding to species (see Table 7 for names) are overlaid on the same plot as shown in (a). Data are pooled across sampling dates. Treatments: no-block control (X), block control (C), nutrient fertilization (F), grazer reduction (R), fertilization + reduction (F+R).

Figure 13. Primary producer responses (mean ± 1 SE) to environmental treatments during Summer Nutrient and Grazer Manipulation. a) Micro-epiphyte biomass. Points are jittered to prevent overlap. Treatments: no-block control (X), block control (C), nutrient fertilization (F), grazer reduction (R), fertilization + reduction (F+R). Prelimary and no-block control treatments are marked with asterisks and where not used in repeated measures ANOVA. Green vertical line denotes the first day of the experiment on 20 June 2009. b) Eelgrass growth rate. Symbols and bars as in (a). Blue lines are minimum, maximum, and mean daily water temperature during the experiment. Samples from Day 10 were not used in repeated measures ANOVA. Inset text is Pearson's product-moment correlation coefficient between mean eelgrass growth and mean daily water temperatures for each sampling day and the preceding 10 days. c) Proportional change in eelgrass shoot density from preliminary sampling to experiment breakdown. Treatments as in a). No-block control treatment was not used in ANOVA to preserve orthogonality.

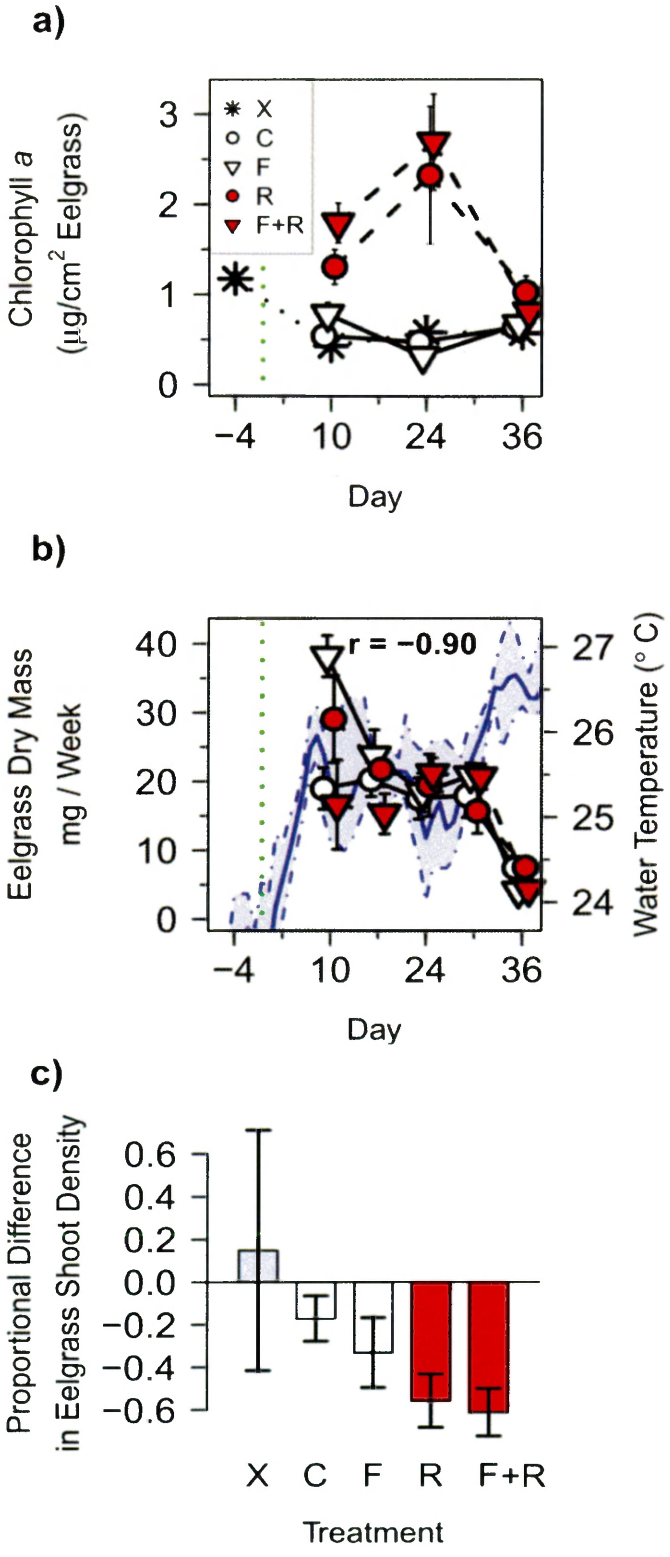
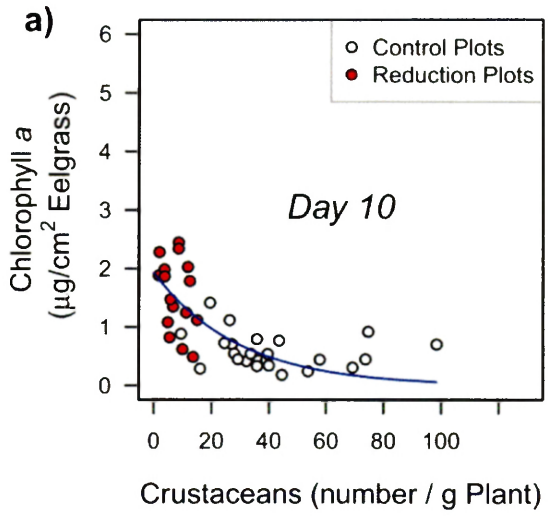
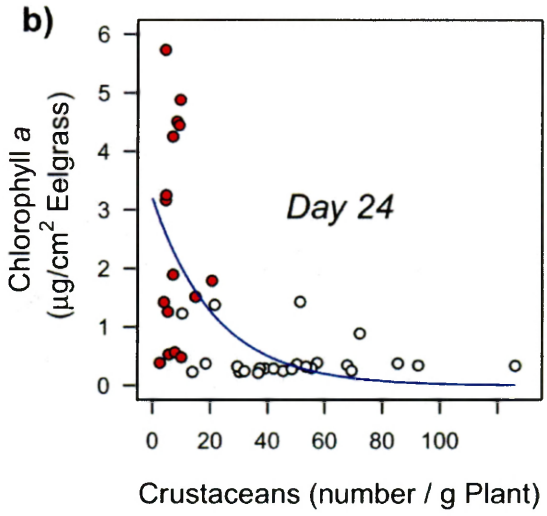
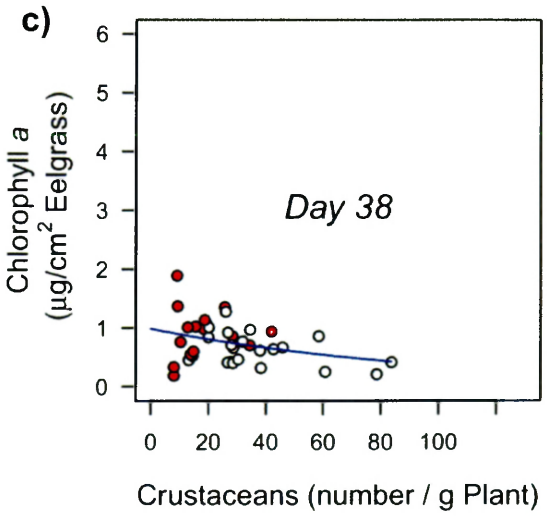

a)
Chlorophyll a
(µg/cm² Eelgrass)
X
C
F
R
F+R
3
2
1
0
−4
10
24
36
Day
b)
Eelgrass Dry Mass
mg / Week
r = −0.90
40
30
20
10
0
27
26
25
24
Water Temperature (° C)
−4
10
24
36
Day
c)
Proportional Difference
in Eelgrass Shoot Density
0.6
0.4
0.2
0.0
−0.2
−0.4
−0.6
X
C
F
R
F+R
Treatment

Figure 14. Bivariate relationship between crustacean mesograzer density and micro-epiphyte biomass at each sampling day of Summer Nutrient and Grazer Manipulation. Color of circles corresponds to the use of chemical deterrent. Blue lines are exponential curves fitted to data at different sampling dates.


a)
Control Plots
Reduction Plots
Day 10
Chlorophyll a
(µg/cm² Eelgrass)
0
1
2
3
4
5
6
0
20
40
60
80
100
Crustaceans (number / g Plant)


b)
Day 24
Chlorophyll a
(µg/cm² Eelgrass)
0
1
2
3
4
5
6
0
20
40
60
80
100
Crustaceans (number / g Plant)


c)
Day 38
Chlorophyll a
(µg/cm² Eelgrass)
0
1
2
3
4
5
6
0
20
40
60
80
100
Crustaceans (number / g Plant)

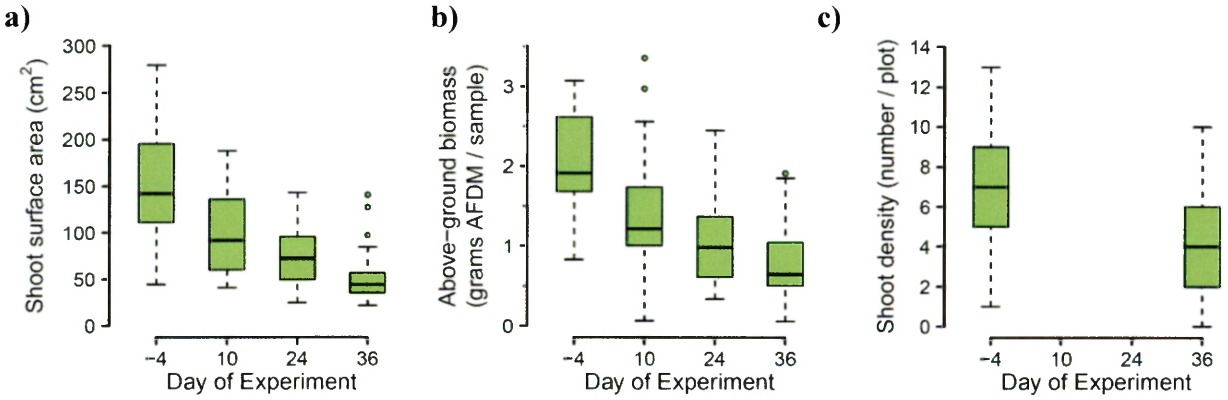


Figure 15. Three eelgrass metrics grouped by sampling date from Summer Nutrient and Grazer Manipulation. (a) Surface area of eelgrass shoots from which micro-epiphytes were scraped. (b) Eelgrass above-ground biomass collected using epibenthic grab sampler. (c) Eelgrass shoot density collected from circular plot within experimental units prior to experiment and at breakdown. Boxes are 25% and 75% quartiles, the line in the middle of the box is the median, and whiskers extend to the extreme data points that are no more than 1.5 times the interquartile range from the box. Data exceeding 1.5 times the interquartile range are shown as filled circles.

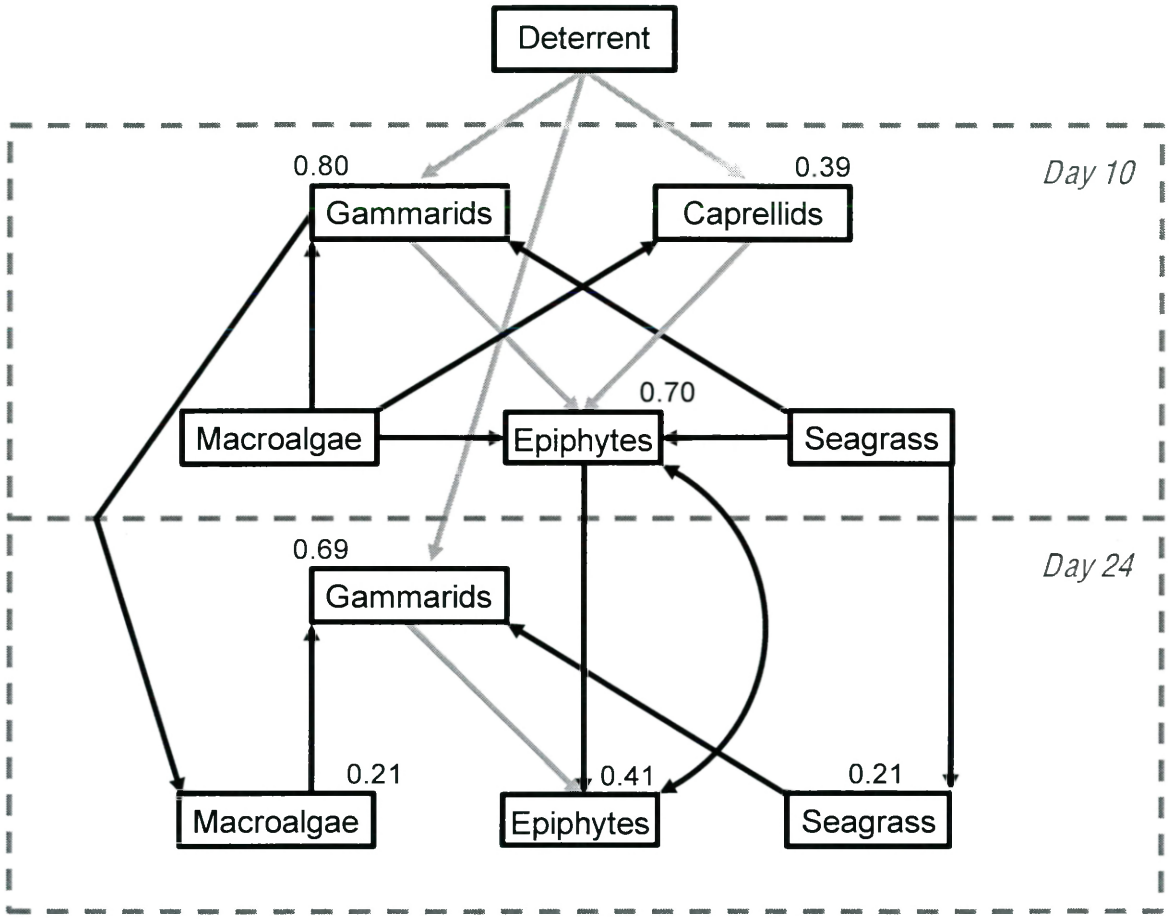


Figure 16. Structural Equation Model for Summer Nutrient and Grazer Removal Manipulation. Data from days 10 and 24 are included. R^2 values are displayed above engogenous variables. All data except Deterrent are natural log-transformed to increase linearity of bivariate relationships. Black and gray single-headed arrows represent positive and negative path coefficients, respectively. Double-headed arrow denotes correlated errors. See Table 12 for estimates of path coefficients.

VITA

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