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# Community dynamics in submersed aquatic vegetation: Intermediate consumers as mediators of environmental change

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# Community dynamics in submersed aquatic vegetation: intermediate consumers as mediators of environmental change

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APPROVAL SHEET

This dissertation is submitted in partial fulfillment of

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## ABSTRACT

Natural ecosystems are strongly affected by changes in resource supply (bottom-up forces) and by changes in upper trophic levels (top-down forces). The extent to which these processes impact a system depends largely on the responses of organisms at middle trophic levels. In seagrass beds, a group of mid-level consumers known as mesograzers form a critical link in the chain of impact, connecting seagrass and epiphytic algae with predatory fishes and crustaceans. I observed dramatic seasonal and interannual changes in mesograzer abundance and species composition in eelgrass (*Zostera marina*) beds of lower Chesapeake Bay, Virginia, and endeavored to explain the top-down and bottom-up causes and consequences of those changes with field studies and controlled experiments. A field cage experiment showed that grazing, predation and nutrient enrichment all had strong effects on the eelgrass community, but that the effects of each factor varied for different community components (Chapter 1). A second experiment delved deeper into the predation dynamic by manipulating the diversity of both predators and mesograzers in macroalgal mesocosms. Increasing predator diversity increased the strength of predation, but increasing mesograzer diversity conferred resistance to some types of predation (Chapter 2). To assess the influence of top-down and bottom-up forces in a more natural context, I analyzed the long-term changes in biotic and abiotic components of an eelgrass bed at the Goodwin Islands National Estuarine Research Reserve. I found that abiotic processes had strong effects on both consumer and resource abundance, and could therefore initiate either top-down or bottom-up control of eelgrass community structure (Chapter 3). To examine this top-down and bottom-up control in more detail I explicitly compared the ecological relationships seen in the field to those observed in mesocosm experiments. Mesocosm experiments tended to find a greater influence of top-down effects and a lesser influence of bottom-up effects, relative to field observations (Chapter 4). Finally, I took a snapshot of the eelgrass food web itself by examining the gut contents and stable carbon and nitrogen isotopic ratios of predators, mesograzers, and plants. I found that direct grazing on eelgrass does occur, but that microalgae and detritus provide the main trophic support for the epifaunal community (Chapter 5). Overall, my results suggest that both top-down and bottom-up forces control eelgrass community structure via mesograzers, but that top-down control in the field is more subtle and more intimately tied with bottom-up control than has been indicated by some manipulative experiments.

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# INTRODUCTION

## Top-down and bottom-up processes in ecology

In the food chain model of ecosystem structure, the propagation of ecological effects from consumers to prey or basal resources is termed "top-down" control, while the influence of basal resources or prey on successively higher consumers is termed "bottom-up" control. Hairston, Smith, & Slobodkin (1960) realized that strong top-down control could lead to alternating patterns of abundance at adjacent levels in the food chain, and theorized that the terrestrial environment was green because abundant predators controlled herbivores, allowing plants to flourish. This type of top-down control is now known as a trophic cascade (Paine 1980). Trophic cascades are well documented in aquatic systems (Shurin et al. 2002), both freshwater (Brett & Goldman 1996, 1997) and marine (Pinnegar et al. 2000). Low diversity, vulnerable primary producers, and strong consumer-prey interactions are thought to facilitate trophic cascades, and are common among the limnetic and rocky intertidal systems where cascades are most often observed (Strong 1992, Cyr & Pace 1993, Polis 1999). Top-down control is often limited by prey defenses, environmental disturbance, and other ecological factors (Polis 1999), however, while bottom-up control is inescapable. Light, water, and nutrients set absolute limits on primary productivity, which in turn limits higher trophic levels. While these observations caution against a strictly top-down view (see also Power 1992, Polis & Strong 1996) there is a growing consensus that both top-down and bottom-up forces have a role in determining productivity, the distribution of biomass, and other aspects of ecosystem structure (Polis 1999, Heck et al. 2000, Worm et al. 2002). Seagrass ecosystems lend themselves to studies of the interplay of top-down and bottom-up processes, because their primary producers

include both algae and vascular macrophytes, which compete with each other and respond differently to grazing and abiotic factors (Jernakoff et al. 1996, Valentine & Duffy 2006).

**The role of mesograzers in seagrass ecosystems**

Seagrasses such as eelgrass (*Zostera marina*) support productive and species-rich communities in coastal areas throughout the world (Hemminga & Duarte 2000). Seagrasses have declined dramatically in many regions, however, apparently in response to increased nutrient and sediment inputs from human activities (Twilley et al. 1984, Duarte 2002, Orth et al. 2006). Chesapeake Bay has been particularly hard-hit, having lost over 80% of its eelgrass beds since aerial surveys began in the middle twentieth century (Orth et al. 2002).

It is believed that grazers directly consume only a small fraction of seagrass primary production in temperate seagrass beds (Nienhuis & Groenendijk 1986), although this may not have been the case prior to the current "anthropocene" era of pervasive human impacts on global ecosystems. Direct seagrass consumption is more prevalent in the tropics (Valentine & Heck 1999), but probably less so now than historically, since large vertebrate grazers have been decimated by humans (Jackson et al. 2001). Hence, Valentine & Duffy (2006, p.1) describe modern seagrass food webs as "seagrass detrital ecosystems":

"Seagrass detrital ecosystems *are mostly devoid of large vertebrates, and herbivory is dominated by small invertebrate* ***mesograzers*** *that feed primarily on algae, indirectly enhancing seagrasses by releasing them from algal competition, resulting in high seagrass biomass, a tendency toward monodominance in the absence of mitigating disturbance, and most seagrass production entering the detrital food chain." p.1.*

Valentine and Duffy's assertion that mesograzers release seagrass from competition with algal epiphytes is supported by numerous experimental studies (reviewed in van Montfrans et al. 1984, Jernakoff et al. 1996, Valentine & Duffy 2006). By maintaining low algal biomass, mesograzers may enhance the ability of seagrass beds to persist in increasingly eutrophic systems. Neckles et al. (1993), using eelgrass planted in mesocosms in greenhouses, showed that nutrient enrichment enhanced epiphyte growth in the absence of grazers but had only minor effects when grazers were abundant. Lin et al. (1996) and Heck et al. (2000, 2006) obtained similar results in outdoor mesocosms and field enclosures, respectively. Noting the growing evidence of the important role of mesograzers, Moore and Wetzel (2000) hypothesized that "... any factor which affects the level of grazer populations on *Z. marina* such as mortality or predation can have important implications for seagrass survival or successful recruitment into formerly vegetated areas."

Though mesograzers generally appear to exert a positive, indirect impact on seagrass, some species of amphipods and isopods are capable of consuming live seagrass and have caused destruction of seagrass in cultures (Kirkman 1978, Short et al. 1995, Duffy & Harvilicz 2001, Duffy et al. 2003, 2005). Mesograzers endogenously produce some enzymes for digesting structural carbohydrate components of macrophytes, and may also digest plant material with the aid of gut endosymbionts (McGrath & Mathews 2000). Destructive overgrazing by small invertebrates has been documented in the field in macroalgal (Harrold & Reed 1985, Salemaa 1987, Davenport & Anderson 2007) and salt marsh (Silliman & Bertness 2002) systems, but has rarely been observed in seagrass systems (but see Zimmerman et al. 2001). What prevents overgrazing on seagrass? By analogy with other systems, grazer populations may be limited by

predation, with overgrazing occurring only when a change in trophic structure releases herbivores from top-down control (i.e. Estes and Duggins 1995). As fisheries activities change the trophic structure of seagrass beds, it is possible that the positive, indirect impacts of mesograzers could shift to negative, direct impacts (overgrazing). Whether mesograzers will stabilize seagrass communities in the future, or hasten their decline, is contingent upon the way mesograzer abundance and species composition respond to simultaneous eutrophication and alteration of higher trophic levels.

Strong variability in mesograzer abundance and species composition has been documented over seasonal and shorter time scales in temperate and subtropical systems (Marsh 1970, Nelson 1979, Nelson et al. 1982, Edgar 1990, this study). The potential causes of this variability include changing top-down and bottom-up forces. Both types of forces exhibit natural variability on diurnal to interannual scales, and are susceptible to human influences such as urban runoff (Orth and Moore 1986) and overfishing of predators (Heck et al. 2007). Top-down control of mesograzer populations is suggested by negative covariance in spatial and temporal abundance patterns of fish and mobile epifauna (Nelson et al. 1982, Orth 1992, Jørgensen et al. 2007), and has sometimes been observed experimentally (Nelson 1981, Leber 1985, Heck et al. 2000, 2006, Duffy et al. 2005). However, problems with the caging experiments designed to exclude or include predators, including colonization of cages by small, predatory crustaceans, have confounded the evidence for top-down control in most of these experiments (see Young et al. 1976, Virnstein 1978). The most rigorous studies to date of the relative influence of top-down and bottom-up forces on mesograzer abundance and secondary productivity have been performed in Japan and Australia by Edgar (1990, 1993, Edgar & Aoki 1993) and in the Gulf of Mexico by Heck et al. (2000, 2006). Edgar found that mesograzers tracked changing resource

levels, and that their size distribution and species composition, but not their secondary productivity, was affected by predation. This led Edgar & Aoki (1993) to the "resource ceiling hypothesis" that total mesograzer productivity was controlled from the bottom up by a limiting resource, probably epiphytic algae. A series of experiments using standardized artificial substrates in locations across the globe seemed to confirm the production ceiling hypothesis. Epifaunal secondary productivity (normalized to 20°C) was low at deep, turbid, and nutrient-depleted sites but did not vary more than two-fold among shallow, clear water sites (Edgar 1993, Edgar & Klump 2003). Edgar interpreted the low variation among the shallow, clear water sites as evidence for the ineffectiveness of top-down control on mesograzer secondary productivity. Necessary to Edgar's conclusion, however, was the untested assumption that predation varied widely among his shallow, clear water sites. Another point of concern regarding the analysis of Edgar & Klump (2003) is their omission of shallow, eutrophic seagrass beds. Eutrophic seagrass beds often have high standing stocks of edible algae and periphyton, suggesting that food resources do not limit their mesograzer inhabitants. Many Northern Hemisphere temperate and subtropical seagrass systems are quite eutrophic, as evidenced by high levels of epiphytic micro- and macroalgae. Increasing coastal development is causing eutrophication even of beds that were once nutrient poor (Cloern 2001, Duarte 2002, Orth et al. 2006). A further distinction between Edgar's systems and many Northern Hemisphere seagrass beds is the diversity of the mesograzer communities in both areas. Upwards of 30 mesograzer species were regular at Edgar's study sites, whereas Northern Hemisphere systems may have less than 5 or 10 abundant mesograzer species (Marsh 1970, Nelson 1980, this study). Recent ecological theory suggests that high herbivore diversity relative to predator diversity may dampen or reduce top-down control of lower trophic levels (Duffy 2002). Diverse, austral mesograzer communities may be

unusually resistant to top-down control. For all these reasons, Edgar's finding that aggregate mesograzer production is predominantly resource-limited should not be assumed to apply to less diverse, more eutrophic seagrass systems.

Secondary production is not the only relevant measure of mesograzer ecosystem function. While aggregate mesograzer productivity may not be strongly affected by predation, mesograzer size distribution and species composition are clearly affected (Nelson 1981, Leber 1985, Edgar 1990, 1993a, Duffy & Hay 2000, Duffy et al. 2005.). Shifts in mesograzer species and sizes could have important implications for predators and plants. Even at an equivalent level of secondary production, a community of small-bodied grazers has less biomass than a community of larger grazers due to scaling constraints of metabolism. Hence equivalent secondary production does not imply equal production entering higher trophic levels. Grazer species composition changes alone could also have significant impacts on ecosystem properties because of strong differences in feeding behavior among species. Experimental manipulations of mesograzer species composition have resulted in dramatic changes in total biomass and species composition of epiphytic algae and macrophytes (Duffy & Hay 2000, Duffy & Harvilicz 2001, Duffy et al. 2003, Spivak et al. 2007). Similar experiments have shown that predators can alter mesograzer species composition, as well. In some of these experiments, overgrazing by mesograzers occurred in the absence of predators but not when predators were present (Duffy et al. 2005) corroborating earlier reports of loss of seagrass cultures to mesograzers in the absence of predators (Kirkman 1978, Short et al. 1995). Occasional observations of overgrazing of large macroalgae following amphipod outbreaks in the field (Kangas et al. 1982, Haahtela 1984, Tegner and Dayton 1987) also suggest that overgrazing occurs when unusual circumstances relax top-down controls.

Thus, while there may be truth to Edgar & Aoki's (1993) resource ceiling hypothesis for the specific case of secondary production of mesograzers, it is nevertheless apparent that top-down control can affect grazer species composition, size distribution, and total biomass, and can have cascading impacts down to the level of primary producers. Recognizing that both top-down and bottom-up control are important in seagrass beds is only a start, however. To predict and manage the consequences of overfishing and eutrophication on seagrass systems we need to develop a detailed understanding of the causes and effects of change in natural mesograzer communities, through comprehensive field studies and realistic, multi-factorial experiments. This dissertation project was therefore undertaken with the following goals:

1) To quantify temporal and spatial variation in the algal epiphyte, mesograzer, and small predator communities associated with *Zostera marina* in lower Chesapeake Bay, and to identify biotic and abiotic controls that underlie that variation (Chapters 3 and 4).

2) To assess trophic relationships among common species and resource pools in eelgrass food webs (Chapter 5), and to examine the impacts of food web structure and diversity on ecosystem properties (Chapter 2).

3) To experimentally test the relative importance and interaction of top down and bottom up control in eelgrass beds (Chapter 1).

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# CHAPTER 1

## Nutrient versus consumer control of community structure in a Chesapeake Bay eelgrass habitat.

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ABSTRACT

Nutrient loading can dramatically alter benthic communities, and has been implicated in the worldwide decline of seagrass beds. Ongoing changes in food webs caused by overfishing could contribute to seagrass decline as well. Yet the interaction of these factors and the role of small invertebrate grazers in mediating them are poorly understood. We examined the relative impacts of nutrient loading and food web alteration on eelgrass (*Zostera marina* L.) community structure in Chesapeake Bay by manipulating nutrients, predatory crabs, and invertebrate grazers in field enclosures over 28 days in summer. Nutrients increased epiphyte accumulation early in the experiment, decreased eelgrass biomass, and reduced the abundance of the colonial tunicate *Botryllus schlosseri*. Grazers decreased epiphyte accumulation, altered the recruitment of sessile invertebrates, and sometimes damaged eelgrass via overgrazing. Crabs reduced the abundance of eelgrass, and changed the species composition and abundance of grazers and sessile invertebrates. On average, the impacts of consumer manipulations and nutrient loading were comparable in magnitude and tended to be additive, rather than interactive. However, the distinct responses of different taxa in the community to the experimental treatments indicated that food web structure interacted with both bottom-up and top-down forces to determine overall community organization. These results highlight the importance of incorporating food web dynamics into seagrass conservation and management efforts.

# INTRODUCTION

A general goal of ecology is to understand how both bottom-up and top-down processes interact with food web structure to determine ecosystem responses (e.g., Hairston et al. 1960, Power 1992, Heck et al. 2000, Worm et al. 2002, Hays 2005). In ecosystems where research has focused disproportionately on either top-down or bottom-up processes, we can often greatly increase our understanding by examining the other type of process, as well. For example, in the rocky intertidal, where early research focused largely on top-down processes like keystone predation (e.g. Paine 1966), recent incorporation of bottom-up processes like changes in nutrient concentration and planktonic food supply has helped explain the system's variability (Menge 2000, Worm & Lotze 2006). In seagrass ecosystems, where the early focus was on the bottom-up influence of nutrients, light, and water flow (Hemminga & Duarte 2000), studies of top-down effects have uncovered the importance of grazing and predation (Williams & Heck 2001, Heck & Orth 2006, Heck et al. 2006, Valentine & Duffy 2006, Jørgensen et al. 2007). Such research has demonstrated the potential for top-down control to strongly affect seagrass growth and biomass (Hughes et al. 2004, Hays 2005, Armitage and Fourqurean 2006). Therefore, in light of the worldwide decline of seagrass habitats (Twilley et al. 1984, Duarte 2002, Orth et al. 2006), it is important to more fully evaluate top-down effects on seagrasses, and their interactions with bottom-up processes.

A meta-analysis of 35 papers that tested grazing and/or nutrient impacts on seagrasses and associated algae found that the top-down effects of grazing were similar in magnitude to the bottom-up effects of nutrients (Hughes et al. 2004). However, few of the studies included in the

meta-analysis were factorial manipulations of both grazing and nutrients that explicitly evaluated their interactive effects (but see Neckles et al. 1993, 1994, McGlathery 1995, Hays 2005, Heck et al. 2006). Neckles et al. (1993, 1994) found that mesograzers (small invertebrates that consume mainly epiphytes and detritus) reduced the negative effect of nutrient enrichment on seagrass biomass. Hays' results were similar; nutrient enrichment negatively affected seagrass in the absence of grazing shrimp and hermit crabs, but enhanced seagrass growth when grazers were present (Hays 2005). In contrast, McGlathery (1995) and Heck et al. (2006) found that nutrients strengthened the negative effects of vertebrate and large invertebrate grazers on seagrass. These qualitatively different results highlight the need to understand better how grazers mediate bottom-up and top-down forces in a variety of seagrass systems.

Most studies of top-down control in seagrass have been restricted to adjacent trophic levels, such as grazer-plant interactions (Orth et al. 1984, Thayer et al. 1984, Jernakoff et al. 1996, Kirsch et al. 2002, Valentine & Duffy 2006), or predator-prey interactions (Heck & Orth 2006). In contrast, the influence of trophic cascades on seagrass has been suggested (Williams & Heck 2001) but rarely tested (Heck et al. 2000, 2006, Duffy et al. 2005). This is surprising, given the recognized importance of trophic cascades in other aquatic and marine systems (Brett & Goldman 1996, 1997, Pinnegar et al. 2000, Shurin et al. 2002, Borer et al. 2006), including freshwater benthic macrophyte habitats (e.g. Martin et al. 1992). A better understanding of trophic cascades in seagrass beds might inform better fisheries management and conservation practices (Jorgensen et al. 2007).

Though top-down control of seagrass health is proximately mediated by grazer-plant interactions, it is essential to put grazing in the context of the larger food web, because the dynamic responses of grazers to changing resource availability and predation intensity can affect

the nature and strength of their impacts (Heck et al. 2000, 2006, Duffy et al. 2005). For example, while mesograzer populations generally respond to epiphyte productivity and maintain low epiphyte levels (van Montfrans et al. 1984, Edgar 1993, Neckles et al. 1994, Hughes et al. 2004, Valentine & Duffy 2006), some mesograzer species are capable of consuming live seagrass blades and have caused destruction of seagrass, or overgrazing, in cultures (Kirkman 1978, Nienhuis & Groenendijk 1986, Short et al. 1995, Duffy et al. 2001, 2003, Boström & Mattila 2005). While overgrazing of seagrass in the field by vertebrates and large invertebrates is well documented (Thayer et al. 1984, McGlathery 1995, Zimmerman et al. 1996, Rose et al. 1999, Kirsch et al. 2002), it has rarely been reported for mesograzers, despite their being the dominant herbivores in many seagrass systems (Valentine & Duffy 2006). In contrast, dramatic overgrazing by mesograzers has been observed in macroalgal systems (Kangas et al. 1982, Haahtela 1984, Saleema 1987, Tegner & Dayton 1987).

One factor contributing to the apparent rarity of seagrass overgrazing by mesograzers in the field may be strong top-down control by predation, which normally prevents mesograzers from reaching densities at which overgrazing can occur. Predators of mesograzers include the young of commercially and recreationally harvested fish and shellfish (Tagatz 1968, Nelson 1981, Leber 1985, Hines et al. 1990, Stoner & Buchanan 1990, Heck et al. 2000). Therefore, overfishing could potentially reduce predation and lead to overgrazing by mesograzers. In Chesapeake Bay juvenile blue crabs, *Callinectes sapidus*, settle in eelgrass (*Zostera marina* L.) beds, where they feed extensively on mesograzers, among other prey (Tagatz 1968, Hines et al. 1990, Stoner & Buchanan 1990, Duffy et al. unpub. data). Thus, over-harvesting of adult blue crabs has the potential to reduce the abundance of juvenile crabs recruiting to eelgrass beds (Lipcius & van Engel 1990), and have cascading effects on mesograzers.

In addition to producing food and shelter for fishes and mobile invertebrates, seagrass beds also support diverse assemblages of sessile invertebrates. Some of these organisms are consumed by mobile fauna like crabs and omnivorous mesograzers, which have been shown to affect the abundance of sessile invertebrates in seagrass mesocosms (Duffy & Harvilicz 2001, Duffy et al. 2003, 2005, France & Duffy 2006). Consumer controls on sessile invertebrate abundance in seagrass beds merit further investigation because sessile invertebrates can strongly affect seagrasses through direct competition for space or through modification of the resource environment (Sewell 1996, Reusch & Williams 1998).

In summary, top-down controls in seagrass beds appear both significant and dynamic, with overgrowth by epiphytes occurring under conditions of high predation and low herbivory, and overgrazing of seagrass possible under low predation and high herbivory. Here we describe a field experiment that examined these two scenarios of top-down control, and evaluated their relative strength and interaction with bottom-up effects of nutrient addition. Using a factorial manipulation of nutrients, mesograzers, and predators in field enclosures we tested the following hypotheses: (1) nutrients will enhance epiphyte growth and reduce seagrass growth; (2) predators will reduce the abundance of mesograzers, indirectly increasing epiphytes and decreasing seagrass growth and biomass; (3) grazer population growth will counteract increased epiphyte growth under nutrient enrichment, but this compensatory response will be inhibited in the presence of predators; (4) in the absence of predators, mesograzers will damage seagrass by overgrazing; and (5) sessile invertebrates will respond negatively to both mesograzers and predators.

# METHODS

**Study Area**: The experiment was conducted at Goodwin Islands, located at the mouth of the York River in Chesapeake Bay (Virginia, USA, 37° 13' N; 76° 23' W). Goodwin Islands is a 315 ha. archipelago of salt-marsh islands surrounded by inter-tidal flats and subtidal seagrass beds (*Zostera marina* and *Ruppia maritima*). The area has been a National Estuarine Research Reserve since 1991. It is closed to development and destructive use, but remains open to commercial and recreational fishing. Average summer and winter water temperatures are 27°C and 7°C, respectively, but temperatures can range from near 0°C to over 30°C. Mean salinity is 23-25 ppt during the summer and fall and 13-15 ppt during the winter and spring. Our experimental area was a shallow, densely vegetated cove in the SE part of the islands.

**Experimental design and treatments:** We designed a factorial manipulation of nutrients, crustacean mesograzers, and predatory blue crabs (*Callinectes sapidus*), with two levels of each factor (presence, absence). Treatments were applied within cages. We also included uncaged plots with and without nutrients as controls for cage effects. Thus, there were a total of 10 unique treatments with 5 replicates each. Experimental design and shorthand for individual treatments is summarized in Table 1.

**Cage design and arrangement:** Cages based on designs by Per-Olav Moksnes (pers. com) were built around rectangular, rebar frames (Fig. 1). They were 51 cm wide and 81 cm deep, with long legs and steel reinforcing plates sunk into the sediment. Frames were padded with foam pipe insulation wrapped in duct tape to minimize the risk of tearing the clear, 250 μm Nytex mesh netting. This mesh size excluded predators and minimized immigration and emigration of mesograzers, while allowing light penetration and the circulation of water, fine

particulates, and propagules of algae and fouling organisms. Access to the cages was through a roll-down opening in the top that was exposed at low tide. Control plots (51 x 51 cm) were marked with a PVC pole at one corner and 3 small, submerged flags at the other corners. Cages and plots were haphazardly distributed and separated from one another by at least 3 m, and treatments were randomly assigned.

**Nutrient additions:** Nitrate, ammonium, and phosphate were administered in the form of Osmocote™ slow-release fertilizer (N:P = 3:1) via perforated PVC tubes that were suspended in the cages or on stakes at uncaged control plots. We determined nutrient loading levels and diffusion distances for appropriate cage spacing based on data from a pilot experiment. Nutrient tubes were replaced weekly immediately after water samples were taken to assess nutrient treatment effectiveness. Nutrient treatments received 200 g of fertilizer during the first week of the experiment. Since there was no significant difference in $[NH_4^+]$ between nutrient and non-nutrient treatments after the first week (0.7μM and 0.5 μM, respectively; one-tailed t-test, $p = 0.189$), we increased the fertilizer delivery to 400 g per cage. During the second and third weeks, the average $[NH_4^+]$ was 4.0 μM and 7.6 μM in nutrient treatments and 0.7 μM and 2.1 μM in non-nutrient treatments, respectively.

**Defaunation and Faunal Additions:** To standardize initial density and species composition of mesograzers among treatments, and to create mesograzer-free controls, all caged and uncaged plots were defaunated by treatment with Sevin™ concentrated liquid insecticide (Carpenter 1986, Duffy & Hay 2000). Sevin™ was applied within large, fiberglass cylinders placed around plots to prevent diffusion of the poison until all enclosed mesograzers were killed. Plots were treated with approximately 0.08 g Sevin™ $L^{-1}$ seawater for 30 minutes, which we determined was sufficient to kill virtually all mesograzers during pilot experiments at the field

site. Haphazard sampling of cages with a small dip net shortly after defaunation verified the absence of live mesograzers. Several days were allowed after defaunation for dispersal of the Sevin™ before mesograzers were added to mesograzer addition treatments. We collected mesograzers from the eelgrass bed surrounding the experimental site using a large dip net. We added 40 *Gammarus mucronatus*, 40 *Idotea balthica*, and 20 *Erichsonella attenuata* to each mesograzer treatment, approximating their relative abundance in the dip net collections. It was not logistically feasible to stock the mesograzers at an initial density equivalent to their natural density. Thus, we considered the initial mesograzer abundance simply an inoculum and expected their populations to grow rapidly during the early part of the experiment, as in similar studies (Duffy et al. 2001, 2003, 2005). Predator addition treatments received two blue crabs, *Callinectes sapidus*, of carapace width 20 - 40 mm. This density of blue crabs was well within the range observed at the field site (Duffy et al. unpub. data).

**Experimental Timeline**: On May 20, 2005, cage locations were marked with stakes. Cages were installed May 27, and defaunated on June 1. Mesograzer, crab, and nutrient treatments were applied on June 7, which we refer to as Day 1 of the experiment. Light measurements were made on June 10, which was Day 4 of the experiment but 14 days after cage placement. Blades were collected for epiphyte measurements on June 15 (Day 9) and June 29 (Day 23). Cages were destructively sampled July 5-6 (Day 28-29).

**Cage Maintenance and Light Measurements:** Twice each week we checked cages and removed epiphytes from the mesh by scrubbing. Small tears in cage mesh were sewn shut when found. If a cage had a large tear or was otherwise compromised it was considered a failed replicate and not included in statistical analyses. To examine light attenuation, photosynthetically active radiation (PAR) was measured within the eelgrass canopy inside and

outside of cages using a Li-Cor Spherical Light Meter. Ten cages were haphazardly selected for light measurements, and 3 readings were recorded within each cage, alternated with 3 recorded outside, approximately 2 m away. This took place in late afternoon of June 10, 2005, at which point cages had been in the water and subjected to fouling for 14 days. Cages were not scrubbed on the day of the light measurements.

**Epiphyte Measurements:** A single eelgrass shoot (approximately 5 blades) was collected from each cage or control plot on days 9 and 23 of the experiment; June 15, 2005 and June 27, 2005, respectively. Fouling material was scraped from the blades and collected on Whatman™ GFF filters, and blade surface areas were determined with a Li-Cor 3100 area meter (Li-Cor, Lincoln, NE). We measured chlorophyll *a* as a proxy for the biomass of photosynthesizing algae on the blades. Filters were extracted in 20 ml 90% acetone at -20° C for 24 hours. The extract was passed through 0.45 μm hydrophilic PTFE membrane filters (Millipore Corporation) and absorbance was monitored at 480, 510, 630, 647, and 750 nm using a Shimadzu UV-1601 spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, MD). Chlorophyll *a* concentration was calculated using the trichromatic equation (Lorenzen 1967), and chlorophyll *a* mass was calculated and normalized to blade area to serve as a proxy for epiphyte density.

**Sample Collection and Processing:** At the end of the experiment, all cages and control plots were destructively sampled. First, a 20 by 20 cm grab sample of eelgrass and epifauna was taken from the center of each plot with a sampling device adapted from Virnstein & Howard (1987). Grab samples were used because: (1) their relatively small size permits more detailed and timely processing than whole plot samples; (2) they allow for a consistent sampling technique between caged and uncaged plots; and (3) they can be directly compared with field

monitoring data collected using the same apparatus (Duffy et al. unpub. data). After a grab sample was taken, the whole plot was swept exhaustively with a dipnet, eelgrass was uprooted, and all contents of the cage were collected in a plastic bag. Both grab samples and whole plot samples were frozen at -20°C until sorting. Whole-plot and grab samples were processed similarly, except that the mobile epifauna and grazing damage were not quantified in whole-plot samples.

During the sorting process, all flora and sessile epifauna were identified to the lowest taxonomic level possible, with the exception of microalgal epiphytes scraped from blades, which were considered en masse. Additionally, eelgrass blades were separated from roots and rhizomes. Sessile organisms were dried at 60°C, weighed, and combusted at 450°C to determine ash-free dry mass (AFDM). Mobile epifauna were sorted by size class with a nested series of sieves (8.0, 5.6, 4.0, 2.8, 2.0, 1.4, 1.0, 0.71, and 0.50 mm screens), then identified to species and counted. Counts of individuals within each size class were multiplied by empirically derived coefficients to convert them to AFDM. The AFDM from each size class were then pooled to get total AFDM for each mesograzer species within a sample (e.g. Edgar 1990).

To measure grazing damage to eelgrass blades, 10 live blades from each grab sample were randomly selected for analysis. The total length of each blade was measured in cm, and grazing damage was recorded as the number of cm per blade that bore ragged scars suggestive of grazing (procedure adapted from Silliman & Newell 2003).

**Statistical Analysis:** A paired sample t-test was used to compare light levels inside and outside of cages. Mesograzer abundance, grass biomass, epiphyte density, sessile invertebrate abundance, and damage to eelgrass blades were compared among caged treatments using fully factorial 3-way ANOVA with nutrients, crabs, and mesograzers as fixed factors. Uncaged

controls were excluded from the ANOVA to allow a fully-crossed design, so comparisons of responses between caged and uncaged treatments were made separately using t-tests. Cages that failed during the course of the experiment (n = 7) were removed from the analysis. Two cages from the crabs + nutrients treatment failed. Otherwise no more than one cage from each treatment was compromised (for final n for each treatment, see Table 1). Data were log-transformed as necessary to achieve homogeneity of variance among treatments, as determined by Cochran's C test. The proportion of variance explained by each factor and interaction was calculated as $\omega^2$ according to Kirk (1995). All statistical analyses were performed in Minitab ver. 14.1.

## RESULTS

**Caging Effects:** Cages reduced light levels within the eelgrass canopy by 66% after being in the water for 14 days. Average PAR was 262 $\mu E\ s^{-1}m^{-2}$ inside cages (range 54-419 $\mu E\ s^{-1}m^{-2}$) and 826 $\mu E\ s^{-1}m^{-2}$ outside cages (range 183-1332 $\mu E\ s^{-1}m^{-2}$). Though light levels inside cages were reduced, they were in the range of saturating irradiance for eelgrass (Larkum et al. 2006).

**Mesograzer Abundance:** At the end of the experiment total mesograzer biomass was higher in uncaged plots than in caged treatments inoculated with mesograzers (two-tailed t-test, $p < 0.0005$, Fig. 2a). Interestingly, *Erichsonella attenuata* was the most abundant mesograzer outside of cages at the end of the experiment (Fig. 2d) despite being relatively rare in the dip net collections made to stock the mesograzer treatments at the beginning of the experiment (see Methods). *Idotea balthica*, on the other hand, was rare outside of cages at the end of the

experiment (Fig. 2c), despite being common in the collections used for stocking. Among caged treatments, total mesograzer biomass was higher in grazer-only treatments than in control treatments or treatments with crabs (Table 2, Fig. 2a). However, there was considerable contamination of cages by the end of the experiment, as evidenced by the presence of mesograzers in caged control plots. A high proportion of these contaminant mesograzers were *Gammarus mucronatus* (Fig. 2b), but one replicate of the control + nutrients treatment had many *E. attenuata* (Fig. 2d). Crabs reduced total mesograzer biomass by 64% and reduced both *G. mucronatus* and *Idotea balthica* biomass (Table 2, Fig. 2). Nutrient effects on total mesograzer biomass were inconsistent, but nutrients decreased *G. mucronatus* biomass (Table 2, Fig. 2b).

**Epiphyte abundance:** Nutrients strongly enhanced epiphyte growth in caged treatments when epiphytic chlorophyll was first measured on Day 9 of the experiment (Table 2, Fig. 3a). At the same time, there was a non-significant trend of reduced epiphyte abundance by mesograzers ($p = 0.069$). By Day 23 the effect of mesograzers had increased, while the nutrient effect had disappeared. Epiphytes on Day 23 were reduced by about half in both mesograzer and mesograzer-crab treatments relative to their mesograzer-free counterparts (Table 2, Fig. 3b).

**Eelgrass biomass:** *Zostera marina* biomass was lower in cages compared to uncaged control plots (Fig. 4). This trend was larger with the whole-plot method of sampling (Fig. 4b), probably due to the inadvertent inclusion of blades from outside the plot in uncaged samples, and reduced grass biomass at the margins of cages where cage walls penetrate the sediment. Among caged treatments, both nutrients and crabs reduced eelgrass biomass (Table 2, Fig. 4). A significant interaction between nutrients and crabs for whole plot *Z. marina* biomass reflects a partially redundant effect of each factor in the presence of the other; when eelgrass was reduced by nutrients it was not reduced much further by crabs, and vice versa (Table 2).

**Mesograzer and Crab Damage to Eelgrass:** Physical damage to blades was low in caged control treatments and uncaged control plots, but high in cages with either mesograzers or crabs (Table 2, Fig. 5). Damage from mesograzers vs. crabs could not be distinguished reliably, but damage in both mesograzers-only and crabs-only treatments suggests that both kinds of consumers damaged eelgrass (Table 2, Fig. 5). ANOVA revealed a significant interaction between crabs and mesograzers, suggesting that in the presence of crabs, mesograzers did not further increase blade damage (Table 2). Nutrients also increased damage, although it is difficult to tell whether the degradation of seagrass by nutrients is due to light reduction by epiphytes, or through interactions with crabs and mesograzers. Artifacts of caging may have played a role in blade damage, because little damage to blades was observed outside of cages despite high mesograzer density and the presence of crabs.

**Sessile Invertebrate Recruitment:** Sessile invertebrates grew on eelgrass blades at our experimental site, and were strongly affected by the experimental manipulations, particularly by crabs (Table 2, Fig. 6). The colonial tunicate *Botryllus schlosseri* was moderately abundant outside of the caged treatments. In cages, *B. schlosseri* was facilitated by the presence of either mesograzers or crabs, and reduced by nutrient enrichment (Table 2). A strongly contrasting pattern was observed with the solitary tunicate *Molgula manhattensis*, which was found only inside cages in the absence of crabs. Crabs strongly depressed bryozoan abundance, and mesograzers reduced bryozoans in the absence of crabs, as shown by a significant crab by mesograzer interaction (Table 2, Fig. 6c). Barnacles were also reduced by crabs (Table 2, Fig. 6d).

**Relative influence of top-down versus bottom-up effects:** ANOVAs conducted on caged treatments partitioned the variance in responses of the community into components

attributable to nutrients, mesograzers, crabs, and their interactions. These models explained between 6% and 57% of the variance for different community components (Table 2). The relative influence of nutrients, mesograzers, and crabs depended strongly on community components and, for epiphytic chlorophyll, the date of sampling. Of the 12 response variables analyzed, nutrients significantly influenced six, crabs influenced nine, and mesograzers four (Table 2). Nutrients and crabs strongly influenced final eelgrass biomass, while mesograzer effects on eelgrass biomass were minor (Table 2). However, nutrients, crabs, mesograzers, and the mesograzer-crab interaction all affected eelgrass blade damage (Table 2). Epiphytic algae were primarily influenced by nutrients at day 9 ($\omega^2 = 0.41$), but by day 23, mesograzers were the main influence on algae ($\omega^2 = 0.43$) and nutrient effects were negligible (Table 2). Initial mesograzer presence was the strongest influence on final mesograzer abundance, followed closely by the top-down effect of crabs; no bottom-up effect of nutrients on mesograzers was apparent (Table 2). Sessile invertebrates tended to be strongly influenced by consumers (mesograzers, crabs, and their interactions), but *Botryllus schlosseri* differed from the other sessile invertebrates in that it was most strongly affected by nutrients (Table 2).

## DISCUSSION

Seagrass beds are often characterized by high spatial and temporal variability of seagrass biomass, epiphytic algae, epifaunal grazers, and sessile invertebrates (Marsh 1970, Nelson et al. 1982, Sewell 1996, Williams & Heck 2001, Jorgensen et al. 2007). The forces generating this variability can be difficult to discern, but controlled experiments testing both top-down and

bottom-up factors in seagrass beds have helped reveal these forces and how they interact (McGlathery 1995, Heck et al. 2000, 2006, Hughes et al. 2004, Hays et al. 2005). In our experiment, crustacean mesograzers, crabs, and nutrients all influenced the eelgrass community within one month. Top-down impacts included the effects of blue crabs on mesograzer biomass, the impacts of crabs and mesograzers on sessile invertebrates, the reduction of epiphytes by mesograzers, and the direct damage to eelgrass blades by crabs and mesograzers. The impact of mesograzers on final eelgrass biomass was negligible, perhaps because the positive, indirect effect of their grazing on epiphytes was counteracted by the negative effect of their direct grazing on eelgrass. Another possible explanation is that other factors affecting seagrass biomass, such as nutrients, crabs, and light limitation by the cages, simply overshadowed the effects of mesograzers. Bottom-up control was evident in the stimulation of epiphyte growth by nutrients early in the experiment and in the negative effect of nutrients on eelgrass biomass by the end. Nutrient effects on total mesograzer biomass were inconsistent, perhaps because nutrients increased food availability for grazers (in the form of epiphytes and N-rich detrital material) but degraded habitat by reducing eelgrass biomass.

The scarcity of experimental field manipulations of mesograzers in the literature reflects the daunting logistical challenges involved. When designing field enclosures, there is an inevitable trade-off between maintaining light and water flow and preventing immigration / emigration of mesograzers. We were not able to completely exclude mesograzers, particularly *Gammarus mucronatus*, from cages in which they were not initially introduced. However, our observations confirmed that the initial defaunation was successful, and suggested that the contamination occurred progressively such that invading mesograzers were relatively scarce until later in the experiment. We attempted to maximize light and water flow by placing our cages in

a shallow, high-light environment, and scrubbing them regularly to keep the mesh open. This approach was successful insofar as the caged treatments were above compensating light intensity for eelgrass, 10 - 40 $\mu E\ s^{-1}m^{-2}$, and were in the range of saturating irradiance, 65 - 290 $\mu E\ s^{-1}m^{-2}$ (Larkum et al. 2006), at least early in the experiment. Nevertheless, eelgrass biomass was significantly lower in caged than in uncaged plots at the end of the experiment, suggesting that light limitation or other artifacts of caging did limit eelgrass growth by the end. We have considered this in our interpretation of the observed effects of nutrients, crabs, and mesograzers.

Despite the cage artifacts inherent in our study and in previous field experiments in seagrass beds (e.g. Young et al. 1976) several of our results corroborate those seen previously in mesocosm experiments in this system (Duffy et al. 2001, 2003, 2005, Duffy and Harvilicz 2001), strengthening conclusions from both types of experiments. For example, in all experiments, mesograzers reduced epiphyte accumulation, and crabs reduced both grazer and sessile invertebrate abundance. We consider these processes in more detail below.

Crabs reduced biomass of total mesograzers, *Gammarus mucronatus*, and *Idotea balthica* (Fig. 2, Table 2). However, predation impacts on the cryptic isopod *Erichsonella attenuata* were weaker in our cages than in mesocosm studies. In our experiment, *E. attenuata* populations in treatments with crabs were reduced by approximately 50%, whereas in Duffy et al. (2005), *E. attenuata* were completely eliminated by crabs. The high density of grass in our field cages may have afforded *E. attenuata* more protection than the relatively sparse plantings used in that mesocosm experiment; increasing habitat complexity or vegetation density has often been associated with reduced predation (Orth et al. 1984, Heck & Crowder 1990, Heck et al. 2006). Also, an important difference between mesocosms and field cages is the lack of infaunal prey for blue crabs, i.e. polychaetes and clams, in the former (Tagatz 1968, Stoner & Buchanan 1990).

Blue crabs may have focused foraging effort more on infaunal prey in field cages relative to mesocosms, and thus had weaker impacts on *E. attenuata*. Furthermore, in uncaged plots, *E. attenuata* abundance was higher than that of any other mesograzer, suggesting that these cryptic isopods are even less susceptible to predation in natural habitats where eelgrass tends to be even denser than in our cages. When *E. attenuata* abundance is normalized to grass biomass, the inside vs. outside cage difference is lessened but remains significant (two-tailed t-test, $p = 0.045$).

Blue crabs, and possibly mesograzers, controlled sessile invertebrates through predation, as seen previously in mesocosm and caging experiments (Seitz 1996, Duffy & Harvilicz 2001, Duffy et al. 2003, 2005, France & Duffy 2006). The solitary tunicate *Molgula manhattensis* grew to 25% of the biomass of the eelgrass itself within our caged control treatments, but was rare in the presence of crabs or mesograzers, as seen previously (Duffy & Harvilicz 2001). Thus, a disruption of the normal consumer community in an eelgrass bed might facilitate overgrowth of eelgrass by *M. manhattensis* or other sessile invertebrates, although such an effect has not been documented in the field.

Consumers did not appear to directly affect the encrusting compound tunicate *Botryllus schlosseri*. However, *B. schlosseri* was rare in controls with no consumers and rare in nutrient addition treatments (Fig. 6a). This pattern might be explained by competition with epiphytic algae for space during the early part of the experiment (Fig 4a). Under low nutrients or high grazing, space may have been available for *B. schlosseri* to settle on the blades. However, with high nutrients or in the absence of effective grazing (i.e. control treatments), epiphytes could have inhibited *B. schlosseri* settlement. A similar effect has been seen in mesocosm experiments where increasing grazer diversity (which corresponds with more intense epiphyte grazing)

enhanced the recruitment of *B. schlosseri* (Duffy et al. 2003). *B. schlosseri* is often extremely abundant in Chesapeake Bay eelgrass beds (Duffy et al. unpub. data) and may pose a threat to eelgrass health.

Reductions of epiphytes by mesograzers in our experiment were similar to those seen in mesocosm studies (Neckles et al. 1993, 1994, Hughes et al. 2004, Duffy et al. 2005), supporting the notion that mesograzers can exert important control on epiphyte abundance in the field (van Montfrans et al. 1984, Jernakoff et al. 1996). We also saw evidence for direct consumption of eelgrass by mesograzers in the form of blade damage, as seen previously in mesocosm experiments (Kirkman 1978, Short et al. 1995, Duffy & Harvilicz 2001, Duffy et al. 2003), and occasionally observed at our study site (Douglass et al., unpub. data). Although we did not manipulate individual mesograzer species in this experiment, the blade damage was probably caused by *Idotea balthica*, which is particularly destructive (Duffy et al. 2003, 2005, Boström & Mattila 2005). However, despite the evidence for overgrazing by mesograzers, their overall contribution to blade damage was no greater than the effects of nutrients or crabs (Table 2). Crabs harmed blades through mechanical means, whereas nutrients probably weakened the eelgrass through epiphyte-mediated light-reduction.

Artifacts of the caging design may have enhanced the strength of both consumer and nutrient impacts on eelgrass through several mechanisms. First, both mesograzers and predators were unnaturally confined. While densities of crabs within the cages were well within the range of observed values in the field at Goodwin Islands (Duffy et al. unpub. data), we speculate that their confinement may have triggered more destructive behavior than would be observed at similar density in the field. Second, cages held only one predator species, while the predators in the field are diverse. We saw evidence of overgrazing by mesograzers in cages with no crabs,

but a loss of crabs in the field would not necessarily cause overgrazing because other predators, such as fishes, could limit mesograzer populations or activity levels. Indeed, it has been suggested that the strength of top-down control depends strongly on the relative diversity of consumer and prey trophic levels (Duffy 2002, Duffy et al. 2007, Douglass et al. *in prep*). Third, cages reduced light levels. Relatively little blade damage was observed in the open eelgrass bed (Fig. 5) despite a high abundance of mesograzers and the presence of crabs outside of cages. Light reduction by the cages may have altered the eelgrass' chemical composition, making it more vulnerable to grazing; nutrient enrichment could have had similar effects. Unfortunately, we cannot verify these speculations because we did not analyze the composition of eelgrass tissues.

The relative magnitudes of consumer and nutrient effects were similar for some community responses, while for others there was a clear predominance of top-down or bottom-up control (Table 2). For example, the impact of consumers was stronger than that of nutrients for most of the sessile invertebrate species (with the exception of *Botryllus schlosseri*), while nutrient effects explained about twice as much of the variation in final eelgrass biomass as did consumer effects (Table 2). Epiphyte abundance was affected equally by nutrients and consumers, but at different times in the experiment. Early on, before populations of stocked mesograzers had had much time to increase, epiphytes bloomed in nutrient addition treatments. Later, nutrients had little effect, with most of the variation in epiphytes among treatments being explained by mesograzers. Some of this change may have arisen from increasing light limitation caused by fouling of cages, but the top-down control by mesograzers is still clear. This result supports the hypothesis that the relative strength of top-down and bottom-up forces in natural systems can fluctuate over time (Boyer et al. 2003). Time of the year may also influence the

relative sensitivity of seagrass communities to top-down and bottom-up perturbations, but this remains to be determined.

Our experiment and others demonstrate the potential for strong effects and interactions of top-down and bottom-up forces in seagrass communities (see Hughes et al. 2004 for review, also Hays 2005, Heck et al. 2006). The effects of consumers and nutrients that we observed took two distinct forms, both with precedent in previous experimental work. First, like Neckles et al. (1993, 1994) and Hays (2005) we observed countervailing effects of nutrients and epifaunal grazers on epiphyte accumulation. Second, like McGlathery (1995) and Heck et al. (2006) we observed negative effects of nutrients and larger consumers (blue crabs) on seagrass itself. However, whereas the negative effects that McGlathery (1995) and Heck et al. (2006) observed occurred when nutrients facilitated consumption of seagrass, the negative effects of nutrients and crabs in our study were apparently independent. The wide variation in consumer effects demonstrated by these and other experiments emphasizes the importance of studying multiple functional groups of consumers in conjunction with bottom-up factors in seagrass beds. However, small-scale experiments alone cannot determine the relative extent to which top-down and bottom-up control are realized over large scales in seagrass beds. Field survey data could be useful in achieving this end, but observational studies relating seagrass health and eutrophication have seldom included faunal abundance as a variable (Duarte 1995, Kemp et al. 2005, but see Jorgensen 2007). Likewise, observations of consumer abundance and distribution in seagrass beds (e.g. Marsh 1970, Nelson et al. 1982, Edgar 1990) have rarely been related to human fisheries or potential top-down impacts on seagrass. Careful analyses of long-term monitoring data with both physical and biological components should be useful in illuminating how top-

down and bottom-up processes affect seagrass beds at natural scales (Hampton & Schindler 2006).

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Table 1. Experimental design and key to the treatment designations used in figures. "Out" refers to uncaged control plots, "Con" refers to caged controls with no crabs or mesograzers, "+N" denotes nutrient addition, "M" indicates mesograzer presence, and "C" signifies the presence of blue crabs. The number of undamaged replicates of each treatment at the end of the experiment is indicated in parentheses.

| | | Cage | | | |
|---|---|---|---|---|---|
| | **No Cage** | **Control** | **Mesograzers** | **Crabs** | **Crabs, Mesograzers** |
| **No Nutrients** | Out | Con | M | C | MC |
| | (5) | (4) | (5) | (4) | (4) |
| **Nutrients** | Out+N | Con+N | M+N | C+N | MC+N |
| | (5) | (4) | (4) | (3) | (5) |

Table 2. ANOVA results for variance in eelgrass community responses among caged treatments. DF = 1 for each main effect and interaction effect, error DF = 25, and total DF = 32. Omega$^2$ is an estimate of the proportion of variance explained by an effect; $\omega^2 = [SS_{effect} - (DF_{effect})(MS_{error})]*(MS_{error} + SS_{total})^{-1}$. Bold $\omega^2$ values denote significant effects ($p < 0.05$). AFDM = Ash free dry mass. Bottom row gives mean $\omega^2$ values for each factor and interaction.

| | | | Effect | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Nutrients | | Crabs | | Mesograzers | | N*C | | N*M | | C*M | | N*C*M | |
| **Response** | **$R^2$ (Adj)** | **MS Error** | **MS** | **$\omega^2$** | **MS** | **$\omega^2$** | **MS** | **$\omega^2$** | **MS** | **$\omega^2$** | **MS** | **$\omega^2$** | **MS** | **$\omega^2$** | **MS** | **$\omega^2$** |
| Total Mesograzer AFDM | 0.448 | 565 | 608 | 0.010 | 5960 | **0.178** | 6080 | **0.192** | 289 | 0.000 | 1010 | 0.017 | 1160 | 0.021 | 1520 | 0.029 |
| *G. mucronatus* AFDM | 0.373 | 247 | 1150 | **0.087** | 1330 | **0.095** | 992 | 0.083 | 911 | 0.059 | 142 | 0.000 | 278 | 0.006 | 779 | 0.042 |
| *E. attenuata* AFDM | 0.056 | 202 | 98.6 | 0.000 | 489 | 0.043 | 726 | 0.076 | 96.9 | 0.000 | 249 | 0.009 | 3.60 | 0.000 | 109 | 0.000 |
| *I. balthica* AFDM | 0.365 | 45.0 | 8.00 | 0.000 | 287 | **0.124** | 444 | **0.186** | 2.39 | 0.000 | 6.62 | 0.000 | 301 | **0.110** | 1.67 | 0.000 |
| Epiphytic Chl a Day 9 | 0.409 | 2.51 | 63.7 | **0.411** | 0.746 | 0.000 | 9.03 | 0.040 | 1.07 | 0.000 | 4.09 | 0.011 | 0.208 | 0.000 | 0.425 | 0.000 |
| Epiphytic Chl a Day 23 | 0.499 | 0.156 | 0.093 | 0.000 | 0.584 | 0.039 | 4.18 | **0.434** | 0.362 | 0.013 | 0.007 | 0.000 | 0.366 | 0.019 | 0.228 | 0.007 |
| *Z. marina* AFDM, Grab Sample | 0.409 | 0.274 | 3.76 | **0.240** | 1.79 | **0.099** | 0.679 | 0.033 | 0.328 | 0.004 | 0.501 | 0.014 | 0.713 | 0.029 | 0.000 | 0.000 |
| *Z. marina* AFDM, Whole Cage | 0.570 | 7.65 | 223 | **0.401** | 69.9 | **0.113** | 11.5 | 0.015 | 42.8 | **0.062** | 3.61 | 0.000 | 3.28 | 0.000 | 0.009 | 0.000 |
| Ln(*Z. marina* Blade Damage / cm) | 0.364 | 0.639 | 4.55 | **0.084** | 4.38 | **0.080** | 3.07 | **0.065** | 0.924 | 0.010 | 0.118 | 0.000 | 5.58 | **0.155** | 0.010 | 0.000 |
| *B. shlosseri* AFDM / *Z. marina* | 0.250 | 0.033 | 0.340 | **0.214** | 0.040 | 0.005 | 0.014 | 0.000 | 0.080 | 0.028 | 0.017 | 0.000 | 0.020 | 0.000 | 0.075 | 0.028 |
| *M. manhattensis* AFDM / *Z. marina* | 0.328 | 0.011 | 0.006 | 0.000 | 0.134 | **0.214** | 0.041 | 0.063 | 0.006 | 0.000 | 0.011 | 0.003 | 0.041 | 0.060 | 0.011 | 0.000 |
| Bryozoan AFDM / *Z. marina* | 0.297 | 0.000 | 0.000 | 0.000 | 0.001 | **0.122** | 0.001 | 0.062 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | **0.129** | 0.000 | 0.033 |
| Ln(Barnacle AFDM / *Z. marina*) | 0.219 | 3.47 | 0.448 | 0.000 | 46.2 | **0.285** | 3.47 | 0.007 | 3.19 | 0.000 | 0.072 | 0.000 | 2.39 | 0.000 | 0.002 | 0.000 |
| Mean $\omega^2$ | ... | ... | ... | 0.111 | ... | 0.108 | ... | 0.097 | ... | 0.014 | ... | 0.004 | ... | 0.041 | ... | 0.011 |

Figure Legends:

Figure 1. Design of field enclosures used in factorial manipulation of nutrients, blue crabs, and mesograzers.

Figure 2. Final mesograzer biomass by treatment from 400 $cm^2$ grab samples, as calculated by the Edgar method. A) Total mesograzer biomass. B) *Gammarus mucronatus* biomass. C) *Erichsonella attenuata* biomass. D) *Idotea balthica* biomass. See table 1 for treatment code and n for each treatment. Error bars are SEM. N*, C*, and M* indicate significant effects ($p < 0.05$) of nutrients, crabs, and mesograzers, respectively.

Figure 3. Epiphytic algal density (μg epiphytic chlorophyll-a * $cm^{-2}$ *Zostera marina* blade) versus treatment. A) Epiphyte density at day 9 of the experiment, June 15, 2005. B) Epiphyte density at day 23 of the experiment, June 29, 2005. See table 1 for treatment code and n for each treatment. Error bars are SEM. N* and M* indicate significant ($p < 0.05$) effects of nutrients and mesograzers, respectively.

Figure 4. Final *Zostera marina* above-ground biomass (g AFDM) measured on June 29-31, 2005. A) *Z. marina* AFDM from 400 $cm^2$ grab samples taken at the center of experimental plots. B) *Z. marina* AFDM from whole plot area (0.268 $m^{-2}$). See table 1 for treatment code and n for each treatment. Error bars are SEM. N*, C*, and M* indicate significant effects ($p < 0.05$) of nutrients, crabs, and mesograzers, respectively.

Figure 5. Physical damage to *Zostera marina* blades versus treatment, measured as the average number of scarred or torn areas per linear cm of live blade. See table 1 for treatment code and n for each treatment. Error bars are SEM. N*, C*, and M* indicate significant effects ($p < 0.05$) of nutrients, crabs, and mesograzers, respectively.

Figure 6. Biomass of selected sessile invertebrates, normalized to *Zostera marina* blade biomass (AFDM), versus treatment. A) Biomass of the colonial tunicate *Botryllus schlosseri* * *Z. marina* $AFDM^{-1}$, by treatment. B) Biomass of the solitary tunicate *Molgula manhattensis* * *Z. marina* $AFDM^{-1}$, by treatment. C) Biomass of encrusting bryozoans * *Z. marina* $AFDM^{-1}$, by treatment. D) Biomass of barnacles *Balanus sp.* * *Z. marina* $AFDM^{-1}$, by treatment. See table 1 for treatment code and n for each treatment. Error bars are SEM. N*, C*, and M* indicate significant effects ($p < 0.05$) of nutrients, crabs, and mesograzers, respectively.

Figure 1.

# Field Cage Design

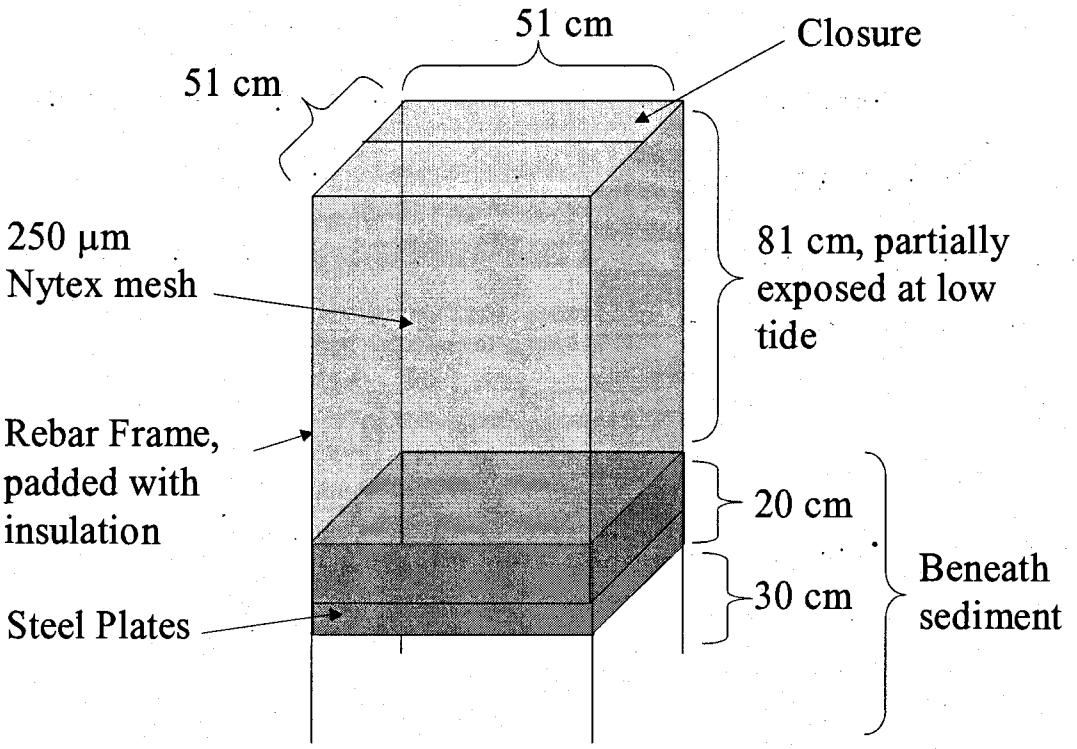


Figure 2.

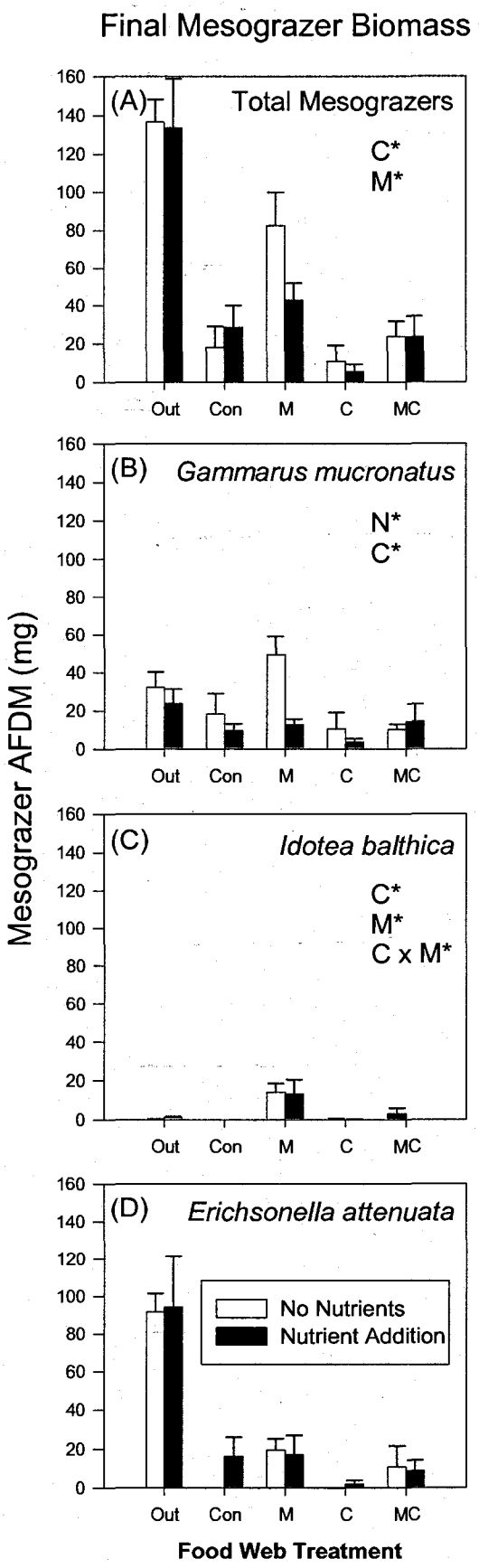

Final Mesograzer Biomass
(A) Total Mesograzers
C*
M*
(B) Gammarus mucronatus
N*
C*
(C) Idotea balthica
C*
M*
C x M*
(D) Erichsonella attenuata
No Nutrients
Nutrient Addition
Mesograzer AFDM (mg)
0
20
40
60
80
100
120
140
160
Out
Con
M
C
MC
Food Web Treatment

Figure 3.

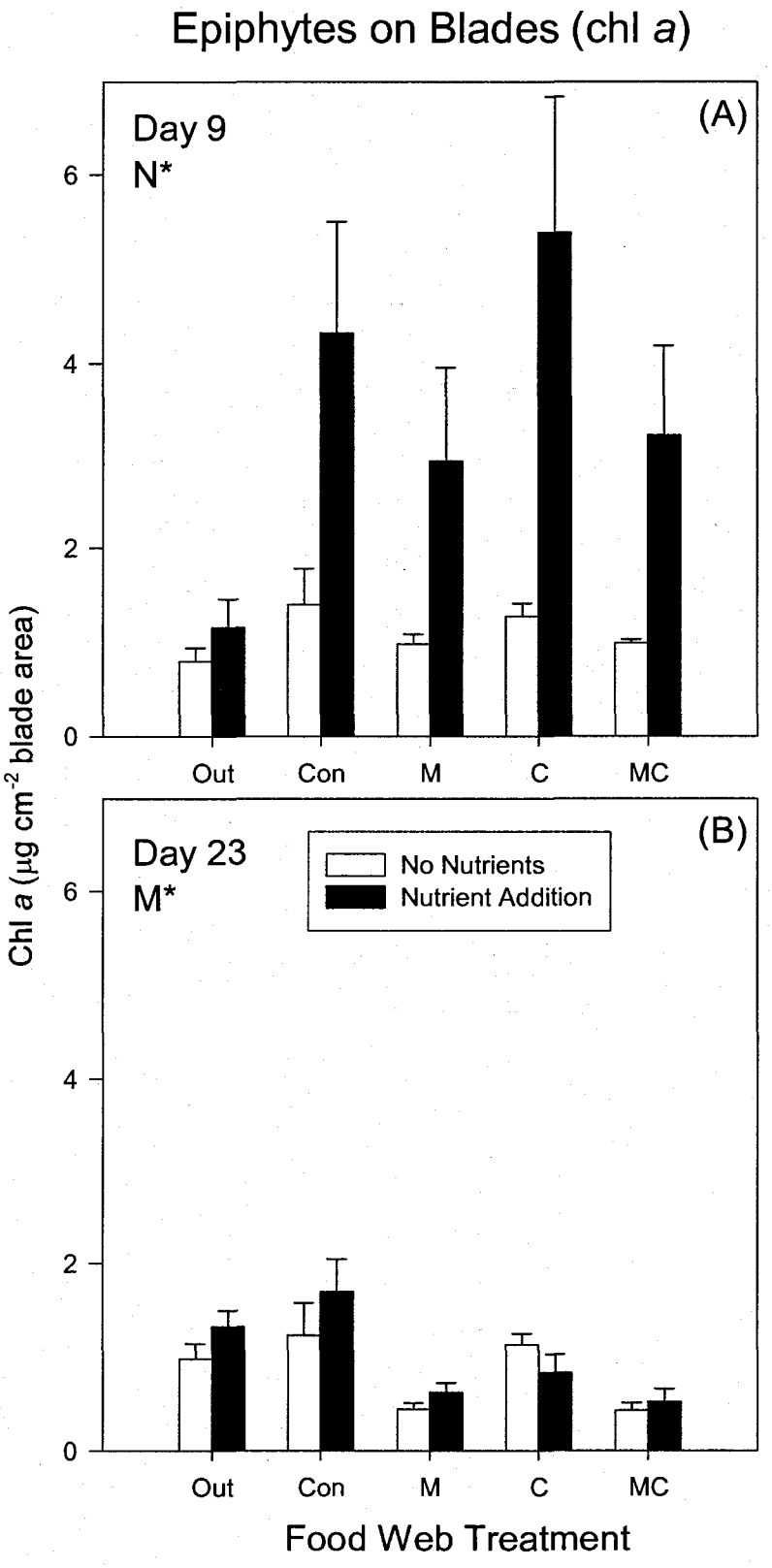

Epiphytes on Blades (chl a)
Day 9
N*
(A)
Day 23
M*
(B)
No Nutrients
Nutrient Addition
Chl a (μg cm-2 blade area)
0
2
4
6
Out
Con
M
C
MC
Food Web Treatment

Figure 4.

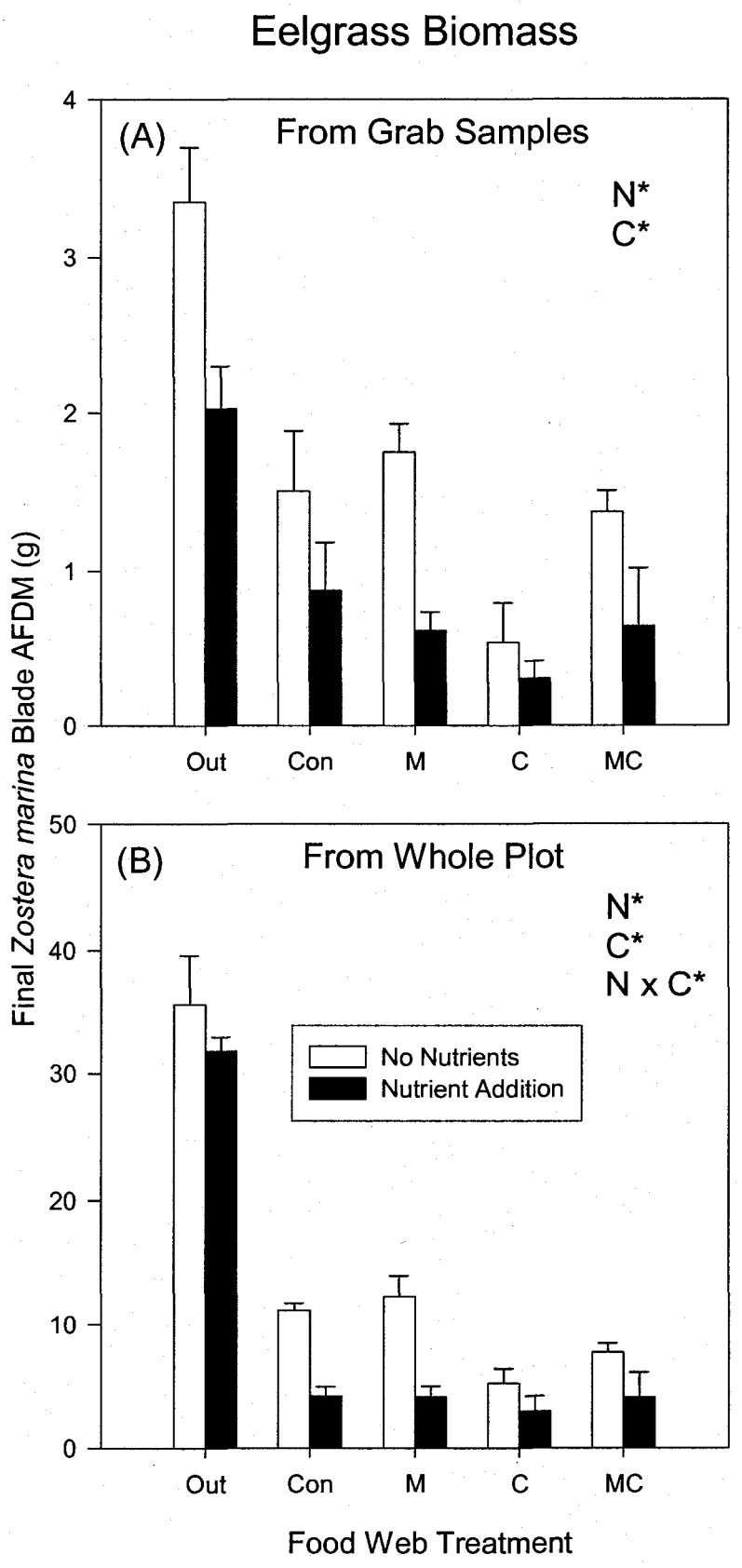

Eelgrass Biomass
(A)
From Grab Samples
N*
C*
4
3
2
1
0
Out
Con
M
C
MC
(B)
From Whole Plot
N*
C*
N x C*
No Nutrients
Nutrient Addition
50
40
30
20
10
0
Out
Con
M
C
MC
Final Zostera marina Blade AFDM (g)
Food Web Treatment

Figure 5.

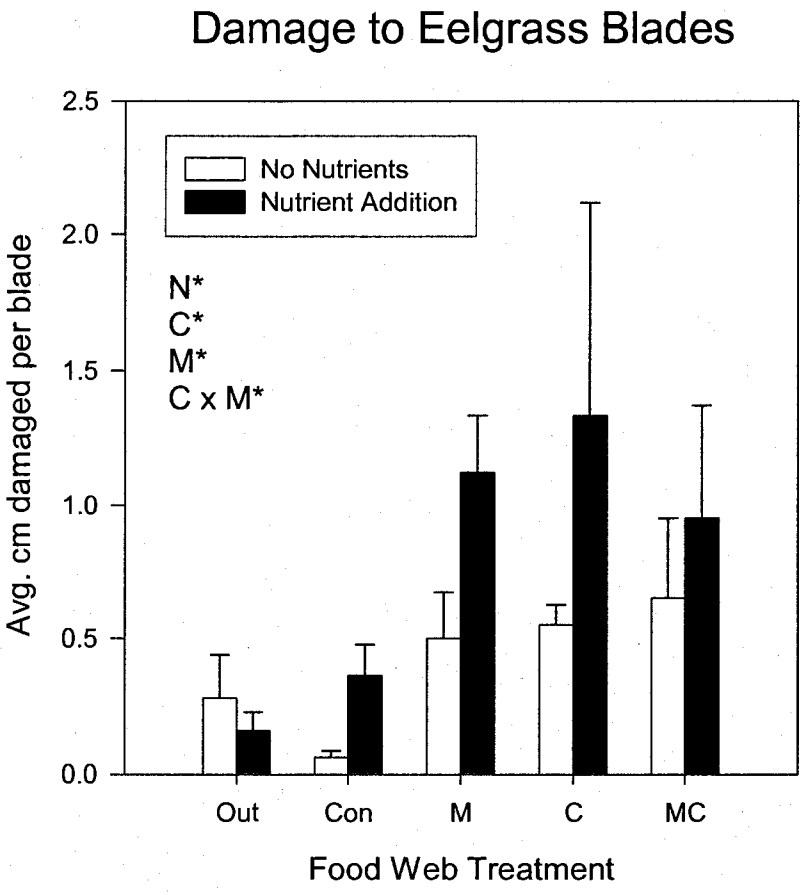
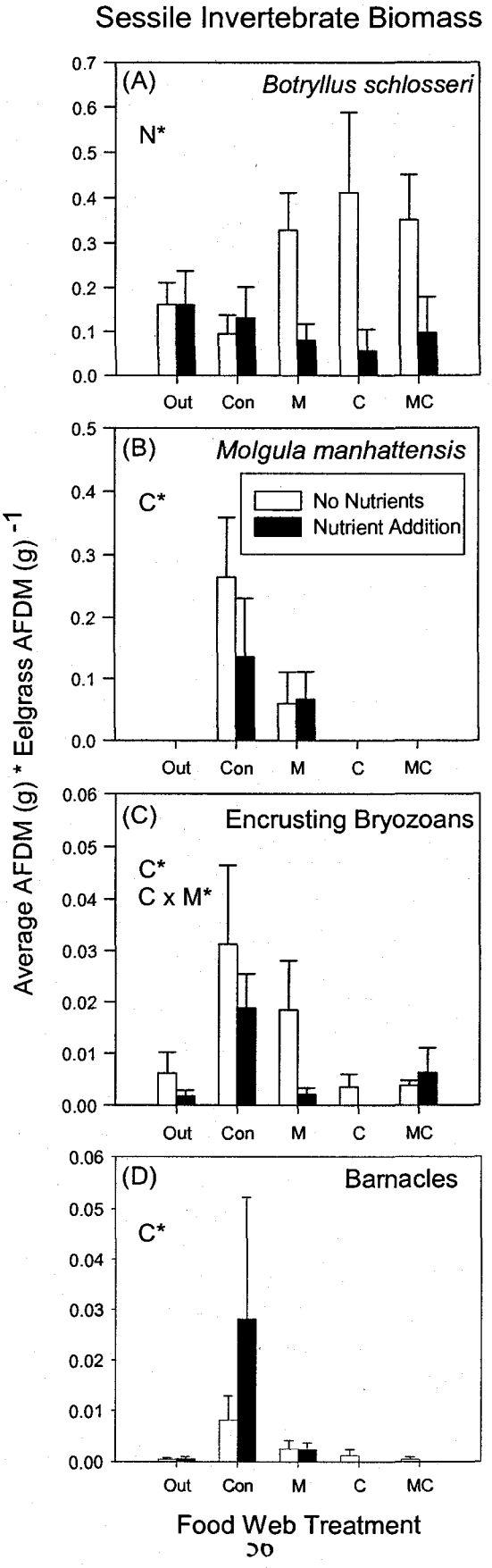

Damage to Eelgrass Blades
No Nutrients
Nutrient Addition
N*
C*
M*
C x M*
Avg. cm damaged per blade
2.5
2.0
1.5
1.0
0.5
0.0
Out
Con
M
C
MC
Food Web Treatment

Figure 6.


Sessile Invertebrate Biomass
(A)
Botryllus schlosseri
N*
(B)
Molgula manhattensis
C*
No Nutrients
Nutrient Addition
(C)
Encrusting Bryozoans
C*
C x M*
(D)
Barnacles
C*
Out
Con
M
C
MC
Average AFDM (g) * Eelgrass AFDM (g) -1
Food Web Treatment

# CHAPTER 2

## Herbivore and predator diversity affect ecosystem properties in an experimental marine community

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## ABSTRACT

Changes in predator and prey diversity likely influence ecosystem properties but have rarely been tested together. We manipulated the species richness of herbivores and predators in an experimental benthic marine community and measured their effects on predator, herbivore and primary producer performance. Predator composition and richness strongly affected several community and population responses, mostly via sampling effects. However, some predators survived better in polycultures than in monocultures, suggesting complementarity due to stronger intra- than interspecific interactions. Predator effects also differed between additive and substitutive designs, emphasizing that the relationship between diversity and abundance in an assemblage can strongly influence whether and how diversity effects are realized. Overall, the presence and richness of predators dominated biotic effects on community and ecosystem properties.

# INTRODUCTION

There is now considerable evidence that species diversity influences a variety of ecosystem processes including productivity, decomposition, and nutrient cycling (reviewed by Hooper et al. 2005, Balvanera et al. 2006, Cardinale et al. 2006a). Experiments with plants and herbivores suggest that in general, increasing diversity within a given trophic level tends to increase the total abundance or production at that level, and to decrease the abundance of organisms or resources at the level below (Balvanera et al. 2006, Cardinale et al. 2006a). However, most experiments testing effects of biodiversity on ecosystem function (BEF) have focused on diversity within a single, lower trophic level (Hooper et al. 2005). In contrast, effects of diversity changes at higher trophic levels, and the interactive effects of changes at multiple trophic levels, are less well understood. Predator diversity effects are especially difficult to generalize because there is intriguing variation in how multiple predators interact to affect prey populations and ecosystem processes (Duffy et al. 2007, Bruno and Cardinale In press, Schmitz 2007). Increasing predator diversity may strengthen total impact on prey through complementary prey choices or emergent multi-predator effects such as risk-enhancement (Losey & Denno 1998, Sih et al. 1998, Byrnes et al. 2006), but it may also reduce impacts on prey due to competition and intra-guild predation among predators, and/or omnivory (Hart 2002, Finke & Denno 2004, Bruno & O'Connor 2005, Jonsson et al. 2007). How changes in diversity at multiple trophic levels interact is also poorly understood, though theory predicts that such interactions can have additive, synergistic, or antagonistic effects on ecosystem processes (Worm & Duffy 2003, Thebault & Loreau 2003, Fox 2004b).

Biodiversity may be thought of as having a horizontal component, which constitutes

diversity within a trophic level, and a vertical component, which constitutes the number and distinctness of trophic levels (Duffy et al. 2007). Changes in number of trophic levels tend to have strong effects on ecosystems in the form of trophic cascades (Shurin et al. 2002). Some have argued that community-wide cascades, which affect abundance and biomass across whole trophic levels, are rare in diverse systems, implying an interaction between effects of changing vertical and horizontal diversity (Strong 1992, Polis 1999, but see Bruno & O'Connor 2005, Borer et al. 2006). Although a growing number of BEF studies have included multiple trophic levels, few have been designed to rigorously evaluate the effects of changing diversity within a level on adjacent levels, or the interactive effects of simultaneous diversity change at multiple levels (reviewed by Duffy et al. 2007, Bruno & Cardinale In press). With the exception of experiments performed in plant-pollinator systems (Fontaine et al. 2006), the only factorial manipulations of diversity of adjacent trophic levels have been done in microbial systems in the laboratory (Naeem et al. 2000, Fox 2004a, Gamfeldt et al. 2005). Naeem et al. (2000) manipulated decomposers and algae, and found that increasing decomposer diversity and algal diversity acted synergistically to increase algal production. Manipulations of ciliates (primary consumers) and microalgae have produced mixed results. Gamfeldt et al. (2005) found that increasing ciliate diversity decreased algal biomass and increased consumer production, and that algal diversity increased consumer production only at the highest diversity of consumers. In contrast, Fox (2004a) found that algal diversity increased algal biomass but did not affect consumer production, and that consumer diversity had no effects. These divergent results call for deeper investigation of how changing diversity at multiple trophic levels affects ecosystem properties, especially in systems with macroscopic animals and plants.

One critical design consideration in experiments investigating functional effects of

diversity is appropriate selection of the abundance of organisms initially stocked. This has been of special concern in studies of larger organisms, which may never reach equilibrium populations during an experiment (Schmitz 2007, O'Connor & Bruno 2007). Two approaches to setting initial abundance are the additive design and the replacement design (Jolliffe 2000). In the additive design the same abundance or biomass of each species is added to polycultures as is added to the monoculture of that species, such that the total initial abundance in a treatment increases with species richness. In the replacement design, the total abundance is initially constant across treatments, but is divided evenly among all the species included, such that the initial abundance of any one species declines with species richness. The extent to which increasing species richness decreases abundance of individual species in nature is difficult to quantify, but is expected to correlate with the degree of niche overlap among species (Ruesink & Srivastava 2001). Thus, the additive design approximates a scenario of zero niche overlap, and the replacement design simulates high niche overlap.

Here we report an experimental test of the effects of herbivore and predator species composition and richness on algal growth, herbivore abundance, and predator growth and survival in a benthic marine community. Small, invertebrate grazers (mostly amphipod and isopod crustaceans hereafter referred to as "grazers") are the most abundant herbivores in this system, as they are in many benthic communities worldwide. Some species have generation times of less than three weeks in warm water (Fredette & Diaz 1986, Edgar 1993, Duffy *et al.* 2003). Grazer species differ in their impacts on macroalgae (Duffy 1990, Duffy & Hay 2000), and increasing grazer diversity decreases macroalgal biomass in seagrass mesocosms (Duffy *et al.* 2003, 2005). Grazers also consume epiphytic microalgae, however, and may thus have a positive influence on some species of macroalgae by reducing competition with microalgae for

light and nutrients (Duffy 1990). Grazers are a primary food source for many larger crustaceans and small fishes, including the prey of commercially harvested species, and are an important link from benthic algal production to fisheries yield (Edgar & Shaw 1995, Williams & Heck 2001). Commercial harvest of large fishes and crustaceans could alter vertical diversity (i.e., effective food chain length) in marine macroalgal systems. This in turn could increase densities of smaller predators, leading to reduced abundance of grazers and increased abundance of algae via a trophic cascade (e.g. Heck *et al.* 2000, Worm & Myers 2003, Frank *et al.* 2005).

To address potential effects of such interacting changes in vertical and horizontal diversity, we conducted a factorial manipulation of predator and grazer diversity to test the following hypotheses: 1) Multi-species predator assemblages have stronger impacts on grazers than predator monocultures, on average, due to complementary effects of different predator species and / or the increased likelihood of including highly effective predator species. 2) Grazer polycultures maintain higher grazer abundance, on average, than grazer monocultures because differences in grazer population growth rates and susceptibility to predation lead to sampling and / or complementarity effects. 3) Predators grow and survive better with diverse assemblages of prey than with prey monocultures (the balanced diet hypothesis, DeMott 1998, Stachowicz *et al.* 2007). 4) Predator and prey assemblages interact such that predator effects differ depending on the prey assemblage, and prey effects differ depending on the predator assemblage

## METHODS

### Mesocosm system

The experiment was conducted in 30 L transparent plastic mesocosms held in outdoor water tables at the University of North Carolina at Chapel Hill's Institute of Marine Science in

Morehead City, North Carolina (USA) from 20 August – 13 September, 2005 (25 days). Mesocosms were open at the top, but covered in 8 mm black Vexar™ screens to provide some shade and to prevent predator escape. Seawater pumped from the adjacent sound was filtered through 150 μm mesh bags and delivered to the mesocosms through a system of dump-buckets suspended over the water tables. Filtration minimized immigration of juvenile grazers, but allowed organisms with planktonic propagules (i.e., the alga *Enteromorpha intestinalis*) to colonize mesocosms during the experiment. (We included settling plates to assay the accumulation of microalgae, but a freezer malfunction ruined those samples before they could be analyzed.) Water flowed out of the mesocosms through holes drilled at 20 cm height and covered in 500 μm mesh to minimize loss of grazers. Experimental treatments were randomly assigned to water table, row, and column positions.

**Experimental design**

All organisms were collected from lagoons, inlets, and tidal creeks in the immediate vicinity of Morehead City (34.43 N, 76.43 W). Grazer and predator diversity treatments were crossed in a factorial design. A simplified gradient of diversity was used for both grazers and predators: monocultures of each of three species, and "diverse" treatments with all three species. Grazers were stocked using a replacement design (Jolliffe 2000), with 60 of one species inoculated in monocultures, or 20 of each species in three-spp. treatments. Predators were stocked in a similar manner, with a total of three individuals per mesocosm. However, we also included a high-density predator treatment based on an additive design, which had three individuals of each predator species. We included both types of three-spp. predator treatments to simulate two potential relationships of predator abundance and predator species richness (see introduction).

We did not incorporate an additive, high-density grazer treatment, because we assumed that rapid population growth would bring grazer communities to near carrying capacity for each species by the end of the experiment, reducing effects of differently sized inocula, as found previously (Duffy & Harvilicz 2001). We included a no-predator treatment but not a no-grazer treatment. Each of the 24 unique treatments was replicated 5 times, for a total of 120 mesocosms.

**Community assembly**

All mesocosms were stocked with the same three macroalgal species: *Sargassum filipendula*, a fleshy, brown alga; *Gracilaria tikvahiae* a coarse, branched red alga; and *Ulva sp.*, a leafy, green alga. These algae were selected because they tend to dominate local hard substratum benthic communities and represent distinct taxonomic groups that have been shown in previous mesocosm experiments to be differently susceptible to consumption by grazers (Duffy and Hay 2000). The macroalgae were added in equal proportions (14 g wet mass each) to each mesocosm by attaching algal thalli to Vexar™ screens at the bottom of the mesocosms (Bruno *et al.* 2005). Algae and screens were defaunated before installation by soaking in a dilute solution of Sevin™ insecticide (43% 1-napthyl-N-methylcarbamate) in seawater (0.1 g Sevin™ $L^{-1}$) for 30 minutes, followed by rinsing in flowing seawater.

The three species of grazers used are generally very common in the system and represented the numerically dominant species in the field at the time of the experiment. All are pericaridean crustaceans with rapid life cycles and direct development of young too large to escape through the outflow screens on the mesocosms. Adults of all species reach a maximum size of about 1 cm length. *Paracerceis caudata* is an isopod that consumes algae and detritus.

*Elasmopus levis* and *Dulichiella appendiculata* are gammaridean amphipods known to consume both micro- and macroalgae (Duffy and Hay 2000).

Predators included the grass shrimp *Palaemonetes vulgaris*, the mud crab *Panopeus herbstii*, and the mummichog *Fundulus heteroclitus*. The grass shrimp is a common estuarine species with an omnivorous diet including grazers (Nelson 1979, 1981). The mud crab is a predator and scavenger known to consume amphipods (Stachowicz and Hay 1999). The mummichog is a predatory killifish with a broad diet including amphipods (Allen *et al.* 1994). Average $\pm$ SD wet masses (g) of individual fish, shrimp, and crabs added to the experiment were $1.32 \pm 0.827$, $0.397 \pm 0.089$, and $0.899 \pm 0.603$, respectively.

**Statistical analyses**

Data were tested for homogeneity of variance using Cochran's C-test and were log transformed as needed. We considered that the locations of individual mesocosms in the outdoor array might have unintended effects on responses due to slight variations in light and water flow. We controlled for mesocosm position effects by running a partially crossed 3-way ANOVA on all responses using water table, row position, column position, and the row by column interaction as factors; if mesocosm position significantly affected a response then we used the residuals from this ANOVA instead of raw data for subsequent analyses on that response.

Predator and grazer treatment effects and their interactions were tested with a fully crossed 2-way, fixed-factor ANOVA, excluding the no-predators treatment unless otherwise noted. The proportion of variance explained by each factor and interaction was calculated as $\omega^2$ according to Kirk (1995). Planned contrasts were included to test for the hypothesized effects of grazer and predator diversity (see Supplemental Table S1). To distinguish non-transgressive

and transgressive effects, we compared the performance of polycultures to the average performance of monocultures, and to the performance of the highest performing monoculture (Hector *et al.* 2002). By necessity, these contrasts were not orthogonal. We considered predator polycultures that used additive density vs. replacement density in separate contrasts. Effects of predator density and diversity on predator survival were tested with a Kruskal-Wallis comparison among predator monocultures, low-density polycultures, and high-density polycultures. Correlations among final predator biomass, final grazer abundance, and algal mass change were assessed with multiple linear regression.

Our study involves several variables and a large number of separate statistical tests, increasing the probability of spurious statistical significance at the nominal $p < 0.05$ level (Rice 1989). Table-wide adjustments to the critical value such as the Bonferroni procedure have sometimes been recommended to address this issue, but such corrections have been widely criticized because they involve arbitrary decisions about how to group the tests, are mathematically suspect, and strongly increase the probability of type II errors, thus obscuring ecologically significant effects (Cabin & Mitchell 2000, Moran 2003, Nakagawa 2004). For these reasons, we opted not to apply table-wide adjustments, and instead use three other approaches to evaluate ecological significance: 1) We interpret a high frequency of nominally significant results among multiple, related tests as stronger evidence of a real effect than a nominally significant result in only one test. 2) We evaluate statistical results strictly in the context of our hypotheses. 3) We report effect size measures alongside nominal p-values whenever possible, and downplay the results of tests that are statistically significant but have ecologically negligible effect sizes.

# RESULTS

## Treatment efficacy

Algae, grazers, and predators generally survived and grew or multiplied over the course of the experiment. Contamination of mesocosms by taxa not initially stocked was minimal, except for some recruitment of the green alga *Enteromorpha intestinalis*. Two replicates of (*Dulichiella appendiculata* + Low-density predator polyculture) treatments had no fish or fish remains at the end of the experiment and extremely high numbers of *D. appendiculata*, whereas all other replicates of that treatment had few grazers. We removed these two replicates from analyses, on the grounds that they had apparently been without fish for most or all of the experiment and their responses did not represent valid observations from the same sample space as the other replicates (Gotelli & Ellison 2004). Mesocosm placement in the outdoor array had no significant effects on grazer or predator responses, but did have substantial effects on algal growth as evidenced by significant effects of position in all ANOVAs on algae growth, with $R^2$ values up to 0.5. This was probably due to differential shading and water delivery at some locations in the mesocosm array. To factor out this variation, tests of treatment effects on algal growth were performed on the residuals from ANOVAs testing effects of mesocosm placement.

## Macroalgae responses

Top-down control by grazers and predators had little influence on macroalgal biomass relative to the inadvertent variation caused by mesocosm position. However, the green seaweed *Enteromorpha intestinalis*, which recruited into the mesocosms, varied significantly among

predator treatments in ANOVA (Supplemental Table S2 in the appendix), and multiple regression suggested that this was because *E. intestinalis* was slightly reduced by the omnivorous grass shrimp (Table S3). Grazer treatment had no effect on algae in ANOVA, but multiple regression found that total grazer abundance slightly reduced the biomass of *Ulva sp.* and *Sargassum filipendula*. Predator and grazer diversity had weak and idiosyncratic effects on macroalgae, never explaining more than 8% of the variance (Table S2).

**Grazer responses**

Total grazer abundance increased roughly 10-fold by the end of the experiment, representing an intrinsic rate of growth $r$ of about 0.1 $d^{-1}$. There was no main effect of grazer treatment on total grazer numbers or individual species' population growth rates. However, grazer and predator treatments interacted to affect total grazer numbers, indicating that grazer species composition and richness both influenced total grazer abundance under some conditions (Table 1, Fig. 1).

Comparisons of individual grazer species population growth rates in monoculture vs. in the all-grazer treatments directly tested whether horizontal diversity influenced grazer populations (Table 1). For *P. caudata* and *D. appendiculata*, final abundances were higher in monocultures than in polycultures (Fig. 1), and population growth rates (PGR) were not affected by grazer treatment (Table 1). Conversely, for *E. levis*, final abundance was similar in monocultures and polycultures (Fig. 1) and PGR was marginally higher in polycultures (Table 1). This suggests that *E. levis* was released from intraspecific competition in the presence of other grazers, whereas *P. caudata* and *D. appendiculata* experienced intra- and interspecific competitors equally. Accordingly, grazer richness tended to increase total grazer abundance

relative to the average of monocultures, although this effect was not statistically significant ($p = 0.102$, Table 1).

In contrast to the generally weak effects of grazer composition and richness, predator treatment and the predator by grazer interaction strongly affected grazer abundance, together explaining 65% of the variance. When the no-predator treatment was excluded from the analysis, predators and the predator by grazer interaction still explained 60% of the variance in grazer abundance (Table 1, $\omega^2$ values), confirming that much of the predator effect was due to variation in predator species composition and richness, rather than presence vs. absence. Among predators, fish were the most effective consumers of all species of grazers (Fig. 1). Predator impacts on all grazers were enhanced in predator polycultures relative to the average of predator monocultures. However, only the grazer *Paracerceis caudata* was reduced more by predator polycultures than by the most effective predator monoculture (fish) (Table 1, Fig. 1).

**Predator responses**

Predator survival was generally high, but differed among predator treatments (Fig. 2, Table 2). Fish and crabs survived best in low-density predator polycultures, where there was just one predator of each species (Fig. 2, Table 2), suggesting that negative intraspecific impacts on these two species were relaxed in the absence of conspecifics. Shrimp, on the other hand, survived best in the absence of other predator species. Surviving predators generally increased in mass during the experiment, though fish sometimes lost mass (Fig. 3).

Proportional mass changes (PMC) of surviving fish and shrimp were unaffected by treatment, but crab growth was significantly influenced by composition and richness of both predators and grazers (Fig. 3, Table 1). Grazer richness increased crab growth in crab

monocultures, and slightly increased crab growth in low-density predator polycultures, but surprisingly decreased crab growth in high-density predator polycultures (Fig. 3).

**Prevalence and Magnitude of Predator and Grazer Effects**

Effects of predator diversity were consistently stronger than those of grazer diversity. In two-way ANOVAs, predator treatment effects and associated planned contrasts were statistically significant far more often than those of grazer treatment or predator by grazer interactions, and only predator diversity had a significant transgressive impact on any response; the reduction of *Paracerceis caudata* (Table 1). More importantly, the magnitude of predator effects also tended to be much greater (ranging to $\omega^2 = 0.717$) than the magnitude of grazer effects (ranging only to $\omega^2 = 0.073$) (Table 1). It should be noted, however, that many of the response variables are interrelated, which may exaggerate the apparent prevalence of predator effects.

## DISCUSSION

By definition, species differ functionally between trophic levels, such that adding or removing a complete trophic level—altering "vertical diversity" (Duffy *et al.* 2007)—often has strong ecosystem effects (Shurin *et al.* 2002, Borer *et al.* 2006). Within a trophic level, relationships between diversity and ecosystem function may be weaker because of greater similarity among species (Strong 1992, Walker 1992). However, species' traits can vary widely even within a trophic level, and changing this horizontal diversity has often been shown to influence ecosystem properties (reviewed by Hooper *et al.* 2005), especially when diversity is initially low (e.g.,

Jonsson *et al.* 2002). In our experimental communities the number of trophic levels varied and species differed functionally within both grazer and predator trophic levels, leading to significant effects of both horizontal and vertical diversity, and their interaction, on ecosystem properties.

In the horizontal dimension, we found weak effects of herbivore diversity (defined broadly to include both species richness and composition) but much stronger effects of predator diversity. One possible contributor to this disparity in the strength of grazer and predator diversity effects is that the predators were more taxonomically and functionally diverse than the grazers, creating greater scope for sampling and complementarity effects at the predator level. Another potential explanation for the strong effects of predators could be the limited space available in the mesocosm system. In nature, predators often range widely, integrating over habitat patches, whereas herbivores may be more localized within a patch. Confinement of predators to a single habitat patch might reduce the prey recovery time of that patch relative to a patch in an open system, inflating the apparent importance of predators (Ellner et al. 2001, Cardinale et al. 2006b). The finding that dispersal corridors reduced the impact of grazers (at high diversity) on algae in a similar mesocosm system (France & Duffy 2006) is perhaps consistent with this possibility, although the diminution of herbivory with dispersal in that experiment was slight.

Only two effects of grazer diversity in our experiment approached statistical significance. First, grazer richness increased predation resistance when all three predators were present at low density, but not when only a single predator species was present (Fig. 1a). The contingency of this grazer diversity effect on the predator community resembles a previous report that grazer diversity enhanced grazer biomass only in the presence of a predator (Duffy *et al.* 2005). The difference in our result, however, is that the grazer diversity effect did not arise in the presence

of a single predator species, but only at high predator diversity. Second, grazer richness interacted with predator richness by increasing crab growth when crabs were the sole predators, but reducing crab growth when other predators were present at high density (Fig. 3, Table 1). These interactions between grazer diversity and predation may reflect the differing vulnerability of grazer species to predation. In the absence of fish, *Elasmopus levis* and *Dulichiella appendiculata* reproduced rapidly. In the presence of fish, however, *Paracerceis caudata* tended to do better, perhaps because its tough cuticle provided resistance to predation, resulting in higher grazer abundance in grazer polycultures than in monocultures without *P. caudata* (Fig. 1). A similar switch in prey species dominance under predation has been observed in previous experiments with marine grazers (Duffy *et al.* 2005). The importance of different grazers' functional traits to total grazer production under different conditions of predation might be considered an example of "response diversity" (Elmqvist *et al.* 2003), in which different species maintain ecosystem function under different environmental conditions.

Changes in horizontal diversity at the level of predators had important community-level impacts in our experiment, i.e. predator polycultures kept grazers at a lower abundance than did predator monocultures, on average. This appeared to be largely a sampling effect driven by the inclusion of the strongest predator (fish) in all three-species predator treatments. Thus it is similar to Straub & Snyder's (2006) finding that predator identity but not predator richness per se, affected prey abundance in an agroecosystem. However, we also detected an element of predator complementarity or facilitation in the reduction of grazers; the combined effects of crabs, fish, and shrimp reduced *P. caudata* to a lower level in predator polycultures than in any single-predator treatment (Fig. 1, Table 1), an analog of "overyielding" in plant studies.

When predators interact negatively via intraguild predation or interference, increasing predator diversity is predicted to reduce their total impacts on prey (Sih *et al.* 1998, Finke & Denno 2004, Schmitz 2007). Intraguild predation by fish likely occurred in our experiment since shrimp survival was reduced in predator polycultures (Fig. 2), probably by fish. Nevertheless, multi-predator assemblages had stronger impacts on prey (Table 1). This can be explained by the fact that the most effective predators, fish, were not the victims of intraguild predation. If predator species with dominant effects on prey are generally beneficiaries rather than victims of intraguild predation, then intraguild predation may not weaken top-down control on lower trophic levels.

The initial abundance of species in experimental manipulations of species richness is an important factor that can potentially be confounded with richness effects, especially with non-microbial animals that may have limited growth and reproduction during an experiment. An often-cited problem with the replacement density design (Joliffe 2000) is that the lower initial abundance of each species in diverse treatments than in monocultures could lead to underestimation of sampling-based diversity effects if the timescale of the experiment does not allow potentially dominant species to increase from their low initial abundance (Weis *et al.* 2007). Replacement designs could also underestimate complementarity effects, because they set overall abundance in diverse treatments at the same level as monocultures, when in nature, niche differentiation may allow higher total abundance in diverse assemblages. Conversely, additive designs may inflate estimates of diversity effects by increasing overall abundance along with diversity, leading to abnormally high density when many species are included in a diverse treatment (Schmitz 2007). Finally, neither replacement nor additive designs are likely to accurately represent the unequal distributions of species abundance that would result for a given

level of diversity after a long term in nature; something that may be impossible to simulate precisely in experiments (Weis *et al.* 2007). By including both low-density and high-density predator diversity treatments we were able to compare the extreme ends of the spectrum, i.e. consequences of replacement versus additive designs. Low and high-density predator polycultures had similar effects on grazers (Fig. 1, Table 1), probably because even a single fish could reduce grazers to a low level. However, predator density did affect crab proportional mass change (Fig. 3, Table 1) and fish survival (Table 2). Fish may have survived better in low-densitypredator polycultures than in high -density polycultures or monocultures due to reduced competition for food and reduced agonistic intraspecific interactions (Weis *et al.* 2007). These results demonstrate that the mode of diversity manipulation can significantly alter experimental outcomes and suggest caution in the interpretation of BEF effects from experiments that used only the additive or the replacement design where species densities were not allowed to change. When performing diversity manipulations with species that cannot adjust their population densities during the course of an experiment, it may prove useful to include both additive and replacement density treatments and perhaps additional density treatments (Ruesink & Srivastava 2001). More broadly, these results emphasize that understanding how abundance and diversity are related within natural assemblages is critical to interpreting how biodiversity, particularly of predators, will influence ecosystem processes.

While we found strong interactions between predators and herbivores, we observed little influence of predator and grazer treatments on macroalgal mass change. This result probably reflects the fact that our grazer assemblages did not include ampithoid amphipods, which were scarce in the field at the time of the experiment but are known to feed heavily on macroalgae (Duffy and Hay 2000). Top-down effects of grazers and trophic cascades from predators to

macroalgae have been documented in experiments that included ampithoids (Duffy and Hay 2000, Bruno and O'Connor 2005). It would be useful to perform joint predator and grazer diversity manipulations with a selection of grazer species with stronger effects on plant biomass, to investigate the hypothesized role of intermediate trophic level diversity in attenuating trophic cascades (Duffy 2002, Hillebrand & Cardinale 2004, Ives *et al.* 2005, Duffy *et al.* 2007).

Perhaps the most intriguing result we observed involved the interactive effects of herbivore and predator treatment on performance of mud crabs. When crabs were the only predator species they grew better in the presence of diverse grazer species than with any grazer monoculture (Fig. 3). This supports the balanced diet hypothesis (Demott 1998, Gamfeldt *et al.* 2005), which appears to hold in many marine consumers (Worm *et al.* 2006, Stachowicz *et al.* 2007). But in high-density polycultures of predators, crabs actually grew more, on average, with monocultures of grazers. We were not able to determine exactly why this happened, but we suspect a trait-mediated indirect interaction (TMII) (Werner & Peacor 2003). For example, crabs may have restricted their foraging behaviors in the presence of high densities of other predators, such that they were unable to fully exploit all prey in three-spp. grazer treatments. The presence of predators and competitors has been shown to change feeding behavior of organisms in other systems, and such TMII's appear to be common (Lima 1998, Werner & Peacor 2003).

Complex, natural communities with their reticulate food webs can be difficult to understand with pairwise predator-prey models and experiments manipulating just one or a few species (Polis & Strong 1996, Ives *et al.* 2005, Duffy *et al.* 2007, but see Schmitz and Sokol-Hessner 2002, Schmitz 2007), and it is virtually impossible to experimentally study or accurately model every trophic link in a community and its indirect effects on ecosystem state. Nevertheless, by factorially manipulating a moderate number of grazer and predator species, we

were able to observe interactions within and between trophic levels that were important in structuring our experimental communities and may be relevant to real ecological systems. As in some previous studies that have manipulated consumer diversity, we found that grazer abundance can be increased by grazer richness (Duffy *et al.* 2005) and decreased by predator richness (Snyder *et al.* 2006). More importantly, by simultaneously manipulating both trophic levels we were able to observe the net effect of increasing diversity at both levels. In this case, as in Gamfeldt *et al.* (2005), the result was consistent, top-down control by the highest trophic level, with ecosystem properties most strongly affected by changing diversity at that level.

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Table 1. Results of 2-way, fully crossed ANOVAs examining effects of grazer and predator species composition and diversity on a) grazer populations and b) predator proportional mass change. Treatments lacking predators are excluded from the analysis. Bold row headings are main treatment effects and interactions, and row headings preceded by > are planned contrasts within those effects or interactions. Omega squared estimates effect size (see Methods), and bold values highlight nominal $p < 0.05$. a) Left: Effects on the natural log of total grazer abundance. Right: Effects on the population growth rate of individual grazer species. PGR = Ln[(Final #)(Initial #)$^{-1}$] $d^{-1}$. b) Left: Effects on the total proportional mass change (PMC) of the predator community. Right: Effects on the PMC of individual predator species. PMC = (Final mass – Initial mass)(Initial mass)$^{-1}$.

Response

| a. | ln(Total Grazer Numbers) | | | | *P. caudata* PGR | | | | *E. levis* PGR | | | | *D. appendiculata* PGR | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Effects | df | F | $\omega^2$ | P | df | F | $\omega^2$ | P | df | F | $\omega^2$ | P | df | F | $\omega^2$ | P |
| **Grazer Treatment** | 3 | 2.14 | 0.01 | 0.102 | 1 | 0.36 | 0.000 | 0.551 | 1 | 4.03 | 0.026 | 0.052 | 1 | 0.09 | 0.000 | 0.772 |
| >Monos. vs. Polyculture | 1 | 1.97 | 0.00 | 0.168 | | | | | | | | | | | | |
| >Best Mono. vs. Polyculture | 1 | 0.22 | ... | 0.638 | | | | | | | | | | | | |
| **Predator Treatment** | 4 | 34.91 | 0.55 | **<0.0001** | 4 | 10.10 | 0.433 | **<0.0001** | 4 | 16.70 | 0.549 | **<0.0001** | 4 | 16.70 | 0.717 | **<0.0001** |
| >Monos. vs. Low Density Poly. | 1 | 39.05 | 0.22 | **<0.0001** | 1 | 19.24 | 0.217 | **<0.0001** | 1 | 14.86 | 0.028 | **0.000** | 1 | 12.16 | 0.063 | **0.001** |
| >Monos. vs. High Density Poly. | 1 | 54.27 | 0.15 | **<0.0001** | 1 | 28.52 | 0.328 | **<0.0001** | 1 | 7.64 | 0.058 | **0.009** | 1 | 50.19 | 0.279 | **<0.0001** |
| >Best Mono. vs. Low Density Poly. | 1 | 0.84 | ... | 0.149 | 1 | 8.75 | ... | **0.005** | 1 | 0.54 | ... | 0.467 | 1 | 3.07 | ... | 0.088 |
| >Best Mono. vs. High Density Poly. | 1 | 2.13 | ... | 0.363 | 1 | 13.96 | ... | **0.001** | 1 | 3.21 | ... | 0.081 | 1 | 0.50 | ... | 0.483 |
| **Grazer Trt. * Predator Trt.** | 12 | 1.95 | 0.05 | **0.041** | 5 | 0.56 | 0.000 | 0.695 | 5 | 0.90 | 0.000 | 0.473 | 5 | 1.70 | 0.016 | 0.170 |

| b. | Total Predator PMC | | | | Shrimp PMC | | | | Crab PMC | | | | Fish PMC | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Effects | df | F | $\omega^2$ | P | df | F | $\omega^2$ | P | df | F | $\omega^2$ | P | df | F | $\omega^2$ | P |
| **Grazer Treatment** | 3 | 1.06 | 0.002 | 0.370 | 3 | 0.25 | 0.000 | 0.863 | 3 | 3.15 | 0.073 | **0.034** | 3 | 0.67 | 0.000 | 0.576 |
| >Monos. vs. Polyculture | 1 | 3.44 | 0.021 | 0.068 | 1 | ... | ... | ... | 1 | 5.00 | 0.041 | **0.030** | 1 | 1.17 | 0.003 | 0.285 |
| >Best Mono. vs. Polyculture | 1 | 0.08 | ... | 0.776 | 1 | 0.62 | ... | 0.440 | 1 | 0.00 | ... | 0.961 | 1 | 1.78 | ... | 0.189 |
| **Predator Treatment** | 4 | 1.24 | 0.005 | 0.295 | 2 | 0.16 | 0.000 | 0.851 | 2 | 5.62 | 0.105 | **0.006** | 2 | 0.30 | 0.000 | 0.740 |
| >Monos. vs. Low Density Poly. | 1 | 2.79 | 0.015 | 0.100 | 1 | ... | ... | ... | 1 | 7.00 | 0.061 | **0.011** | 1 | 0.80 | 0.000 | 0.376 |
| >Monos. vs. High Density Poly. | 1 | 0.43 | 0.000 | 0.514 | 1 | 0.07 | 0.000 | 0.798 | 1 | 3.17 | 0.022 | 0.082 | 1 | 0.00 | 0.000 | 0.996 |
| >Best Mono. vs. Low Density Poly. | 1 | 2.26 | ... | 0.137 | | | | | | | | | | | | |
| >Best Mono. vs. High Density Poly. | 1 | 12.32 | ... | **0.001** | | | | | | | | | | | | |
| **Grazer Trt. * Predator Trt.** | 12 | 0.63 | 0.000 | 0.708 | 5 | 4.02 | 0.054 | 0.373 | 6 | 3.46 | 0.179 | **0.007** | 6 | 1.82 | 0.098 | 0.117 |

Table 2. Kruskal-Wallis comparisons of predator survival versus predator diversity treatment (high density polyculture, low density polyculture, or monoculture). Df = 2. Bold values indicate nominal $p < 0.05$.

| Proportional | | High density polyculture | | | Low density polyculture | | | Monoculture | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Survival** | **P** | **Median** | **Rank** | **Z** | **Median** | **Rank** | **Z** | **Median** | **Rank** | **Z** |
| Fish | **0.041** | 0.67 | 25.30 | -1.62 | 1.00 | 38.50 | 2.49 | 0.83 | 27.70 | -0.87 |
| Shrimp | **0.001** | 0.00 | 21.20 | -2.91 | 0.00 | 28.20 | -0.72 | 1.00 | 42.10 | 3.63 |
| Crab | 0.058 | 1.00 | 27.60 | -0.93 | 1.00 | 38.10 | 2.37 | 0.83 | 25.90 | -1.44 |

# FIGURE LEGENDS

Figure 1. A) Final mean (± 1 SEM) total grazer abundance as a function of grazer and predator treatment. B) Final, relative abundance of grazer species in treatments initially stocked with equal numbers of each grazer species. Error bars are SEM of total grazer abundance.

Figure 2. Mean (± 1 SEM) proportion of predators of a given species surviving the duration of the experiment). Monocultures had three individuals, low-density polycultures had one individual of each species, and high-density polycultures had three individuals of each species.

Figure 3. Mean (± 1 SEM) proportional change in mass of surviving predators, by predator and grazer treatment.

Figure 1.

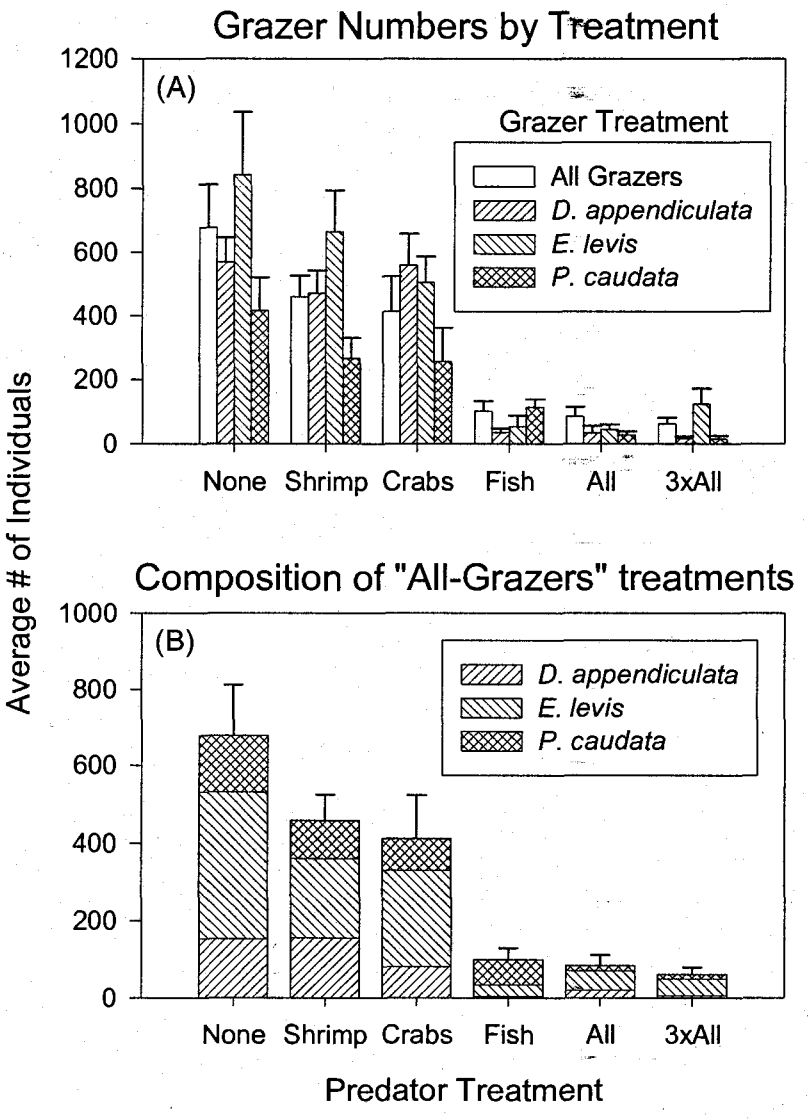

Grazer Numbers by Treatment
(A)
Grazer Treatment
All Grazers
D. appendiculata
E. levis
P. caudata
1200
1000
800
600
400
200
0
None
Shrimp
Crabs
Fish
All
3xAll
Composition of "All-Grazers" treatments
(B)
D. appendiculata
E. levis
P. caudata
1000
800
600
400
200
0
None
Shrimp
Crabs
Fish
All
3xAll
Average # of Individuals
Predator Treatment

Figure 2.

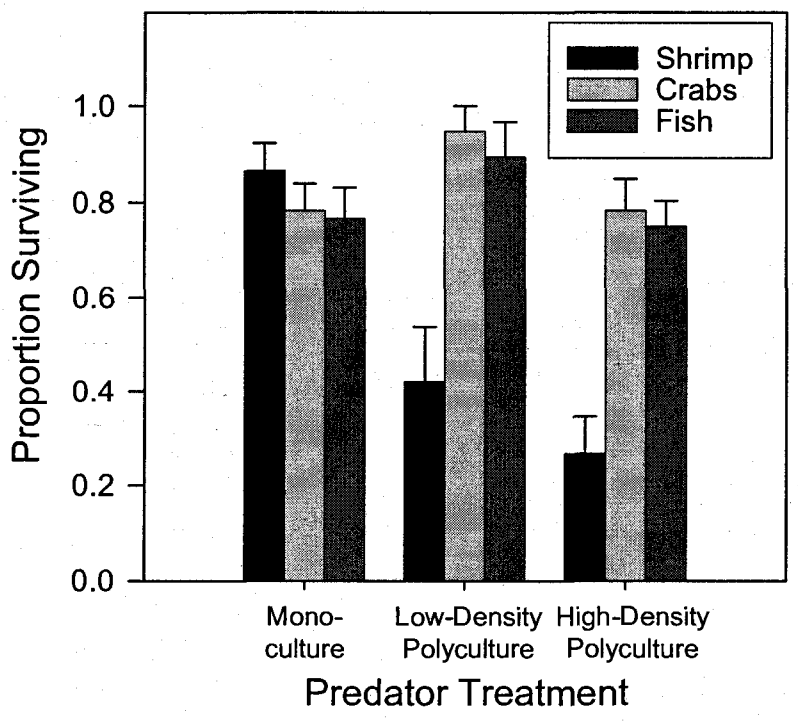
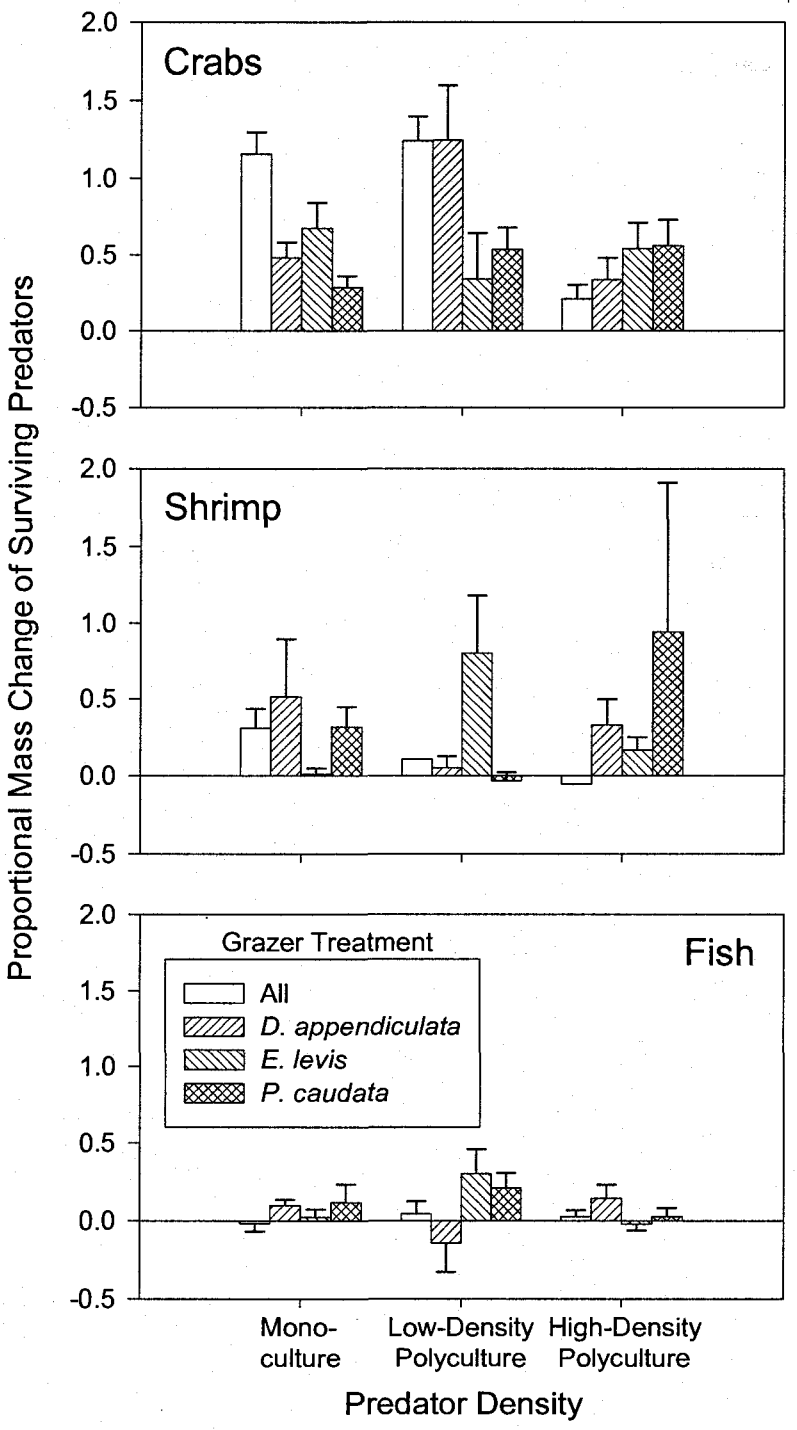

Shrimp
Crabs
Fish
Proportion Surviving
1.0
0.8
0.6
0.4
0.2
0.0
Mono-culture
Low-Density Polyculture
High-Density Polyculture
Predator Treatment

Figure 3.


Crabs
Shrimp
Fish
Proportional Mass Change of Surviving Predators
2.0
1.5
1.0
0.5
0.0
-0.5
Grazer Treatment
All
D. appendiculata
E. levis
P. caudata
Mono-culture
Low-Density Polyculture
High-Density Polyculture
Predator Density

# CHAPTER 3

## Annual cycles, inter-annual variation, and correlation among biotic and abiotic factors in a Chesapeake Bay eelgrass community

## ABSTRACT

The results of numerous mesocosm and caging experiments suggest that seagrass-associated biota interact strongly with seagrass, with each other, and with changes in abiotic conditions to influence the health and persistence of the seagrass itself. The sign and strength of these interactions appear to depend on the number of trophic levels in a community and the functional diversity within trophic levels. However, the dynamics of seagrass-associated communities in nature have rarely been assessed to validate these experimental findings. We characterized the seasonal and interannual variation in macrophytes, epiphytes, invertebrate grazers, small demersal predators, and physicochemical characteristics of an eelgrass (*Zostera marina*) bed in Chesapeake Bay, Virginia, USA over ten years to evaluate the following hypotheses: 1) physicochemical drivers affect both producer and consumer trophic levels, 2) top-down control generates inverse patterns of abundance at epiphyte, grazer, and predator trophic levels, and 3) grazer diversity contributes to the stability over time of total grazer abundance. We observed strong variation in abundance and species composition of eelgrass-associated species on both seasonal and interannual scales, and found that temperature and salinity explained much of that variation, consistent with hypothesis 1. We found minimal support for hypotheses 2 and 3, however. These results suggest either that top-down effects of consumer abundance and diversity are unimportant in this seagrass system, that variation in consumer abundance and diversity are insufficient to detect significant effects on top-down control, or that consumer effects are too complex to detect with this type of observational study.

# INTRODUCTION

Productivity, the distribution of biomass, and other aspects of ecosystem structure can be determined by "top-down" effects, which propagate from consumers to prey or basal resources, or by "bottom-up" effects, which propagate from basal resources or prey to successively higher consumers. While the relative importance and generality of top-down versus bottom-up control has been the subject of vigorous debate (Power 1992, Cyr & Pace 1993, Polis & Strong 1996), there is a growing consensus that both top-down and bottom-up processes affect most ecosystems (Polis 1999, Heck et al. 2000, Worm et al. 2002). Consequently, some ecosystems previously studied from a predominantly top-down or bottom-up perspective are now being evaluated from the alternative, or an integrated, view (Menge 2000, Frank et al. 2006, 2007). For example, ecological studies of seagrass ecosystems, which have historically focused on the bottom-up influence of water quality and physical conditions, are increasingly considering the importance of consumer effects (Hughes et al. 2004, Heck & Valentine 2007).

Seagrasses form productive and species-rich habitats in coastal areas throughout the world (Hemminga & Duarte 2000), where they contribute to human well-being by stabilizing sediments, improving water quality, and enhancing fisheries resources (Costanza et al. 1997, Worm et al. 2006). Unfortunately, seagrasses have declined dramatically in many regions (Duarte 2002, Orth et al. 2006). Bottom-up impacts of human activities have been implicated in most of these declines; increased sediment and nutrient inputs lead to high turbidity and eutrophic overgrowth of seagrass by epiphytes (Twilley et al. 1985, Kemp et al. 2005), and anthropogenic changes in climate and hydrography can exceed seagrasses' physiological

tolerances (Moore & Jarvis 2008). Yet, the abundance and species composition of herbivores also affect seagrass (Williams & Heck 2001, Hughes et al. 2004, Valentine & Duffy 2006) and these top-down factors may be changing in nature as a result of overfishing, contributing to seagrass declines (Jackson et al 2001, Heck & Valentine 2007).

Seagrass herbivory can be divided into two broad classes; direct grazing, which removes seagrass biomass, and epiphyte grazing, which removes algal competitors and indirectly benefits seagrass (Jernakoff et al. 1996, Valentine & Duffy 2006). Meta-analyses of mesocosm and field experiments manipulating grazers show that the effects of both types of grazing on seagrass growth and biomass tend to be equal to or greater in magnitude than nutrient enrichment effects (Hughes et al. 2004), and that epiphyte grazing in particular can counteract some negative effects of nutrients (i.e. Neckles et al. 1993). This has lead some to hypothesize that eutrophic overgrowth of seagrass by epiphytes can only occur when healthy grazer communities have been disturbed by outside factors, such as trophic cascades stemming from overharvesting of top predators by humans (Wetzel and Neckles 1986, Heck and Valentine 2007). Overfishing of predatory species could either increase or decrease grazing intensity, depending on the structure of the seagrass-associated food web and the trophic position of the predators harvested (Valentine & Duffy 2006, Heck & Valentine 2007). To most effectively conserve and restore seagrass beds, we need a better understanding of such top-down effects and how they interact with physicochemical factors to determine seagrass survival and persistence. An important step towards achieving this understanding will be connecting the results of small-scale, manipulative experiments to our interpretations of seagrass dynamics at landscape scales.

Controlled experiments have been useful in evaluating the relative importance of top-down and bottom-up factors affecting seagrass at local scales (e.g. Moore & Wetzel 2000, Heck

et al. 2006, Douglass et al. 2007), including the important role of consumer species composition and diversity in determining ecosystem properties and buffering against disturbance (Duffy et al. 2005, France & Duffy 2006, Blake et al. in prep). These experiments have begun to converge on the conclusion that top-down control by grazers is of potentially equal or greater importance than bottom-up control by nutrients (Hughes et al. 2004). However, unavoidable artifacts of small-scale experiments, including short duration, simplified food webs, and lack of dispersal and recruitment, raise questions as to how well their interpretations of the effects of consumers and physicochemical conditions can be extrapolated to the scale of real seagrass beds, as in ecosystems generally (Carpenter 1996). Field survey data can help to assess the relevance of experimental findings to large-scale community dynamics, and to identify differences that may reveal other, important processes occurring at natural scales. For instance, comparison of the temporal variation in total mesograzer abundance to that of individual mesograzer species could test the "insurance hypothesis", which states that diversity helps to maintain consistent biomass or function through changing conditions (Naeem & Li 1997). Unfortunately, mobile epifauna have seldom been included in observational studies designed to relate seagrass performance to physicochemical conditions, making it difficult evaluate the contributions of consumers to seagrass ecosystem properties (Dennison et al. 1993, Stevenson et al. 1993, Duarte 1995, Kemp et al. 2005, but see Jørgensen et al. 2007). Likewise, observations of consumer abundance and distribution in seagrass beds (e.g. Marsh 1970, Nelson et al. 1982, Edgar 1990b) have rarely assessed their top-down impacts on seagrass health or their relationships with physicochemical conditions. Analyses of long-term monitoring data with both physical and biological components is one approach to more fully understanding how top-down and bottom-up processes interact to affect seagrass beds in natural settings.

The dominant forcing in temperate seagrass beds is likely to be seasonality, with many components of the community experiencing winter minima as direct demographic or indirect behavioral responses to reduced light and temperature. We believe it is important to assess the relationships of faunal abundance and diversity to physicochemical forcing (e.g. Edgar & Barrett 2002), because changes in climate and water quality could produce consumer-mediated indirect effects on seagrass as well as direct effects. If top-down control occurs then it will probably be superimposed on top of the broader, seasonal patterns, and might reveal itself in either of two ways: 1) Inverse correlations in the abundance of adjacent trophic levels across spatial and / or interannual temporal scales, or 2) Asynchronous peaks in abundance of adjacent trophic levels within years. These patterns have been hypothesized and suggested experimentally (i.e. Heck et al. 2000, 2006, Duffy et al. 2005) but rarely documented in natural seagrass beds (but see Jørgensen et al. 2007).

In the Chesapeake Bay estuary (Virginia, USA), submerged aquatic vegetation including eelgrass (*Zostera marina* L.) has been monitored extensively since the 1970s (Moore et al. 2000, Kemp et al. 2005). While this monitoring has included both seagrass areal coverage and water quality parameters to address the bottom-up relationship between physicochemical conditions and seagrass, surveys of seagrass-associated fauna and epiphytes are rare and inconsistent prior to 1998, precluding analysis of top-down dynamics. Since 1998, however, we have maintained a semi-monthly monitoring program, keeping track of lower levels of the food web, including seagrass, epiphytic algae, mesograzers and small demersal predators, in an eelgrass bed in the polyhaline region of the lower bay. Here we assemble these data, together with climate and water quality information, to assess and compare the variation in both biotic and abiotic aspects of an eelgrass community. We sought to test the following hypotheses: 1) Among years,

physicochemical indicators of poor water quality correlate negatively with eelgrass abundance. 2) Seasonal patterns of variation in adjacent trophic levels are inversely correlated, and consistent with top-down control superimposed over bottom-up control. 3) Among years, epifaunal abundance is negatively correlated with predator abundance. 4) Mesograzer species vary in a complementary fashion in response to changing physicochemical conditions, such that temporal variance in the diverse mesograzer assemblage is less than that of individual species abundances.

## METHODS

**Study Location**- Our data were collected at the Goodwin Islands National Estuarine Research Reserve, located at the mouth of the York River in Chesapeake Bay (Virginia, USA, 37° 13' N; 76° 23' W). Goodwin Islands is a 315 hectare archipelago of salt-marsh islands surrounded by intertidal flats and subtidal seagrass beds (*Zostera marina* and *Ruppia maritima*) extending to a maximum of about 1 m mean-low-water depth. The area is closed to development and destructive use, but remains open to commercial and recreational fishing. Surveys were performed in an area of seasonally dense eelgrass on the SE side of the islands.

**Data Collection Overview**- Water quality and meteorological data have been monitored semi-continuously at Goodwin Islands since October 1997 by the Chesapeake Bay National Estuarine Research Reserve Program. Water temperature, salinity, dissolved oxygen, pH, and turbidity are recorded at 15 minute intervals from a permanent monitoring station in the eelgrass bed by a YSI 6600 EDS data sonde, following standard YSI (YSI, Inc., Yellow Springs, Ohio) and NERRS System-wide Monitoring Program protocols

(http://nerrs.noaa.gov/Monitoring/Water.html). Water column nutrient and chlorophyll *a* concentration data are collected monthly in the same area by CBNERR staff. The springtime extent of the Goodwin Islands eelgrass bed is mapped annually by aerial photo surveys and incorporated into a database of seagrass coverage throughout the Chesapeake Bay region that extends back to 1984 (Moore et al. 2000). For the years from 1998 – 2006, areal coverage of eelgrass was categorized into four estimated density classes, and empirically-derived relationships between aerial photo-based density class and ground-based density surveys were used to convert areal coverage to an indexed value; "density adjusted eelgrass cover" (Marion *pers. com.*). We have collected data on eelgrass community structure and composition approximately monthly at Goodwin Islands since 1998. This monitoring program has been modified since its inception, and now includes the following data: eelgrass biomass, eelgrass cover, epiphytic algae abundance, abundance of mobile and sessile epifauna, and abundance of small, resident predators. Table 1 summarizes the years for which each type of data is available. Collection methods for the data are described in more detail below.

**Sampling Design**- Eelgrass community sampling is based around two, 50 m transects roughly parallel to the shore; one set near the inshore edge of the contiguous eelgrass bed, and one near the offshore edge. A stratified random draw is used to position five sampling spots along each transect. At each location, epifaunal samples are collected, eelgrass blades are harvested to determine epiphytic chlorophyll levels, eelgrass cover is estimated, a core is taken to determine eelgrass density (above- and below-ground biomass), and dip net sweep is performed to sample small predator density.

**Epifaunal Sample Collection and Processing**- From 1998 through the spring of 2004, epifaunal samples were collected using 12 cm diameter, 50 cm long acrylic core tubes. A 500

μm mesh bag was attached to one end of the tube, and the other end slipped over an eelgrass patch and onto the sediment. Eelgrass blades were cut off at the base, a plastic plate was slipped under the tube to seal it, and the contents (blades and epifauna) were collected into the mesh bag. Beginning in April, 2004, epifaunal samples were taken with a grab sampler based on a design by Virnstein and Howard (1987). The grab sampler collects eelgrass blades and associated fauna from a 20 x 20 cm bottom area, with the advantage that it does not sample sediment and infauna. We normalized epifaunal abundance to eelgrass above-ground biomass to facilitate comparison of samples taken with core and grab samples; a paired, one-tailed t-test comparing the density of epifauna from ten adjacent core and grab samples taken in April 2004 found no significant differences ($n = 20$, $P = 0.21$). Epifaunal samples were frozen at -20°C until sorting. During sorting, eelgrass blades were separated from roots and rhizomes and all flora and sessile epifauna were identified to the lowest taxonomic level possible, usually species. Sessile organisms including eelgrass were dried at 60°C, weighed, and combusted to determine ash-free dry mass (AFDM). Mobile epifauna were sorted by size class with a nested series of sieves (8.0, 5.6, 4.0, 2.8, 2.0, 1.4, 1.0, 0.71, and 0.50 mm screens), then identified to species and counted. Counts of individuals within each size class were multiplied by empirically derived coefficients to convert them to biomass (mg AFDM) and production (μg AFDM $day^{-1}$) (Edgar 1990). Figures and statistical analyses of mesograzer abundance all use biomass unless otherwise noted.

**Epiphyte Sampling-** A single eelgrass shoot (approximately 5 blades) was collected from each of the five sampling spots along a transect. Fouling material was later scraped from the blades and collected on Whatman™ GFF filters, and blade surface area was determined with a Li-Cor 3100 area meter (Li-Cor, Lincoln, NE). We measured chlorophyll *a* as a proxy for the biomass of photosynthesizing algae on the blades. Filters with algae were extracted in 20 ml

90% Acetone at -20° C for 24 hours. The extract was passed through a 0.45 μm hydrophilic PTFE membrane filter (Millipore Corporation) and absorbance was monitored at 480, 510, 630, 647, and 750 nm using a Shimadzu UV-1601 spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, MD). Chlorophyll *a* concentration was calculated using the trichromatic equation (Lorenzen 1967), and chlorophyll *a* mass was calculated and normalized to blade area to serve as a proxy for epiphyte density.

**Eelgrass Biomass Sampling**- At each of the five sampling spots along a transect, a core of eelgrass and rhizomes approximately 15 cm deep was collected with a 20 cm diameter tube. Cores were taken even if no above-ground eelgrass biomass was apparent. Blades and rhizomes from the core were separated, dried, and combusted to determine above and below ground biomass (AFDM) for the core.

**Eelgrass Cover and Small Predator Sampling**- At each sampling spot, a five m rope was stretched perpendicular to the transect line. A 52 cm wide dip net was swept once along the five m rope to collect small, demersal predators such shrimp and juvenile fish. Numbers of each predator species in the net were recorded, and fish and crabs were measured to the nearest millimeter. Concurrent with the predator sweep, grass presence or absence was recorded as one or zero for each one m interval along the five m rope. A zero was recorded if less than 10% of the sediment along the 1 m stretch of rope was occluded by eelgrass. Binary data were later converted to proportional cover (0, 0.2, 0.4, 0.6, 0.8, or 1.0) by adding up the ones and dividing by five. This measure was intended to capture meso-scale patchiness of the bed that was not detected by aerial surveys.

**Data Analysis**- Time series of biological data were generated from the mean and standard error of all samples for each sampling date. Typical annual cycles of temperature,

salinity and turbidity were estimated by averaging daily values for each day of the year across all years of the CBNERRVA continuous monitoring dataset. For chemical and biological data, which were recorded at less frequent than daily intervals, we used linear interpolation to estimate daily values within years, before averaging across years to generate typical annual cycles.

To quantitatively compare trends in biological and physicochemical components of the community, we calculated differences of measured responses from interannual averages for the same day of year, and then used multiple linear regressions to assess correlation among these "deviations". The multiple linear regression models used were a small subset of all possible models; we only tested for correlations that could be interpreted in light of our hypotheses about the system (Burnham & Anderson 2002). Decisions about what factors to include in models were also dictated by the temporal extent of the data (Table 1); we opted to include epiphytic chl *a* in most models, limiting the usable data to the years 2001 – 2006. We excluded eelgrass shoot biomass from most models in which it was not the response variable, because it was only monitored after 2004. However, we did include shoot biomass in some models of predator abundance, to examine the influence of habitat on predators (Orth & Heck 1980). We conducted analyses using all available data from the whole year, and separate analyses using only spring (days 70-150), and only summer (days 180-260) data, under the assumption that deviations in certain factors (i.e. temperature) might have different effects during the early versus late season. In addition to regressions using data from individual sampling dates, we evaluated models predicting spring eelgrass extent at Goodwin Islands, for which we had only one datum per year. The predictor variables for these regressions were not deviations from a mean, but rather averages of daily values from each of two periods we hypothesized to be relevant to spring eelgrass abundance; days 70-120 of the same year, when much growth occurs (Orth & Moore

1986), and days 200 – 250 of the preceding year, when much senescence occurs (Moore & Jarvis 2008). R-squared values were calculated for all models, and the relative likelihood of models was compared using Akaike's Information Criterion (AIC). We considered "good" models to be those that explained a sizeable portion of the variance in a response based on R-squared, and which were weighted favorably relative to the other models by AIC (Burnham & Anderson 2002). Only results for models in which both $R^2$ and weighted model probability ($w_i$) were > 0.1 are presented here, but full model results are presented in the supplemental tables (Tables 1-14 in the appendix).

The relationship of mesograzer species diversity to the temporal variance in total mesograzer abundance was assessed by comparing the ratio of the variance in total mesograzers to the sum of the variances in each mesograzer species; $(S^2_{\text{Total Mesograzer Abundance}})/(\Sigma\, S^2_{\text{Mesograzer Species 1-n Abundance}})$. A ratio > 1 indicates that mesograzers of different species tend to vary synchronously, whereas a ratio < 1 indicates that mesograzers vary in a compensatory manner, reducing the variance of total mesograzers (AL Downing *pers. comm.*). Variances were assessed over 4 timescales: all sample dates (n = 58), annual averages (n = 9), monthly averages across years (n = 9, Dec., Jan., Feb. excluded), and monthly averages within years (n = 58, ratio of variances calculated for each year then averaged across years). The statistical significance of variance ratios was tested with resampling, over 10000 iterations.

Statistical analyses were done using Minitab, Resampling Stats for MS Excel, and our own calculations in MS Excel.

## RESULTS

**Physicochemical conditions-** Thirty-day average water temperature at Goodwin Islands exhibited strong, unimodal, annual cycles, ranging from zero to 30 degrees C (Figs. 1, 2). Interannual variation was apparent both in minimum winter temperatures and in maximum summer temperatures, with the warmest summer occurring in 2005 (Fig. 1). Thirty-day average salinity ranged from 13 to 26 ppt with a weak seasonal cycle and high variability on an interannual scale (Figs. 1, 2). Notably, the period between 1999 and 2003 had an average salinity of around 22 ppt, while before and after that period, the salinity averaged about 18 ppt. Daily averages for turbidity ranged from near zero to over 200 NTU after storm events. This stochastic variability remained apparent in thirty day-averages, which ranged from three to 30 NTU (Fig. 1). Despite this high variation, turbidity exhibited a distinct seasonal trend, with higher values usually occurring in the late summer or fall (Fig. 2).

**Eelgrass and epiphytes-** Density-adjusted eelgrass coverage on the Southeast side of Goodwin Islands increased from 36 to 56 hectares from 1998 – 2002, then fell to 23 hectares by 2006 (Fig. 1). These data came from aerial photographs taken only in the spring of each year when eelgrass was usually dense, and did not capture within-year variation in eelgrass. However, our ground-based eelgrass biomass monitoring, which began in March 2004 and extended through October 2007, captured some of that seasonal variation (Fig 3). In 2004 and 2005, eelgrass biomass and cover peaked in the spring and early summer and declined in late summer; a cycle previously documented for Chesapeake Bay (Orth & Moore 1986). The late summer decline was severe in 2005, however, and the seasonal cycle appeared disrupted in 2006 (Moore & Jarvis 2008). Interestingly, in 2006, shoot biomass reached its highest level quite late in the year. Seasonal cycles were clear in the time series of epiphyte density (Fig. 3), but relative

to eelgrass, epiphytes peaked much later in the year (Fig 4). The 2005 eelgrass dieback was followed by a surge in epiphyte density; the sparse eelgrass remaining after the event was heavily epiphytized.

**Mobile Epifauna-** From 1998 – 2006 we collected and sorted 52511 mobile epifauna individuals from core and grab samples, representing 29 species or lowest-taxonomic level categories (Table 2). Incidentally-collected sessile and / or infaunal species, and larger fish and decapod predators, were not included in this count. The average species richness of mobile epifauna for a single sample date was 11.5 (range 3 – 17). The most abundant species in terms of both numbers and estimated biomass was the caprellid amphipod *Caprella penantis*, comprising 32% of total individuals by number and 34% by biomass (Table 2, Figs. 4, 5). Though *C. penantis* exhibits both grazing and suspension-feeding behaviors (Caine 1974, Guerra-Garcia et al. 2004), we categorized it along with 16 other epifaunal species as mesograzers, based on their reported or inferred feeding mode (Table 2). The second most abundant mesograzer in terms of biomass was the gammaridean amphipod *Gammarus mucronatus*, with 16% of the total, followed by the isopod *Erichsonella attenuata* with 14% (Table 2, Figs. 4, 5). In total, mesograzers comprised 96% of mobile epifauna, with the remainder consisting of filter feeders, micropredators, and omnivores. Numeric density of total mesograzers averaged about 205 individuals $g^{-1}$ plant dry mass (Fig 5), but ranged from near absence to more than 1000 individuals $g^{-1}$ (Fig. 6). Total mesograzer biomass and production averaged 61 mg AFDM * g plant $DM^{-1}$ and 1.4 mg AFDM * g plant $DM^{-1} * d^{-1}$, respectively. The average seasonal cycle in total mesograzer density was variable and weakly patterned (Fig. 5). Trends in numbers and biomass were qualitatively similar, with no consistent seasonal peak (Fig. 5a, b), whereas estimated secondary production, which is related to water temperature, was

greatest during the summer (Fig. 5c). The seasonal trends of individual mesograzer species differed from one another (Fig. 4). *Caprella penantis* was most abundant during the winter, *Gammarus mucronatus* in the spring, and most other mesograzers during the summer and into the fall (Figs. 4, 6). There was strong interannual variation in the species composition and abundance of mesograzers. For instance, *Gammarus mucronatus* was most abundant in spring, and in the low salinity years beginning in 2003, whereas *Elasmopus levis* was abundant only in the high-salinity years between 1999 and 2003 (Fig. 6). The large isopod *Idotea balthica* was rare before 2002, seasonally dominant between 2002 and 2005, and virtually absent from collections after the 2005 eelgrass dieoff (Fig 6).

The variance of total mesograzer biomass was significantly greater than the summed variances of individual mesograzer species when calculated over the entire record of sample dates (Ratio = 1.94, P = 0.0001). This was also the case for annual averages of mesograzer abundance (Ratio = 2.81, P = 0.0001), and for months within years averaged across years (Ratio = 1.47, P = 0.0004). However, in monthly averages across all years (akin to the seasonal cycles of abundance in Fig. 4) mesograzers tended to vary in an asynchronous manner, and the variance of total mesograzers tended to be less than the summed variances of the individual species, but not significantly so (Ratio = 0.65, P = 0.1114).

**Predators-** From 1998 – 2006, we collected 14624 small predators from dip net sweeps, representing 19 species or lowest-taxa determinations (Table 3). Grass shrimp *Palaemonetes* spp. (mostly *P. vulgaris* but also including some *P. pugio* and *P. intermedius*) were the most abundant predators collected, followed by sand shrimp (*Crangon* spp.), blue crabs (*Callinectes sapidus)* pipefish (*Syngnathus* spp., mostly *S. fuscus* with some *S. floridae*), and gobies (*Gobiesoma bosci*). Other small fishes comprised about 3% of total predator individuals.

Predator abundance varied strongly on both seasonal (Fig. 4) and interannual (Fig. 7) scales. Fish abundance exhibited the most consistent patterns, with unimodal peaks occurring in mid summer (pipefish) and late summer (other fish). The abundance of shrimp and blue crabs was more variable between and within years, often peaking in both spring and fall, but shrimp exhibited relatively consistent spring peaks after 2002. A dramatic decline is apparent after 2001 for these crustaceans (Fig. 7), but the decline in shrimp should be interpreted cautiously because very small (< 2 cm TL) shrimp were not counted after 2001, whereas blue crabs of all sizes were counted throughout the survey period.

**Regression analyses of eelgrass community control-** The spring eelgrass index at Goodwin Islands, a landscape-scale measure incorporating both area and density, was best predicted by the regression model based solely on spring turbidity, which was negatively related to the index (Table 4a). However, several other models also explained a substantial fraction of the eelgrass index and had similar weighted probability based on AIC. Spring mesograzer density and salinity were each positively related to the index, while summer temperature was negatively related (Table 4a, Appendix Table 1a). Eelgrass shoot biomass was measured only between 2004 and 2007, and was measured approximately monthly. Perhaps not surprisingly, it had largely different model relationships than did the spring eelgrass index (Table 4b, Appendix Table 1b). While deviations in shoot biomass were positively related to mesograzer density (Appendix Table 1b), their strongest association was a negative relationship with salinity, in contrast to the positive association for the landscape scale spring eelgrass index, which used only a single value (spring) per year (Table 4b). Deviations in monthly epiphytic chlorophyll density were not predicted well by the regression models in spring, but in summertime, there was a strong negative effect of turbidity on epiphytic chlorophyll (Table 5, Appendix Table 2).

Deviations in total mesograzer density were negatively related to turbidity, as well, both in the spring and through the whole year (Table 5, Appendix Table 3). Mesograzer density was also correlated with total fish abundance, but the relationship was positive, in contrast with the negative effect predicted by our top-down control hypothesis (Table 5). In summer, the best model for mesograzer density included *Palaemonetes* spp. shrimp and blue crabs as well as fish, with the crustaceans having a modest negative correlation with mesograzers in the regression (Table 5). Deviations in the density of the most abundant mesograzer, *Caprella penantis*, were not explained well by the models tested, except during the summer when higher than normal temperatures were associated with lower than normal density of this cold-weather amphipod (Table 5, Appendix Table 4). *Gammarus mucronatus*, the most abundant gammaridean amphipod, was negatively associated with salinity and water temperature when the whole year was considered, and was negatively associated with blue crabs in spring (Table 5, Appendix Table 5). As with total mesograzer density, *Erichsonella attenuata* density was positively associated with total fish abundance, suggesting bottom-up control, but was sometimes negatively associated with predatory crustaceans (Table 5, Appendix Table 6). *Idotea balthica* had a positive association with total fish and other predators in spring, although in the summer, a negative association with salinity was a stronger predictor of *I. balthica* (Table 5, Appendix Table 7). *Ampithoe longimana* abundance was predicted well by epiphyte abundance in spring (Table 5, Appendix Table 8), but on a whole-year basis, most of the variation in *A. longimana* density was unexplained by the regression models. *Elasmopus levis* abundance was positively associated with salinity overall and during summer, but in spring *E. levis* abundance was best predicted by a multi-predator model with positive relationships to fish and crabs and a negative relationship with shrimp (Table 5, Appendix Table 9). Epifaunal species richness was best

modeled by salinity in summer and by epiphytic chlorophyll in spring, while on a whole-year basis it was best predicted by a heavily-parameterized model including all physical factors, epiphytes, mesograzer density, and total fish abundance (Table 5, Appendix Table 10). The positive relationship between epifaunal species richness and salinity was the most consistent result.

Total fish abundance was positively associated with total mesograzer density in all seasons, although a negative relationship with turbidity was a stronger predictor in spring (Table 6, Appendix Table 11). Pipefish were also positively associated with total mesograzer density, although only in summer, which is the season when they were most abundant (Table 6, Appendix Table 12). Blue crabs were positively associated with salinity in spring, but otherwise poorly predicted (Table 6, Appendix Table 13). *Palaemonetes spp.* grass shrimp were strongly positively associated with eelgrass shoot biomass and negatively associated with turbidity in the post-2004 data, and were negatively related to spring turbidity in the full dataset, as well (Table 6, Appendix Table 14).

## DISCUSSION

Our data revealed a dynamic seagrass community in which strong seasonal cycles in species composition and abundance were at odds with equally-strong interannual trends. The most obvious drivers of this biological variation were temperature, salinity, and turbidity, although correlations among producers and consumers also suggested that bottom-up trophic relationships affected community structure. Evidence for top-down control was inconclusive;

some mesograzers were negatively related to some predators, but relationships among adjacent trophic levels were mostly weak or positive. These results differ strongly from the results of experimental manipulations in mesocosms (Duffy et al. 2005) and field cages (Heck et al. 2006, Douglass et al. 2007), which have found strong top-down effects of grazing and predation on seagrass community structure, including negative correlations of abundance at adjacent trophic levels.

The spatial confinement, low consumer diversity, and whole-trophic-level presence / absence treatments in experiments may exaggerate the apparent influence of top-down effects in those artificial settings relative to what can be observed in the field (Christie & Kraufvelin 2004, France & Duffy 2006). Also, whereas the entire consumer community is known in an experiment, our field sampling methods only quantified the abundance of those organisms that were effectively captured in our sampling gear. Larger and / or faster-swimming fishes than those collected in our dip-net sweeps could potentially exert strong top-down controls on mesograzers, and it would be useful to quantify those predators with additional surveys. Finally, the absence of negative correlation between predators and grazers at the scale of our sampling does not necessarily imply the absence of top-down control, as it is quite conceivable that mobile predators congregate where mesograzers are abundant and quickly disperse to other habitat patches after depleting their prey. The abundance of mesograzers in the gut contents of demersal fishes in Chesapeake Bay eelgrass beds at least demonstrates the potential of predation to influence mesograzer populations (Teixeira & Musick 1994, Douglass et al. *in prep*), and grazer consumption of epiphytes is well known from studies both in the lab and the field (Jernakoff et al. 1996). Therefore, despite the fact that we did not detect strong top-down effects in this first

analysis of the field data, it would be premature for ecosystems managers to dismiss the potential of food web changes to affect eelgrass growth and survival.

Chesapeake Bay is one of the most variable aquatic environments in the world in terms of physicochemical conditions, so it comes as no surprise that the biotic components of the Goodwin Islands eelgrass bed exhibited such high variation on annual and interannual scales. Eelgrass growth, biomass, and density, which respond to changing temperature and light (Orth & Moore 1986, Olesen & Sand-Jensen 1994), are clearly primary drivers of many of the annual patterns in eelgrass-associated species, which have been shown to respond strongly to habitat availability (Orth & Heck 1980). The seasonal cycles of abundance and density of eelgrass that we observed between 2004 and 2007 are similar to patterns described previously for the lower Chesapeake Bay (Orth & Moore 1986, Moore & Jarvis 2008); sparse eelgrass in the winter, increasing in density through the late spring, and senescing through midsummer into the fall (Figs. 3a,b, 4j). The annual cycle of epiphyte density on eelgrass was nearly the exact inverse of the pattern of eelgrass density (Figs. 3c, 4i). Epiphytes increased throughout the summer and into the fall and winter, but were scarce during the spring, a pattern similar to that seen in a previous study of eelgrass and epiphyte growth in the York River (Moore et al. 1996). A simple explanation for the inverse pattern of eelgrass and epiphytes is that epiphyte accumulation is reduced when eelgrass blades are growing and being replaced rapidly in the spring (Borum 1987), but that epiphyte growth continues to increase during the summer as the temperature exceeds the optimum for eelgrass growth. It could also be that eelgrass growth is high in spring because epiphytes are scarce at that time for reasons other than substrate turnover, such as the absence of top-down control by predatory fish, many of which do not migrate or recruit into Chesapeake Bay until later in the season (Fig 4a,b, Lazzari & Able 1990). Our data did not seem

to support this top-down hypothesis, however, because total mesograzer abundance was usually lower in the spring than in the summer and fall (Figs. 4e, 5), and correlation between deviations in mesograzer and epiphyte density tended to be minor and positive (Appendix Tables 3-9). Thus, within the range of grazer densities found in this survey, variation in epiphyte density appears to be controlled primarily by bottom-up factors, including light (turbidity) and stability of the substratum (eelgrass leaf growth rate).

The occurrence of high densities of mesograzers despite abundant predators in the late summer may be attributable in part to the often low clarity of water at that time of year (Fig. 2c,f), which could reduce predation rates. Another explanation for the simultaneous occurrence of high mesograzer density and high predator abundance is increased cryptic or defensive behavior by mesograzers in the presence of predators. The high epiphyte density observed in late summer and fall might result as mesograzers increase predator avoidance and reduce epiphyte grazing in a trait-mediated indirect interaction (TMII, sensu Werner & Peacor 2003). Such a TMII was evident in an eelgrass mesocosm experiment in which the presence of predatory blue crabs strongly, indirectly enhanced algae biomass without strongly depressing the numerical abundance of mesograzers (Duffy et al 2005). A simpler, but not mutually exclusive, explanation of mesograzer abundance in spite of summer predators is that high secondary production overwhelms losses to predation. Water temperatures at Goodwin Islands often exceed 25 degrees C for three months or more (Fig. 2), during which time mesograzers can grow and reproduce very rapidly (Fredette et al. 1990, Duffy et al. 2003, 2005).

Pipefish and small fish abundance varied from year to year, but there was no clear trend in the variation across multiple years. With blue crabs and shrimp, however, abundance was distinctly higher before 2001 than after (Fig. 7). The decline in shrimp is probably an artifact of

a change in survey methods, because very small (< 2 cm) shrimp were not counted after 2001. The decline in blue crabs is more likely to reflect real trends in abundance, though, because all sizes of crabs were counted throughout the survey period. The blue crab decline may be related more to variation in recruitment at the landscape scale than to processes occurring within the eelgrass bed, since the bay-wide spawning stock of adult female blue crabs reached an historic low in 2000 (Lipcius & Stockhausen 2002) and has remained at low levels. This explanation is also consistent with the steady decline in cover of eelgrass, a nursery habitat for blue crabs, after 2002 (Fig. 1d).

Of all taxa studied, mesograzers showed some of the greatest interannual variability, presumably because their populations are not buffered by pelagic larval recruitment from outside the study area. Two of the most abundant species, *Gammarus mucronatus* and *Elasmopus levis*, had nearly inverse patterns of abundance among years (Fig. 6) and opposite correlations with salinity (Table 5, Appendix Tables 5, 9). *G. mucronatus* appears to be an opportunistic species that can capitalize on low salinity and cooler spring waters, while *E. levis* apparently requires high salinity to flourish. Mesocosm experiments (Blake unpub. data) also suggest that *E. levis* are intolerant of freshwater shock relative to *G. mucronatus* and *Erichsonella attenuata*. The positive correlation of epifaunal species richness and salinity suggests that some of the rarer epifaunal species that we observed could have a similar dependence on high salinity and recruit from more marine waters during high salinity conditions (Table 5, Appendix Table 10). Negative responses of mesograzers to freshwater disturbance on landscape scales have been documented previously in this system. Prior to intense freshwater flooding associated with Hurricane Agnes in 1972, the isopod *Paracerceis caudata* was by far the most abundant crustacean mesograzer in an eelgrass bed of the York River estuary (Marsh 1973) and

presumably throughout Chesapeake Bay (Anderson et al. 1973). While the species is still common in the salty coastal bays of Virginia's Eastern Shore, it has apparently never returned to abundance within Chesapeake Bay; we have collected only 18 individuals of *P. caudata* in 9 years of sampling. In contrast, other species that were relatively rare in earlier documented collections were abundant in ours, for instance, *Idotea balthica* was nearly absent from a survey done at Goodwin Islands in the late 1990s (Parker et al 2001), but was a prominent component of the community in our samples, especially between 1999 and 2005. However, *I. balthica* have been virtually absent from our collections since the 2005 eelgrass dieback, further evidence that a large disturbance can have lasting changes on the epifaunal community even after the eelgrass itself recovers. The shrinking distribution and increasing patchiness of eelgrass beds in Chesapeake Bay may alter population dynamics within mesograzer communities, increasing their interannual variability and increasing the chance of species extirpation (France & Duffy 2006).

Whether of the documented changes in mesograzer species diversity will influence the health and productivity of eelgrass beds is contingent upon the relationship between mesograzer diversity and the ecological functions of mesograzers, i.e. epiphyte grazing and secondary production. Does the presence of more species of mesograzers equate with higher or more consistent levels of grazing or production in the field? There are two potential lines of evidence in support of this: 1) Mesocosm experiments have established that there is considerable variation in population growth rates and in the strength and selectivity of algal grazing among the mesograzer species of our system. These differences lead to higher grazer biomass and lower algae biomass in experimental treatments with diverse mesograzers, relative to the average of single species mesograzer treatments (Duffy & Harvilicz 2001, Duffy et al. 2005). 2) This study

and field studies in other vegetated benthic systems (i.e. Edgar 1990b) demonstrate that seasonal and interannual patterns in abundance differ among mesograzer species at the same location. In theory, this could lead to complementary patterns of grazing and production when some species are at low and others are at high abundance. However, our quantitative tests of the variance in total mesograzer abundance versus individual species abundance provide little evidence for such compensation. In fact, at most timescales the temporal variance in total mesograzer biomass was significantly higher, not lower, than the sum of the temporal variances in individual species biomass, indicating positive covariance among most of the species in the community. Only the average annual patterns across all years of sampling provided some indication of complementarity in the seasonal timing of abundance of different species (Fig. 4e,f,g,h). It appears that the asynchronous mesograzer seasonal cycles are often overwhelmed by non-seasonal abiotic forcings that generate synchronous change across many species, highlighting the importance of temporal scale and environmental variability in judging how diversity relates to stability. For example, most of the abundant mesograzers including *Caprella penantis*, *Gammarus mucronatus*, *Erichsonella attenuata*, *Idotea balthica*, and *Elasmopus levis*, which all have different seasonal cycles, had similar, negative correlations with turbidity, presumably reflecting their universal dependence on algal production, which requires light.

In our comparison of explanatory models of variation in biological components of the Goodwin Islands eelgrass community, there was a surprising bias towards simple models based on a single, abiotic or bottom-up factor (Tables 5, 6). This is likely due, at least in part, to the small size of the datasets used in the models. Both the AIC and the adjusted-$R^2$ calculation introduce a penalty for larger numbers of explanatory variables, and this penalty is more severe for models with a small sample size. Thus, the best single factor models are likely to be favored

in a small dataset, even if more complex models including biological interactions explain more of the variation in the data. Multicollinearity among predictive factors could also bias against multivariate models, although this seems unlikely in our data set because correlations among temperature, salinity, and turbidity were all below 0.12. Continuation of the Goodwin Islands monitoring program will allow more complex models to be evaluated fairly in the future as a larger dataset is accumulated. In particular, it will be useful to include eelgrass density as a predictor in the models in order to evaluate the extensive theoretical and experimental work on the relationship of vegetation density to trophic interactions in seagrass (Heck & Orth 2006). Another benefit of an extended time series is the ability to capture community responses to unusual natural and anthropogenic events that strongly impact seagrass communities.

Major eelgrass dieback events in Chesapeake Bay have been attributed to a variety of factors, such as the *Labyrinthula sp.* slime mold wasting disease in the 1930s (Muehlstein et al 1988), turbidity and freshwater shock after Hurricane Agnes (Anderson et al 1973), physical disturbance and burial in Hurricane Isabel (*pers. obs.*), and abnormally high water temperatures in 2005 (Moore & Jarvis 2008). All these types of disturbance have the potential not only to affect eelgrass directly but also to affect eelgrass indirectly by altering the epifaunal community. For instance, freshwater inputs from a storm may simultaneously increase nutrients and sediments, and decrease the abundance and diversity of mesograzers, reducing their capacity to control epiphytes. Thus, a single disturbance may generate both top-down and bottom-up effects, which act synergistically to compound the damage experienced by seagrass. An awareness of the synergy between top-down and bottom-up aspects of seagrass ecology will enhance the ability to diagnose and address the seagrass declines so apparent in observational data from around the world (Orth et al. 2006).

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## FIGURE LEGENDS

Figure 1. Time series of 30-d average water temperature (a.), salinity (b.), and turbidity (c.) at the Goodwin Islands eelgrass study site from January 1998 – Jan 2007. d. Time series of aerial-photo-based springtime eelgrass bed area on the southeast side of Goodwin Islands, corrected for vegetation density (see text).

Figure 2. Typical annual cycles of water column conditions in the Goodwin Islands eelgrass bed, calculated as averages for each day of the year over 8 years from 1998 through 2006. Solid lines are mean values. Dotted lines are mean +/- 1 SD.

Figure 3. a. Mean $\pm$ SEM eelgrass shoot dry mass per core (314 $cm^2$) from monthly samples taken at the Goodwin Islands eelgrass bed. b. Mean $\pm$ SEM proportional cover. c. Mean $\pm$ SEM epiphyte density ($\mu g$ chl *a* * $cm^{-2}$) from eelgrass shoots collected at Goodwin Islands.

Figure 4. Typical annual cycles of major primary producers, mesograzers, and predators at the Goodwin Islands eelgrass bed, calculated as averages for each day of the year. Predator averages are based on sampling from 1998-2007; mesograzer averages are from 1998-2006, epiphyte averages are from 2001-2007, eelgrass averages are from 2004-2007. Dotted lines are mean +/- 1 SD. Total mesograzers includes 17 species (see Table 1). a. *Syngnathus* spp. pipefish. b. All other fish. c. *Palaemonetes sp.* shrimp. d. Blue crabs, *Callinectes sapidus*. e. Total mesograzer biomass. f. *Caprella penantis* biomass. g. *Gammarus mucronatus* biomass. h. *Erichsonella attenuata* biomass. i. Epiphytic algae density. j. Eelgrass, *Zostera marina* shoot biomass.

Figure 5. Mesograzer abundance per g eelgrass shoot dry mass, versus day of year. Solid black lines are averages for the day of year, and dotted black lines are mean +/- 1 SD. Grey dots are actual values for particular sample dates. a. Raw counts of individuals. b. Mesograzer biomass estimated from size fractionated counts (Edgar 1990). c. Mesograzer daily secondary production estimated from size fractionated counts and water temperature (Edgar 1990).

Figure 6. Mean $\pm$ SEM biomass (mg AFDM * g plant $DM^{-1}$) for the most abundant mesograzer species at Goodwin Islands.

Figure 7. Mean $\pm$ SEM abundance of small predators from 2.65 $m^2$ dipnet sweeps. a. Grass shrimp (*Palaemonetes spp.*) and sand shrimp (*Crangon septemspinosa*) abundance. b. Blue crab (*Callinectes sapidus*) abundance. c. Pipefish (*Syngnathus sp.*) abundance. d. Abundance of all other fish. Asterisks indicate a long gap in the data set between 26 June and 24 October 2000.

Figure 1.

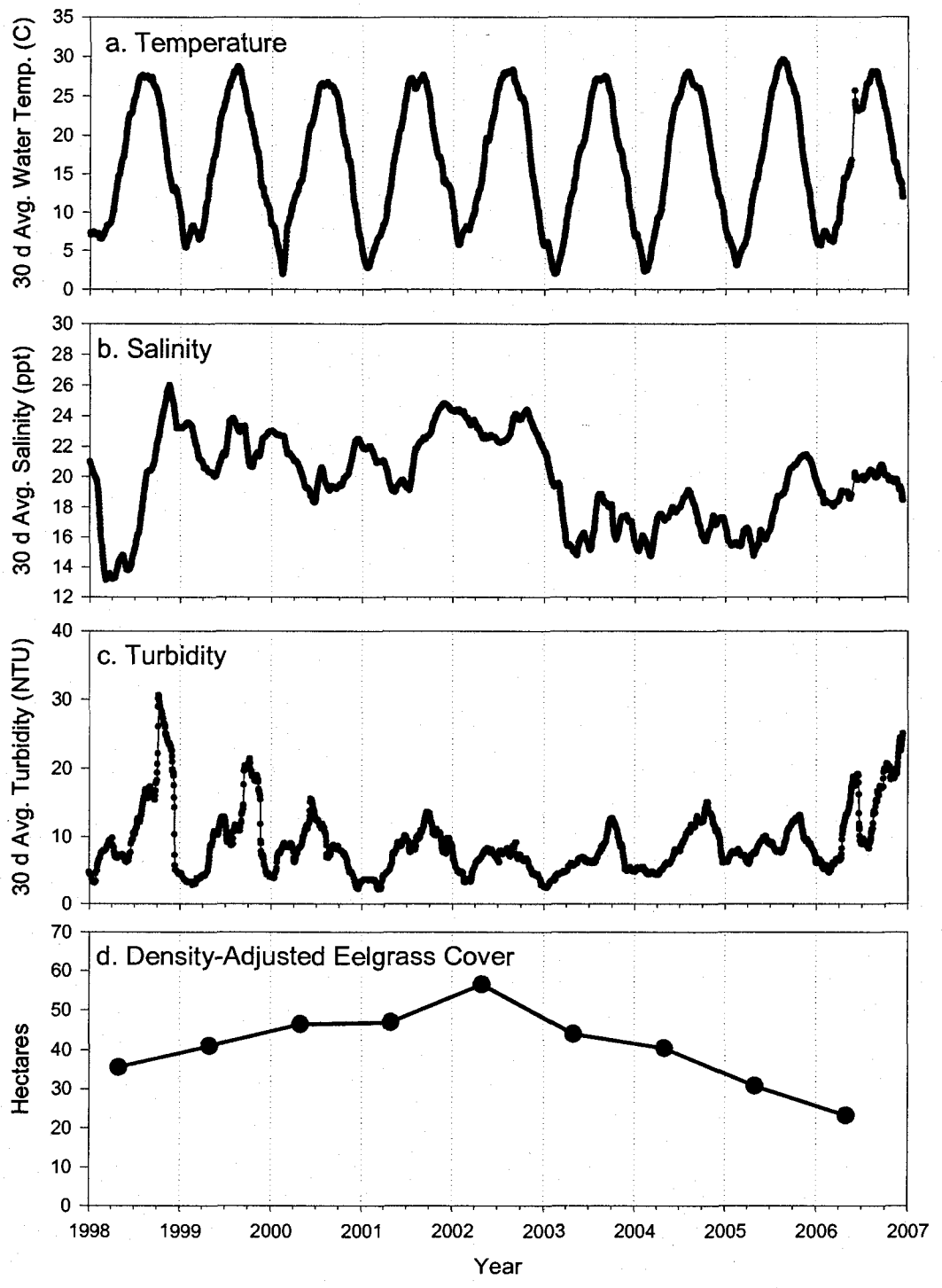
a. Temperature
30 d Avg. Water Temp. (C)
b. Salinity
30 d Avg. Salinity (ppt)
c. Turbidity
30 d Avg. Turbidity (NTU)
d. Density-Adjusted Eelgrass Cover
Hectares
Year
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007

Figure 2.

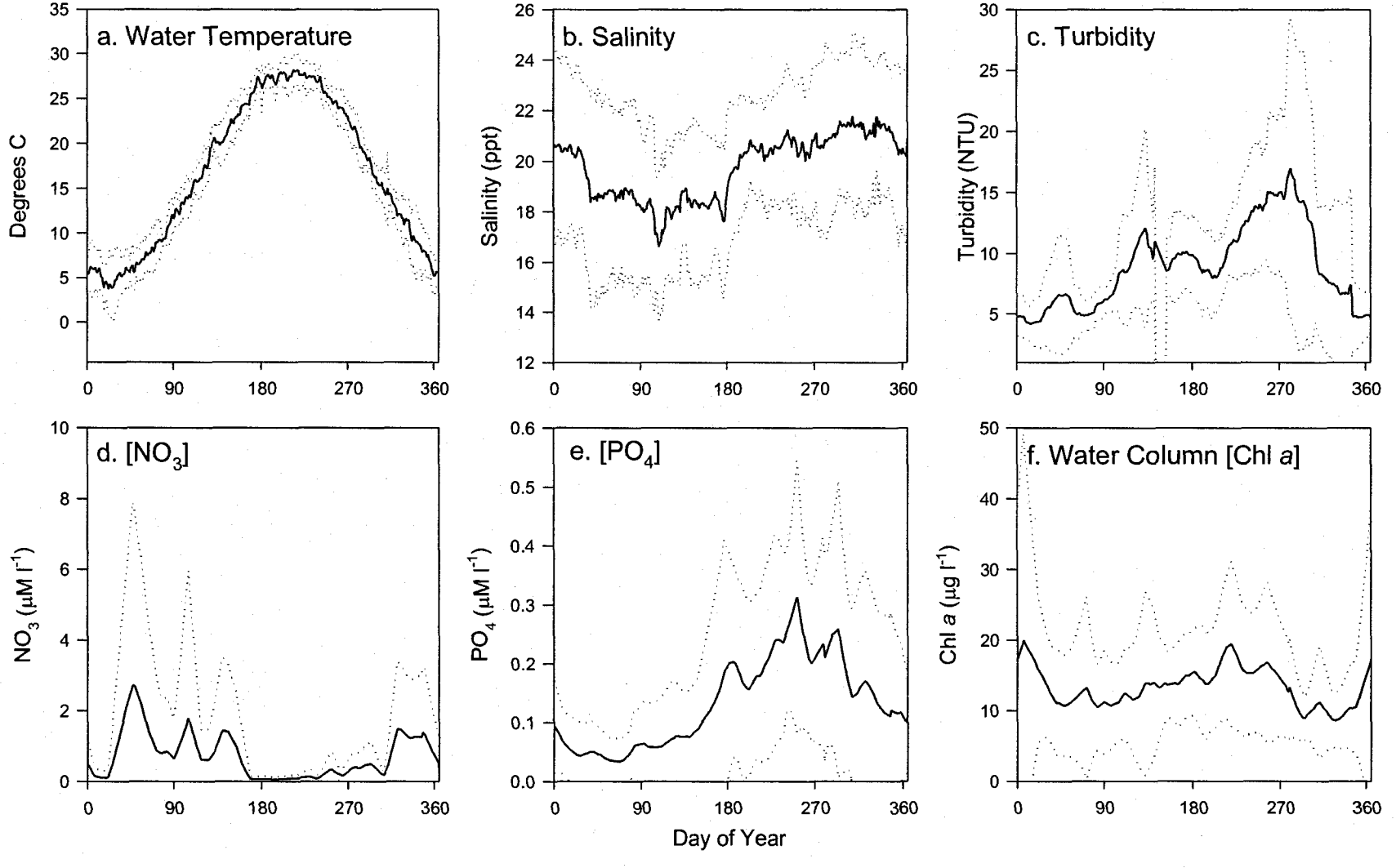
a. Water Temperature
Degrees C
b. Salinity
Salinity (ppt)
c. Turbidity
Turbidity (NTU)
d. [NO3]
NO3 (µM l-1)
e. [PO4]
PO4 (µM l-1)
f. Water Column [Chl a]
Chl a (µg l-1)
Day of Year

Figure 3.

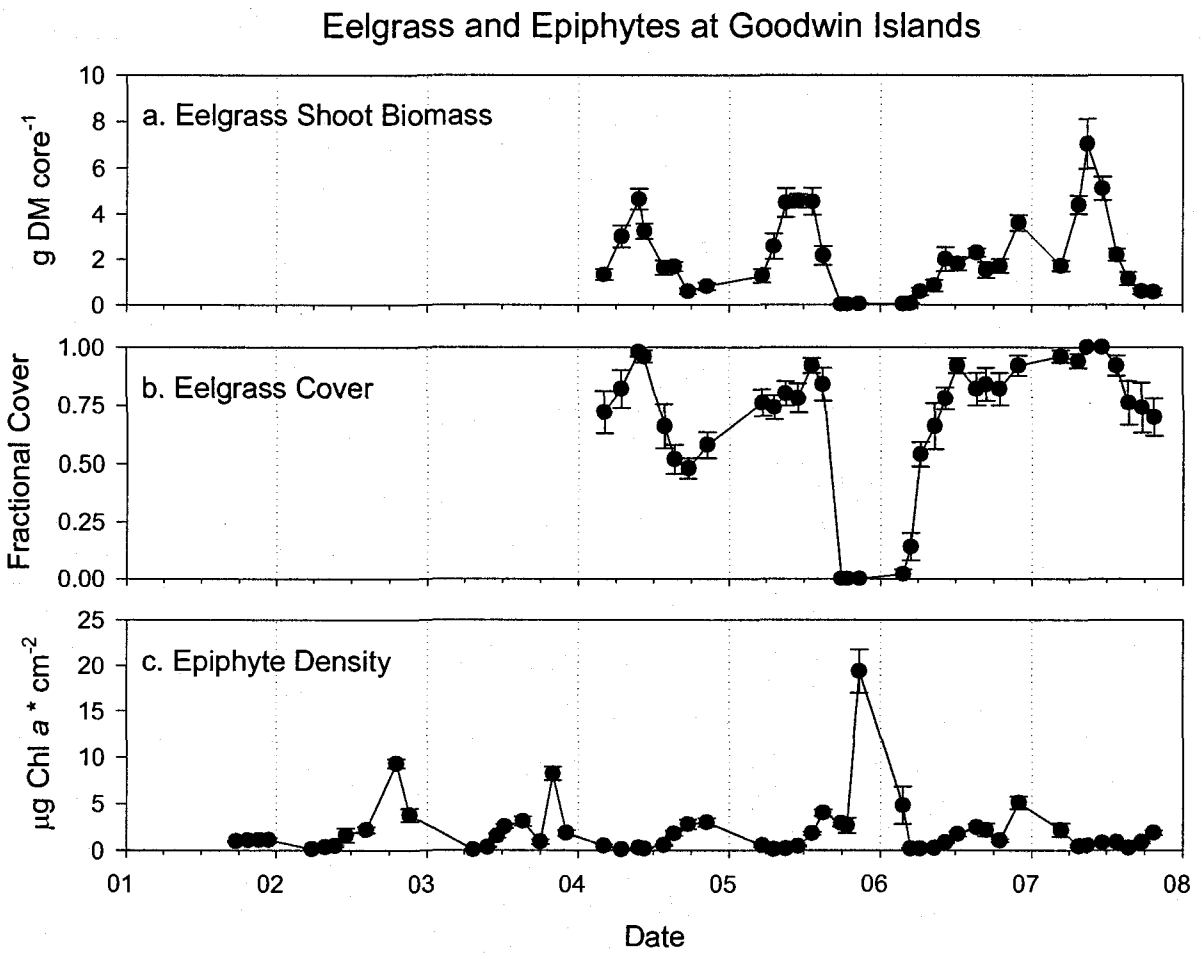
Eelgrass and Epiphytes at Goodwin Islands
a. Eelgrass Shoot Biomass
g DM core-1
b. Eelgrass Cover
Fractional Cover
c. Epiphyte Density
µg Chl a * cm-2
Date
01
02
03
04
05
06
07
08

Figure 4.

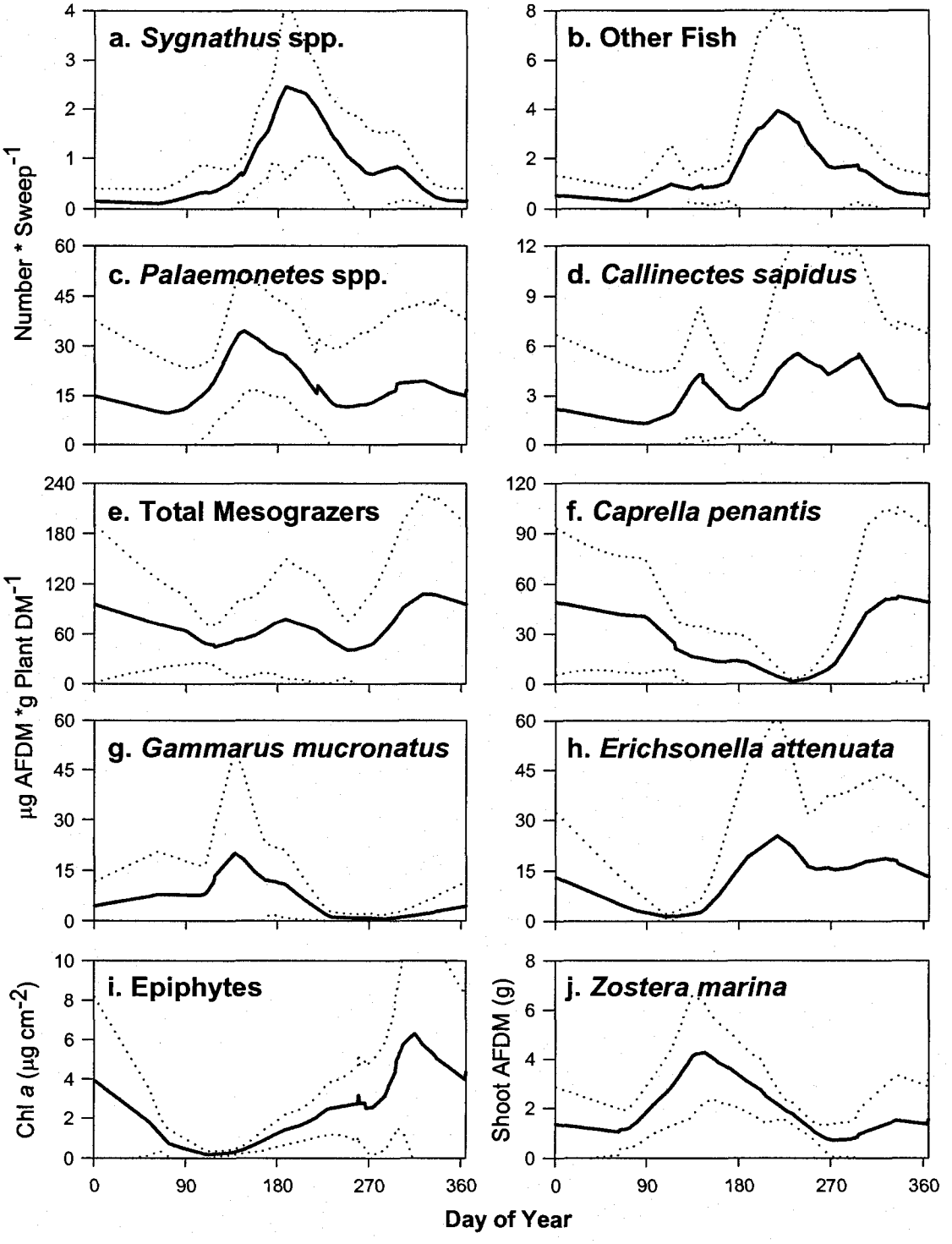
a. Sygnathus spp.
b. Other Fish
c. Palaemonetes spp.
d. Callinectes sapidus
e. Total Mesograzers
f. Caprella penantis
g. Gammarus mucronatus
h. Erichsonella attenuata
i. Epiphytes
j. Zostera marina
Number * Sweep-1
µg AFDM *g Plant DM-1
Chl a (µg cm-2)
Shoot AFDM (g)
Day of Year

Figure 5.

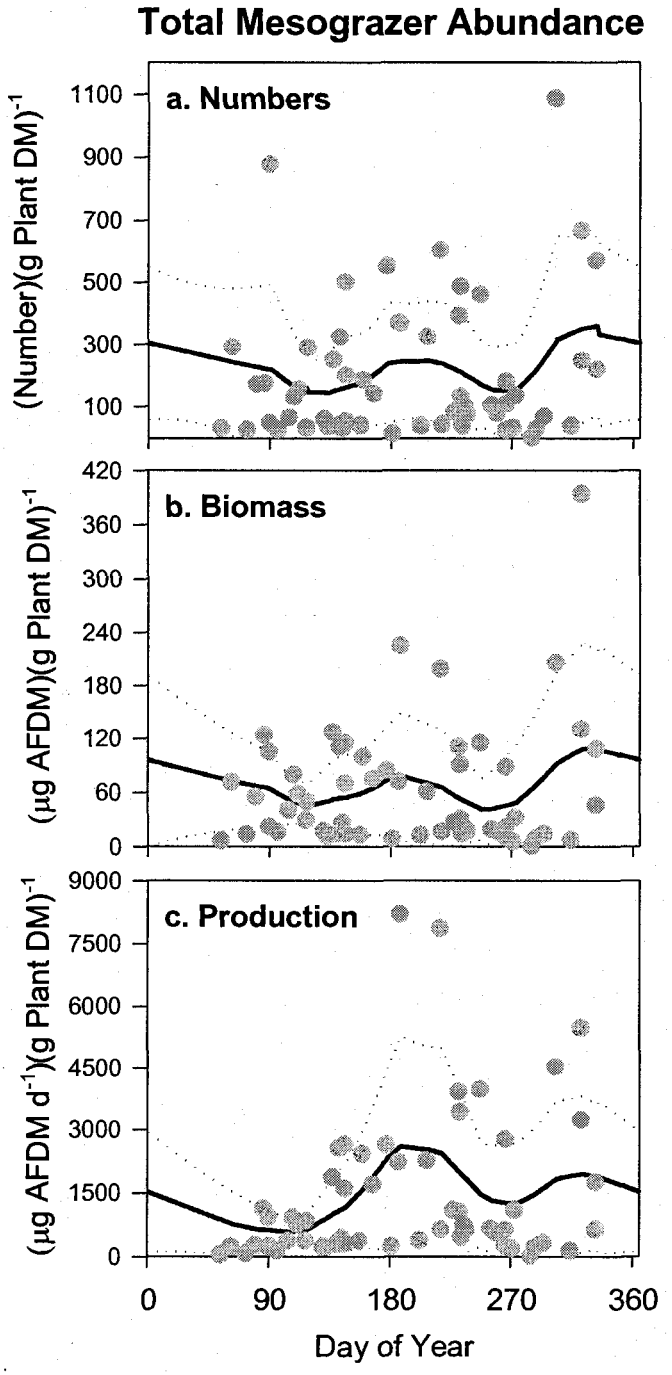
Total Mesograzer Abundance
a. Numbers
b. Biomass
c. Production
(Number)(g Plant DM)$^{-1}$
(µg AFDM)(g Plant DM)$^{-1}$
(µg AFDM d$^{-1}$)(g Plant DM)$^{-1}$
1100
900
700
500
300
100
420
360
300
240
180
120
60
0
9000
7500
6000
4500
3000
1500
0
0
90
180
270
360
Day of Year

Figure 6.

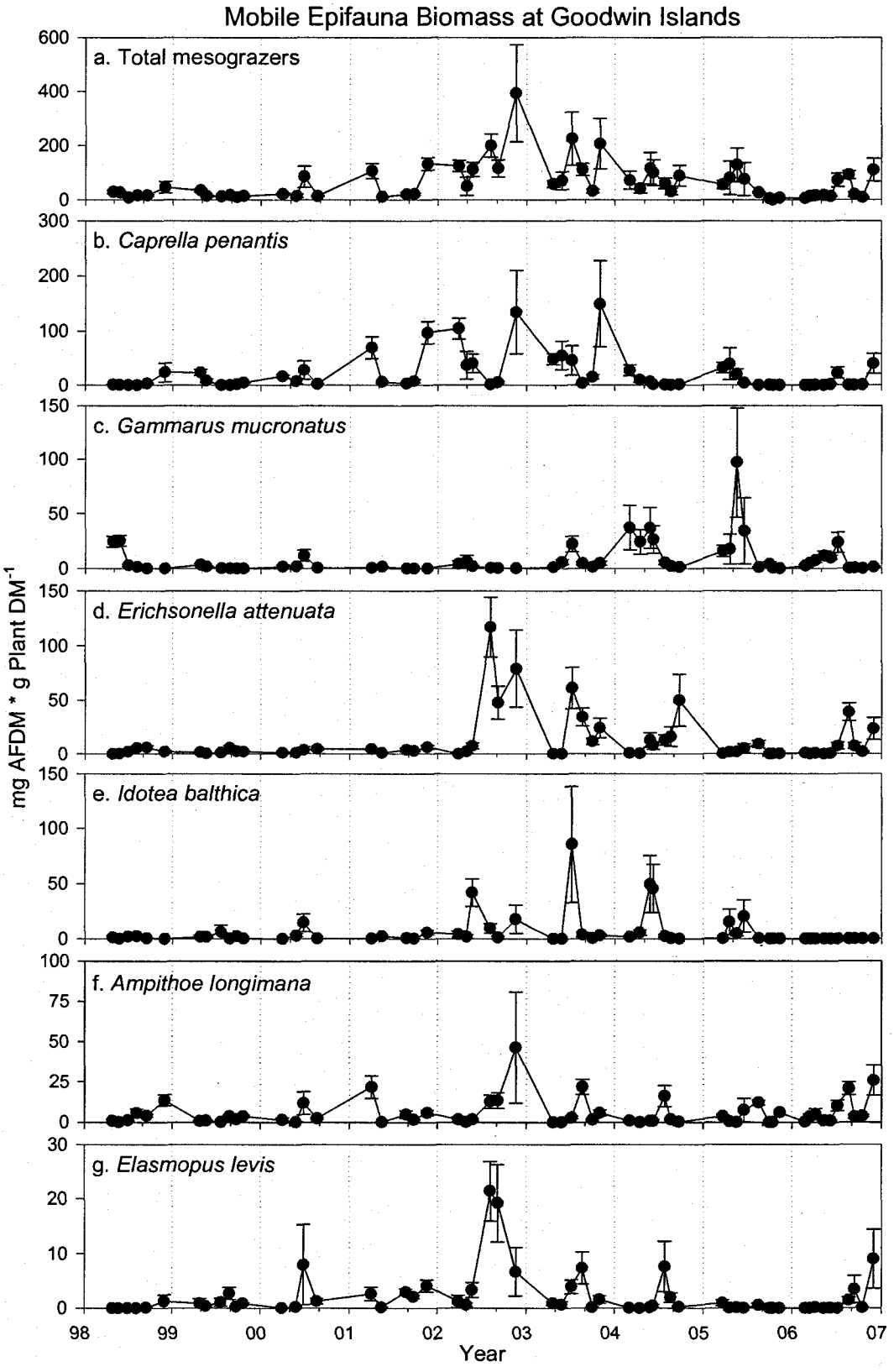
Mobile Epifauna Biomass at Goodwin Islands
a. Total mesograzers
b. Caprella penantis
c. Gammarus mucronatus
d. Erichsonella attenuata
e. Idotea balthica
f. Ampithoe longimana
g. Elasmopus levis
mg AFDM * g Plant DM-1
Year
98
99
00
01
02
03
04
05
06
07

Figure 7.

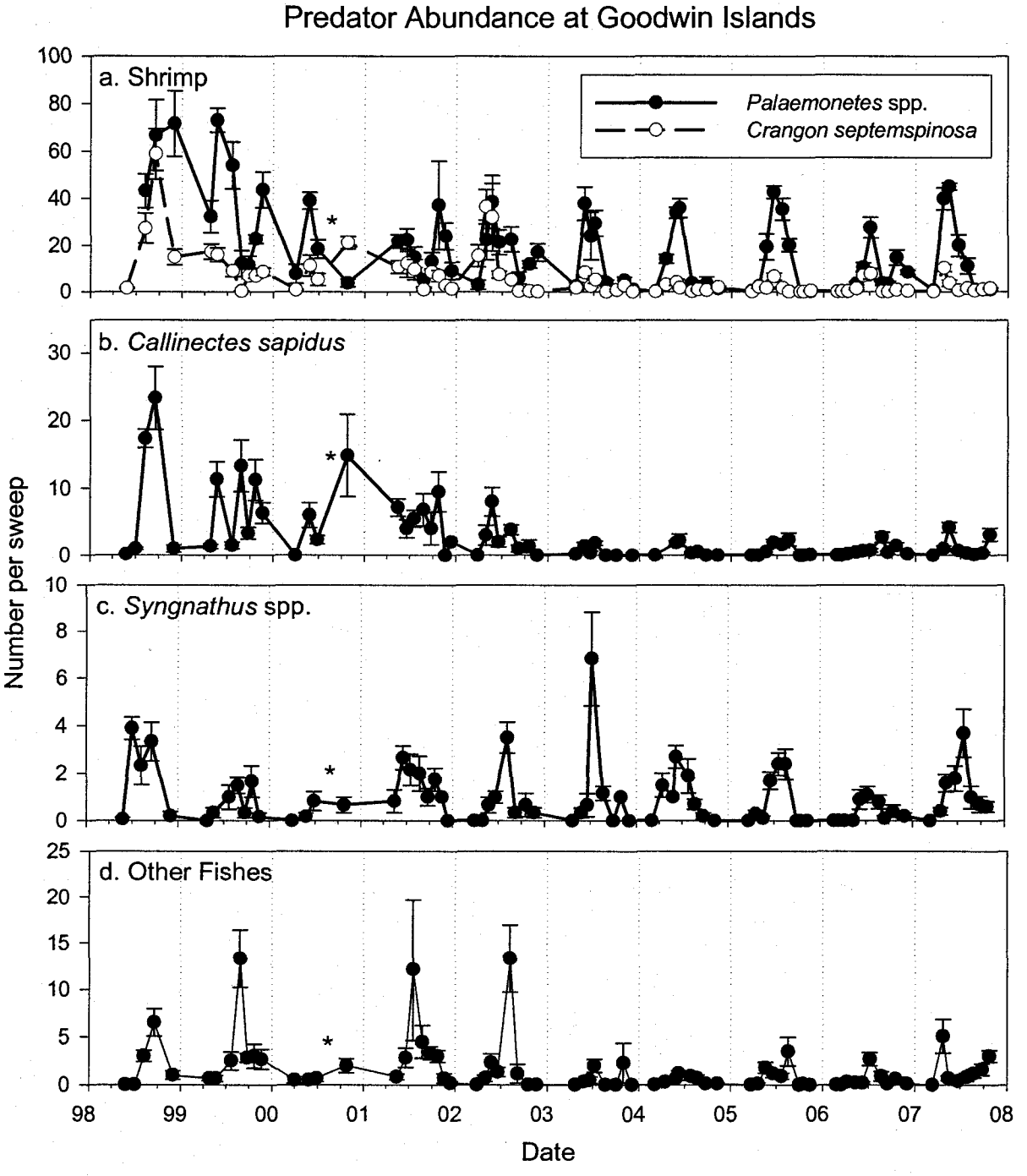
Predator Abundance at Goodwin Islands
a. Shrimp
Palaemonetes spp.
Crangon septemspinosa
b. Callinectes sapidus
c. Syngnathus spp.
d. Other Fishes
Number per sweep
Date
98
99
00
01
02
03
04
05
06
07
08

Table 1. Temporal extent of the types of data collected at Goodwin Islands and presented in this study. An "x" indicates the availability of a given data type in a given year. The frequency of measurements within years for each data type is described in the "Data Collection Overview" section of the methods.

| | Data Availability | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Year | Water Temp | Salinity | Turbidity | $NO_3$ | $PO_4$ | Water column Chl *a* | Aerial eelgrass cover estimate | Eelgrass shoot biomass | Eelgrass percent cover | Epiphytic Chl *a* | Epifauna | Predators |
| 1998 | x | x | x | x | x | x | x | | | | x | x |
| 1999 | x | x | x | x | x | x | x | | | | x | x |
| 2000 | x | x | x | x | x | x | x | | | | x | x |
| 2001 | x | x | x | x | x | x | x | | | x | x | x |
| 2002 | x | x | x | x | x | x | x | | | x | x | x |
| 2003 | x | x | x | x | x | x | x | | | x | x | x |
| 2004 | x | x | x | x | x | x | x | x | x | x | x | x |
| 2005 | x | x | x | x | x | x | x | x | x | x | x | x |
| 2006 | x | x | x | | | | x | x | x | x | x | x |
| 2007 | | | | | | | | x | x | x | | x |

Table 2. Total biomass and proportional abundance of mobile epifaunal taxa collected from eelgrass core and grab samples in the Goodwin Islands eelgrass bed from 1998 - 2006. Incidentally collected infaunal taxa are not included, nor are larger predatory epifauna that were sampled with dip net sweeps, however the latter are presented in table 2.

| Taxon | Category | Feeding mode | References | Biomass (mg AFDM) | Percent of Total |
|---|---|---|---|---|---|
| *Caprella penantis* | Caprellid amphipod | Mesograzer / Filter | Caine 1974, Guerra-Garcia et al. 2004 | 5613.104 | 34.16% |
| *Gammarus mucronatus* | Gammaridean amphipod | Mesograzer | Zimmerman et al. (1979), Neckles et al. (1993, 1994) | 2644.487 | 16.09% |
| *Erichsonella attenuata* | Isopod | Mesograzer | Marsh (1973), van Montfrans et al. (1984), Howard & Short (1986) | 2271.941 | 13.82% |
| *Idotea balthica* | Isopod | Mesograzer / Omnivore | Marsh (1973), van Montfrans et al. (1984), Borum (1987), Hauxwell et al. (1998) | 1579.492 | 9.61% |
| *Ampithoe longimana* | Gammaridean amphipod | Mesograzer | Nagle (1968), Bousfield (1973), Duffy & Hay (2000), Nelson (1979) | 1525.098 | 9.28% |
| *Elasmopus levis* | Gammaridean amphipod | Mesograzer | Nelson (1979), Duffy & Hay (2000) | 584.092 | 3.55% |
| *Edotea triloba* | Isopod | Mesograzer / Scavenger | Orth (1973) | 487.471 | 2.97% |
| *Cymadusa compta* | Gammaridean amphipod | Mesograzer | Zimmerman et al. (1979), Nagle (1968), Hauxwell et al. (1998) | 482.55 | 2.94% |
| *Bittium varium* | Snail | Mesograzer | Marsh (1973, 1976), van Montfrans et al. (1982), Kitting (1984) | 244.082 | 1.49% |
| *Ampithoe valida* | Gammaridean amphipod | Mesograzer | Douglass et al. unpub. data | 191.724 | 1.17% |
| *Paracaprella tenuis* | Caprellid amphipod | Filter | Caine (1974) | 153.176 | 0.93% |
| *Nassarius vibex* | Snail | Omnivore | Hurst (1965) | 153.08 | 0.93% |
| *Microprotopus raneyi* | Gammaridean amphipod | Mesograzer | Douglass et al. unpub. data | 139.134 | 0.85% |
| *Hippolyte pleuracanthus* | Decapod shrimp | Mesograzer | Howard & Short 1986 | 135.495 | 0.82% |
| *Caprella equilibria* | Caprellid amphipod | Filter | Guerra-Garcia et al. 2004 (filter) | 72.581 | 0.44% |
| Nudibranchs | Sea slug | Various | | 39.466 | 0.24% |
| *Dulichiella appendiculata* | Gammaridean amphipod | Mesograzer | Duffy & Hay (2000) | 26.104 | 0.16% |
| *Odostomia bisuturalis* | Snail | Predator | Allen (1958), Marsh (1973, 1976), Robertson & Mau-Lastovicka (1979) | 23.933 | 0.15% |
| *Nassarius obsoletus* | Snail | Omnivore | Scheltema (1964) | 23.6 | 0.14% |
| *Eupleura caudata* | Snail | Predator | Manzi (1970) | 11.8 | 0.07% |
| *Erichthonius brasiliensis* | Gammaridean amphipod | Mesograzer | Duffy (1990) | 8.382 | 0.05% |
| *Mitrella lunata* | Snail | Predator | Osman et al. (1992) | 6.608 | 0.04% |
| *Haminoea solitaria* | Snail | ? | | 5.706 | 0.03% |
| *Epitonium rupicolum* | Snail | ? | | 5 | 0.03% |
| *Triphora nigrocincta* | Snail | ? | | 2.438 | 0.01% |
| *Paracerceis caudata* | Isopod | Mesograzer | Marsh (1973), Duffy & Hay (2000) | 1.856 | 0.01% |
| *Melita nitida* | Gammaridean amphipod | Mesograzer | Zimmerman et al. (1979) | 1.482 | 0.01% |
| *Odostomia impressa* | Snail | Predator | Allen (1958), Marsh (1973, 1976), Robertson & Mau-Lastovicka (1979) | 0.166 | 0.00% |
| *Hydrobia sp.* | Snail | Mesograzer | Hootsmans & Vermaat (1985), Borum (1987) | 0.028 | 0.00% |

Table 3. Total numbers and proportional abundance of small predators collected in dipnet sweeps at the Goodwin Islands eelgrass bed from 1998 – 2006.

| Taxon | Common Name | Total Number | Percent of total | Length (mm) Mean ± SD |
|---|---|---|---|---|
| *Palaemonetes* spp. | Grass shrimp | 9363 | 63.16% | |
| *Crangon septemspinosa* | Sand Shrimp | 2904 | 19.59% | |
| *Callinectes sapidus* | Blue Crab | 1191 | 8.03% | 22 ± 19 |
| *Sygnathus* spp. | Pipefish | 558 | 3.76% | 101 ± 39 |
| *Gobiosoma bosci* | Goby | 378 | 2.55% | 25 ± 8 |
| Unidentified juvenile fishes | Fish | 105 | 0.71% | 23 ± 9 |
| *Fundulus* spp. | Killifish | 88 | 0.59% | 27 ± 9 |
| *Gobiesox strumosus* | Skilletfish | 54 | 0.36% | 28 ± 16 |
| Gasterosteidae | Stickleback | 49 | 0.33% | 33 ± 12 |
| *Anguilla rostrata* | American Eel | 29 | 0.20% | 55 ± 14 |
| *Symphurus plagiusa* | Tonguefish | 25 | 0.17% | 32 ± 11 |
| *Micropogonias undulatus* | Croaker | 21 | 0.14% | 30 ± 16 |
| *Menida menida* | Atlantic silversides | 19 | 0.13% | 44 ± 22 |
| Pleuronectiformes | Flounder | 13 | 0.09% | 48 ± 29 |
| *Bairdiella chrysura* | Silver Perch | 9 | 0.06% | 39 ± 20 |
| Blennidae | Blenny | 8 | 0.05% | 21 ± 4 |
| *Anchoa mitchilli* | Bay Anchovy | 5 | 0.03% | 31 ± 7 |
| *Pomatomus saltatrix* | Bluefish | 4 | 0.03% | 30 ± 9 |
| *Leiostomus xanthurus* | Spot | 1 | 0.01% | 40 ± n/a |

Table 4. Comparison of selected linear regression models of eelgrass abundance. Predictor variables in models are indicated by their T values (regression coefficient / std. error of coefficient) in columns 2-7. Blanks cells indicate that the predictor variable denoted by that column was not included in the model presented in that row. Model fit statistics indicate the explanatory power and parsimony of a model. The model with the lowest Corrected AIC value is considered the "best" model of those evaluated, and $w_i$ gives the proportional weight or confidence that model is given among the set of models. $R^2$, which is adjusted for sample size in this formulation, gives the proportion of the data's variance explained by a given model. Only models with $w_i > 0.1$ and $R^2 > 0.1$ are shown here; other models are in the supplemental tables (see Appendix). a. Models for density-adjusted eelgrass area at Goodwin Islands, as determined by aerial photos taken in the late spring of 1998 through 2006. The predictor variables used in these models; water temperature, salinity, turbidity, and mesograzer density (biomass / g eelgrass shoot dry mass) are average values from days 200 – 250 of the preceding year ("summer") or days 70 – 120 of the focal year ("spring"). b. Models for deviation from the mean value of eelgrass shoot biomass for monthly samples taken between 2004 and 2007. Predictor variables are themselves deviations from the mean value of the selected variable for the day of the year on which the sample was taken. Additionally, temperature, salinity, and turbidity are based on average values from 30 days prior to the sample date.

a.

| | | Predictor variables with T values | | | | | | | | | | Model Fit Statistics | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Response** | Model # | Constant | Spring Water Temperature | Spring Salinity | Spring Turbidity | Spring Mesograzers | Prev. Summer Water Temp. | Prev. Summer Salinity | Prev. Summer Turbidity | Prev. Summer Mesograzers | | **n** | **K** | **RSS** | **$AIC_c$** | **$w_i$** | **$R^2$** |
| Spring Eelgrass Index | 4 | 0.13 | | 1.54 | | | | | | | | 8 | 3 | 527.7 | 45.51 | 0.13 | 0.16 |
| " | 5 | 6.11 | | | -1.88 | | | | | | | 8 | 3 | 463.4 | 44.47 | 0.22 | 0.27 |
| " | 6 | 4.48 | | | | 1.7 | | | | | | 8 | 3 | 496.0 | 45.02 | 0.17 | 0.21 |
| " | 9 | 2.04 | | | | | -1.71 | | | | | 8 | 3 | 494.1 | 44.99 | 0.17 | 0.22 |
| " | 10 | -0.49 | | | | | | 1.73 | | | | 8 | 3 | 491.0 | 44.94 | 0.18 | 0.22 |

b.

| | | Predictor variables with T values | | | | | | Model Fit Statistics | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Response** | Model # | Constant | Water Temperature | Salinity | Turbidity | Epiphytic Chl *a* | Mesograzer Total Density | **n** | **K** | **RSS** | **$AIC_c$** | **$w_i$** | **$R^2$** |
| Post-2004 Shoot Biomass | 6 | -2.74 | | -2.9 | | | | 26 | 3 | 23.3 | 4.29 | 0.71 | 0.23 |

Table 5. Comparison of selected linear regression models of epiphyte density (μg chl * $cm^{-2}$ eelgrass blade), mesograzer biomass (mesograzer ash free dry mass over plant dry mass; mg AFDM * g $DM^{-1}$), biomass of individual mesograzer species; *Caprella penantis*; *Gammarus mucronatus*; *Erichsonella attenuata*; *Idotea balthica*; *Ampithoe longimana*; *Elasmopus levis*, and species richness of mobile epifauna. Predictor variables in models are indicated by their T values (regression coefficient / std. error of coefficient). Model fit statistics are as described in table 4. Only models with $w_i > 0.1$ and $R^2 > 0.1$ are shown here; other models are in the appendix tables. The three sets of models for each response (all dates, spring, summer) incorporate all dates in which mesograzers, epiphytic chlorophyll, and predators were sampled, only those dates between day 70-150 of a year, and only those dates between 180 and 260 of year, respectively.

| | | Predictor variables with T values | | | | | | | | | | Model Fit Statistics | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Response** | **Model #** | **Constant** | **Water Temperature** | **Salinity** | **Turbidity** | **Epiphytic Chl *a*** | **Mesograzer Total Density** | **Palaemonetes shrimp** | **Blue Crabs** | **Total Fish** | **n** | **K** | **RSS** | **$AIC_c$** | **$w_i$** | **$R^2$** |
| Epiphytic Chl *a*, All dates | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| Epiphytic Chl *a*, Spring | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| Epiphytic Chl *a*, Summer | 5 | 2.20 | | | -2.78 | | | | | | 12 | 3 | 8.87 | 5.37 | 0.87 | 0.38 |
| Mesograzers, All dates | 6 | 1.99 | | | -2.53 | | | | | | 48 | 3 | 204763 | 407.7 | 0.35 | 0.10 |
| " | 8 | 2.07 | | | | | | 1.05 | -2.06 | 2.65 | 48 | 5 | 188490 | 408.7 | 0.23 | 0.14 |
| Mesograzers, Spring | 6 | 0.98 | | | -1.53 | | | | | | 13 | 3 | 17475 | 102.3 | 0.15 | 0.10 |
| " | 11 | 2.52 | | | | | | | | 2.29 | 13 | 3 | 14352 | 99.8 | 0.53 | 0.26 |
| Mesograzers, Summer | 8 | 2.21 | | | | | | -1.07 | -0.12 | 4.06 | 12 | 5 | 13860 | 104.6 | 0.18 | 0.56 |
| " | 11 | 1.81 | | | | | | | | 2.16 | 12 | 3 | 29581 | 102.7 | 0.47 | 0.25 |
| *C. penantis*, All dates | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| *C. penantis*, Spring | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| *C. penantis*, Summer | 4 | 0.38 | -1.78 | | | | | | | | 12 | 3 | 951.08 | 61.47 | 0.36 | 0.17 |
| *G. mucronatus*, All dates | 3 | 1.72 | -1.30 | -2.15 | -0.71 | | | | | | 48 | 5 | 6672 | 248.3 | 0.19 | 0.16 |
| " | 4 | 2.15 | -2.56 | | | | | | | | 48 | 3 | 7398 | 248.4 | 0.18 | 0.11 |
| " | 5 | 1.48 | | -2.98 | | | | | | | 48 | 3 | 7084 | 246.3 | 0.51 | 0.14 |
| *G. mucronatus*, Spring | 10 | -0.33 | | | | | | | -1.86 | | 13 | 3 | 4744 | 85.36 | 0.35 | 0.17 |
| *G. mucronatus*, Summer | 4 | 1.75 | -1.62 | | | | | | | | 12 | 3 | 228.8 | 44.38 | 0.32 | 0.13 |
| *Erich. attenuata*, All dates | 8 | 1.63 | | | | | | 0.22 | -1.78 | 3.44 | 48 | 5 | 17268 | 293.9 | 0.56 | 0.18 |
| " | 11 | 2.37 | | | | | | | | 2.70 | 48 | 3 | 19497 | 294.9 | 0.35 | 0.12 |
| *Erich. attenuata*, Spring | 9 | 0.76 | | | | | | 1.52 | | | 13 | 3 | 129.7 | 38.57 | 0.15 | 0.10 |
| " | 11 | 1.56 | | | | | | | | 2.01 | 13 | 3 | 114.8 | 36.99 | 0.33 | 0.20 |
| *Erich. attenuata*, Summer | 8 | 2.35 | | | | | | -1.13 | -0.35 | 5.57 | 12 | 5 | 2245 | 82.78 | 0.58 | 0.72 |
| " | 11 | 1.75 | | | | | | | | 2.86 | 12 | 3 | 6131 | 83.83 | 0.35 | 0.40 |
| *Idotea balthica*, All dates | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| *Idotea balthica*, Spring | 9 | 1.74 | | | | | | 1.51 | | | 13 | 3 | 2097 | 74.75 | 0.15 | 0.10 |
| " | 10 | 1.88 | | | | | | | 1.83 | | 13 | 3 | 1940 | 73.74 | 0.24 | 0.16 |
| " | 11 | 2.14 | | | | | | | | 1.88 | 13 | 3 | 1918 | 73.59 | 0.26 | 0.17 |
| *Idotea balthica*, Summer | 5 | 0.19 | | -1.81 | | | | | | | 12 | 3 | 3658 | 77.64 | 0.38 | 0.17 |
| *A. longimana*, All dates | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| *A. longimana*, Spring | 7 | -1.11 | | | | 2.46 | | | | | 13 | 3 | 17.16 | 12.27 | 0.66 | 0.30 |
| *A. longimana*, Summer | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| *Elasmopus levis*, All dates | 5 | 1.25 | | 3.25 | | | | | | | 48 | 3 | 702 | 135.3 | 0.71 | 0.17 |
| *Elasmopus levis*, Spring | 8 | 3.05 | | | | | | -1.61 | 4.09 | 2.29 | 13 | 5 | 2.55 | -2.62 | 0.12 | 0.68 |
| " | 10 | 2.10 | | | | | | | 4.26 | | 13 | 3 | 4.05 | -6.48 | 0.83 | 0.59 |
| *Elasmopus levis*, Summer | 5 | 1.45 | | 2.61 | | | | | | | 12 | 3 | 342.2 | 49.21 | 0.71 | 0.35 |
| Epifaunal SR, All dates | 1 | 1.97 | -0.33 | 3.12 | 1.98 | -1.12 | 2.44 | | | 1.83 | 48 | 8 | 103 | 56.19 | 0.70 | 0.34 |
| " | 5 | 2.02 | | 3.32 | | | | | | | 48 | 3 | 144 | 59.14 | 0.16 | 0.18 |
| Epifaunal SR, Spring | 5 | 0.29 | | 2.52 | | | | | | | 13 | 3 | 24.78 | 17.05 | 0.13 | 0.31 |
| " | 7 | 2.57 | | | | 3.39 | | | | | 13 | 3 | 19.13 | 13.69 | 0.72 | 0.47 |
| Epifaunal SR, Summer | 5 | 0.78 | | 1.53 | | | | | | | 12 | 3 | 26.24 | 18.39 | 0.22 | 0.11 |

Table 6. Comparison of selected linear regression models of predator abundance, measured as the average number of individuals per net sweep per collection date, at Goodwin Islands. Predictor variables in models are indicated by their T values (regression coefficient / std. error of coefficient). Model fit statistics are as described in table 4. Only models with $w_i > 0.1$ and $R^2 > 0.1$ are shown here; other models are in the supplemental tables. The three sets of models for each response (all dates, spring, summer) incorporate all dates in which mesograzers, epiphytic chlorophyll, and predators were sampled, only those dates between day 70-150 of a year, and only those dates between 180 and 260 of year, respectively.

| Response | Model # | Constant | Water Temperature | Salinity | Turbidity | Eelgrass density | Epiphytic Chl *a* | Mesograzer Total Density | n | K | RSS | $AIC_c$ | $w_i$ | $R^2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Total Fish, Post-2004 | . | . | . | . | . | . | . | . | . | | . | . | . | . |
| Total Fish, All dates | . | . | . | . | . | . | . | . | . | | . | . | . | . |
| Total Fish, Spring | 5 | -7.80 | | | -2.82 | | | | 13 | 3 | 3.84 | -7.18 | 0.69 | 0.37 |
| " | 6 | -7.58 | | | | | | 2.29 | 13 | 3 | 4.48 | -5.19 | 0.25 | 0.26 |
| Total Fish, Summer | 6 | -1.24 | | | | | | 2.16 | 12 | 3 | 181.2 | 41.58 | 0.72 | 0.25 |
| Pipefish, Post-2004 | . | . | . | . | . | . | . | . | . | | . | . | . | . |
| Pipefish, All dates | . | . | . | . | . | . | . | . | . | | . | . | . | . |
| Pipefish, Spring | . | . | . | . | . | . | . | . | . | | . | . | . | . |
| Pipefish, Summer | 6 | 0.01 | | | | | | 1.96 | 12 | 3 | 22.06 | 16.30 | 0.58 | 0.21 |
| Blue Crab, Post-2004 | . | . | . | . | . | . | . | . | . | | . | . | . | . |
| Blue Crab, All dates | . | . | . | . | . | . | . | . | . | | . | . | . | . |
| Blue Crab, Spring | 4 | -2.96 | | 2.73 | | | | | 13 | 3 | 22.79 | 15.96 | 0.81 | 0.35 |
| Blue Crab, Summer | . | . | . | . | . | . | . | . | . | | . | . | . | . |
| *Palaemonetes*, Post-2004 | 2 | -1.97 | 0.15 | 1.43 | -2.97 | 4.71 | | | 26 | 6 | 927.0 | 109.34 | 0.12 | 0.51 |
| " | 5 | -4.11 | | | -2.80 | 4.56 | | | 26 | 4 | 1032 | 105.60 | 0.76 | 0.50 |
| *Palaemonetes*, All dates | . | . | . | . | . | . | . | . | . | | . | . | . | . |
| *Palaemonetes*, Spring | 5 | -4.79 | | | -3.97 | | | | 13 | 3 | 341.3 | 51.15 | 0.78 | 0.55 |
| *Palaemonetes*, Summer | . | . | . | . | . | . | . | . | . | | . | . | . | . |

# CHAPTER 4

## Consumer versus resource control of seagrass community structure; using path analysis to compare field observations with mesocosm experiments

## ABSTRACT

Experimental and observational approaches offer potentially complementary advantages in understanding ecological processes. Manipulative experiments can isolate effects of factors, such as resource supply and predation intensity, on community structure.. But these factors may not induce the same responses, to the same degree, in natural communities. In seagrass ecosystems, mesocosm and caging experiments have demonstrated both top-down impacts of grazing and trophic cascades, and bottom-up impacts of light and nutrients, on community structure. However, it is uncertain to what degree seagrass communities respond similarly to naturally-occurring variation in these top-down and bottom-up processes. We created structural equation models based on the natural history of a seagrass food web and tested their ability to explain variation both in results of two seagrass mesocosm experiments that simultaneously manipulated top-down and bottom-up factors, and in long term time-series data from natural eelgrass (*Zostera marina*) beds. Both the strength and the sign of path coefficients differed between models fit to experimental versus observational data, with the observational data suggesting a relatively stronger influence of bottom-up factors and weaker influence of top-down factors than the mesocosm experiments. The limited temporal and spatial scale, low environmental variability, absence of large predators, and high light levels in the mesocosm experiments probably contributed to these differences. Conversely, the field time series may have had insufficient variance in consumer density and unmeasured variance in large predator density to detect strong top-down forcing. These results

suggest caution in making simple extrapolations between natural and experimental systems, and highlight the relative strengths and weaknesses of the two approaches.

# INTRODUCTION

The relative influence of top-down and bottom-up forces in structuring ecosystems has been actively debated since Hairston, Smith, & Slobodkin (1960) presented their "green world hypothesis," which suggested that predator control of herbivores maintained high plant biomass in terrestrial systems. Strong top-down control resulting in inverse patterns of abundance or biomass at adjacent trophic levels is now known as a "trophic cascade" (Paine 1980, Pace et al. 1999). Trophic cascades are well documented in aquatic systems (Shurin et al. 2002), both freshwater (Brett and Goldman 1996, 1997) and marine (Pinnegar et al. 2000, Duffy *in press*). Low diversity, vulnerable primary producers, and strong consumer-prey interactions are thought to facilitate aquatic trophic cascades, and are common among the limnetic and rocky intertidal systems where cascades are most often observed (Strong 1992, Cyr & Pace 1993, Polis 1999).

Seagrasses such as eelgrass (*Zostera marina*) support productive and species-rich communities in coastal areas around the world (Hemminga & Duarte 2000), but seagrasses have declined in many regions (Orth et al. 2006). These declines have been attributed mainly to bottom-up stressors, such as increased nutrient and sediment inputs from human activities (Twilley et al. 1984, Duarte 2002). However, top-down control mediated by herbivores has the potential to strongly impact seagrasses, as well (Heck & Valentine 2007). Top down control in seagrass beds is complicated by the fact that the habitat includes two competing groups of benthic primary producers: the seagrasses themselves, which are relatively resistant to grazing, and epiphytic algae, which are readily consumed by small invertebrate mesograzers (Van Montfrans et al. 1984,

Jernakoff et al. 1996, Valentine & Duffy 2006). Thus, seagrasses could experience negative effects of a trophic cascade either in the form of direct consumption by abundant grazers, or in the form of epiphyte overgrowth in the absence of grazers. Despite the potential for these forms of top-down control, the bottom-up perspective emphasizing the influence of light, nutrients, and other physicochemical factors, has dominated seagrass research (e.g. Hemminga & Duarte 2000). Recent research has questioned that focus on bottom-up forcing, however, with recent meta-analyses suggesting that grazing effects are just as strong as nutrient effects on seagrass (Hughes et al. 2004), and that top-down control of grazers by predators can also be important (Duffy et al. 2005, Heck & Valentine 2007). Syntheses from a variety of systems suggest that predator effects often reach farther down the food chain than productivity effects reach up the food chain (Borer et al. 2006), such that even the harvest of apex-level consumers in coastal oceans could have significant impacts on shallow water benthic communities (Myers et al. 2007). These findings emphasize the need to better incorporate the understanding of top-down control in real-world seagrass ecosystems, in order to effectively manage consumer communities for seagrass conservation.

Most of what we know now about top-down and bottom-up effects in seagrass beds comes from manipulative experiments (Hughes et al. 2004, Valentine & Duffy 2006, but see Jørgensen et al. 2007). Experiments can clearly identify ecological mechanisms of cause and effect in a controlled, replicable environment (Carpenter 1996), whereas complexity and uncontrolled variation in nature can make it difficult or impossible to infer causality from observational studies alone (Ch. 3). However, most experiments are performed in simplified model venues like mesocosms or small

enclosures, for which the ecological realism tends to be unknown or unquantified (Skelly 2002). Though causality may be accurately determined within the context of an experiment, it does not necessarily follow that the same mechanism of causality operates in the field. Thus, the effects of top-down and bottom-up treatments observed in experimental systems may differ from the realized effects of changing consumer communities and physicochemical conditions in nature (Douglass et al. 2007).

One way to assess how well mesocosm experiments model natural dynamics is to analyze experimental results and field data with a similar, statistical framework. If the covariation among consumers and resources in observational field data is similar to the covariation in mesocosm experiment results, then it would support the inference that similar ecological interactions occur in mesocosms and in nature. If not, then the factors that generate patterns among community components in mesocosms are either different from, or a subset of, the factors at work in the natural environment.

Path analysis, or "structural equations modeling" is a regression-based approach to data analysis that can evaluate interactions among multiple variables, i.e. among several species in a biological community. The "paths" in a structural equations model are the hypothesized interactions among variables, which the researcher specifies a priori (Hatcher 1994). When the model is run with empirical data, it returns standardized coefficients for each path in the model, which represent the sign and strength of direct and indirect interactions among the community components being compared. The degree to which a path model effectively explains the covariance structure of the data, its "fit", can be evaluated statistically and compared to the fit of alternative path models (Burnham & Anderson 2002). For example, the explanatory power of a model representing the

bottom-up control paradigm for seagrass ecology could be compared to that of a model incorporating top-down control.

Our aim for this study was to see how well the evolving paradigm of interacting top-down and bottom-up control in seagrass beds, developed with the results of experiments (Heck et al. 2000, Hughes et al. 2004, Duffy et al. 2005, Heck et al. 2006, Douglass et al. 2007, Spivak et al. 2007), could explain naturally-occurring variation in a seagrass community. We created simple path models based on the designs of two seagrass mesocosm experiments (Duffy et al. 2005, Spivak et al. 2007). The experiments were manipulations of predator presence and light or season, which measured the effects of these top-down and bottom-up factors on mesograzers, epiphytes, and seagrass (Duffy et al. 2005, Spivak et al. 2007). We then compared the models' power to explain results of these experiments with their power to explain variation in observational data from eelgrass beds in lower Chesapeake Bay. After the initial comparison, we modified models to better describe the experimental and field data. We discuss how differences in the structure of the models that best describe the experimental versus the field data affect the interpretation of observational and experimental data in general, and lend more or less support to the proposed importance of top-down processes in seagrass ecology (Heck & Valentine 2007).

## METHODS

**Study system:** Eelgrass (*Zostera marina*) beds in lower Chesapeake Bay (Virginia, USA) historically covered large areas of the shallow subtidal zone down to 2 m below mean low water depth. Deteriorating water quality in the Bay over the last century has greatly reduced the areal extent and maximum depth range of eelgrass, which has high light requirements for growth (Kemp et al. 2004, 2005). In addition to phytoplankton and suspended sediment in the water column, algal epiphytes on eelgrass blades block incident light. These epiphytes can be reduced by invertebrate mesograzers (van Montfrans 1984, Jernakoff 1996), which are believed to be critical to eelgrass' persistence, especially in marginal habitats nearer to terrestrial and riverine inputs of sediment and nutrients (Moore et al. 1996).

**Experimental data:** The three eelgrass mesocosm experiments from which the data and path model structures for this analysis were obtained were performed at the Virginia Institute of Marine Science from 29 October – 14 December 2001, 17 May – 2 July 2002, and 16 May – 20 June 2003. The 2001 and 2002 experiments repeated the same design, and are therefore analyzed together with "season" as an additional factor (Table 1). Methods and results of the experiments are only briefly outlined here, but are fully described for the 2002 experiment in Duffy et al. (2005), and for the 2003 experiment in Spivak et al. (2007).

All experiments were performed with live eelgrass planted in 113 liter outdoor tanks supplied with flowing, filtered seawater from the York River estuary and stocked with mesograzers collected from nearby eelgrass beds. The 2001 / 2002 experiments, hereafter the "season experiment", manipulated mesograzer diversity and predator presence, and consisted of replicated experiments in the fall and the spring to include

season as a factor. We coded the fall as "0" and the spring as "1", so a positive influence of season on a measured response would indicate that it was strongest in spring (Table 1). The experiment had five mesograzer diversity treatments; monocultures of each of four species; the amphipods *Ampithoe longimana* and *Gammarus mucronatus*, and the isopods *Erichsonella attenuata* and *Idotea balthica*, and polycultures with all four species. We used only data from the polyculture treatments for our analysis, to simulate the multispecies assemblages characteristic of the field and to establish total mesograzer abundance as an endogenous (dependent) variable in the models. A total of 80 mesograzers was added to each mesocosm at the beginning of each experiment and allowed to reproduce throughout the six week study (Duffy et al. 2005). For the predator treatment, each tank received either three juvenile blue crabs (*Callinectes sapidus*) between 20 and 40 mm carapace width, or received no blue crabs. Each treatment in the experiment was replicated five times, giving us a total sample size of 20 (Table 1). Measured responses from the experiment included final ash-free dry mass of eelgrass, total mesograzer numbers, and final epiphyte density ($\mu$g chl *a* * $cm^{-2}$).

The design of the 2003 mesocosm experiment (hereafter the "light experiment") was similar to that of the season experiment in that it manipulated mesograzers and crabs, but its third factor was light level (Table 1). Also, the mesograzer species composition and levels of diversity were different, with 0, 2 and 6 species of mesograzers per treatment. The full complement of mesograzer species included the gastropod *Bittium varium* and the caprellid amphipod *Caprella penantis* in addition to the four species used in the season experiment. These differences were minimized, however, because we again used only the results from high mesograzer diversity treatments, and because *B. varium*

and *C. penantis* fared so poorly in mesocosms as to be negligible in abundance by the end of the experiment. Measured responses from the light experiment were identical to those from the season experiment, except that total mesograzer biomass, as estimated by size fractionated counts (Edgar 1990), was used in lieu of mesograzer numbers. (Biomass data were not available for the season experiment).

**Field data:** Data from the field included estimated eelgrass density (percent cover), biomass of mesograzers (*Ampithoe longimana*, *Gammarus mucronatus*, *Erichsonella attenuata*, and *Idotea balthica*)(mg ash free dry mass per g dry mass of plant), epiphyte density (μg chl *a* * $cm^{-2}$), total fish abundance per net sweep (Douglass Ch. 3), and a turbidity-light index. Total fish abundance in the field was selected as the most appropriate metric of predation in the field (in contrast to the crab predation factor used in experiments), because we believe based on abundance and gut contents analyses that fishes are the functionally dominant predators on mesograzers in the system (Douglass et al Chs. 3, 5, Teixeira & Musick 1994). The eelgrass community monitoring study from which these data were obtained is described in more detail in Douglass (Ch. 3), but is summarized here. All field data were collected from seagrass beds in lower Chesapeake Bay, which were comprised predominantly of eelgrass (*Zostera marina*) with some widgeon grass (*Ruppia maritima*). Most data were collected from the Goodwin Islands National Estuarine Research Reserve, located at the mouth of the York River in Chesapeake Bay (Virginia, USA, 37° 13' N; 76° 23' W), but several other eelgrass beds were monitored at less frequent intervals. Average values of eelgrass density, mesograzer biomass, epiphyte density, and fish abundance from the inshore versus offshore transects from a site were considered as separate data points because these data

often varied significantly between transects within a given site on a given date (Douglass *unpub data*). A full list of sites and sample dates used for our analyses is provided in Table 2. Note that only sample dates corresponding with the time of the year of the mesocosm experiments were selected from a larger data set. We matched field data to the end of the second week of each experiment, which was June 1$^{st}$ for the light experiments and the spring portion of the season experiment, and November 14$^{th}$ for the fall portion of the season experiment. For Goodwin Islands, which was sampled at approximately monthly intervals, interpolated values of eelgrass density, mesograzer biomass, epiphyte density, and fish abundance between the nearest prior and post sample dates were used. For sites that were not sampled at such frequent intervals, the date nearest to June 1$^{st}$ or November 14$^{th}$ was used without interpolation, providing it occurred within 30 days of the target date.

To come up with a field analog of the light factor in the light experiment we developed an index based on measured turbidity, estimated site quality, and depth rank. Turbidity data for Goodwin Islands from 1997 – 2006 were available from the NERRS continuous monitoring station. These data were taken with a YSI 6600 EDS data sonde, following standard YSI (YSI, Inc., Yellow Springs, Ohio) and NERRS System-wide Monitoring Program protocols (http://nerrs.noaa.gov/Monitoring/Water.html). Comparable data were not available for the other eelgrass sites, so the light environment at all sites was estimated by multiplying an ordinal rank of site quality, an adjustment for the depth of the portion of the eelgrass bed surveyed, and the inverse of the average turbidity at Goodwin Islands from the 30 days prior to sampling (Table 2). In the ordinal ranking system for site quality, a score of three was assigned if a bed had turbidity

conditions apparently marginal for eelgrass survival (i.e. the bed at VIMS), a five was assigned for turbidity conditions similar to Goodwin Islands (which has high water quality for Chesapeake Bay), and a four was assigned for intermediate conditions (i.e. the bed at Allen's Island). The adjustment for depth was 1.0 for the shallow inshore region of a bed, and 0.7 for the deeper, offshore region of a bed. If the offshore sampled portion of the bed was not deeper than the inshore region, as for Goodwin Islands, then 1.0 (no adjustment) was used (Table 2).

Eelgrass shoot biomass was directly monitored after 2004 in our eelgrass community surveys, but these data alone did not constitute a sufficiently large sample size for our analysis. Thus, we estimated eelgrass density by using a Chesapeake Bay – wide GIS database of yearly spring eelgrass density from digitized aerial photographs (Moore et al. 2000). In the GIS database, the entire area of seagrass in the bay is divided into irregularly shaped and sized polygons of less than 1 Ha to over 100 Ha, which designate habitat patches of similar seagrass density. Each patch was assigned to one of four estimated density classes. Empirically-derived relationships between the aerial photo-based density class and ground-based shoot density surveys (S.R. Marion *pers. com.*) were used to estimate an area-weighted average shoot density for the polygons in the immediate vicinity of our sample sites. A linear regression between these density estimates for our sites and the measured shoot biomass from our post-2004 monitoring dates in spring had an $R^2$ value of 0.63 ($n = 11$). Estimating fall eelgrass density was more involved, but was accomplished by taking the average of the previous and following spring's photo-based density estimate, and reducing it by a factor of 0.75 in accordance with typical fall declines in eelgrass shoot biomass (Ch. 3). With the fall data

added, a linear regression between estimated density and observed shoot biomass had an $R^2$ value of 0.48 (n = 11).

**Data Analysis:** Initial path models (Figure 1) were constructed based on the designs of the experiments (Table 1) and on the causal relationships inferred from the experimental results (Duffy et al. 2005, Spivak et al. 2007). By necessity, the factors that were manipulated in the experiments (predators, season, and light) were coded as exogenous variables in all models of experimental data, while the measured responses (mesograzer abundance, epiphytes, and eelgrass) were endogenous variables. In the initial model based on the season experiment (Figure 1a) we included bottom-up paths from season to epiphytic algae, final eelgrass biomass, and total grazer abundance, and top-down paths (implying consumption) from crabs to mesograzers, mesograzers to epiphytic algae, and mesograzers to eelgrass. We also included a path from epiphytic algae to eelgrass, representing light-mediated competition (Figure 1a). The initial path model for the light experiment (Figure 1b) had a similar structure to the model for the season experiment, but with light treatment substituted for season and no direct bottom-up path from light to mesograzers.

Models were run using the CALIS procedure in SAS (SAS Institute Inc., Cary, NC, USA) with maximum likelihood estimation. Models were evaluated with a variety of statistics assessing fit and parsimony (Table 3), and by logical assessment of how well standardized path coefficients (Figures 1, 2) could be explained by plausible ecological interactions. The probability of the $\chi^2$ statistic for a model actually gives the degree of support for the null model of no covariance along the designated paths; thus, non-significant values close to 1.0 indicate a good model. The normed-fit index (NFI, Bentler

& Bonnett 1980) is an alternative to the $\chi^2$ statistic which can be thought of as the proportion of the covariance among the observed measures that is explained by the model, compared with a null model with unstructured covariance. NFI values closer to 1.0 indicate a better model fit, with 0.9 or greater considered an acceptable model (Hatcher 1994). The NFI is susceptible to underestimating fit in models with small samples, like ours, but another index, the non-normed fit index (NNFI, Bentler & Bonnett 1980) is supposed to be more effective for smaller sample sizes (Hatcher 1994). Unlike the NFI, which is constrained between 0 and 1, the NNFI can have values less than 0 or greater than 1, but like the NFI, any value of NNFI greater than 0.9 indicates a good model fit. Finally, the comparative fit index (CFI, Bentler 1989) is said to combine the qualities of the NFI and NNFI by being effective with small sample sizes, but also remaining constrained between 0 and 1. We used Akaike's Information Criterion (AIC)(Burnham & Anderson 2002) to assess the explanatory power of the model versus its complexity. Among models predicting the same responses with the same data set, models with lower AIC are considered to be more likely to be true, given the data. The $R^2$ values for endogenous variables give the proportion of the variation in those variables that is accounted for by a model.

## RESULTS

**Path Analysis: Season Experiment**: Our initial path model for the season experiment (SE1, Figure 2a) was a good fit to the data (Table 3). For the most part, the

path coefficients in SE1 supported the causal relationships among eelgrass community variables that we hypothesized in figure 1. This was expected, since the hypothesized model was based largely on insights from those experiments. Predatory blue crabs reduced total mesograzers, and mesograzers reduced epiphytes and eelgrass. Season had a positive influence on final eelgrass biomass and total grazers, meaning that plants grew more and mesograzers reproduced more rapidly in spring than in fall. However, in contrast with our predictions, season had a modest negative influence on epiphytes, indicating that algae on eelgrass blades were somewhat denser in the fall than in the spring. Also, the relationship between epiphytes and eelgrass was positive, rather than negative as predicted; eelgrass was apparently not negatively affected by competition from epiphytes in the season experiment.

Though the initial model fit for SE1 was good according to the $\chi^2$, NFI, NNFI, and CFI, a large residual covariance between crabs and eelgrass in the results of this model motivated us to include an additional, direct, top-down path from crabs to eelgrass in a second version of the model (SE2, Figure 2b). Our rationale for including this path was that crabs might have a negative impact on eelgrass by tearing it up or feeding on it. This was apparently not the case, however, as the crab - eelgrass path coefficient was actually positive in SE2, and when this path was included the negative relationship between mesograzers and eelgrass was reduced to a negligible level in the model. The addition of the crab - eelgrass path to the model improved most measures of fit, but increased AIC slightly (i.e. it yielded a less negative value), indicating that it may have made the model unnecessarily complex.

The initial field analogue model for the season experiment (SF1, Figure 2c) was a moderately good fit to the field data, although some of the fit statistics were below the "good fit" threshold (Hatcher 1994)(Table 3). However, the pattern of path coefficients in SF1 was quite different from that in the results for SE1 (Figure 2a, c). Though season had a positive effect on eelgrass and a negative effect on epiphytes in both field and experimental data, it had a slight negative effect on mesograzer abundance (density) in the field, meaning lower mesograzer abundances in spring, versus a strong positive effect in the experiment. Also, mesograzers were positively associated with eelgrass in the field, versus negatively in the experiment, and were only weakly negatively associated with epiphytes in the field, suggesting minimal top-down control by grazing. The hypothesized top-down pathway between predators and mesograzers in the field was positive, in sharp contrast with the strongly negative predator – mesograzer relationship in the experiment. The epiphyte – eelgrass pathway was negative in the field model (Fig. 2c), however, which agreed with our general prediction (Fig. 1a) but contrasted with the experimental results (Fig. 2a).

A second version of the field model (SF2, Figure 2d) included fish as an endogenous (i.e. dependent) variable to account for their potential responses to the bottom-up influences of season and eelgrass density. These relationships turned out to be weak, however, and detracted from the fit of the model, indicating that the addition of those paths decreased the degrees of freedom of the model, they did not cause a corresponding decrease in the unexplained covariance in the data. We kept fish as an endogenous variable in the third and fourth iterations of the seasonal field model (SF3 and SF4, Figure 2e, f), but used only mesograzer abundance as a bottom-up predictor of

fish. We did this because we knew from the results of SF1 that there was a positive relationship between mesograzers and fish that would make more sense as a bottom-up path. In SF3 (Figure 2e) we also included bottom-up paths from epiphytes to mesograzers and from eelgrass to mesograzers, because the top-down mesograzer-epiphyte path in SF1 and SF2 was weak, and because the positive top-down relationship between mesograzers and eelgrass in that model was difficult to interpret as a direct (not epiphyte – mediated) interaction. This bottom-up model (SF3) agreed well with the data, with high indices of fit and a low value of AIC (Table 3). However, the relationship between epiphytes and mesograzers was weak, and we opted to leave it out of a fourth and final, revised version of the model (SF4, Figure 2f). This model was similar to SF3 in its fit statistics and its ability to explain the variation in the endogenous variables, but had a lower AIC value reflecting its more parsimonious structure.

**Path Analysis: Light Experiment**: The light experiment models (LE1 and LE2, Figure 3a, b) effectively explained much of the variation in mesograzers, epiphytes, and eelgrass but were not evaluated as good fits by the statistical indices, with $p(\chi^2) < 0.0001$ for both models (Table 3). Nevertheless, the models' path coefficients were mostly in agreement with our predictions that light would have positive effects on eelgrass and epiphytes, mesograzers would have a negative effect on epiphytes, and crabs would have a negative effect on mesograzers (Figure 1b). As in the season experiment, the relationship between epiphytes and eelgrass, which we predicted to be negative, was positive and large in LE1 (Figure 3a). However, in contrast with the initial model for the season experiment (SE1, Figure 2a), which found a negative relationship between mesograzers and eelgrass, LE1 (Figure 3a) found a weak positive effect. As in the initial

season experiment model (SE1, Figure 2a) there was a large residual covariance between crabs and eelgrass in LE1, which we attempted to account for with a direct path from crabs to eelgrass in a modified model. The resultant model (LE2, Figure 3b) calculated a strong positive path coefficient between crabs and eelgrass, reducing the strength of the positive path from epiphytes to eelgrass, and increasing the strength of the positive path from mesograzers to eelgrass. However, the fit of LE2 was little better than the fit of the initial model (Table 3).

The light experiment path models had better fit statistics when used with the observational field data than when they were applied to the experimental data, but like the field tests of the season experiment models, the signs of their path coefficients often disagreed with our predictions (Figure 3c-f). The initial adaptation of the light experiment model to the field data (LF1, Figure 3c) found a positive effect of site quality / turbidity (the proxy for light level) and eelgrass, similar to the light – eelgrass relationship in the experimental data. A positive relationship between mesograzer abundance and eelgrass was also shared between experiment and field models. That was where the similarities ended, however; light negatively affected epiphytes in the field, and the crab – mesograzer and mesograzer – epiphyte relationships were positive. A modification of LF1 designated fish an endogenous variable by adding a bottom-up path between eelgrass and fish (LF2, Figure 3d). This model had greatly improved fit characteristics (Table 3), and calculated a positive association between eelgrass and fish. A second revision was made to LF2 to account for the bottom-up forcing suggested by the positive associations between resources and consumers (LF3, Figure 3e). This model had only slightly better fit than LF2 (Table 3) but made more sense from the perspective

of causality. As in the third field model based on the season experiment (SF3), the link between epiphytes and eelgrass was poorly supported in LF3. We eliminated this path to create the final light-based field model (LF4, Figure 3f). The fit of this model was further improved, and its AIC value was reduced by this change.

## DISCUSSION

This study faced two major challenges with respect to data collection and assembly: 1) achieving sufficiently large sample sizes to properly run path analyses, and 2) insuring good quality, comparable data from both the experiments and the field. Measures taken to address the first challenge necessarily interfered with the second, and vice versa. For example, no direct analog of the experimental light manipulation was available for all field sites, hence our use of ordinal depth and site quality classes in conjunction with turbidity data from one site to create the hybrid index of estimated light level in the field. Likewise, the estimation of eelgrass density in the field from aerial photos instead of ground-based measurements introduced uncertainty in the field data which was not present in the direct experimental measurements of eelgrass. Larger data sets and more direct measurements of light and eelgrass cover would increase our confidence in the model results. Nevertheless, our results were fairly consistent and the selected models fit the data well despite these constraints.

Not surprisingly, we found that path models based on the insights from experimental manipulations in eelgrass mesocosms were good at explaining the

interactions among eelgrass, epiphytes, mesograzers, and predators within those mesocosms. In general, the models run with experimental data supported our predicted causal relationships among eelgrass community components better than the models run with field data. All the models from experimental data showed the classic top-down relationships expected and found in the experiments; small predators (blue crabs) reduced mesograzers and mesograzers reduced epiphytes (van Montfrans et al. 1984, Duffy et al. 2005, Heck & Valentine 2007). Interestingly, though, these models failed to show a negative effect of epiphytes on eelgrass (Figs. 1a-b, 2a-b), in contrast with other mesocosm studies (Jernakoff et al. 1996). It is likely that the high light environment of the shallow, outdoor mesocosms supplied the eelgrass with adequate light for growth regardless of epiphyte load, and epiphytes protected the blades from damage by ultraviolet light (Jernakoff et al. 1996). It is also possible that a common factor which affected both epiphytes and eelgrass, but was not fully included in the models, led to a positive covariance that only *appeared* to be a positive effect of epiphytes on eelgrass. Changing mesograzer species composition, which has been shown to affect eelgrass and epiphytes irrespective of total mesograzer abundance (Duffy & Harvilicz 2001), could have been that factor.

While our models captured the negative effect of crab predation on total mesograzer abundance in both the season experiment and the light experiment, they could not capture the changes in mesograzer species composition that were induced by crab predation (Duffy et al. 2005, Spivak et al. 2007). Crabs almost completely eliminated the isopods *Idotea balthica* and *Erichsonella attenuata* in these experiments, while leaving fair numbers of *Gammarus mucronatus*, and in some cases increasing the

abundance of ampithoid amphipods. The isopod species are stronger grazers, and *I. balthica* is particularly prone to overgraze eelgrass itself as well as epiphytes (Duffy et al. 2003). So the inclusion of the crab – eelgrass interaction path in models SE2 (Figure 2b) and LE2 (Figure 3b) is probably not representing a direct causal relationship, but rather the result of crab predation preventing overgrazing by *I. balthica* via a trophic cascade. The more positive mesograzer - eelgrass path coefficients in SE2 and LE2, which included a direct path from crabs to eelgrass, relative to SE1 and LE1, which did not, also support the idea that eelgrass is affected not by mesograzer numbers themselves, but by some other aspect of the mesograzer community, which is affected by crabs. A larger dataset would allow us to confirm this by including individual mesograzer species as separate endogenous variables with unique relationships to crabs and epiphytes.

The model results for field data were plainly divergent from the experimental data results in that they found positive relationships between predators and mesograzers and between mesograzers and epiphytes, among other differences. Possible reasons for these discrepancies can be grouped into two categories; 1) differences in the type of data representing analogous boxes in field versus experimental models and 2) differences in the types of ecological interactions occurring in the mesocosms versus in the field. At one or the other extreme, the differences in the model results could be explained entirely by the differences in the types of data, or entirely by the differences in ecological interactions, but the reality is probably a combination of both. Possible problems with the estimating eelgrass density and light intensity in the field, mentioned earlier, could have affected results. For example, in the field analog models for the season experiment (SF1 – SF4, Figure 2c-f), the path from "season" to eelgrass is biased by our mode of

estimation of fall eelgrass biomass as the average of prior spring and post spring eelgrass reduced by a factor of 0.75.

Another type of data that was somewhat different in experiment and field was mesograzer abundance. Number of mesograzers was used as a measure of abundance in the season experiment, while estimated biomass (based on size fractionated counts, Edgar 1990) was used in the light experiment and in the field data. The $R^2$ for a linear regression of biomass against count from the light experiment was 0.68, however, indicating an acceptably close relationship between the two measures of abundance. A separate possible problem with the type of mesograzer data used is that, although the four species of mesograzers in the experiments include four of the five most abundant (by biomass) species in the field (Ch. 3), they exclude the most abundant species; the caprellid amphipod *Caprella penantis*. Though *C. penantis* comprises 34% of the mesograzer biomass in the overall field dataset versus 49% by *Gammarus mucronatus*, *Erichsonella attenuata*, *Idotea balthica*, and *Ampithoe longimana* together, it is thought to mix filter feeding with grazing (Caine 1974, Guerra- Garcia et al. 2004), is typically scarce in the summer (Ch. 3) and likely makes a smaller contribution to overall epiphyte grazing than indicated by its proportional biomass. For these reasons, we believe that the sum of *G. mucronatus*, *E. attenuata*, *I. balthica*, and *A. longimana* abundance in experiments and field data was an acceptable measure for this comparison of field data and experiments.

A final difference in data type between experiment and field that merits discussion is the measurement of predators. Blue crabs were obviously the only relevant predator in the mesocosm experiments, whereas there were predators in the field besides

the small fishes we captured in our dip net surveys (Ch. 3). Top-down control could be masked in our models by compensatory predation in the absence of fish predators, or even enhanced predation if smaller predators like *Palaemonetes sp.* shrimp are more abundant at times that fish are scarce (e.g. Nelson 1979). Unfortunately, because we lacked data on per capita rates of predation by different taxonomic groups of predators (i.e. shrimp, crabs, and fishes), we felt it was not feasible to combine predators into a "total predators" category, and our field dataset was too small to support the complexity of models including three or more groups of predators. A useful solution to this problem that could employed with a larger dataset in the future would be to model "predation" itself as a latent variable (sensu Hatcher 1994) predicted in part by each group of predators.

While it is difficult to know exactly how the differences in variable type discussed above influenced the results of experiment versus field models, some of the observed differences in the models are easily attributable to different ecological processes in nature versus in the short-term mesocosm experiments. For example, the positive effect of season on mesograzer abundance in the experiment is likely the result of increased growth and reproduction rates by mesograzers in the warmer water temperatures of the spring versus the fall. This would have allowed initially equal mesograzer populations to reach a higher level in mesocosms during the six week spring experiment than during the equally long fall experiment. In the field, the effect of season on mesograzer abundance was slightly negative. This likely indicates that mesograzer density was not directly limited by temperature-based population growth rate in either the spring or the fall, but that mesograzers were "concentrated" in the fall as high summer populations sought

refuge in shrinking amounts of senescent eelgrass. Also, there are many environmental differences between seasons in different years in the field data set, and natural eelgrass beds do not necessarily begin the season with a standardized density of mesograzers.

The simplified ecology of mesocosms could also explain the strong predation and grazing effects in the experiments, which were not detected in the field. Of course the goals of the particular experiments used in this study were not to simulate the ecological dynamics of eelgrass beds in the most realistic manner possible, but rather to test the general hypotheses about relationships of consumer biodiversity and ecosystem function in a simplified, multi-trophic model system (Duffy et al. 2005, Spivak et al. 2007). The same characteristics of the mesocosm system that made the original experimental goals possible to achieve; a limited set of consumers, and their confinement to a single habitat patch, might have compromised the ability of the experiment to be extrapolated to real eelgrass bed dynamics. The prevention of consumer dispersal among multiple habitat patches reduces the potential recovery time of prey populations under consumptive pressure (Ellner et al. 2001, Cardinale et al. 2006, France & Duffy 2006) and may thereby predispose mesocosm experiments to detecting strong top-down effects (Douglass et al. 2007, 2008). Additionally, it is unclear if the time scales over which consumptive effects occur in the field would generate a negative correlation of consumer and resource abundance in a sample of "snapshot" data points such as ours, even given strong top-down control in the system at large. In other words, the positive path coefficients we detected between consumers and resources in our path models of field data might merely reflect short-term aggregations of consumers and resources, whilst the ecosystem scale effects are actually negative. The alternative is that we were truly

observing a bottom-up controlled system, where consumptive effects were not strong or long enough to overwhelm the effects of variation in the resource environment. The good fits of our bottom-up path models for the field data (SF3, SF4, LF3, LF4, Table 3) support the idea of bottom-up control at the scale of our sampling, but cannot distinguish whether this is a system wide result of bottom-up forcing or a more local effect of aggregation. These results emphasize the importance of validating experiment-based hypotheses of top-down control of mesograzers and epiphytes with compelling evidence from the field (Heck et al. 2007, Jørgensen et al. 2007).

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# FIGURE LEGENDS

Figure 1. Initial hypothesized models for relationships among eelgrass community components and top-down and bottom-up forcing factors. Arrows indicate the hypothesized direction of causality, minus signs indicate a negative interaction, and plus signs indicate a positive interaction. a. Model based on the design of the season experiment and applied both to the experimental data and to analogous field data. b. Model based on the design of the light experiment and applied both to the experimental data and to analogous field data.

Figure 2. Path analysis results for models based on the design of the season experiment. Arrows identify the interaction paths specified by the model, and numbers on the arrows are standardized path coefficients giving the proportional change in the receiving box associated with a unit change in the donor box. a. Initial model run with data from the experiment. b. Revised model run with experiment data. c. Initial model run with data from field observations. d. Revised model run with field observations, considering predator abundance as an endogenous variable. e. Revised model with only bottom-up control pathways. f. Revised model with bottom-up control and no epiphyte-mesograzer connection.

Figure 3. Path analysis results for models based on the design of the light experiment. Arrows identify the interaction paths specified by the model, and numbers on the arrows are standardized path coefficients giving the proportional change in the receiving box

associated with a unit change in the donor box. a. Initial model run with data from the experiment. b. Revised model run with experiment data. c. Initial model run with data from field observations. d. Revised model run with field observations, considering predator abundance as an endogenous variable. e. Revised model with only bottom-up control pathways. f. Revised model with bottom-up control and no epiphyte-mesograzer connection.

Figure 1.

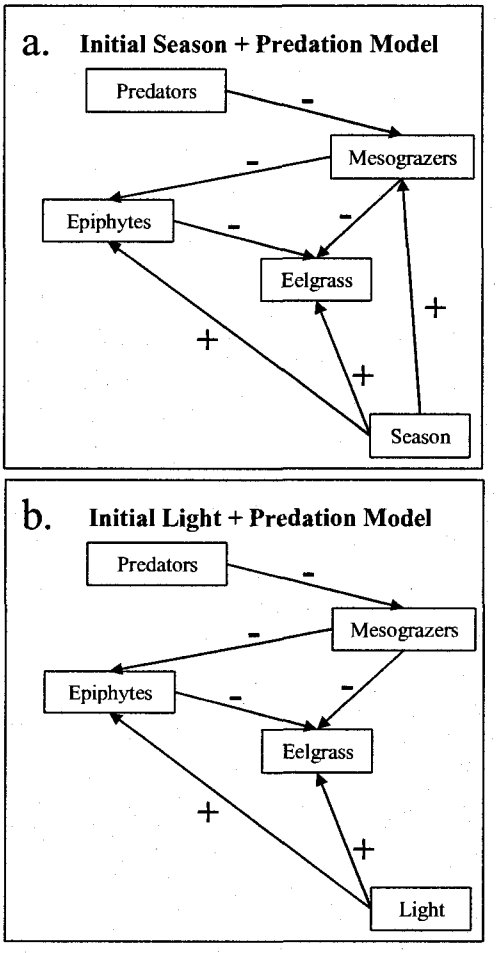
a. Initial Season + Predation Model
Predators
Mesograzers
Epiphytes
Eelgrass
Season
b. Initial Light + Predation Model
Predators
Mesograzers
Epiphytes
Eelgrass
Light

Figure 2.

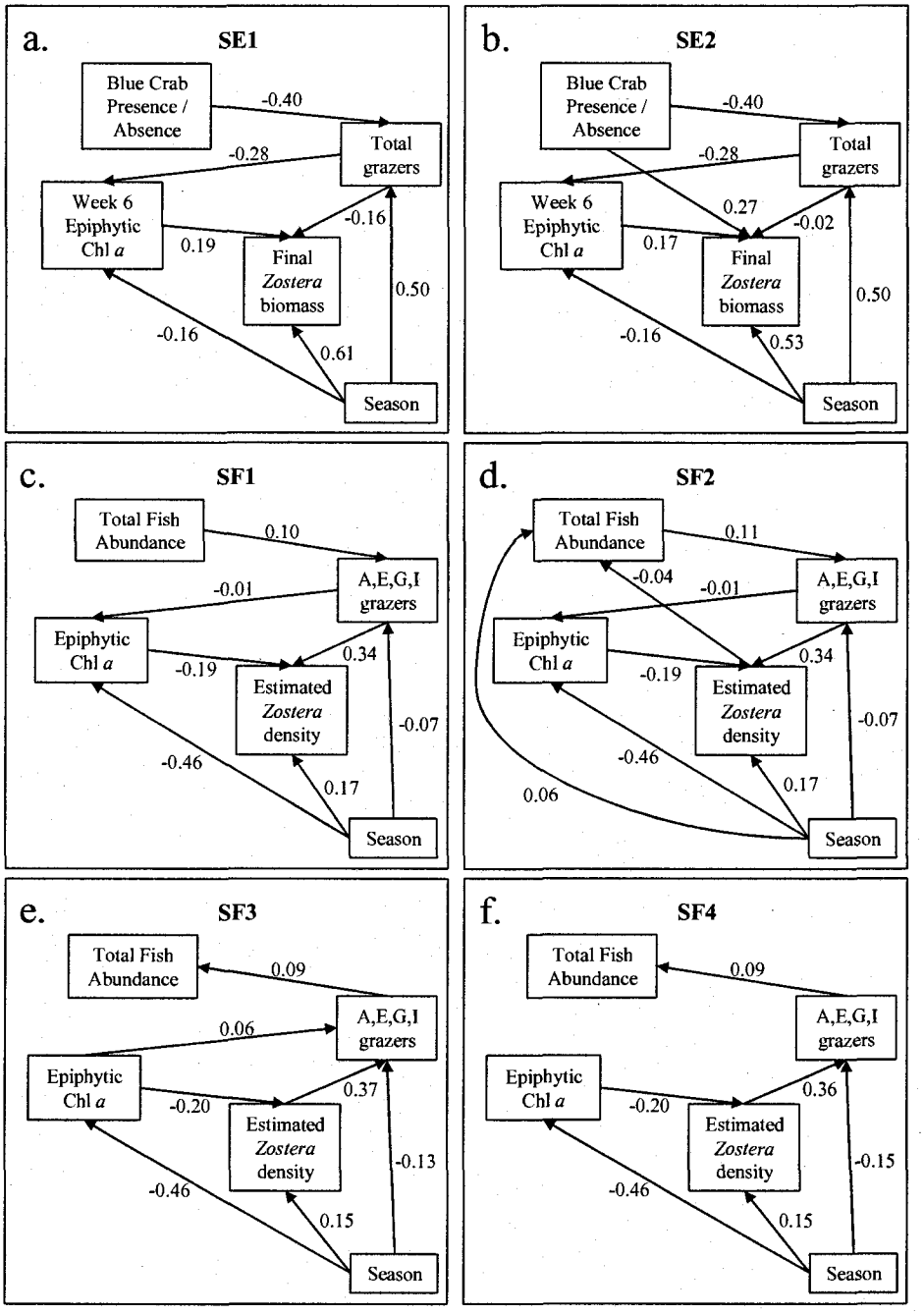
a.
SE1
Blue Crab Presence / Absence
Total grazers
Week 6 Epiphytic Chl a
Final Zostera biomass
Season
-0.40
-0.28
-0.16
0.19
0.50
-0.16
0.61
b.
SE2
Blue Crab Presence / Absence
Total grazers
Week 6 Epiphytic Chl a
Final Zostera biomass
Season
-0.40
-0.28
0.27
-0.02
0.17
0.50
-0.16
0.53
c.
SF1
Total Fish Abundance
A,E,G,I grazers
Epiphytic Chl a
Estimated Zostera density
Season
0.10
-0.01
0.34
-0.19
-0.07
-0.46
0.17
d.
SF2
Total Fish Abundance
A,E,G,I grazers
Epiphytic Chl a
Estimated Zostera density
Season
0.11
-0.04
-0.01
0.34
-0.19
-0.07
-0.46
0.17
0.06
e.
SF3
Total Fish Abundance
A,E,G,I grazers
Epiphytic Chl a
Estimated Zostera density
Season
0.09
0.06
0.37
-0.20
-0.13
-0.46
0.15
f.
SF4
Total Fish Abundance
A,E,G,I grazers
Epiphytic Chl a
Estimated Zostera density
Season
0.09
0.36
-0.20
-0.15
-0.46
0.15

Figure 3.

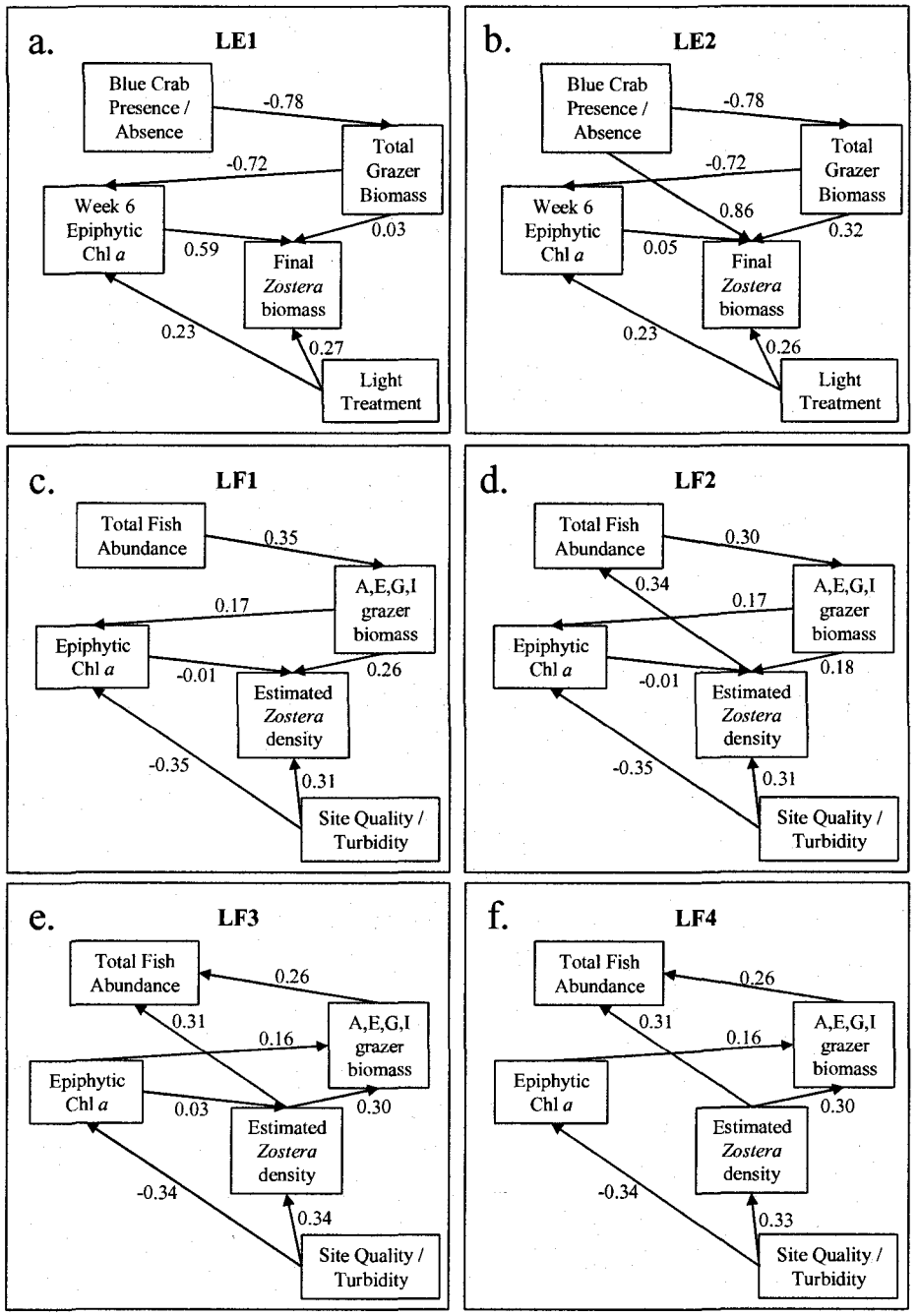
a.
LE1
Blue Crab Presence / Absence
-0.78
Total Grazer Biomass
-0.72
Week 6 Epiphytic Chl a
0.59
0.03
Final Zostera biomass
0.23
0.27
Light Treatment
b.
LE2
Blue Crab Presence / Absence
-0.78
Total Grazer Biomass
-0.72
Week 6 Epiphytic Chl a
0.86
0.05
0.32
Final Zostera biomass
0.23
0.26
Light Treatment
c.
LF1
Total Fish Abundance
0.35
A,E,G,I grazer biomass
0.17
Epiphytic Chl a
-0.01
0.26
Estimated Zostera density
-0.35
0.31
Site Quality / Turbidity
d.
LF2
Total Fish Abundance
0.30
0.34
A,E,G,I grazer biomass
0.17
Epiphytic Chl a
-0.01
0.18
Estimated Zostera density
-0.35
0.31
Site Quality / Turbidity
e.
LF3
Total Fish Abundance
0.26
0.31
A,E,G,I grazer biomass
0.16
Epiphytic Chl a
0.03
0.30
Estimated Zostera density
-0.34
0.34
Site Quality / Turbidity
f.
LF4
Total Fish Abundance
0.26
0.31
A,E,G,I grazer biomass
0.16
Epiphytic Chl a
0.30
Estimated Zostera density
-0.34
0.33
Site Quality / Turbidity

Table 1. Design of the eelgrass mesocosm experiments as used in path analyses. Each experiment manipulated predator presence (blue crabs, *Callinectes sapidus*) and a bottom-up factor (season or light level). Each experiment also manipulated mesograzer diversity, but that factor is not shown, because only the full-diversity mesograzer treatments were used in our analyses. Alphanumeric codes in cells are abbreviations for treatments, followed by the number of usable replicates from each treatment.

**2001/2002 Season * Predation Exp.**
n = 20

| | **Predation** | |
|---|---|---|
| **Season** | - Crabs | + Crabs |
| Fall 2001 | S0C0 x5 | S0C1 x5 |
| Spring 2001 | S1C0 x5 | S1C1 x5 |

**2003 Light * Predation Experiment**
n = 19

| | **Predation** | |
|---|---|---|
| **Light** | - Crabs | + Crabs |
| Shaded | L0C0 x5 | L0C1 x5 |
| Unshaded | L1C0 x5 | L1C1 x4 |

Table 2. Summary of field monitoring data used in path analyses. The "Fall Dates" and "Spring Dates" columns give the number of years for which field data corresponding with experiment dates (November 14 and June 1, respectively) are available for that site and transect position. The ordinal quality rank (OQR) is an estimate of a site's water clarity as it relates to light level and eelgrass survival, with a rank of three being assigned to marginal sites, and a rank of five assigned to the most pristine sites. The depth adjustment (DA) is a 70% reduction in the OQR applied to account for inshore-offshore differences in depth at an eelgrass site.

| Site Name | Transect Position | Location | Fall Dates | Spring Dates | Ordinal Quality Rank | Depth Adjustment | OQR * DA |
|---|---|---|---|---|---|---|---|
| Allen's Island | Inshore | 37°15'12.41"N, 76°25'39.57"W | 2 | 3 | 4 | 1.0 | 4.0 |
| Allen's Island | Offshore | 37°15'9.44"N, 76°25'38.15"W | 4 | 2 | 4 | 0.7 | 2.8 |
| Brown's Bay | Inshore | 37°18'13.77"N, 76°23'38.27"W | 1 | | 4 | 1.0 | 4.0 |
| Brown's Bay | Offshore | 37°18'11.92"N, 76°23'35.01"W | 1 | | 4 | 0.7 | 2.8 |
| Cape Charles | Inshore | 37°16'45.83"N, 76° 0'47.85"W | 1 | | 3 | 1.0 | 3.0 |
| Cape Charles | Offshore | 37°16'46.62"N, 76° 0'50.30"W | 1 | | 3 | 0.7 | 2.1 |
| Goodwin Islands | Inshore | 37°13'5.50"N, 76°23'41.23"W | 9 | 9 | 5 | 1.0 | 5.0 |
| Goodwin Islands | Offshore | 37°13'3.10"N, 76°23'37.03"W | 9 | 9 | 5 | 1.0 | 5.0 |
| Messick Point | Offshore | 37° 6'23.52"N, 76°17'47.75"W | 1 | 1 | 5 | 0.7 | 3.5 |
| New Point Comfort | Inshore | 37°18'56.93"N, 76°16'49.55"W | 2 | 1 | 5 | 1.0 | 5.0 |
| New Point Comfort | Offshore | 37°18'53.42"N, 76°16'56.44"W | 2 | 1 | 5 | 0.7 | 3.5 |
| Onancock | Inshore | 37°43'48.65"N, 75°49'29.47"W | 1 | | 4 | 1.0 | 4.0 |
| Onancock | Offshore | 37°43'46.94"N, 75°49'28.24"W | 1 | | 4 | 0.7 | 2.8 |
| Sandy Point | Offshore | 37°15'46.91"N, 76°23'15.39"W | 1 | 1 | 4 | 0.7 | 2.8 |
| VIMS Waterfront | Offshore | 37°14'55.32"N, 76°29'49.18"W | 1 | 1 | 3 | 0.7 | 2.1 |

Table 3. Path analysis fit statistics for empirical data fit to the models in figures 2 and 3. SE1 and SE2 are models run with data from the season mesocosm experiment (Figure 2a, b) and SF1 through SF4 are similar models based on field data (Figure 2c-f). LE1 and LE2 are models run with data from the light mesocosm experiment (Figure 3a, b) and LF1 through LF4 are similar models based on field data (Figure 3c-f). Columns 2-6 give model design characteristics, and columns 7-12 give model fit characteristics. See methods for explanation of fit statistics.

| | | | | | | | | | | | | $R^2$ for Endogenous Vars. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Model | Observations | Variables | Informations | Parameters | □²DF | p(□²) | NFI | NNFI | CFI | AIC | Largest Normalized Residual | Eelgrass | Epiphytes | Mesograzers | Predators |
| SE1 | 19 | 5 | 15 | 12 | 2 | 0.45 | 0.92 | 1.20 | 1.00 | -2.39 | 0.94 | 0.30 | 0.15 | 0.39 | |
| SE2 | 19 | 5 | 15 | 13 | 1 | 0.85 | 1.00 | 2.00 | 1.00 | -1.96 | 0.16 | 0.35 | 0.15 | 0.39 | |
| SF1 | 44 | 5 | 15 | 12 | 2 | 0.25 | 0.88 | 0.72 | 0.94 | -1.26 | -1.36 | 0.21 | 0.21 | 0.01 | |
| SF2 | 44 | 5 | 15 | 14 | 1 | 0.10 | 0.88 | -0.26 | 0.87 | 0.69 | -1.40 | 0.21 | 0.21 | 0.01 | 0.00 |
| SF3 | 44 | 5 | 15 | 12 | 3 | 0.41 | 0.88 | 1.03 | 1.00 | -3.13 | -1.53 | 0.09 | 0.21 | 0.13 | 0.01 |
| SF4 | 44 | 5 | 15 | 11 | 4 | 0.56 | 0.87 | 1.19 | 1.00 | -5.01 | -1.50 | 0.09 | 0.21 | 0.13 | 0.01 |
| LE1 | 19 | 5 | 15 | 11 | 3 | 0.00 | 0.64 | -0.24 | 0.63 | 15.84 | 1.28 | 0.47 | 0.56 | 0.59 | |
| LE2 | 19 | 5 | 15 | 12 | 2 | 0.00 | 0.71 | -0.56 | 0.69 | 13.88 | 1.13 | 0.50 | 0.56 | 0.59 | |
| LF1 | 17 | 5 | 15 | 11 | 3 | 0.32 | 0.68 | -0.49 | 0.55 | -2.46 | 1.16 | 0.17 | 0.15 | 0.13 | |
| LF2 | 17 | 5 | 15 | 12 | 3 | 0.63 | 0.85 | 4.48 | 1.00 | -4.26 | 1.08 | 0.16 | 0.15 | 0.12 | 0.15 |
| LF3 | 17 | 5 | 15 | 12 | 3 | 0.63 | 0.85 | 4.49 | 1.00 | -4.27 | 1.27 | 0.11 | 0.11 | 0.11 | 0.22 |
| LF4 | 17 | 5 | 15 | 11 | 4 | 0.78 | 0.84 | 5.65 | 1.00 | -6.25 | 1.23 | 0.11 | 0.11 | 0.11 | 0.22 |

# CHAPTER 5

## Food web structure in a Chesapeake Bay eelgrass bed as determined through gut contents and $^{13}C$ and $^{15}N$ isotope analysis

# ABSTRACT

Food web structure determines how biological communities respond to top-down and bottom-up perturbations, like changes in abundance of consumers and resources. For example, the structure of seagrass food webs is thought to make seagrass communities prone to shifts in the competitive balance of vascular macrophytes and algal epiphytes. Yet, trophic relationships in many seagrass systems remain poorly resolved, clouding predictions about the ultimate consequences of disturbances. We estimated the food web linkages among small predators, invertebrate mesograzers, and primary producers in a Chesapeake Bay eelgrass (*Zostera marina*) bed by analyzing gut contents and stable C and N isotope ratios of these organisms. Isotopic signatures varied widely among primary producers, but herbivores and predators grouped into relatively distinct trophic levels with respect to $\delta^{15}N$, indicating the potential for trophic cascades in this system. However, variation in $\delta^{13}C$ and gut contents among both predator and mesograzer species implied significant differences in diet within trophic levels, Pipefish consumed mostly mesograzers and copepods, other fishes consumed mesograzers and infauna, and crustacean predators consumed infauna, sessile epifauna, and plant material. Periphyton and benthic detritus formed the major portion of the diet for most mesograzers, but macrophytes were consumed by Ampithoid amphipods and by the isopod *Idotea balthica*, which also showed evidence of omnivory. These findings challenge the simple model that mesograzers have uniformly positive effects on eelgrass by epiphyte reduction, and emphasize the need for taxonomic resolution and ecological information within seagrass epifaunal communities.

# INTRODUCTION

Biological communities can be affected by changes in resource supply (bottom-up forces), and by changes in upper trophic levels (top-down forces). The manner in which such changes propagate throughout an ecosystem depends on food web structure, i.e., the direct and indirect trophic connections among species (Hunter & Price 1992, Cardinale et al. 2006). The diversity and feeding behaviors of consumers at intermediate trophic levels, namely herbivores and their predators, are especially important in determining how the effects of a perturbation are compounded or attenuated within a community (Heck et al. 2000, Duffy 2002, Douglass et al. 2008, Moksnes et al. 2008). As human activities alter both top-down and bottom-up forces in ecosystems all over the world, it is increasingly important that we understand how intermediate level consumers process these changes.

Unfortunately, the feeding ecology of middle trophic levels remains poorly resolved in many ecosystems. The lack of species-specific information about consumptive behavior is especially pronounced in some marine ecosystems, perhaps because their intermediate consumers are often small in body size, high in species diversity, not subject to direct harvest by humans, and not implicated in agricultural pest control or pollination. When describing and modeling these ecosystems, intermediate consumers have often been lumped in broadly-defined functional categories (e.g. Wetzel & Neckles 1986, Cerco & Moore 2001), which may obscure important differences in their food web connections and their other functional attributes. For example, in seagrass beds, small invertebrate grazers, hereafter "mesograzers", are generally believed to

benefit seagrass by consuming algal epiphytes (van Montfrans et al. 1984). While this pattern is supported by a number of studies (reviewed by Jernakoff et al. 1996, Hughes et al. 2004, Valentine & Duffy 2006), there is evidence of ecologically significant differences in feeding behavior among the diverse taxa of mesograzers. Some mesograzers effectively consume mostly epiphytic microalgae, while others graze directly on seagrass and have caused destruction of seagrass in cultures (Kirkman 1978, Short et al. 1995, Duffy & Harvilicz 2001). Certain mesograzer species, i.e. *Gammarus mucronatus*, have also been shown to facilitate growth of macroalgae, and others, i.e. *Idotea balthica*, have been shown to exhibit intraguild predation (Duffy & Hay 2000, Duffy et al. 2003, 2005, Spivak et al. 2007). These findings come mainly from mesocosm and laboratory experiments, however, making it difficult to assess the prevalence and importance of these diverse mesograzer trophic behaviors in the wild.

While direct consumption of seagrass in nature is regularly documented for large vertebrate and invertebrate grazers in the tropics (McGlathery 1995, Valentine & Heck 1999, Hughes et al. 2004, Valentine & Duffy 2006), it has rarely been described for small mesograzers in temperate seagrass beds (but see Nienhuis & Groenendijk 1986, Zimmerman et al. 2001). The apparent rarity of direct grazing by mesograzers in seagrass beds might reflect the real situation, but it could also be an artifact of infrequent observations. If overgrazing by mesograzers in the field is truly rare, what factors prevent it from occurring? One possibility is that top-down control normally limits mesograzer populations, with overgrazing occurring only when an unusual, perhaps anthropogenic, change in food web structure releases herbivores from predation (i.e.

Estes & Duggins 1995, Duffy et al. 2005, Valentine & Duffy 2006, Heck & Valentine 2007).

In Chesapeake Bay, mesograzers in eelgrass (*Zostera marina*) beds are consumed by a suite of small predators including demersal fishes and decapod crustaceans (Heck & Orth 1980, Orth & Heck 1980, Orth et al. 1984, Teixeira & Musick 1994). These predators vary in the strength and selectivity of their mesograzer consumption, and most do not appear to be exclusive consumers of mesograzers. For example, in seagrass beds, blue crabs, *Callinectes sapidus*, have been variously documented to prey upon infaunal bivalves (Virnstein 1977, Mansour 1992), mesograzers (Tagatz 1968, Stoner & Buchanan 1990, Hines et al. 1990), and seagrass and epiphytes (Perkins-Visser et al. 1996), while pipefish, *Syngnathus fuscus* and *S. floridae* have a stronger predilection towards mesograzer consumption (Teixeira & Musick 1994). Opportunistic feeding by predators on abundant mesograzers might help to prevent the latter from overpopulating and overgrazing, while obligate consumption would be more likely to suppress mesograzers to a level at which they could no longer control the growth of epiphytes.

We used gut contents and stable C and N isotope ratios to assess the trophic links connecting mesograzers and small predators to each other and to the primary producers and basal resource pools in a Chesapeake Bay eelgrass bed. Gut contents data provide a "snapshot" of the items consumed by an organism within the brief period prior to its collection. However, to make conclusions about the overall diet composition of a species based on gut contents alone requires extensive sampling, given that the various food sources utilized by an organism are often spatially and temporally heterogeneous and unlikely to be equally represented in the gut of an individual at any one point in time.

Analysis of the relative abundance of stable isotope biomarkers in organismal tissues provides a complementary approach to quantifying trophic connections, which give a less specific but more spatially and temporally integrated picture of diet.

The most commonly used stable isotope biomarkers for food web studies are $^{13}C$ and $^{15}N$. The former is expressed as a units per mil deviation, $\delta^{13}C$, which is the ratio of $^{13}C$ to $^{12}C$ in a sample minus the ratio of $^{13}C$ to $^{12}C$ in a standard material (Pee Dee Belemnite), divided by the ratio in the standard and multiplied by 1000. The latter is also expressed as a units per mil deviation, $\delta^{15}N$, which is the ratio of $^{15}N$ to $^{14}N$ in a sample minus the ratio of $^{15}N$ to $^{14}C$ in a standard material (air), divided by the ratio in the standard and multiplied by 1000. The first step in a stable isotope study of trophic linkages is to identify the $\delta^{13}C$ and $\delta^{15}N$ values of resources at the base of the food web which can then be matched to the isotopic signatures of consumers to identify the food resources utilized by those consumers, after the appropriate corrections for trophic fractionation are applied (Vander Zanden & Rasmussen 1999, Post 2002, Fry 2006). Carbon isotope ratios tend to be enriched by approximately +1.0‰ $\delta^{13}C$ with successive trophic levels, while the nitrogen isotopic ratio is enriched by approximately $3 \pm 1$‰ with each trophic step (Peterson and Fry 1987, Currin et al. 1995, Fry 2006). The relative contributions of multiple food resources to a consumer can be determined as long as the isotope ratios of food items differ significantly, and the number of possible food items exceeds the number of isotope biomarkers by no more than one (Phillips 2001). For example, the proportions of epiphytes, eelgrass, and macroalgae assimilated by a particular mesograzer could be calculated with data for $\delta^{13}C$ and $\delta^{15}N$. However, if there are more potential resources than there are isotopic biomarkers, then there may be more

than one proportional combination of resources that could result in the isotopic signatures observed in the consumer. In this case it is not possible to unambiguously determine diet composition, but a distribution of possible compositions can be calculated using specialized software and reported along with error or range estimates (Phillips & Gregg 2003).

There are many potential basal food sources in lower Chesapeake Bay eelgrass beds, including eelgrass, periphyton (operationally defined as the amorphous film of microalgae and other organic material covering eelgrass blades), macroalgae, and surficial sediments (including benthic microalgae and detrital material from various sources). $\delta^{13}C$ and $\delta^{15}N$ values for these food sources in other estuaries have been shown to differ appreciably (Thayer et al. 1978, Currin et al. 1995, Riera et al. 1999, Cloern et al. 2002, Orth & Canuel unpub. data). Phytoplankton is also a potential food source in the system, but was not directly measured in this study. Based on previous studies of mesograzer feeding (Kitting 1984, van Montfrans et al. 1984, Jernakoff et al. 1996) we hypothesized that periphyton, detritus, and macroalgae would be the primary food sources for most species of mesograzers, with eelgrass making a lesser contribution. This hypothesis was supported by the results of $\delta^{13}C$ isotope analyses in a North Carolina, USA eelgrass bed (Thayer et al. 1978). Also, we expected pipefish to have gut contents and isotope ratios reflecting a mesograzer diet (Teixeira & Musick, 1994), while we expected other fishes and predatory crustaceans to show a more varied diet. In particular, we thought that the reportedly omnivorous grass shrimp *Palaemonetes* sp., might show evidence of being at a lower trophic level than the other predators (Nelson 1979).

# METHODS

**Study Location**- Samples were collected in an area of perennially dense eelgrass at the Goodwin Islands National Estuarine Research Reserve, located near the mouth of the York River estuary in Chesapeake Bay (Virginia, USA, 37° 13' N; 76° 23' W). Goodwin Islands is a 315 hectare archipelago of salt-marsh islands surrounded by intertidal flats and subtidal seagrass beds (*Zostera marina* and *Ruppia maritima*) extending to a maximum of about 1 m mean-low-water depth. Salinity averages around 20 ppt, but ranges from 10 - 27 ppt. Mean annual water temperature is 17 degrees C, but ranges from zero to more than 31 degrees C in some years (Douglass Ch. 3). Water column $[NO_x]$ and $[NH_4^+]$ average 1.04 μm $L^{-1}$ and 0.65 μm $L^{-1}$, respectively, but range from below detection limits to over 20 μm $L^{-1}$ and 10 μm $L^{-1}$, respectively. Water column phytoplankton concentrations average 13 μg chl *a* $L^{-1}$ but can reach as high as 91 μg chl *a* $L^{-1}$ during blooms, and algal epiphyte densities on eelgrass blades average 2.5 μg chl *a* $cm^{-2}$ but can reach 19 μg chl *a* $cm^{-2}$ during late summer peaks (Douglass Ch. 3). Macroalgal epiphytes and drift algae of various species are occasionally abundant. The area is closed to development and destructive use, but remains open to commercial and recreational fishing.

**Sample Collections**- Collections for gut contents and stable isotope analyses were made on 21 April 2005, 19 May 2006, and 21 August 2006. Primary producers and hypothesized basal resource pools sampled for $\delta^{13}C$ and $\delta^{15}N$ analysis included surficial sediment (the surface layer down to approximately 1 cm depth), live eelgrass shoots, all

abundant macroalgae species, and periphyton (the matrix of microalgal epiphytes, sediment, and other organic material adhered to eelgrass shoots). Periphyton was scraped from shoots in the lab immediately following field collections. For April 2005, periphyton on shoots was too scarce for analysis, so periphyton and eelgrass were obtained from frozen samples taken in March 2005. Phytoplankton were not sampled, but $\delta^{13}C$ and $\delta^{15}N$ values for this basal resource group were indirectly estimated by back-calculating from the signatures of sessile filter feeders (see Canuel et al. 1995 for a similar approach using other biomarkers). The back-calculated values were within the range of $\delta^{13}C$ values reported for estuarine phytoplankton (Chanton & Lewis 1999). Mobile epifauna were collected with a dip net and quickly placed on ice or in 10% formalin. For April 2005 samples, organisms for gut contents analysis were preserved in formalin, while organisms and materials for isotopic analysis were put on ice, then frozen. Preliminary analyses determined that the quality of gut contents was similar for frozen and formalin-preserved organisms, so May and August 2006 samples were frozen regardless of their future fate. The number of species and individuals sampled at each date was dictated by their abundance at the time of sampling. some species were not present in all months, or were represented only by one or a few individuals (Tables 1, 2).

**Gut contents analysis-** Fish stomachs were dissected according to the procedures of Teixeira & Musick (1994) and blotted on a petri dish under a dissecting microscope at 16x magnification. Identifiable prey individuals were counted and the percent cover of all items in the blot, including mineral grains and organic "unidentified material", was estimated visually by a single observer. Other techniques exist for quantifying fish gut contents (Teixeira & Musick 1994) but the percent cover method was used because it was

convenient, it worked well for the small organisms examined, and it could be applied to all predator and mesograzer taxa. Crab stomach contents were also dissected and quantified at 16x. Shrimp stomachs were too small for analysis under the dissecting scope, and were therefore blotted on a glass slide and examined under a compound microscope at 100x according to the same procedure that was used for mesograzer guts (Kitting 1984).

**Isotope sample preparation and analysis-** Organisms and materials for isotope analysis were cleaned with deionized water, if appropriate, and dried for five days at 60 degrees C. They were then homogenized with a mortar and pestle, acidified with hydrochloric acid (0.1 N), and dried for several more days at 60 degrees C. For samples of mesograzers, and the shrimps *Palaemonetes vulgaris* and *Crangon septemspinosa*, multiple individuals were pooled and homogenized. The larger predators were analyzed as separate individuals, except for the April 2005 sample in which three *Syngnathus fuscus* between 100 and 120 mm in length were pooled. One to three procedural replicates from each homogenized sample were weighed, packed into tin capsules, and shipped to the University of California Davis Stable Isotope Facility for dual $\delta^{13}C$ and $\delta^{15}N$ analyses. The analyses were performed using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). We report isotopic composition as $\delta$ values (units per mil) based on the following formula: $\delta = (R_{sample}/R_{standard} - 1)*1000$. $R_{sample}$ is the raw ratio of heavy to light isotopes in a sample, and $R_{standard}$ is the raw ratio of heavy to light isotopes in a standard reference material. For $\delta^{13}C$, $R_{standard}$ was the isotopic ratio of $^{13}C/^{12}C$ in the

fossil carbonate rock "Pee Dee Belemnite", and for $\delta^{15}N$, $R_{standard}$ was the isotopic ratio of $^{15}N/^{14}N$ in the earth's atmosphere.

**Statistical analyses-** Mean and standard error of $\delta^{13}C$ and $\delta^{15}N$ within procedural replicates were calculated for each item analyzed, and were graphed on dual isotope plots (Figs. 1-3). Between one and five possible food items were inferred for each consumer based on gut contents, the ecological literature, and the items' position relative to the consumer on the dual isotope plot (filled cells in Table 3). The relative percent composition of each candidate food item in a consumer's diet was estimated using mixing equations that assumed isotopic fractionations of +1 $\delta^{13}C$ and +3 $\delta^{15}N$ per trophic level (Fry 2006). If there were only two candidate food items a weighted-distance estimate of diet composition was made with $\delta^{13}C$ values (Table 3, estimation method 1). The formulae used for this estimation method were: $F1 = (C_{org} - C_{F2})/(C_{F1} - C_{F2})$, and $F2 = 1 - F1$, where F1 and F2 are the proportional contributions to the organism's diet of food types 1 and 2, respectively, $C_{org}$ is the $\delta^{13}C$ value of the organism, and $C_{F1}$ and $C_{F2}$ are the $\delta^{13}C$ values of food types 1 and 2, respectively, corrected for isotopic enrichment. Diet mixing between three candidate food sources was calculated in one of two ways. When a single solution was possible, a linear mixing model based on both $\delta^{13}C$ and $\delta^{15}N$ was used (Tables 3, estimation method 2) (Phillips 2001). The formulae used for this estimation were: $F1 = [(N_{F3} - N_{F2})(C_{org} - C_{F2}) - (C_{F3} - C_{F2})(N_{org} - N_{F2})]/[(N_{F3} - N_{F2})(C_{F1} - C_{F2}) - (C_{F3} - C_{F2})(NF1 - N_{F2})]$, $F2 = [(C_{org} - C_{F3}) - (C_{F2} - C_{F3})(F1)]/(C_{F2} - C_{F3})$, $F3 = 1 - F1 - F2$, where F1, F2, and F3 are proportional contributions of each food source 1 - 3, $C_{org}$, $C_{F1}$, $C_{F2}$, and $C_{F3}$ are the $\delta^{13}C$ values of the organism and each food source, and $N_{org}$, $N_{F1}$, $N_{F2}$, and $N_{F3}$ are the $\delta^{15}N$ values of the organism and each food source. When

no unique solution was possible, either because the fractionation-corrected signatures of the food items did not form a triangle enclosing the consumer in $\delta^{13}C$ - $\delta^{15}N$ space, or because there were more than three candidate food items, a distribution of possible mixing solutions was calculated with IsoSource (Phillips & Gregg 2003) (Table 3, estimation method 3). For each candidate food item assessed in IsoSource we reported mean $\pm$ SD percent diet composition and also the tolerance for the estimate. Tolerance is the percent uncertainty value incorporated in the mixing estimates. We chose successively higher tolerance values of 0.05, 0.1, 0.5, 1, 2, or 3 until the tolerance was high enough for the IsoSource program to calculate solutions to a particular mixing problem. Estimates with high tolerance tend to make less distinction between food sources, assigning a similar diet contribution to each candidate food item.

For gut contents data, mean $\pm$ SEM percent composition for each food item was calculated for each consumer species for each sample date (Tables 1, 2). Blue crabs, *Callinectes sapidus*, were divided into three size classes based on carapace width, which were treated as separate consumer species. Crabs < 20 mm, between 20 and 40 mm, and > 40 mm carapace width formed size classes one, two and three, respectively.

All statistics were performed in MS Excel and IsoSource.

## RESULTS

**Gut contents-** Mesograzer guts contained mostly amorphous material and sediment, with varying amounts of other materials, including multicellular plants, single-

celled algae, and masticated crustacean parts (Table 1). The guts of ampithoid amphipods *Ampithoe longimana*, *A. valida*, and *Cymadusa compta* had the highest proportions of macrophytes among the mesograzers, with *A. valida* in May 2006 appearing to consume almost entirely multicellular plant material. The only mesograzer that clearly consumed significant amounts of eelgrass was the isopod *Idotea balthica*, in which an average of 27% of gut contents from the sampled individuals in April 2005 were coarse chunks of *Zostera marina*. It could not be positively determined whether this had been live or detrital eelgrass at the time of consumption, but intact cellular structure relative to eelgrass detritus suggests that it was likely live or standing dead material (pers. obs.). The most abundant mesograzer at Goodwin Islands, the caprellid amphipod *Caprella penantis*, did not appear to consume macrophytes, but its gut contents contained unidentified material, sediment, and microalgae in proportions similar to those in the gammaridean amphipod mesograzers (Table 1). Three mesograzer species, *I. balthica*, the amphipod *Elasmopus levis*, and the decapod shrimp *Hippolyte pleuracantha*, had some crustacean exoskeletal material in their guts, indicating possible intra-guild predation or zooplanktivory. Only for *I. balthica*, however, was a significant portion of crustacean material found in multiple individuals.

Predator gut contents varied among species, among size classes within species, and among dates sampled (Table 2). Blue crabs, *Callinectes sapidus*, in size class one (less than 20 mm carapace width) had diets similar to mesograzers, with guts largely full of sediment, unidentifiable material, and macrophytes. However, they also included a few amphipods, crustacean parts, and polychaetes, and the single small blue crab examined in May 2006 had barnacle plates and cirri in its gut. Blue crabs in size class

two had a greater proportion of crustacean parts in their guts, but this material appeared to come from other blue crabs or from barnacles rather than from mesograzers. Only five blue crabs in size class 3 were examined, and none contained mesograzers. Barnacles were the dominant component of blue crab diet in May 2006, suggesting opportunistic feeding on the heavy recruitment of barnacles to the eelgrass beds at that time (pers. obs.). In August 2006, one medium and one large crab each had a small fish in its stomach. *Palaemonetes vulgaris* and *Crangon septemspinosa* shrimp were collected in large numbers, but with the exception of the *P. vulgaris* samples taken in August 2006, most individuals had empty guts at the time of dissection. The *C. septemspinosa* individuals that had full guts often contained polychaetes, along with a significant portion of sediment and unidentifiable material. *Palaemonetes vulgaris* guts contained mostly sediment and amorphous material, but also showed evidence of consumption of several taxa of epifaunal crustaceans (Table 2). A single *Penaeus aztecus* shrimp (rare in the Goodwin Islands eelgrass bed) had a gut full of polychaete segments. Relative to the shrimp and crabs, the small predatory fishes had lower proportions of sediment and plant material in their guts, and higher proportions of small crustaceans. With the exception of the naked goby, *Gobiesoma bosci*, which appeared to consume primarily polychaetes, mesograzers were the main component of fish gut contents. The dusky pipefish *Syngnathus fuscus*, the longnose pipefish, *Syngnathus floridae*, and the skilletfish *Gobiesox strumosus* could be distinguished by the other crustacean types they consumed in addition to mesograzers. *S. fuscus* preyed heavily on copepods, *S. floridae* consumed juvenile shrimp (though this was only seen in one individual with 17 *Palaemonetes sp.* individuals in its gut), and *G. strumosus* ate ostracods.

**Isotopic signatures-** The $\delta^{13}C$ signatures of the primary producers and other basal resource pools examined at Goodwin Islands varied appreciably; usually by at least 1-2 units per mil (‰), satisfying a prerequisite for determination of consumers' food sources (Figs. 1, 2, 3). *Zostera marina* tended to be more enriched in $^{13}C$ than other producers and basal food sources, with $\delta^{13}C$ around -10‰, versus approximately -18‰ for surficial sediment and -21‰ estimated for phytoplankton (Figs. 1-3). However, periphyton had a similarly enriched signature in May 2006 (-9.3‰), however, and the green macroalga *Ulva* sp. had a more enriched $\delta^{13}C$ signature in August 2006 (-6.8‰). $\delta^{15}N$ signatures for periphyton, *Z. marina*, and surficial sediment, were usually more depleted than those of the mesograzers by 1-3‰, as would be predicted if they were mesograzer food sources (Figs. 1-3). The macroalgae *Cladophora* sp., *Gracilaria* sp., and *Ulva* sp., tended to be enriched in $\delta^{15}N$ at the same or even a higher level than the mesograzers ($\delta^{15}N$ 9-12‰). In May and August, some of the macroalgae had $\delta^{15}N$ signatures even higher than those of the predators (Figs. 2, 3).

The isopod *Erichsonella attenuata* was enriched in $\delta^{13}C$ relative to the other mesograzers, with signatures similar to *Z. marina*, between -8 and -12‰. However, most of the other consumer $\delta^{13}C$ signatures were more depleted than *Z. marina* and periphyton, suggesting that these animals assimilated much of their carbon from sources with depleted $\delta^{13}C$, such as surficial sediments or phytoplankton from the water column. Depleted $\delta^{13}C$ signatures of known filter-feeding organisms; the tunicate *Botryllus schlosseri* in April 2005, the hydroid *Hydractinia* sp. in May 2006, and the sponge in August 2006, support our estimated positioning of phytoplankton as the most $\delta^{13}C$ depleted food source in each month (Figs. 1-3).

The big, $\delta^{13}C$ standard error bar for *Bittium varium* snails in May 2006 (Fig. 2) is probably due to incomplete acidification of some parts of the sample. Calcium carbonate in shells is enriched in $\delta^{13}C$ because dissolved carbonate has $\delta^{13}C$ around 0 ppt. Thus, unreacted bits of shell would make the $\delta^{13}C$ of *B. varium* appear more positive.

The range in isotopic signatures among predator species ($\delta^{13}C$ -21 to -16 , $\delta^{15}N$ 10 to 15) was less than that among basal food sources ($\delta^{13}C$ -22 to -6 , $\delta^{15}N$ 6 to 18) and herbivore species ($\delta^{13}C$ -20 to -7 , $\delta^{15}N$ 7 to 11). Predators were consistently clustered together for each sample about 3 ‰ $\delta^{15}N$ above the mesograzers, and most similar to *Caprella penantis* and the ampithoid amphipods with respect to $\delta^{13}C$ values. The only predator that deviated from this pattern was a small (20 mm) blue crab in August 2006, which appeared to be at a lower trophic level closer to the mesograzers.

**Diet composition estimates**- Estimates based on combined data from gut contents and stable isotopes suggest that surficial sediment figured strongly in the diets of most mesograzers, with the exception of *Erichsonella attenuata* (Table 3). This suggests that benthic microalgae and detritus, whether on the bottom, resuspended, or loosely associated with eelgrass blades, complement firmly attached epiphytes as a main source of mesograzer nutrition. Aside from the high weight given to surficial sediment and periphyton in the estimates for mesograzer diet, macroalgae appeared to be a important components in April 2005, particularly for *Cymadusa compta* and other ampithoid amphipods, which have often been documented feeding upon and living in association with macroalgae (Duffy 1990, Duffy & Hay 2000) (Table 3). *Zostera marina* was an apparently important component in the diet of some mesograzers, particularly in August 2006 when it was the only food web component (of those we analyzed) that had a low

enough $\delta^{15}N$ signature to match the mesograzer signatures after trophic enrichment. Sessile filter feeders, represented by *Botryllus schlosseri*, *Hydractinia sp.* hydroids, and sponge for April 2005, May 2006, and August 2006, respectively, appeared important in the diet of predators. It is very unlikely, based on the gut contents evidence, that these animals were actually consumed by the predators, so this result may indicate that zooplankton or infauna, which were not analyzed but which also feed on phytoplankton or phytodetritus, were consumed by the predators. This is inference is supported by the gut contents data for *Syngnathus* spp., which include a sizeable fraction of copepods. Other items of apparent importance for predators were the mesograzers *Caprella penantis* and *Gammarus mucronatus* in spring, and ampithoid amphipods and *Erichsonella attenuata* in August.

## DISCUSSION

Together, our isotope and gut contents analyses paint a picture of an upwardly narrowing eelgrass food web, which channels energy and materials from a broad spectrum of basal food sources, into a more integrative guild of primary consumers, and finally into a coherent group of secondary consumers. Although the isotope analyses suggest a sizeable contribution of eelgrass itself to the diets of mesograzers, especially in August 2006 (Table 3), this finding is not borne out by the gut contents analyses, which suggest that only a few species consumed detectable amounts of eelgrass (Table 1). Thus, eelgrass itself does not appear to be a major food resource for most of the primary

consumers we examined. Rather, our results support the view of the Goodwin Island eelgrass bed as a "seagrass detrital ecosystem" (Valentine & Duffy 2006) in which macrophytes provide structure and some trophic support, but detritus and microalgae on eelgrass blades, in the sediment, and in the water column are more important as food for primary consumers.

Some caveats to our interpretations merit discussion. Small sample sizes and limited spatial and temporal extent of sampling may misrepresent the apparent importance of what could actually be rare or season-dependent trophic relationships. For example, the predominance of barnacles in the gut contents of blue crabs in May 2006 should not be interpreted as typical, because barnacles are rare in the Goodwin Islands seagrass bed except during occasional high-recruitment events (pers. obs.). On the other hand, this may be fortuitous evidence that crabs can have a big effect on community organization by wiping out episodic barnacle recruitments. Conversely, the abundance of *Palaemonetes* sp. shrimp that we found in the gut of one *Syngnathus floridae* pipefish might have been wrongly deemed atypical, if not for the extensive documentation of *S. floridae* feeding on shrimp by Teixeira & Musick (1994). Aside from these issues of low replication, the well-known limitations of gut contents analysis could also affect our results. With mesograzers, for example, it was impossible to determine if the amorphous detritus in guts was a result of indiscriminate feeding on detritus, or if fine mastication and rapid digestion obscured the structure of ingested macrophytes and algal cells. The similar gut contents yet widely different carbon isotope ratios of *Gammarus mucronatus* and *Erichsonella attenuata* (Table 1, Figs 1, 2, 3) suggest that feeding is more selective than indicated by gut contents alone. In the future it would be useful to do gut contents

analysis in conjunction with feeding assays (i.e. Zimmerman 1979) to determine what types of consumed materials are or are not distinguishable in guts.

Limitations to the isotopic analysis methods are obvious, as well, most notably the incomplete representation of all possible resource pools. For example, we did not sample phytoplankton, zooplankton, or infauna, and we did not separate microalgae from other types of organic material in our bulk samples of sediments and periphyton. Expanding the scope and the level of detail of sampling to include these resources in future studies would help to account for the connections between the eelgrass epifaunal food web and the pelagic and soft-bottom benthic food webs with which it is associated (Williams & Heck 2001). With so many potential food sources, however, it might be difficult to resolve ambiguities in diet composition. Here, the use of additional biomarkers, such as polyunsaturated fatty acids (Canuel et al. 2007, Spivak et al. 2007) and / or naturally-occurring isotopes of sulfur, might be of assistance to future researchers (Fry 2006). Another point to note regarding stable isotopes is that quantitative diet composition estimates of the sort we have made in table 3 are susceptible to influence not only from incomplete selection of candidate food sources, but also inaccurate measurements, inexact trophic fractionation factors, and disproportionate assimilation of C and N from different food sources (Phillips & Gregg 2003, Fry 2006).

Keeping these caveats in mind, there were nevertheless some interesting and surprising trends in our data, including the differences seen between small and large *Callinectes sapidus* in their gut contents and isotope ratios. Our results support earlier studies finding that *C. sapidus*, though they are omnivorous and opportunistic at all life stages, undergo marked ontogenetic diet shifts (Tagatz 1968, Mansour 1992). Like

Tagatz (1968) and Stoner & Buchanan (1990) we found that *C. sapidus* between 20 and 40 mm carapace width had a greater proportion of mesograzers in their diets than did larger and smaller crabs (Fig. 3, Table 3). No size group of *C. sapidus* preyed as strongly and selectively on mesograzers as did fishes, however. *Syngnathus* spp. pipefish consumed almost exclusively amphipods and isopods, except in April 2005 when copepods made up a modest fraction of pipefish diet. A single *S. fuscus* individual collected in April 2005 had 108 intact amphipods in its gut; another had 97. If these gut contents pass quickly and pipefish are abundant, i.e. population feeding rate is high, it follows that fishes could have strong top-down demographic impacts on the mesograzer community in this system. However, such impacts have thus far been difficult to demonstrate outside of mesocosm experiments (Chs. 2, 3, 4).

A surprising characteristic of the isotope data from May and August 2006 was the high $^{15}N$ isotope ratios in some macroalgal taxa, especially *Gracilaria* sp. in May 2006 ($\delta^{15}N$ 16.4‰, Fig. 2). These opportunistic macroalgae probably exploited a different, more enriched, nitrogen source than did the other photoautotrophs in the system. The enriched nitrogen may have come from intense microbial processing and recycling of organic matter, such as occurs during sewage treatment (Anderson & Cabana 2005). Plants exploiting nitrogen from enriched sources tend to have high tissue $\delta^{15}N$ values reflecting the source (Evans 2001, Robinson 2001). It is unclear whether the macroalgae in our samples obtained their enriched nitrogen from a source within the Goodwin Islands eelgrass bed, or whether they grew in another environment then drifted into the eelgrass bed.

The overall goal of this study was to characterize the middle trophic level connections in an eelgrass food web, in order to better understand and predict the influence of top-down and bottom-up forces on the system. Though our data are by no means complete, they can help us make more refined estimates of the likely consequences of changes in the food web. Taking a bottom-up example, a decrease in the production of periphyton or of palatable material on the sediment surface could reduce mesograzer secondary production and, in turn, the production of predators. This would be consistent with negative influences of turbidity on mesograzers reported for this system (Douglass Ch. 3). However, such a change might also favor mesograzers like *Idotea balthica*, which are able to consume eelgrass itself (Table 1). From a top-down perspective, a reduction in the abundance of juvenile blue crabs via overharvesting of adults (Lipcius & Stockhausen 2002) might have comparatively minor effects on mesograzer abundance. On the other hand, an increase in the abundance of some predator that preyed effectively on pipefish might increase mesograzers by releasing them from predation. Correlations in abundance of pipefish and mesograzers in the field tend to be positive, however, (Douglass Ch. 3), suggesting that the actual ecological impact of pipefish predation may be weak compared to bottom-up controls on mesograzers. Also, a missing link in our ability to draw these top-down scenarios is a firm establishment of the connections between the small predators in this study and larger, commercially harvested species (i.e. *Morone saxatilis*, *Cynoscion nebulosus*, etc.) in the region. Making this connection would be a useful aim for future research efforts.

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Table 1. Gut contents of mesograzers sampled at Goodwin Islands on 21 April 2005, 19 May 2006, and 21 August 2006. Values in cells are mean and SEM (in parentheses) percent cover of each type of item or material as observed in a blot of total gut contents on a microscope slide at 100x magnification. The "macrophytes" category includes multicellular plant or algal material that could not be positively identified as *Zostera marina* or macroalgae.

| Sample Date | Species | n | Unidentified Material | Sediment | *Zostera marina* | Macrophyte | Macroalgae | Diatoms | Dinoflagellates | Unidentified protists | Crustacean parts |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 21-Apr-05 | *Ampithoe longimana* | 3 | 58 (3.3) | 32 (1.7) | | | 3 (2.7) | 3 (1.7) | 4 (3.1) | | |
| 19-May-06 | *Ampithoe longimana* | 9 | 57 (2.6) | 34 (4.4) | | 8 (3.3) | 1 (0.6) | 0 (0.2) | | | |
| 21-Aug-06 | *Ampithoe longimana* | 3 | 56 (4.5) | 25 (5.8) | | 18 (8.3) | | 0 (0.3) | | | |
| 21-Apr-05 | *Ampithoe valida* | 1 | 60 | 40 | | | | | | | |
| 19-May-06 | *Ampithoe valida* | 8 | 14 (3.2) | 8 (2.5) | | 78 (4.4) | | | | | |
| 21-Apr-05 | *Caprella penantis* | 9 | 79 (3.5) | 13 (2.5) | | | | 1 (0.6) | 1 (0.7) | 6, 2.4 | |
| 19-May-06 | *Caprella penantis* | 11 | 69 (1.6) | 31 (1.6) | | | | | 0 (0.1) | | |
| 21-Apr-05 | *Cymadusa compta* | 6 | 58 (4.2) | 30 (4.7) | 1 (0.8) | | 3 (1.9) | 1 (1.2) | 6 (2.2) | | |
| 19-May-06 | *Cymadusa compta* | 1 | 35 | 5 | | | 60 | | | | |
| 21-Aug-06 | *Cymadusa compta* | 9 | 63 (5.2) | 36 (5) | | 2 (1.7) | | | | | |
| 21-Aug-06 | *Elasmopus levis* | 3 | 53 (8.8) | 23 (6.7) | | 18 (10.1) | | | | | 5 (5.0) |
| 21-Apr-05 | *Erichsonella attenuata* | 10 | 58 (2.5) | 31 (2.3) | 1 (0.5) | | 1 (0.5) | 2 (2.0) | 7 (2.0) | | |
| 21-Aug-06 | *Erichsonella attenuata* | 12 | 87 (2.3) | 9 (2.1) | | | 4 (1.5) | | | | |
| 21-Apr-05 | *Gammarus mucronatus* | 10 | 72 (3.8) | 27 (4) | 1 (0.5) | | | | 2 (0.5) | | |
| 19-May-06 | *Gammarus mucronatus* | 11 | 67 (2.6) | 32 (2.7) | | 0 (0.5) | | | 1 (0.5) | | |
| 19-May-06 | *Hippolyte pleuracantha* | 1 | 55 | 45 | | | | | | | |
| 21-Aug-06 | *Hippolyte pleuracantha* | 10 | 69 (3.5) | 29 (3.7) | | | | | | | 2 (2.0) |
| 21-Apr-05 | *Idotea balthica* | 10 | 43 (9.1) | 5 (1.1) | 27 (13) | | 1 (1.0) | | 1 (0.5) | | 23 (7.4) |
| 19-May-06 | *Idotea balthica* | 1 | 90 | 10 | | | | | | | |

Table 2. Gut contents of small predators sampled at Goodwin Islands on 21 April 2005, 19 May 2006, and 21 August 2006. *Callinectes sapidus* 1, 2, and 3 are crabs of carapace widths < 20 mm, 20-40 mm, and > 40 mm, respectively. Values in cells are mean and SEM (in parentheses) percent cover of each type of item or material as observed in a blot of total gut contents on a glass dish at 16x magnification.

| Sample Date | Species | n | Unidentified Material | Sediment | *Zostera marina* | Macroalgae | *Cladophora* sp. | Diatoms | Copepoda | Ostracoda | Gammaridean amphipods | Caprellid amphipods | Isopods | *Palaemonetes* spp. | Crustacean parts | Polychaetes | Barnacles | Bivalves | Fish |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 19-May-06 | *Callinectes sapidus 1* | 1 | 75 | 20 | | | | | | | | | | | | | 15 | | |
| 21-Aug-06 | *Callinectes sapidus 1* | 12 | 56 (5.3) | 38 (4.5) | 1 (0.9) | | 0 (0.4) | | | | 2 (1.7) | | | | 1 (1.3) | 1 (0.9) | | | |
| 21-Apr-05 | *Callinectes sapidus 2* | 1 | | 10 | | | | | | | | | | | | 90 | | | |
| 19-May-06 | *Callinectes sapidus 2* | 8 | 17 (1.9) | 8 (3.0) | 2 (1.0) | 3 (2.5) | | | | | | | | | 5 (2.5) | | 65 (3.5) | 2 (1.4) | |
| 21-Aug-06 | *Callinectes sapidus 2* | 25 | 47 (3.7) | 29 (3.0) | 7 (2.4) | | 6 (1.9) | | | 1 (0.3) | 2 (1.6) | | 1 (1.0) | | 1 (0.7) | 0 (0.4) | 1 (0.8) | 0 (0.4) | 1 (1.0) |
| 19-May-06 | *Callinectes sapidus 3* | 2 | 20 | 13 (2.5) | 3 (2.5) | | | | | | | | | | | | 60 (10) | 5 (5.0) | |
| 21-Aug-06 | *Callinectes sapidus 3* | 3 | 37 (10) | 18 (16) | 26 (13) | | | | | | | | | | 11 (3.0) | | | 3 (3.3) | 3 (3.3) |
| 21-Apr-05 | *Crangon septemspinosa* | 5 | 32 (10) | 24 (15) | | 8 (8.0) | | | | | | | | | | 36 (19) | | | |
| 19-May-06 | *Crangon septemspinosa* | 1 | 60 | 40 | | | | | | | | | | | | | | | |
| 21-Aug-06 | *Gobiesoma bosci* | 10 | 52 (6.3) | 11 (3.6) | | | | | | | 3 (3.0) | 3 (2.0) | 1 (1.0) | | | 29 (5.4) | | | |
| 21-Aug-06 | *Gobiesox strumosus* | 2 | 45 (5.0) | | | | | | 10 (10) | 18 (2.5) | 28 (7.5) | | | | | | | | |
| 21-Apr-05 | *Palaemonetes vulgaris* | 1 | 10 | | | | | | | 90 | | | | | | | | | |
| 19-May-06 | *Palaemonetes vulgaris* | 5 | 73 (17) | 3 (1.9) | | | | | | | 16 (16) | | | | 8 (8.0) | | | | |
| 21-Aug-06 | *Palaemonetes vulgaris* | 17 | 62 (2.7) | 26 (2.7) | | | | 4 (1.3) | | 2 (2.4) | | | 0 (0.4) | | 5 (2.5) | | | | |
| 21-Aug-06 | *Penaeus aztecus* | 1 | 15 | 15 | | | | | | | | | | | | 70 | | | |
| 19-May-06 | *Sygnathus floridae* | 1 | 20 | | | | | | | | 20 | | | 60 | | | | | |
| 21-Aug-06 | *Sygnathus floridae* | 4 | 53 (4.8) | | | | | | | | 36 (5.5) | 5 (2.9) | 4 (3.8) | | | | | | |
| 21-Apr-05 | *Sygnathus fuscus* | 13 | 30 (3.6) | | | | | | 26 (5.5) | 0 (0.1) | 38 (5.5) | 0 (0.4) | | | 2 (2.3) | | | | |
| 19-May-06 | *Sygnathus fuscus* | 8 | 38 (3.8) | | | | | | 0 (0.3) | | 60 (3.8) | 1 (0.6) | | 1 (0.6) | | | | | |
| 21-Aug-06 | *Sygnathus fuscus* | 6 | 58 (7.5) | | | | 0 (0.2) | | 9 (6.6) | 0 (0.2) | 26 (4.5) | 1 (0.8) | 4 (2.7) | | | | | | |

Table 3- Diet composition estimates [mean % composition] for consumers based on $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures from organisms and resource pools at the Goodwin Islands eelgrass bed. Estimates assumed a fractionation of +1 $\delta^{13}C$ and +3 $\delta^{15}N$ per trophic level. Diet mixing estimates for two inferred sources used only $\delta^{13}C$ (estimation method 1). When possible, mixing between three sources was calculated with a linear mixing model based on both $\delta^{13}C$ and $\delta^{15}N$ (estimation method 2) (Phillips 2001). When a mixing problem could have multiple solutions, the distribution of possible sources was calculated with IsoSource (estimation method 3) (Phillips & Gregg 2003) and reported as [mean % composition, SD]. Tolerance is the minimum percent uncertainty value (from 0.05, 0.1, 0.5, 1, 2, or 3) that was necessary for the IsoSource program to calculate solutions. Question marks denote where there was only a single feasible food source and % composition could not be calculated.

| Sample Date | Sample Date, Consumer, Size (mm) | Estimated Percent Diet Composition | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Surficial Sediment | Periphyton | Phytoplankton (Estimated) | *Zostera marina* | Filter Feeders | *Cladophora* sp. | *Ulva* sp. | *Ectocarpus* sp. | *Caprella penantis* | *Gammarus mucronatus* | *Erichsonella attenuata* | *Idotea balthica* | Ampithoids | Estimation Method | Tolerance |
| | 22-Mar-05 | | | | | | | | | | | | | | | |
| 22-Mar-05 | *Botrylus schlosseri* | | | ? | | | | | | | | | | | | |
| | 21-Apr-05 | | | | | | | | | | | | | | | |
| 21-Apr-05 | *Crepidula* spp. | 40 | | 60 | | | | | | | | | | | 1 | |
| 21-Apr-05 | *Caprella penantis* | 2 | 65 | 33 | | | | | | | | | | | 2 | |
| 21-Apr-05 | *Gammarus mucronatus* | 65 | 35 | | | | | | | | | | | | 1 | |
| 21-Apr-05 | *Erichsonella attenuata* | | | | ? | | | | | | | | | | | |
| 21-Apr-05 | *Idotea balthica* | 32 (4) | 36 (21) | | 16 (7) | | | | | | 17 (10) | | | | 3 | 0.05 |
| 21-Apr-05 | *Cymadusa compta* | 57 (1) | 0 | | | | 41 (1) | 2 (2) | | | | | | | 3 | 1.00 |
| 21-Apr-05 | Ampithoid amphipods | 50 (15) | 12 (8) | | | | 22 (15) | 17 (12) | | | | | | | 3 | 2.00 |
| 21-Apr-05 | *Palaemonetes vulgaris* | | | | | 63 (7) | | | | 29 (18) | 2 (2) | | 4 (3) | 3 (3) | 3 | 0.50 |
| 21-Apr-05 | *Crangon septemspinosa* | | | | | 27 (13) | | | | 26 (18) | (14) 11 | | 12 (9) | 21 (16) | 3 | 0.50 |
| 21-Apr-05 | *C. septemspinosa*, Lg. Indiv. | | | | | 57 (17) | | | | 16 (13) | 7 (6) | | 6 (5) | 14 (11) | 3 | 0.50 |
| 21-Apr-05 | *Callinectes sapidus*, 28 | | | | | 35 (14) | | | | 25 (18) | 12 (9) | | 10 (7) | 19 (2) | 3 | 0.50 |
| 21-Apr-05 | *Callinectes sapidus*, 45 | | | | | ? | | | | | | | | | | |
| 21-Apr-05 | *Sygnathus fuscus*, 100-120 | | | | | 48 (15) | | | | 19 (16) | 9 (8) | | 7 (6) | 17 (14) | 3 | 2.00 |
| | 19-May-06 | | | | | | | | | | | | | | | |
| 19-May-06 | Hydroid | | | ? | | | | | | | | | | | | |
| 19-May-06 | *Caprella penantis* | 35 (2) | | 35 (1) | | | | | 30 (1) | | | | | | 3 | 0.05 |
| 19-May-06 | *Gammarus mucronatus* | 67 (1) | 24 (1) | | | | | | 9 (2) | | | | | | 3 | 0.05 |
| 19-May-06 | *Erichsonella attenuata* | | 1 | | 98 | | | | 1 | | | | | | 2 | |
| 19-May-06 | *Ampithoe longimana* | 32 (8) | 21 (15) | | 35 (15) | | | | 12 (9) | | | | | | 3 | 1.00 |
| 19-May-06 | *Hippolyte pleuracantha* | 87 | | | | | | | 13 | | | | | | 1 | |
| 19-May-06 | *Bittium varium* | 44 (11) | 14 (10) | | 33 (16) | | | | 8 (6) | | | | | | 3 | 2.00 |
| 19-May-06 | *Palaemonetes vulgaris* | | | | | 60 (8) | | | | 26 (16) | 14 (9) | | | | 3 | 0.50 |
| 19-May-06 | *Crangon septemspinosa* | | | | | 25 (10) | | | 23 (2) | 35 (20) | 17 (10) | | | | 3 | 0.50 |
| 19-May-05 | *Callinectes sapidus*, 35 | 30 | | | | 56 | | | | 14 | | | | | 2 | |
| 19-May-05 | *Callinectes sapidus*, 55 | 28 | | | | 67 | | | | 4 | | | | | 2 | |
| 19-May-05 | *Sygnathus fuscus*, 115 | | | | | 13 (8) | | | | 34 (19) | 42 (17) | | | 11 (8) | 3 | 0.50 |
| 19-May-05 | *Sygnathus fuscus*, 137 | | | | | 23 (12) | | | | 47 (20) | 24 (15) | | | 7 (5) | 3 | 0.50 |
| | 21-Aug-06 | | | | | | | | | | | | | | | |
| 21-Aug-06 | *Membranipora* sp. | | | ? | | | | | | | | | | | | |
| 21-Aug-06 | Porifera | | | ? | | | | | | | | | | | | |
| 21-Aug-06 | *Gammarus mucronatus* | 49 | 51 | | | | | | | | | | | | 1 | |
| 21-Aug-06 | *Erichsonella attenuata* | 27 (18) | 5 (3) | | 69 (17) | | | | | | | | | | 3 | 3.00 |
| 21-Aug-06 | *Ampithoe longimana* | 65 (15) | 5 (3) | | 30 (14) | | | | | | | | | | 3 | 2.00 |
| 21-Aug-06 | *Hippolyte pleuracantha* | 42 (15) | 5 (4) | | 53 (14) | | | | | | | | | | 3 | 2.00 |
| 21-Aug-06 | *Palaemonetes vulgaris* | | 25 (4) | | | | | | | | 20 (12) | 26 (3) | | 30 (17) | 3 | 0.10 |
| 21-Aug-06 | *Callinectes sapidus*, 20 | 19 (14) | | | | | | | | | 28 (21) | 31 (19) | | 22 (16) | 3 | 2.00 |
| 21-Aug-06 | *Callinectes sapidus*, 35 | 61 (10) | | | | | | | | | 27 (16) | 11 (6) | | 2 (1) | 3 | 0.05 |
| 21-Aug-06 | *Callinectes sapidus*, 56 | 49 (10) | | | | | | | | | 27 (16) | 11 (6) | | 14 (2) | 3 | 0.05 |
| 21-Aug-06 | *Sygnathus floridae*, 120 | | | | | | | | | | 14 (8) | 2 (2) | | 84 (8) | 3 | 0.50 |
| 21-Aug-06 | *Sygnathus fuscus*, 190 | | | | | | | | | | 25 (13) | 4 (3) | | 71 (14) | 3 | 1.00 |
| 21-Aug-06 | *Gobiosoma bosci*, 28 | | | | | | | | | | 29 (2) | 0 (0) | | 71 (3) | 3 | 0.05 |
| 21-Aug-06 | *Gobiosoma bosci*, 36 | | | | | | | | | | 37 (18) | 5 (4) | | 58 (20) | 3 | 1.00 |
| 21-Aug-06 | *Gobiosoma bosci*, 38 | | | | | | | | | | 80 (12) | 2 (2) | | 18 (13) | 3 | 1.00 |
| 21-Aug-06 | *Gobiesox strumosus*, 17 | | | | | | | | | | 52 (26) | 8 (5) | | 41 (27) | 3 | 2.00 |
| 21-Aug-06 | *Gobiesox strumosus*, 30 | | | | | | | | | | 80 (12) | 2 (2) | | 18 (13) | 3 | 1.00 |

# FIGURE LEGENDS

Figure 1. Plot of $\delta^{15}N$ and $\delta^{13}C$ stable isotope signatures from biota collected at the Goodwin Islands eelgrass bed on 21 April, 2005. Algae and plants are shaded green, putative grazers are shaded yellow or orange, putative predators are shaded red or pink, and filter feeders are shaded blue. Error bars are calculated from procedural replicates of pooled tissue; not true replicates.

Figure 2. Plot of $\delta^{15}N$ and $\delta^{13}C$ stable isotope signatures from biota collected at the Goodwin Islands eelgrass bed on 19 May 2006. Algae and plants are shaded green, putative grazers are shaded yellow or orange, putative predators are shaded red or pink, and filter feeders are shaded blue. Error bars are calculated from procedural replicates of pooled tissue; not true replicates.

Figure 3. Plot of $\delta^{15}N$ and $\delta^{13}C$ stable isotope signatures from biota collected at the Goodwin Islands eelgrass bed on 21 August 2006. Algae and plants are shaded green, putative grazers are shaded yellow or orange, putative predators are shaded red or pink, and filter feeders are shaded blue. Error bars are calculated from procedural replicates of pooled tissue; not true replicates.

Figure 1.

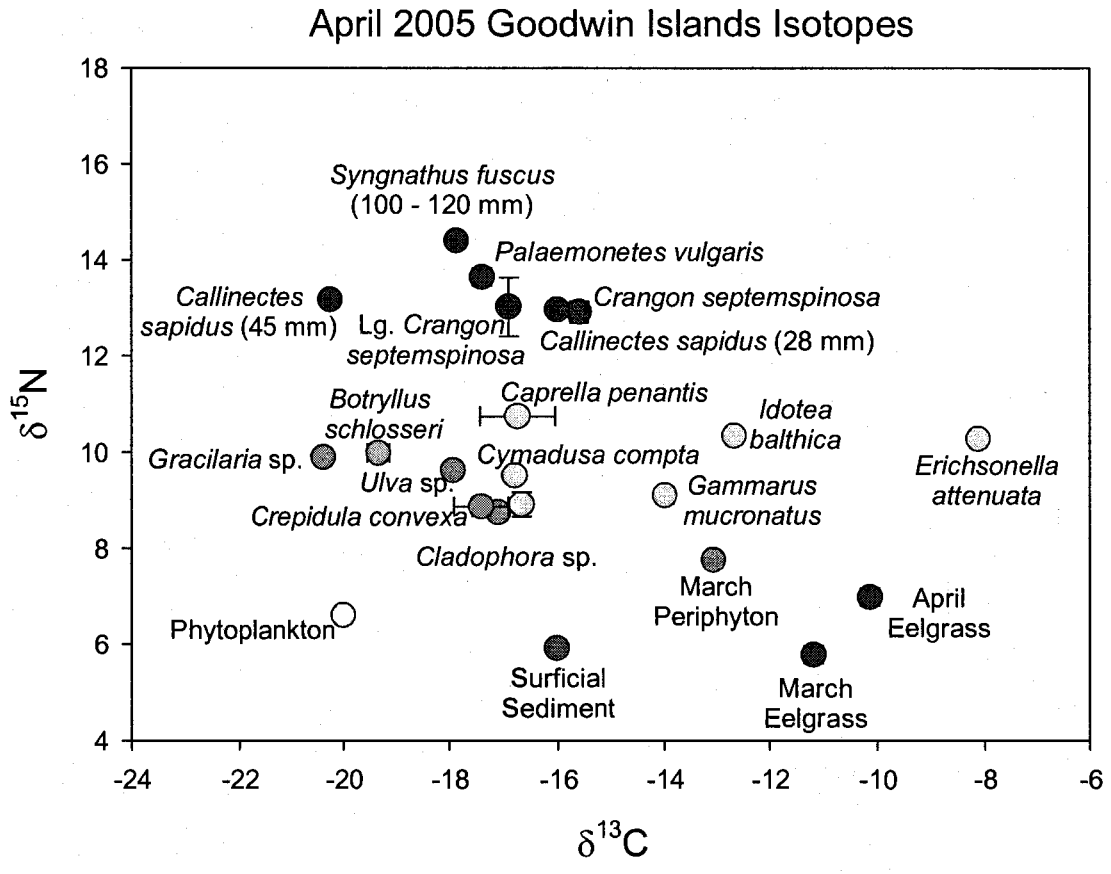

April 2005 Goodwin Islands Isotopes
18
16
14
12
10
8
6
4
-24
-22
-20
-18
-16
-14
-12
-10
-8
-6
δ15N
δ13C
Syngnathus fuscus
(100 - 120 mm)
Palaemonetes vulgaris
Callinectes
sapidus (45 mm)
Lg. Crangon
septemspinosa
Crangon septemspinosa
Callinectes sapidus (28 mm)
Botryllus
schlosseri
Caprella penantis
Idotea
balthica
Gracilaria sp.
Cymadusa compta
Erichsonella
attenuata
Ulva sp.
Gammarus
mucronatus
Crepidula convexa
Cladophora sp.
March
Periphyton
April
Eelgrass
Phytoplankton
Surficial
Sediment
March
Eelgrass

Figure 2.

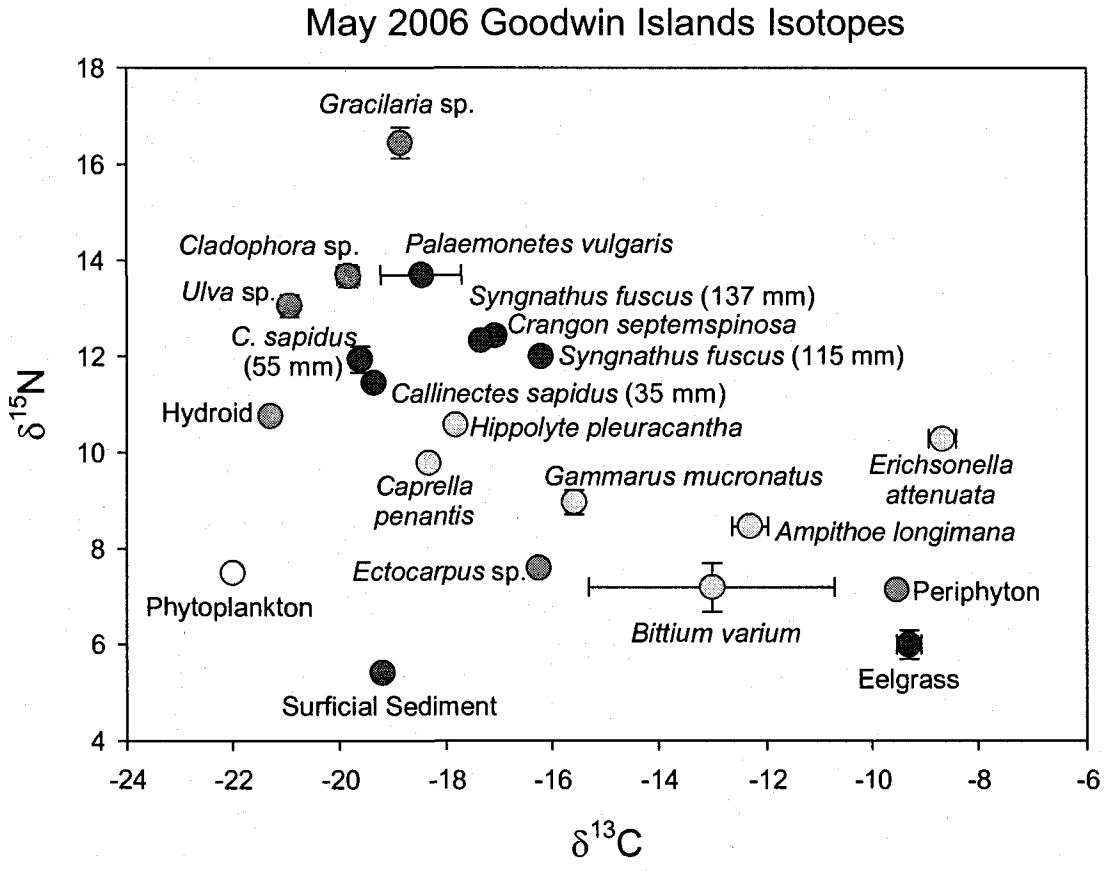
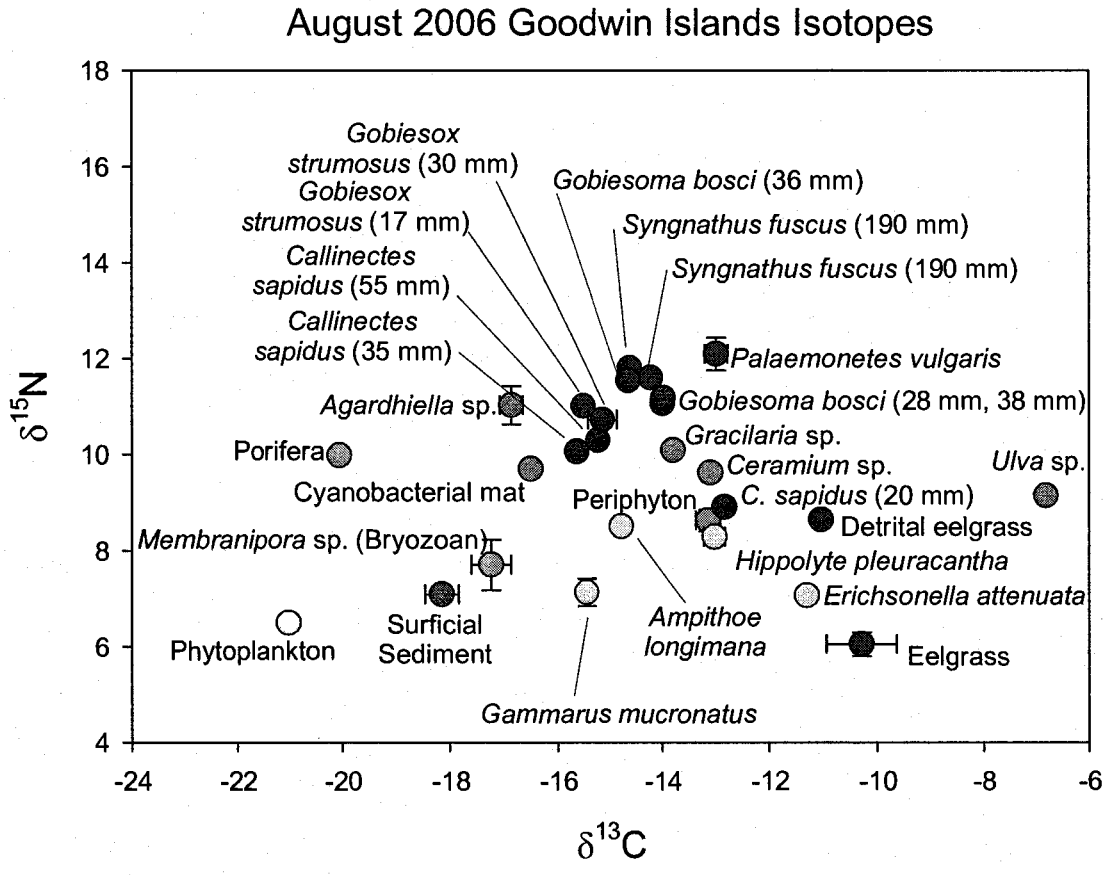

May 2006 Goodwin Islands Isotopes
18
16
14
12
10
8
6
4
-24
-22
-20
-18
-16
-14
-12
-10
-8
-6
δ15N
δ13C
Gracilaria sp.
Cladophora sp.
Palaemonetes vulgaris
Ulva sp.
Syngnathus fuscus (137 mm)
Crangon septemspinosa
C. sapidus (55 mm)
Syngnathus fuscus (115 mm)
Callinectes sapidus (35 mm)
Hydroid
Hippolyte pleuracantha
Erichsonella attenuata
Caprella penantis
Gammarus mucronatus
Ampithoe longimana
Ectocarpus sp.
Periphyton
Phytoplankton
Bittium varium
Eelgrass
Surficial Sediment

Figure 3.


August 2006 Goodwin Islands Isotopes
18
16
14
12
10
8
6
4
δ15N
-24
-22
-20
-18
-16
-14
-12
-10
-8
-6
δ13C
Gobiesox strumosus (30 mm)
Gobiesox strumosus (17 mm)
Callinectes sapidus (55 mm)
Callinectes sapidus (35 mm)
Agardhiella sp.
Porifera
Cyanobacterial mat
Membranipora sp. (Bryozoan)
Phytoplankton
Surficial Sediment
Gobiesoma bosci (36 mm)
Syngnathus fuscus (190 mm)
Syngnathus fuscus (190 mm)
Palaemonetes vulgaris
Gobiesoma bosci (28 mm, 38 mm)
Gracilaria sp.
Ceramium sp.
Periphyton
C. sapidus (20 mm)
Detrital eelgrass
Hippolyte pleuracantha
Erichsonella attenuata
Ulva sp.
Ampithoe longimana
Eelgrass
Gammarus mucronatus

## SUMMARY

This dissertation project explored the resource- and consumer-mediated dynamics of submersed macrophyte communities through an array of approaches; detailed food web characterizations (Ch. 5), long-term observational studies (Ch. 3), manipulative experiments (Chs. 1, 2), and critical comparisons of experimental and observational findings (Ch. 4). The final chapter of the project is a good place to start synthesizing the results, because it addressed the core question underlying consumer-resource dynamics in the eelgrass system: what eats what? Gut contents of consumers and stable carbon and nitrogen isotopic ratios of predators, mesograzers, and plants suggested that some eelgrass and macroalgae were consumed directly, but that microalgae and detritus supplied most of the trophic support for the epifaunal community, e.g. Thayer et al. (1978), Zimmerman et al. (1979). The structure of the Goodwin Islands food web therefore fits well with Valentine & Duffy's (2006) definition of a "seagrass detrital ecosystem". Despite this general trophic pattern, differences in diet among consumers were clear at both the mesograzer and predator levels. Changes in species composition and diversity among either primary or secondary consumers could therefore alter ecosystem functions like predation and grazing rate. This was demonstrated, albeit in a slightly different marine macrophyte system, in chapter two. The strength of top-down control in the model communities examined in that experiment was contingent upon the particular combinations of mesograzer and predator species present in each mesocosm. The effects of predation were greatest when fish, *Fundulus heteroclitus*, were paired with gammaridean amphipods. Similarly, small *Syngnathus* spp. fish in the Goodwin Islands eelgrass bed displayed an impressive appetite

for mesograzers, though the ecosystem scale impact of that predation was unclear, because Syngnathids actually had a positive covariance with mesograzers in the field (Chs. 3, 4).

Indeed, the long-term patterns of species co-occurrence in the field, described in chapter 3, suggested that the community composition at Goodwin Islands was determined more by abiotic forces interacting with the physiological tolerances of eelgrass, mesograzers, and predators than by predation. However, given the strong linkages among consumers evident from their gut contents, stable isotope ratios, and demographic relationships in experiments (Ch.3, Duffy et al. 2005, Heck et al. 2000, 2006), it may be fairly assumed that the direct effect of an abiotic forcing on any one consumer species has indirect effects radiating both up and down through the food web. For bottom-up effects, this assumption was supported in the regression model results for chapter 3 (Table 4a). There we saw that that turbidity and summer heat had a negative bottom-up influence on eelgrass density on an interannual scale (e.g. Moore et al. 1996, Moore & Jarvis 2008). We also saw positive bottom-up influences, like the substantial positive effect of eelgrass density on *Palaemonetes* spp. shrimp abundance, which is probably mediated by habitat availability rather than food (Ch. 3, Table 6). Similar, beneficial effects of seagrass habitat on the abundance, survival and production of demersal fish and crustaceans have been demonstrated numerous times in this system and others, supporting the "nursery role hypothesis", a bottom-up paradigm for seagrass – animal interactions (Heck & Orth 1980, Orth & Heck 1980, Heck et al. 2003). With respect to potential top-down effects, however, the covariation in predator, mesograzer, and epiphyte abundance that we observed in the field did not much resemble the opposite trends at adjacent trophic levels expected for systems with strong top-down control (Hairston, Smith, & Slobodkin 1960). Indeed, while the path analysis models in chapter 4 nicely predicted the classic patterns of top-down control seen in the eelgrass

mesocosm experiments (Duffy et al. 2005, Spivak et al. 2007), they failed to describe the actual patterns of species co-occurrence in the field, which were better described by bottom-up path models. It may be that the temporal and spatial scales of top-down interactions in the field are just too different from those in experiments to be detected in the same way. When not confined to an experimental enclosure or mesocosm, consumers can move among habitat patches to avoid competition and predation, or to opportunistically exploit abundant food sources while depleted food sources recover (France & Duffy 2006). This may reduce the impact of top-down control, or at least change it from an overwhelming demographic impact, as is typically observed in mesocosm experiments, to a more moderate impact in the form of altered behavior or species composition in the prey guild (Duffy et al. 2005). Overall, our results suggest that both top-down and bottom-up forces control eelgrass community structure via mesograzers, but that top-down control in the field is more subtle than has been indicated by some manipulative experiments.

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# APPENDIX

## *Chapter 2 Supplemental Material*

Table S1. Experimental design and explanation of planned contrasts. Rows with number headings denote grazer treatments, and columns with letter headings denote predator treatments. The additive density predator polyculture treatment (3xAll) is not shown in this set of contrasts, but it would take the place of column D. The contrast for predator diversity effects compares the average performance of predator monocultures (shaded) to predator polycultures (not shaded). The grazer diversity contrast compares the average performance of grazer monocultures (shaded) to grazer polycultures (not shaded).

| Experimental Design | | Predator Treatment | | | |
|---|---|---|---|---|---|
| | | Fish | Shrimp | Crabs | All |
| Grazer Treatment | | A | B | C | D |
| *D. appendiculata* | 1 | A1 | B1 | C1 | D1 |
| *E. levis* | 2 | A2 | B2 | C2 | D2 |
| *P. caudata* | 3 | A3 | B3 | C3 | D3 |
| All Grazers | 4 | A4 | B4 | C4 | D4 |

| Contrast: Predator Diversity | | | | |
|---|---|---|---|---|
| | A1 | B1 | C1 | D1 |
| | A2 | B2 | C2 | D2 |
| | A3 | B3 | C3 | D3 |
| | A4 | B4 | C4 | D4 |

| Contrast: Grazer Diversity | | | | |
|---|---|---|---|---|
| | A1 | B1 | C1 | D1 |
| | A2 | B2 | C2 | D2 |
| | A3 | B3 | C3 | D3 |
| | A4 | B4 | C4 | D4 |

Table S2. Results of 2-way, fully crossed ANOVAs examining effects of grazer and predator species composition and diversity on macroalgae mass change. See text regarding statistical treatment of macroalgae data prior to this analysis. Bold row headings are main grazer and predator treatment effects and interactions, and row headings preceded by > are planned contrasts within those effects or interactions. Omega squared estimates effect size (see Methods), and bold values highlight nominal $p < 0.05$.

| | | Response | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ln(Total Algae Res.) | | | Ln(*Entero.* Res.) | | | Ln(*Ulva* Res.) | | | Ln(*Gracilaria* Res.) | | | Ln(*Sargassum* Res.) | | |
| Effects | df | F | $\square^2$ | P | F | $\square^2$ | P | F | $\square^2$ | P | F | $\square^2$ | P | F | $\square^2$ | P |
| **Grazer Treatment** | 3 | 0.29 | 0.000 | 0.834 | 0.48 | 0.000 | 0.696 | 0.82 | 0.000 | 0.489 | 0.21 | 0.000 | 0.893 | 0.38 | 0.000 | 0.769 |
| >Monos. vs. Polyculture | 1 | 0.08 | 0.000 | 0.775 | 1.12 | 0.001 | 0.292 | 0.21 | 0.000 | 0.649 | 0.00 | 0.000 | 0.962 | 0.18 | 0.000 | 0.675 |
| >Best Mono. vs. Polyculture | 1 | 0.58 | 0.000 | 0.448 | 1.42 | 0.004 | 0.237 | 1.17 | 0.002 | 0.283 | 0.11 | 0.000 | 0.744 | 0.81 | 0.000 | 0.371 |
| **Predator Treatment** | 4 | 0.54 | 0.000 | 0.706 | 2.97 | 0.077 | **0.025** | 0.60 | 0.000 | 0.664 | 0.77 | 0.000 | 0.546 | 1.76 | 0.031 | 0.146 |
| >Monos. vs. Low Density Poly. | 1 | 0.00 | 0.000 | 0.959 | 2.99 | 0.019 | 0.088 | 0.37 | 0.000 | 0.547 | 0.81 | 0.000 | 0.372 | 0.03 | 0.000 | 0.858 |
| >Monos. vs. High Density Poly. | 1 | 0.38 | 0.000 | 0.540 | 1.63 | 0.006 | 0.205 | 0.31 | 0.000 | 0.581 | 0.22 | 0.000 | 0.637 | 3.43 | 0.025 | 0.068 |
| >Best Mono. vs. Low Density Poly. | 1 | 0.49 | 0.000 | 0.484 | 5.96 | 0.049 | **0.017** | 0.76 | 0.000 | 0.385 | 0.81 | 0.000 | 0.372 | 0.64 | 0.000 | 0.426 |
| >Best Mono. vs. High Density Poly. | 1 | 1.44 | 0.005 | 0.234 | 0.00 | 0.000 | 0.994 | 0.00 | 0.000 | 0.949 | 0.22 | 0.000 | 0.637 | 4.83 | 0.039 | **0.031** |
| **Grazer Trt. * Predator Trt.** | 12 | 0.84 | 0.000 | 0.605 | 0.91 | 0.000 | 0.538 | 1.06 | 0.008 | 0.403 | 0.50 | 0.000 | 0.907 | 0.91 | 0.000 | 0.537 |

Table S3. Results of multiple linear regression analyses of top-down effects on grazers and algae. Algae mass change is the residual of Ln(Final wet weight (g) – Initial wet weight (g)) after ANOVA on mesocosm position effects. The predator response is final wet mass (g). In all tables, "Response" is the dependent variable, $R^2$ is the total variance explained, P is the nominal significance of the whole model, top column headings are independent variables, $\beta_0$ is the intercept, and $\beta_1$- $\beta_3$ are the regression coefficients, which are in **bold** if significant at $P < 0.05$. a) Effects of shrimp, crabs, and fish. b) Effects of grazers considered by species. c) Effects of total grazers.

a.

| | | | | Shrimp | Crabs | Fish |
|---|---|---|---|---|---|---|
| **Response** | **$R^2$** | **P** | **$\beta_0$** | **$\beta_1$** | **$\beta_2$** | **$\beta_3$** |
| Δ ΣAlg. wt. | 0.00 | 0.965 | 0.011 | -0.012 | -0.004 | -0.001 |
| Δ *Ulva* wt. | 0.00 | 0.581 | -0.031 | 0.088 | -0.012 | 0.016 |
| Δ *Sarg.* wt. | 0.01 | 0.304 | -0.028 | 0.040 | -0.011 | 0.024 |
| Δ *Grac.* wt. | 0.00 | 0.435 | -0.037 | 0.026 | 0.024 | -0.002 |
| Δ *Ent.* wt. | 0.03 | 0.096 | 0.183 | **-0.253** | -0.010 | -0.073 |
| *E. levis* | 0.36 | **< 0.0005** | 495.8 | -66.24 | -28.69 | **-135.6** |
| *D. appen.* | 0.34 | **< 0.0005** | 339.3 | -26.42 | -16.06 | -101.6 |
| *P. caudata* | 0.03 | 0.215 | 59.81 | -7.440 | -7.803 | -10.28 |
| Σ Grazers | 0.41 | **< 0.0005** | 507.4 | -50.98 | **-31.55** | **-119.8** |

b.

| | | | | *D. appen.* | *E. laevis* | *P. caud.* |
|---|---|---|---|---|---|---|
| **Response** | **$R^2$** | **P** | **$\beta_0$** | **$\beta_1$** | **$\beta_2$** | **$\beta_3$** |
| Δ ΣAlg. wt. | 0.00 | 0.653 | 0.013 | -1.01E-04 | -4.10E-05 | 2.73E-05 |
| Δ *Ulva* wt. | 0.04 | **0.043** | 0.102 | **-5.02E-04** | **-3.45E-04** | -5.90E-05 |
| Δ *Sarg.* wt. | 0.02 | 0.134 | 0.048 | -1.72E-04 | -7.28E-05 | -3.82E-04 |
| Δ *Grac.* wt. | 0.00 | 0.604 | -0.036 | 9.62E-05 | 1.08E-04 | 1.62E-04 |
| Δ *Ent.* wt. | 0.00 | 0.687 | -0.074 | 4.03E-04 | 1.56E-04 | 1.01E-04 |

c.

| | | | | **Σ Grazers** |
|---|---|---|---|---|
| **Response** | **$R^2$** | **P** | **$\beta_0$** | **$\beta_1$** |
| Δ ΣAlg. wt. | 0.00 | 0.372 | 0.014 | -5.05E-05 |
| Δ *Ulva* wt. | 0.05 | **0.01** | 0.109 | **-3.58E-04** |
| Δ *Sarg.* wt. | 0.02 | 0.082 | 0.038 | -1.36E-04 |
| Δ *Grac.* wt. | 0.01 | 0.184 | -0.034 | 1.11E-04 |
| Δ *Ent.* wt. | 0.00 | 0.316 | -0.072 | 2.20E-04 |

*Chapter 3 Supplementary Material*

Appendix Table 1. Comparison of selected linear regression models of eelgrass abundance. Predictor variables in models are indicated by their T values (regression coefficient / std. error of coefficient) in columns 2-7. Model fit statistics are as described in table 4. a. Models for density-adjusted eelgrass area at Goodwin Islands, as determined by aerial photos taken in the late spring of 1998 through 2006. The predictor variables used in these models; water temperature, salinity, turbidity, and mesograzer biomass (mg AFDM * g Plant $DM^{-1}$)are average values from days 200 – 250 of the preceding year ("summer") or days 70 – 120 of the focal year ("spring"). b. Models for deviation from the mean value of eelgrass shoot biomass for monthly samples taken between 2004 and 2007. Predictor variables are themselves deviations from the mean value of the selected variable for the day of the year on which the sample was taken. Additionally, temperature, salinity, and turbidity are based on average values from 30 days prior to the sample date.

a.

| | | Predictor variables with T values | | | | | | | | | Model Fit Statistics | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Response** | **Model #** | Constant | Spring Water Temperature | Spring Salinity | Spring Turbidity | Spring Mesograzers | Prev. Summer Water Temp. | Prev. Summer Salinity | Prev. Summer Turbidity | Prev. Summer Mesograzers | **n** | **K** | **RSS** | **$AIC_c$** | **$w_i$** | **$R^2$** |
| Spring Eelgrass Index | 1 | -2.07 | 2.42 | 3.24 | -4.08 | 0.05 | | | | | 8 | 6 | 33.32 | 107.41 | 0.00 | 0.89 |
| " | 2 | -2.51 | 3.35 | 3.88 | -7.68 | | | | | | 8 | 5 | 33.35 | 51.42 | 0.01 | 0.92 |
| " | 3 | -0.01 | 0.59 | | | | | | | | 8 | 3 | 696.0 | 47.73 | 0.04 | 0.00 |
| " | **4** | **0.13** | | **1.54** | | | | | | | **8** | **3** | **527.7** | **45.51** | **0.13** | **0.16** |
| " | **5** | **6.11** | | | **-1.88** | | | | | | **8** | **3** | **463.4** | **44.47** | **0.22** | **0.27** |
| " | **6** | **4.48** | | | | **1.7** | | | | | **8** | **3** | **496.0** | **45.02** | **0.17** | **0.21** |
| " | 7 | 2.38 | | | | | -2.58 | 2.67 | 0.29 | 0.01 | 8 | 6 | 146.0 | 119.23 | 0.00 | 0.54 |
| " | 8 | 2.77 | | | | | -3.07 | 3.08 | 0.47 | | 8 | 5 | 146.0 | 63.23 | 0.00 | 0.65 |
| " | **9** | **2.04** | | | | | **-1.71** | | | | **8** | **3** | **494.1** | **44.99** | **0.17** | **0.22** |
| " | **10** | **-0.49** | | | | | | **1.73** | | | **8** | **3** | **491.0** | **44.94** | **0.18** | **0.22** |
| " | 11 | 2.63 | | | | | | | -0.17 | | 8 | 3 | 732.2 | 48.13 | 0.04 | 0.00 |
| " | 12 | 7.35 | | | | | | | | -0.08 | 8 | 3 | 734.9 | 48.16 | 0.04 | 0.00 |

b.

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Response** | **Model #** | Constant | Water Temperature | Salinity | Turbidity | Epiphytic Chl *a* | Mesograzer Total Density | **n** | **K** | **RSS** | **$AIC_c$** | **$w_i$** | **$R^2$** |
| Post-2004 Shoot Biomass | 1 | -1.53 | -0.57 | -1.78 | 0.55 | -0.13 | 0.78 | 26 | 7 | 21.75 | 15.59 | 0.00 | 0.14 |
| " | 2 | -1.58 | -0.58 | -1.85 | 0.59 | | 0.89 | 26 | 6 | 21.77 | 11.81 | 0.02 | 0.18 |
| " | 3 | -2.03 | -0.64 | -2.15 | 0.51 | -0.42 | | 26 | 6 | 22.41 | 12.56 | 0.01 | 0.15 |
| " | 4 | -2.25 | -0.63 | -2.39 | 0.57 | | | 26 | 5 | 22.60 | 9.36 | 0.06 | 0.19 |
| " | 5 | -0.8 | -1.54 | | | | | 26 | 3 | 28.69 | 9.65 | 0.05 | 0.05 |
| " | **6** | **-2.74** | | **-2.9** | | | | **26** | **3** | **23.34** | **4.29** | **0.71** | **0.23** |
| " | 7 | -1.21 | | | 0.4 | | | 26 | 3 | 31.34 | 11.94 | 0.02 | 0.00 |
| " | 8 | -0.49 | | | | 1.61 | -0.35 | 26 | 4 | 27.11 | 10.99 | 0.02 | 0.07 |
| " | 9 | -0.49 | | | | | 1.94 | 26 | 3 | 27.26 | 8.32 | 0.09 | 0.10 |
| " | 10 | -1.06 | | | | -1.04 | | 26 | 3 | 30.17 | 10.96 | 0.03 | 0.00 |

Appendix Table 2. Comparison of selected linear regression models of epiphyte density (μg chl * $cm^{-2}$ eelgrass blade). Model fit statistics are as described in table 4. The three sets of models (all dates, spring, summer) incorporate all dates in which mesograzers, epiphytic chlorophyll, and predators were sampled, only those dates between day 70-150 of a year, and only those dates between 180 and 260 of year, respectively.

| | | Predictor variables with T values | | | | | Model Fit Statistics | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Response** | Model # | Constant | Water Temperature | Salinity | Turbidity | Mesograzer Total Density | **n** | **K** | **RSS** | **$AIC_c$** | **$w_i$** | **$R^2$** |
| **Epiphytic Chl *a*, All dates** | 1 | 0.17 | 0.29 | 0.07 | -1.41 | -0.74 | 48 | 6 | 303.4 | 102.6 | 0.01 | 0.00 |
| " | 2 | -0.06 | 0.37 | -0.04 | -1.25 | | 48 | 5 | 307.3 | 100.5 | 0.04 | 0.00 |
| " | 3 | 0.01 | 0.19 | | | | 48 | 3 | 318.3 | 97.4 | 0.18 | 0.00 |
| " | 4 | 0.06 | | 0.21 | | | 48 | 3 | 318.2 | 97.3 | 0.18 | 0.00 |
| " | 5 | **0.02** | | | **-1.23** | | **48** | **3** | **308.4** | **95.8** | **0.39** | **0.01** |
| " | 6 | 0.12 | | | | -0.29 | 48 | 3 | 318.0 | 97.3 | 0.19 | 0.00 |
| **Epiphytic Chl *a*, Spring** | 1 | -3.13 | -0.89 | 0.96 | 0.69 | 0.81 | 13 | 6 | 0.315 | -22.36 | 0.00 | 0.00 |
| " | 2 | -3.08 | -0.90 | 1.13 | 0.39 | | 13 | 5 | 0.341 | -28.76 | 0.00 | 0.00 |
| " | 3 | -3.18 | -0.14 | | | | 13 | 3 | 0.391 | -36.90 | 0.20 | 0.00 |
| " | 4 | -3.25 | | 0.76 | | | 13 | 3 | 0.372 | -37.55 | 0.28 | 0.00 |
| " | 5 | -3.18 | | | 0.13 | | 13 | 3 | 0.391 | -36.89 | 0.20 | 0.00 |
| " | 6 | -3.40 | | | | 0.87 | 13 | 3 | 0.366 | -37.74 | 0.31 | 0.00 |
| **Epiphytic Chl *a*, Summer** | 1 | 1.88 | 0.57 | 0.88 | -2.27 | 0.12 | 12 | 6 | 7.63 | 23.37 | 0.00 | 0.24 |
| " | 2 | 2.27 | 0.60 | 0.95 | -2.55 | | 12 | 5 | 7.65 | 14.59 | 0.01 | 0.33 |
| " | 3 | 1.51 | 0.63 | | | | 12 | 3 | 15.15 | 11.80 | 0.04 | 0.00 |
| " | 4 | 1.71 | | 0.97 | | | 12 | 3 | 14.40 | 11.19 | 0.05 | 0.00 |
| " | 5 | **2.20** | | | **-2.78** | | **12** | **3** | **8.87** | **5.37** | **0.87** | **0.38** |
| " | 6 | 1.18 | | | | 0.54 | 12 | 3 | 15.30 | 11.91 | 0.03 | 0.00 |

Appendix Table 3. Comparison of selected linear regression models of total mesograzer biomass (mg AFDM * g plant $DM^{-1}$). Model fit statistics are as described in table 4. The three sets of models (all dates, spring, summer) incorporate all dates in which mesograzers, epiphytic chlorophyll, and predators were sampled, only those dates between day 70-150 of a year, and only those dates between 180 and 260 of year, respectively.

| | | Predictor variables with T values | | | | | | | | Model Fit Statistics | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Response | Model # | Constant | Water Temperature | Salinity | Turbidity | Epiphytic Chl a | Palaemonetes shrimp | Blue Crabs | Total Fish | n | K | RSS | $AIC_c$ | $w_i$ | $R^2$ |
| **Mesograzers, All dates** | 1 | 2.66 | -0.53 | 0.85 | -2.03 | -0.67 | | | 1.78 | 48 | 7 | 183692 | 412.8 | 0.03 | 0.12 |
| " | 2 | 2.11 | -0.60 | 0.97 | -2.36 | -0.74 | | | | 48 | 6 | 197619 | 413.5 | 0.02 | 0.07 |
| " | 3 | 2.13 | -0.64 | 0.98 | -2.27 | | | | | 48 | 5 | 200150 | 411.5 | 0.05 | 0.08 |
| " | 4 | 1.99 | -0.61 | | | | | | | 48 | 3 | 231415 | 413.6 | 0.02 | 0.00 |
| " | 5 | 2.01 | | 0.86 | | | | | | 48 | 3 | 229571 | 413.2 | 0.02 | 0.00 |
| " | 6 | **1.99** | | | **-2.53** | | | | | **48** | **3** | **204763** | **407.7** | **0.35** | **0.10** |
| " | 7 | 1.92 | | | | -0.29 | | | | 48 | 3 | 232878 | 413.9 | 0.02 | 0.00 |
| " | 8 | **2.07** | | | | | **1.05** | **-2.06** | **2.65** | **48** | **5** | **188490** | **408.7** | **0.23** | **0.14** |
| " | 9 | 2.03 | | | | | 0.66 | | | 48 | 3 | 231080 | 413.6 | 0.02 | 0.00 |
| " | 10 | 1.55 | | | | | | -0.40 | | 48 | 3 | 232474 | 413.8 | 0.02 | 0.00 |
| " | 11 | **2.73** | | | | | | | **2.33** | **48** | **3** | **208668** | **408.7** | **0.23** | **0.09** |
| **Mesograzers, Spring** | 1 | 1.51 | 0.26 | 0.17 | -0.43 | 0.35 | | | 1.01 | 13 | 7 | 13369 | 126.6 | 0.00 | 0.00 |
| " | 2 | 1.21 | 0.26 | 0.17 | -1.45 | 0.81 | | | | 13 | 6 | 15332 | 117.9 | 0.00 | 0.00 |
| " | 3 | 0.91 | 0.03 | 0.49 | -1.39 | | | | | 13 | 5 | 16596 | 111.5 | 0.00 | 0.00 |
| " | 4 | 0.88 | -0.03 | | | | | | | 13 | 3 | 21196 | 104.8 | 0.04 | 0.00 |
| " | 5 | 0.90 | | 0.55 | | | | | | 13 | 3 | 20636 | 104.5 | 0.05 | 0.00 |
| " | 6 | **0.98** | | | **-1.53** | | | | | **13** | **3** | **17475** | **102.3** | **0.15** | **0.10** |
| " | 7 | 1.26 | | | | 0.87 | | | | 13 | 3 | 19834 | 104.0 | 0.06 | 0.00 |
| " | 8 | 2.23 | | | | | 0.13 | -0.48 | 1.59 | 13 | 5 | 13984 | 109.3 | 0.00 | 0.12 |
| " | 9 | 1.60 | | | | | 1.33 | | | 13 | 3 | 18252 | 102.9 | 0.11 | 0.06 |
| " | 10 | 0.95 | | | | | | 0.38 | | 13 | 3 | 20925 | 104.7 | 0.05 | 0.00 |
| " | 11 | **2.52** | | | | | | | **2.29** | **13** | **3** | **14352** | **99.8** | **0.53** | **0.26** |
| **Mesograzers, Summer** | 1 | 1.41 | -1.08 | 0.05 | -0.52 | -0.33 | | | 1.91 | 12 | 7 | 23411 | 132.9 | 0.00 | 0.01 |
| " | 2 | 0.88 | -0.74 | 0.16 | -0.46 | 0.12 | | | | 12 | 6 | 37628 | 125.4 | 0.00 | 0.00 |
| " | 3 | 1.31 | -0.79 | 0.22 | -0.77 | | | | | 12 | 5 | 37699 | 116.6 | 0.00 | 0.00 |
| " | 4 | 1.32 | -0.78 | | | | | | | 12 | 3 | 40925 | 106.6 | 0.07 | 0.00 |
| " | 5 | 1.32 | | 0.29 | | | | | | 12 | 3 | 43080 | 107.2 | 0.05 | 0.00 |
| " | 6 | 1.39 | | | -0.80 | | | | | 12 | 3 | 40820 | 106.6 | 0.07 | 0.00 |
| " | 7 | 0.95 | | | | 0.54 | | | | 12 | 3 | 42201 | 107.0 | 0.06 | 0.00 |
| " | 8 | **2.21** | | | | | **-1.07** | **-0.12** | **4.06** | **12** | **5** | **13860** | **104.6** | **0.18** | **0.56** |
| " | 9 | 1.34 | | | | | -0.43 | | | 12 | 3 | 42667 | 107.1 | 0.05 | 0.00 |
| " | 10 | 1.20 | | | | | | -0.49 | | 12 | 3 | 42429 | 107.0 | 0.05 | 0.00 |
| " | 11 | **1.81** | | | | | | | **2.16** | **12** | **3** | **29581** | **102.7** | **0.47** | **0.25** |

Appendix Table 4. Comparison of selected linear regression models of *Caprella penantis* biomass (mg AFDM * g plant DM-1). Model fit statistics are as described in table 4. The three sets of models (all dates, spring, summer) incorporate all dates in which mesograzers, epiphytic chlorophyll, and predators were sampled, only those dates between day 70-150 of a year, and only those dates between 180 and 260 of year, respectively.

| | | Predictor variables with T values | | | | | | | | Model Fit Statistics | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Response | Model # | Constant | Water Temperature | Salinity | Turbidity | Epiphytic Chl a | Palaemonetes shrimp | Blue Crabs | Total Fish | n | K | RSS | $AIC_c$ | $w_i$ | $R^2$ |
| ***C. penantis*, All dates** | 1 | 1.81 | 0.01 | 0.79 | -2.05 | -1.79 | | | 1.58 | 48 | 7 | 38457 | 337.7 | 0.07 | 0.13 |
| " | 2 | 1.31 | -0.06 | 0.90 | -2.35 | -1.84 | | | | 48 | 6 | 40757 | 337.8 | 0.07 | 0.10 |
| " | 3 | 1.29 | -0.16 | 0.88 | -2.00 | | | | | 48 | 5 | 43952 | 338.8 | 0.04 | 0.05 |
| " | 4 | 1.20 | -0.09 | | | | | | | 48 | 3 | 49307 | 339.4 | 0.03 | 0.00 |
| " | 5 | 1.32 | | 0.99 | | | | | | 48 | 3 | 48279 | 338.4 | 0.05 | 0.00 |
| " | 6 | **1.22** | | | **-2.15** | | | | | **48** | **3** | **44800** | **334.8** | **0.30** | **0.07** |
| " | 7 | 1.24 | | | | -1.34 | | | | 48 | 3 | 47451 | 337.6 | 0.07 | 0.02 |
| " | 8 | 1.46 | | | | | 0.48 | -1.17 | 2.19 | 48 | 5 | 43416 | 338.2 | 0.05 | 0.06 |
| " | 9 | 1.35 | | | | | 0.62 | | | 48 | 3 | 48903 | 339.0 | 0.04 | 0.00 |
| " | 10 | 1.08 | | | | | | 0.00 | | 48 | 3 | 49315 | 339.4 | 0.03 | 0.00 |
| " | 11 | **1.93** | | | | | | | **2.08** | **48** | **3** | **45079** | **335.1** | **0.25** | **0.07** |
| ***C. penantis*, Spring** | 1 | 0.37 | -0.41 | 0.94 | -0.39 | 0.27 | | | 0.13 | 13 | 7 | 8421 | 120.56 | 0.00 | 0.00 |
| " | 2 | 0.51 | -0.43 | 1.01 | -0.68 | 0.37 | | | | 13 | 6 | 8440 | 110.19 | 0.00 | 0.00 |
| " | 3 | 0.37 | -0.59 | 1.28 | -0.67 | | | | | 13 | 5 | 8584 | 102.98 | 0.00 | 0.00 |
| " | 4 | 0.36 | 0.04 | | | | | | | 13 | 3 | 10932 | 96.21 | 0.09 | 0.00 |
| " | 5 | 0.39 | | 1.14 | | | | | | 13 | 3 | 9781 | 94.77 | 0.19 | 0.02 |
| " | 6 | 0.38 | | | -0.85 | | | | | 13 | 3 | 10255 | 95.38 | 0.14 | 0.00 |
| " | 7 | 0.79 | | | | 0.76 | | | | 13 | 3 | 10388 | 95.55 | 0.13 | 0.00 |
| " | 8 | 0.74 | | | | | 0.63 | -0.11 | 0.16 | 13 | 5 | 9702 | 104.57 | 0.00 | 0.00 |
| " | 9 | 1.07 | | | | | 1.16 | | | 13 | 3 | 9743 | 94.72 | 0.19 | 0.03 |
| " | 10 | 0.62 | | | | | | 0.55 | | 13 | 3 | 10636 | 95.86 | 0.11 | 0.00 |
| " | 11 | 0.98 | | | | | | | 0.91 | 13 | 3 | 10172 | 95.28 | 0.15 | 0.00 |
| ***C. penantis*, Summer** | 1 | -0.25 | -2.44 | -1.88 | -0.40 | 0.82 | | | 1.22 | 12 | 7 | 431.0 | 84.97 | 0.00 | 0.37 |
| " | 2 | -0.50 | -2.24 | -1.73 | -0.40 | 1.11 | | | | 12 | 6 | 538.0 | 74.44 | 0.00 | 0.33 |
| " | 3 | 0.24 | -2.02 | -1.43 | -1.52 | | | | | 12 | 5 | 632.9 | 67.58 | 0.02 | 0.31 |
| " | 4 | **0.38** | **-1.78** | | | | | | | **12** | **3** | **951.1** | **61.47** | **0.36** | **0.17** |
| " | 5 | 0.14 | | -1.13 | | | | | | 12 | 3 | 1110 | 63.32 | 0.14 | 0.03 |
| " | 6 | 0.46 | | | -1.06 | | | | | 12 | 3 | 1125 | 63.49 | 0.13 | 0.01 |
| " | 7 | 0.00 | | | | 0.78 | | | | 12 | 3 | 1180 | 64.06 | 0.10 | 0.00 |
| " | 8 | 0.61 | | | | | -0.54 | 0.21 | 1.22 | 12 | 5 | 1051 | 73.67 | 0.00 | 0.00 |
| " | 9 | 0.37 | | | | | -0.18 | | | 12 | 3 | 1249 | 64.74 | 0.07 | 0.00 |
| " | 10 | 0.33 | | | | | | -0.12 | | 12 | 3 | 1251 | 64.76 | 0.07 | 0.00 |
| " | 11 | 0.47 | | | | | | | 0.91 | 12 | 3 | 1157 | 63.82 | 0.11 | 0.00 |

Appendix Table 5. Comparison of selected linear regression models of *Gammarus mucronatus* biomass (mg AFDM * g plant $DM^{-1}$). Model fit statistics are as described in table 4. The three sets of models (all dates, spring, summer) incorporate all dates in which mesograzers, epiphytic chlorophyll, and predators were sampled, only those dates between day 70-150 of a year, and only those dates between 180 and 260 of year, respectively.

| | | Predictor variables with T values | | | | | | | | Model Fit Statistics | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Response | Model # | Constant | Water Temperature | Salinity | Turbidity | Epiphytic Chl a | Palaemonetes shrimp | Blue Crabs | Total Fish | n | K | RSS | $AIC_c$ | $w_i$ | $R^2$ |
| ***G. mucronatus*, All dates** | 1 | 1.59 | -1.25 | -1.25 | -0.75 | -0.46 | | | 0.04 | 48 | 7 | 6638 | 253.4 | 0.01 | 0.12 |
| " | 2 | 1.70 | -1.26 | -2.13 | -0.78 | -0.47 | | | | 48 | 6 | 6638 | 250.7 | 0.06 | 0.14 |
| " | 3 | **1.72** | **-1.30** | **-2.15** | **-0.71** | | | | | **48** | **5** | **6672** | **248.3** | **0.19** | **0.16** |
| " | 4 | **2.15** | **-2.56** | | | | | | | **48** | **3** | **7398** | **248.4** | **0.18** | **0.11** |
| " | 5 | **1.48** | | **-2.98** | | | | | | **48** | **3** | **7084** | **246.3** | **0.51** | **0.14** |
| " | 6 | 1.65 | | | -0.78 | | | | | 48 | 3 | 8344 | 254.1 | 0.01 | 0.00 |
| " | 7 | 1.66 | | | | -0.41 | | | | 48 | 3 | 8423 | 254.6 | 0.01 | 0.00 |
| " | 8 | 1.22 | | | | | 0.69 | -1.06 | 0.29 | 48 | 5 | 8240 | 258.4 | 0.00 | 0.00 |
| " | 9 | 1.53 | | | | | -0.05 | | | 48 | 3 | 8453 | 254.8 | 0.01 | 0.00 |
| " | 10 | 1.21 | | | | | | -0.66 | | 48 | 3 | 8375 | 254.3 | 0.01 | 0.00 |
| " | 11 | 0.09 | | | | | | | 1.57 | 48 | 3 | 8452 | 254.7 | 0.01 | 0.00 |
| ***G. mucronatus*, Spring** | 1 | 0.49 | -0.56 | -0.41 | 0.20 | -0.37 | | | 0.39 | 13 | 7 | 4865 | 113.4 | 0.00 | 0.00 |
| " | 2 | 0.33 | -0.59 | -0.43 | -0.08 | -0.25 | | | | 13 | 6 | 4969 | 103.3 | 0.00 | 0.00 |
| " | 3 | 0.77 | -0.57 | -0.59 | -0.12 | | | | | 13 | 5 | 5009 | 95.97 | 0.00 | 0.00 |
| " | 4 | **0.84** | **-1.48** | | | | | | | **13** | **3** | **5200** | **86.56** | **0.19** | **0.09** |
| " | 5 | **0.84** | | **-1.43** | | | | | | **13** | **3** | **5258** | **86.70** | **0.18** | **0.08** |
| " | 6 | 0.79 | | | -0.48 | | | | | 13 | 3 | 6113 | 88.66 | 0.07 | 0.00 |
| " | 7 | 0.29 | | | | -0.40 | | | | 13 | 3 | 6153 | 88.74 | 0.06 | 0.00 |
| " | 8 | 1.02 | | | | | -0.33 | -1.88 | 1.34 | 13 | 5 | 3817 | 92.44 | 0.01 | 0.18 |
| " | 9 | 0.22 | | | | | -0.53 | | | 13 | 3 | 6086 | 88.60 | 0.07 | 0.00 |
| " | 10 | **-0.33** | | | | | | **-1.86** | | **13** | **3** | **4744** | **85.36** | **0.35** | **0.17** |
| " | 11 | 0.81 | | | | | | | 0.49 | 13 | 3 | 6108 | 88.65 | 0.07 | 0.00 |
| ***G. mucronatus*, Summer** | 1 | 1.17 | -1.50 | -1.26 | -0.94 | -0.12 | | | -0.07 | 12 | 7 | 152.5 | 72.51 | 0.00 | 0.03 |
| " | 2 | 1.31 | -1.64 | -1.37 | -1.02 | -0.15 | | | | 12 | 6 | 152.7 | 59.32 | 0.00 | 0.17 |
| " | 3 | 1.66 | -1.82 | -1.60 | -1.31 | | | | | 12 | 5 | 153.2 | 50.56 | 0.01 | 0.27 |
| " | 4 | **1.75** | **-1.62** | | | | | | | **12** | **3** | **228.8** | **44.38** | **0.32** | **0.13** |
| " | 5 | **1.41** | | **-1.35** | | | | | | **12** | **3** | **244.3** | **45.16** | **0.22** | **0.07** |
| " | 6 | 1.71 | | | -0.90 | | | | | 12 | 3 | 267.1 | 46.23 | 0.13 | 0.00 |
| " | 7 | 1.46 | | | | -0.05 | | | | 12 | 3 | 288.6 | 47.16 | 0.08 | 0.00 |
| " | 8 | 0.57 | | | | | 0.78 | -0.77 | -0.38 | 12 | 5 | 265.2 | 57.14 | 0.00 | 0.00 |
| " | 9 | 1.58 | | | | | -0.04 | | | 12 | 3 | 288.6 | 47.16 | 0.08 | 0.00 |
| " | 10 | 1.51 | | | | | | -0.30 | | 12 | 3 | 286.0 | 47.05 | 0.08 | 0.00 |
| " | 11 | 1.54 | | | | | | | -0.31 | 12 | 3 | 285.9 | 47.05 | 0.08 | 0.00 |

Appendix Table 6. Comparison of selected linear regression models of *Erichsonella attenuata* biomass (mg AFDM * g plant $DM^{-1}$). Model fit statistics are as described in table 4. The three sets of models (all dates, spring, summer) incorporate all dates in which mesograzers, epiphytic chlorophyll, and predators were sampled, only those dates between day 70-150 of a year, and only those dates between 180 and 260 of year, respectively.

| | | Predictor variables with T values | | | | | | | | Model Fit Statistics | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Response | Model # | Constant | Water Temperature | Salinity | Turbidity | Epiphytic Chl a | Palaemonetes shrimp | Blue Crabs | Total Fish | n | K | RSS | $AIC_c$ | $w_i$ | $R^2$ |
| ***Erich. attenuata*, All dates** | 1 | 2.30 | -0.50 | 0.69 | -0.60 | 0.42 | | | 2.36 | 48 | 7 | 18881 | 303.6 | 0.00 | 0.07 |
| " | 2 | 1.50 | -0.58 | 0.83 | -1.02 | 0.29 | | | | 48 | 6 | 21378 | 306.8 | 0.00 | 0.00 |
| " | 3 | 1.51 | -0.57 | 0.84 | -1.10 | | | | | 48 | 5 | 21421 | 304.3 | 0.00 | 0.00 |
| " | 4 | 1.43 | -0.43 | | | | | | | 48 | 3 | 22503 | 301.8 | 0.01 | 0.00 |
| " | 5 | 1.45 | | 1.45 | | | | | | 48 | 3 | 22339 | 301.4 | 0.01 | 0.00 |
| " | 6 | 1.37 | | | -1.30 | | | | | 48 | 3 | 21788 | 300.2 | 0.02 | 0.02 |
| " | 7 | 1.38 | | | | 0.50 | | | | 48 | 3 | 22469 | 301.7 | 0.01 | 0.00 |
| " | 8 | **1.63** | | | | | **0.22** | **-1.78** | **3.44** | **48** | **5** | **17268** | **293.9** | **0.56** | **0.18** |
| " | 9 | 1.34 | | | | | 0.16 | | | 48 | 3 | 22580 | 301.9 | 0.01 | 0.00 |
| " | 10 | 0.99 | | | | | | -0.57 | | 48 | 3 | 22432 | 301.6 | 0.01 | 0.00 |
| " | 11 | **2.37** | | | | | | | **2.70** | **48** | **3** | **19497** | **294.9** | **0.35** | **0.12** |
| ***Erich. attenuata*, Spring** | 1 | 2.06 | 3.29 | -1.75 | -1.28 | 1.97 | | | 1.40 | 13 | 7 | 39.9 | 50.99 | 0.00 | 0.56 |
| " | 2 | 1.53 | 3.11 | -1.66 | -2.77 | 2.59 | | | | 13 | 6 | 51.1 | 43.80 | 0.01 | 0.51 |
| " | 3 | -0.37 | 1.94 | -0.62 | -1.93 | | | | | 13 | 5 | 93.9 | 44.28 | 0.01 | 0.20 |
| " | 4 | -0.36 | 1.39 | | | | | | | 13 | 3 | 133.5 | 38.94 | 0.13 | 0.07 |
| " | 5 | -0.35 | | 0.67 | | | | | | 13 | 3 | 150.8 | 40.53 | 0.06 | 0.00 |
| " | 6 | -0.36 | | | -1.10 | | | | | 13 | 3 | 141.3 | 39.68 | 0.09 | 0.02 |
| " | 7 | 0.69 | | | | 1.39 | | | | 13 | 3 | 133.5 | 38.95 | 0.12 | 0.07 |
| " | 8 | 1.38 | | | | | 0.04 | 0.47 | 1.14 | 13 | 5 | 111.1 | 46.47 | 0.00 | 0.06 |
| " | 9 | **0.76** | | | | | **1.52** | | | **13** | **3** | **129.7** | **38.57** | **0.15** | **0.10** |
| " | 10 | 0.40 | | | | | | 1.24 | | 13 | 3 | 137.8 | 39.36 | 0.10 | 0.04 |
| " | 11 | **1.56** | | | | | | | **2.01** | **13** | **3** | **114.8** | **36.99** | **0.33** | **0.20** |
| ***Erich. attenuata*, Summer** | 1 | 1.84 | -0.58 | 1.06 | -0.44 | -0.85 | | | 2.57 | 12 | 7 | 4587 | 113.35 | 0.00 | 0.25 |
| " | 2 | 0.99 | -0.22 | 0.93 | -0.35 | -0.17 | | | | 12 | 6 | 9655 | 109.08 | 0.00 | 0.00 |
| " | 3 | 1.21 | -0.28 | 0.99 | -0.34 | | | | | 12 | 5 | 9694 | 100.33 | 0.00 | 0.00 |
| " | 4 | 1.06 | -0.25 | | | | | | | 12 | 3 | 11089 | 90.95 | 0.01 | 0.00 |
| " | 5 | 1.32 | | 1.12 | | | | | | 12 | 3 | 9914 | 89.60 | 0.02 | 0.02 |
| " | 6 | 1.10 | | | -0.43 | | | | | 12 | 3 | 10957 | 90.80 | 0.01 | 0.00 |
| " | 7 | 0.81 | | | | 0.35 | | | | 12 | 3 | 11024 | 90.88 | 0.01 | 0.00 |
| " | 8 | **2.35** | | | | | **-1.13** | **-0.35** | **5.57** | **12** | **5** | **2245** | **82.78** | **0.58** | **0.72** |
| " | 9 | 1.07 | | | | | -0.18 | | | 12 | 3 | 11121 | 90.98 | 0.01 | 0.00 |
| " | 10 | 0.99 | | | | | | -0.30 | | 12 | 3 | 11062 | 90.92 | 0.01 | 0.00 |
| " | 11 | **1.75** | | | | | | | **2.86** | **12** | **3** | **6131** | **83.83** | **0.35** | **0.40** |

Appendix Table 7. Comparison of selected linear regression models of *Idotea balthica* biomass (mg AFDM * g plant $DM^{-1}$). Model fit statistics are as described in table 4. The three sets of models (all dates, spring, summer) incorporate all dates in which mesograzers, epiphytic chlorophyll, and predators were sampled, only those dates between day 70-150 of a year, and only those dates between 180 and 260 of year, respectively.

| | | Predictor variables with T values | | | | | | | | Model Fit Statistics | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Response (All dates, cut to chl | Model # | Constant | Water Temperature | Salinity | Turbidity | Epiphytic Chl a | Palaemonetes shrimp | Blue Crabs | Total Fish | n | K | RSS | $AIC_c$ | $w_i$ | $R^2$ |
| ***Idotea balthica*, All dates** | 1 | 2.09 | 0.30 | -0.47 | -1.38 | -0.06 | | | 1.93 | 48 | 7 | 8364 | 264.5 | 0.01 | 0.04 |
| " | 2 | 1.46 | 0.21 | -0.31 | -1.73 | -0.15 | | | | 48 | 6 | 9108 | 265.8 | 0.00 | 0.00 |
| " | 3 | 1.48 | 0.21 | -0.31 | -1.75 | | | | | 48 | 5 | 9112 | 263.2 | 0.02 | 0.00 |
| " | 4 | 1.64 | -0.21 | | | | | | | 48 | 3 | 9750 | 261.6 | 0.04 | 0.00 |
| " | 5 | 1.60 | | -0.15 | | | | | | 48 | 3 | 9755 | 261.6 | 0.04 | 0.00 |
| " | 6 | **1.64** | | | **-1.78** | | | | | **48** | **3** | **9134** | **258.5** | **0.18** | **0.04** |
| " | 7 | 1.62 | | | | 0.17 | | | | 48 | 3 | 9754 | 261.6 | 0.04 | 0.00 |
| " | 8 | 2.17 | | | | | 0.46 | -0.50 | 1.86 | 48 | 5 | 8719 | 261.1 | 0.05 | 0.05 |
| " | 9 | 2.00 | | | | | 1.29 | | | 48 | 3 | 9419 | 259.9 | 0.09 | 0.01 |
| " | 10 | 1.86 | | | | | | 0.89 | | 48 | 3 | 9596 | 260.8 | 0.06 | 0.00 |
| " | 11 | **2.42** | | | | | | | **2.27** | **48** | **3** | **8773** | **256.5** | **0.48** | **0.08** |
| ***Idotea balthica*, Spring** | 1 | 1.83 | 2.02 | -0.71 | -0.86 | 0.49 | | | 1.19 | 13 | 7 | 1112 | 94.24 | 0.00 | 0.25 |
| " | 2 | 1.48 | 1.98 | -0.70 | -2.12 | 1.01 | | | | 13 | 6 | 1337 | 86.23 | 0.00 | 0.21 |
| " | 3 | 1.08 | 1.76 | -0.37 | -2.01 | | | | | 13 | 5 | 1508 | 80.37 | 0.01 | 0.21 |
| " | 4 | 0.97 | 1.32 | | | | | | | 13 | 3 | 2185 | 75.28 | 0.11 | 0.06 |
| " | 5 | 0.92 | | 0.82 | | | | | | 13 | 3 | 2385 | 76.42 | 0.06 | 0.00 |
| " | 6 | 0.95 | | | -1.25 | | | | | 13 | 3 | 2219 | 75.48 | 0.10 | 0.04 |
| " | 7 | 1.03 | | | | 0.55 | | | | 13 | 3 | 2465 | 76.85 | 0.05 | 0.00 |
| " | 8 | 2.04 | | | | | -0.18 | 1.10 | 1.06 | 13 | 5 | 1667 | 81.67 | 0.00 | 0.12 |
| " | 9 | **1.74** | | | | | **1.51** | | | **13** | **3** | **2097** | **74.75** | **0.15** | **0.10** |
| " | 10 | **1.88** | | | | | | **1.83** | | **13** | **3** | **1940** | **73.74** | **0.24** | **0.16** |
| " | 11 | **2.14** | | | | | | | **1.88** | **13** | **3** | **1918** | **73.59** | **0.26** | **0.17** |
| ***Idotea balthica*, Summer** | 1 | -0.20 | -1.52 | -2.60 | 0.05 | 0.82 | | | 1.84 | 12 | 7 | 1639 | 101.00 | 0.00 | 0.38 |
| " | 2 | -0.51 | -1.15 | -2.14 | 0.03 | 1.13 | | | | 12 | 6 | 2559 | 93.15 | 0.00 | 0.17 |
| " | 3 | 0.24 | -0.92 | -1.84 | -0.97 | | | | | 12 | 5 | 3028 | 86.37 | 0.00 | 0.14 |
| " | 4 | 0.50 | -0.81 | | | | | | | 12 | 3 | 4564 | 80.29 | 0.10 | 0.00 |
| " | 5 | **0.19** | | **-1.81** | | | | | | **12** | **3** | **3658** | **77.64** | **0.38** | **0.17** |
| " | 6 | 0.56 | | | -0.68 | | | | | 12 | 3 | 4646 | 80.51 | 0.09 | 0.00 |
| " | 7 | 0.19 | | | | 0.63 | | | | 12 | 3 | 4675 | 80.58 | 0.09 | 0.00 |
| " | 8 | 0.67 | | | | | -0.38 | 0.06 | 1.54 | 12 | 5 | 3742 | 88.91 | 0.00 | 0.00 |
| " | 9 | 0.48 | | | | | 0.14 | | | 12 | 3 | 4851 | 81.02 | 0.07 | 0.00 |
| " | 10 | 0.51 | | | | | | 0.11 | | 12 | 3 | 4854 | 81.03 | 0.07 | 0.00 |
| " | 11 | 0.71 | | | | | | | 1.40 | 12 | 3 | 4063 | 78.90 | 0.20 | 0.08 |

Appendix Table 8. Comparison of selected linear regression models of *Ampithoe longimana* biomass (mg AFDM * g plant $DM^{-1}$). Model fit statistics are as described in table 4. The three sets of models (all dates, spring, summer) incorporate all dates in which mesograzers, epiphytic chlorophyll, and predators were sampled, only those dates between day 70-150 of a year, and only those dates between 180 and 260 of year, respectively.

| | | Predictor variables with T values | | | | | | | | Model Fit Statistics | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Response | Model # | Constant | Water Temperature | Salinity | Turbidity | Epiphytic Chl a | Palaemonetes shrimp | Blue Crabs | Total Fish | n | K | RSS | $AIC_c$ | $w_i$ | $R^2$ |
| ***A. longimana*, All dates** | 1 | 0.21 | -0.53 | 1.78 | -0.04 | 0.87 | | | -0.78 | 48 | 7 | 2626 | 208.9 | 0.01 | 0.00 |
| " | 2 | 0.52 | -0.50 | 1.73 | 0.11 | 0.91 | | | | 48 | 6 | 2665 | 206.9 | 0.01 | 0.00 |
| " | 3 | 0.51 | -0.45 | 1.73 | -0.06 | | | | | 48 | 5 | 2717 | 205.2 | 0.03 | 0.00 |
| " | 4 | 0.19 | 0.32 | | | | | | | 48 | 3 | 2906 | 203.5 | 0.08 | 0.00 |
| " | 5 | **0.44** | | **1.75** | | | | | | **48** | **3** | **2730** | **200.5** | **0.36** | **0.04** |
| " | 6 | 0.24 | | | -0.24 | | | | | 48 | 3 | 2909 | 203.6 | 0.08 | 0.00 |
| " | 7 | 0.24 | | | | 0.95 | | | | 48 | 3 | 2856 | 202.7 | 0.12 | 0.00 |
| " | 8 | -0.15 | | | | | 1.79 | -1.46 | -0.89 | 48 | 5 | 2688 | 204.6 | 0.05 | 0.01 |
| " | 9 | 0.42 | | | | | 0.55 | | | 48 | 3 | 2894 | 203.3 | 0.09 | 0.00 |
| " | 10 | 0.00 | | | | | | -0.50 | | 48 | 3 | 2897 | 203.4 | 0.09 | 0.00 |
| " | 11 | 0.00 | | | | | | | -0.63 | 48 | 3 | 2888 | 203.2 | 0.09 | 0.00 |
| ***A. longimana*, Spring** | 1 | 1.28 | 2.11 | -1.58 | 1.43 | 2.33 | | | 2.12 | 13 | 7 | 7.11 | 28.56 | 0.00 | 0.54 |
| " | 2 | -0.73 | 1.77 | -1.32 | 0.04 | 2.87 | | | | 13 | 6 | 11.69 | 24.62 | 0.00 | 0.34 |
| " | 3 | -2.98 | 0.73 | -0.25 | 0.30 | | | | | 13 | 5 | 23.77 | 26.42 | 0.00 | 0.00 |
| " | 4 | -3.25 | 1.03 | | | | | | | 13 | 3 | 24.26 | 16.78 | 0.07 | 0.00 |
| " | 5 | -3.14 | | 0.40 | | | | | | 13 | 3 | 26.20 | 17.78 | 0.04 | 0.00 |
| " | 6 | -3.19 | | | 0.69 | | | | | 13 | 3 | 25.46 | 17.41 | 0.05 | 0.00 |
| " | 7 | **-1.11** | | | | **2.46** | | | | **13** | **3** | 17.16 | **12.27** | **0.66** | **0.30** |
| " | 8 | -0.29 | | | | | -1.04 | 0.60 | 1.43 | 13 | 5 | 20.90 | 24.75 | 0.00 | 0.00 |
| " | 9 | -2.17 | | | | | 0.18 | | | 13 | 3 | 26.51 | 17.93 | 0.04 | 0.00 |
| " | 10 | -2.27 | | | | | | 0.59 | | 13 | 3 | 25.78 | 17.57 | 0.05 | 0.00 |
| " | 11 | -0.56 | | | | | | | 1.21 | 13 | 3 | 23.47 | 16.34 | 0.09 | 0.04 |
| ***A. longimana*, Summer** | 1 | 1.28 | 0.79 | 0.91 | -0.12 | -0.09 | | | -0.90 | 12 | 7 | 276.2 | 79.64 | 0.00 | 0.00 |
| " | 2 | 1.52 | 0.70 | 0.86 | -0.11 | -0.32 | | | | 12 | 6 | 313.5 | 67.96 | 0.00 | 0.00 |
| " | 3 | 1.80 | 0.69 | 0.85 | 0.14 | | | | | 12 | 5 | 318.1 | 59.33 | 0.00 | 0.00 |
| " | 4 | 1.80 | 0.74 | | | | | | | 12 | 3 | 346.8 | 49.37 | 0.14 | 0.00 |
| " | 5 | 1.96 | | 0.92 | | | | | | 12 | 3 | 337.4 | 49.04 | 0.17 | 0.00 |
| " | 6 | 1.73 | | | 0.03 | | | | | 12 | 3 | 366.1 | 50.01 | 0.10 | 0.00 |
| " | 7 | 1.55 | | | | 0.05 | | | | 12 | 3 | 366.0 | 50.01 | 0.10 | 0.00 |
| " | 8 | 1.37 | | | | | -0.39 | 0.18 | -0.22 | 12 | 5 | 330.3 | 59.78 | 0.00 | 0.00 |
| " | 9 | 1.91 | | | | | -0.98 | | | 12 | 3 | 334.0 | 48.91 | 0.18 | 0.00 |
| " | 10 | 1.63 | | | | | | -0.84 | | 12 | 3 | 341.8 | 49.19 | 0.16 | 0.00 |
| " | 11 | 1.69 | | | | | | | -0.79 | 12 | 3 | 344.4 | 49.28 | 0.15 | 0.00 |

Appendix Table 9. Comparison of selected linear regression models of *Elasmopus levis* biomass (mg AFDM * g plant $DM^{-1}$). Model fit statistics are as described in table 4. The three sets of models (all dates, spring, summer) incorporate all dates in which mesograzers, epiphytic chlorophyll, and predators were sampled, only those dates between day 70-150 of a year, and only those dates between 180 and 260 of year, respectively.

| | | Predictor variables with T values | | | | | | | | Model Fit Statistics | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Response | Model # | Constant | Water Temperature | Salinity | Turbidity | Epiphytic Chl a | Palaemonetes shrimp | Blue Crabs | Total Fish | n | K | RSS | $AIC_c$ | $w_i$ | $R^2$ |
| ***Elasmopus levis*, All dates** | 1 | 2.04 | -0.73 | 3.12 | 0.33 | 0.62 | | | 2.04 | 48 | 7 | 625 | 140.0 | 0.07 | 0.19 |
| " | 2 | 1.36 | -0.79 | 3.17 | -0.06 | 0.50 | | | | 48 | 6 | 687 | 141.8 | 0.03 | 0.13 |
| " | 3 | 1.37 | -0.77 | 3.19 | -0.15 | | | | | 48 | 5 | 691 | 139.4 | 0.09 | 0.15 |
| " | 4 | 0.72 | 0.59 | | | | | | | 48 | 3 | 857 | 144.9 | 0.01 | 0.00 |
| " | 5 | **1.25** | | **3.25** | | | | | | **48** | **3** | **702** | **135.3** | **0.71** | **0.17** |
| " | 6 | 0.81 | | | -0.45 | | | | | 48 | 3 | 860 | 145.0 | 0.01 | 0.00 |
| " | 7 | 0.82 | | | | 0.56 | | | | 48 | 3 | 858 | 144.9 | 0.01 | 0.00 |
| " | 8 | 1.01 | | | | | 0.01 | -1.21 | 2.67 | 48 | 5 | 733 | 142.3 | 0.02 | 0.09 |
| " | 9 | 0.80 | | | | | 0.09 | | | 48 | 3 | 863 | 145.2 | 0.00 | 0.00 |
| " | 10 | 0.56 | | | | | | -0.40 | | 48 | 3 | 861 | 145.1 | 0.01 | 0.00 |
| " | 11 | 1.59 | | | | | | | 2.15 | 48 | 3 | 784 | 140.6 | 0.05 | 0.07 |
| ***Elasmopus levis*, Spring** | 1 | 2.04 | -0.59 | 1.82 | 0.40 | 1.12 | | | 1.69 | 13 | 7 | 3.37 | 18.86 | 0.00 | 0.46 |
| " | 2 | 1.04 | -0.52 | 1.63 | -0.85 | 1.75 | | | | 13 | 6 | 4.75 | 12.90 | 0.00 | 0.34 |
| " | 3 | -0.27 | -0.96 | 2.17 | -0.57 | | | | | 13 | 5 | 6.55 | 9.66 | 0.00 | 0.19 |
| " | 4 | -0.24 | 0.28 | | | | | | | 13 | 3 | 10.67 | 6.10 | 0.00 | 0.00 |
| " | 5 | -0.27 | | 1.97 | | | | | | 13 | 3 | 7.95 | 2.28 | 0.01 | 0.19 |
| " | 6 | -0.24 | | | -0.71 | | | | | 13 | 3 | 10.27 | 5.60 | 0.00 | 0.00 |
| " | 7 | 1.35 | | | | 2.25 | | | | 13 | 3 | 7.35 | 1.25 | 0.02 | 0.25 |
| " | 8 | **3.05** | | | | | **-1.61** | **4.09** | **2.29** | **13** | **5** | **2.55** | **-2.62** | **0.12** | **0.68** |
| " | 9 | 0.88 | | | | | 1.58 | | | 13 | 3 | 8.75 | 3.52 | 0.01 | 0.11 |
| " | 10 | **2.10** | | | | | | **4.26** | | **13** | **3** | **4.05** | **-6.48** | **0.83** | **0.59** |
| " | 11 | 1.84 | | | | | | | 2.27 | 13 | 3 | 7.32 | 1.20 | 0.02 | 0.26 |
| ***Elasmopus levis*, Summer** | 1 | 1.90 | -0.11 | 2.47 | -1.22 | -1.17 | | | 1.25 | 12 | 7 | 225.2 | 77.19 | 0.00 | 0.28 |
| " | 2 | 1.61 | 0.03 | 2.47 | -1.19 | -0.86 | | | | 12 | 6 | 283.9 | 66.76 | 0.00 | 0.23 |
| " | 3 | 1.40 | -0.16 | 2.36 | -0.85 | | | | | 12 | 5 | 313.6 | 59.16 | 0.00 | 0.25 |
| " | 4 | 0.72 | -0.05 | | | | | | | 12 | 3 | 575.7 | 55.45 | 0.03 | 0.00 |
| " | 5 | **1.45** | | **2.61** | | | | | | **12** | **3** | **342.2** | **49.21** | **0.71** | **0.35** |
| " | 6 | 0.82 | | | -0.90 | | | | | 12 | 3 | 532.8 | 54.52 | 0.05 | 0.00 |
| " | 7 | 0.44 | | | | 0.52 | | | | 12 | 3 | 560.8 | 55.13 | 0.04 | 0.00 |
| " | 8 | 0.99 | | | | | -0.61 | -0.17 | 2.32 | 12 | 5 | 331.3 | 59.82 | 0.00 | 0.21 |
| " | 9 | 0.78 | | | | | -0.55 | | | 12 | 3 | 558.9 | 55.09 | 0.04 | 0.00 |
| " | 10 | 0.62 | | | | | | -0.62 | | 12 | 3 | 554.8 | 55.00 | 0.04 | 0.00 |
| " | 11 | 0.94 | | | | | | | 1.35 | 12 | 3 | 486.6 | 53.43 | 0.09 | 0.07 |

Appendix Table 10. Comparison of selected linear regression models of epifaunal species richness, measured as the total number of mobile epifaunal taxa recorded at a given collection date, at Goodwin Islands. Model fit statistics are as described in table 4. The three sets of models (all dates, spring, summer) incorporate all dates in which mesograzers, epiphytic chlorophyll, and predators were sampled, only those dates between day 70-150 of a year, and only those dates between 180 and 260 of year, respectively.

| | | Predictor variables with T values | | | | | | | | | Model Fit Statistics | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Response | Model # | Constant | Water Temperature | Salinity | Turbidity | Epiphytic Chl a | Mesograzer Total Density | Palaemonetes shrimp | Blue Crabs | Total Fish | n | K | RSS | $AIC_c$ | $w_i$ | $R^2$ |
| **Epifaunal SR, All dates** | 1 | **1.97** | **-0.33** | **3.12** | **1.98** | **-1.12** | **2.44** | | | **1.83** | **48** | **8** | **103** | **56.19** | **0.70** | **0.34** |
| " | 2 | 2.13 | -0.58 | 3.29 | 0.74 | -1.35 | | | | | 48 | 6 | 134 | 63.34 | 0.02 | 0.18 |
| " | 3 | 2.12 | -0.65 | 3.27 | 1.00 | | | | | | 48 | 5 | 140 | 62.73 | 0.03 | 0.16 |
| " | 4 | 1.36 | 0.93 | | | | | | | | 48 | 3 | 175 | 68.56 | 0.00 | 0.00 |
| " | 5 | **2.02** | | **3.32** | | | | | | | **48** | **3** | **144** | **59.14** | **0.16** | **0.18** |
| " | 6 | 1.53 | | | 0.65 | | | | | | 48 | 3 | 176 | 69.02 | 0.00 | 0.00 |
| " | 7 | 1.54 | | | | -1.30 | | | | | 48 | 3 | 172 | 67.73 | 0.00 | 0.01 |
| " | 8 | 1.43 | | | | | 2.11 | | | 1.55 | 48 | 4 | 146 | 62.21 | 0.03 | 0.15 |
| " | 9 | 2.02 | | | | | | 0.12 | -0.40 | 2.05 | 48 | 5 | 159 | 68.98 | 0.00 | 0.05 |
| " | 10 | 1.77 | | | | | | 0.96 | | | 48 | 3 | 175 | 68.50 | 0.00 | 0.00 |
| " | 11 | 1.67 | | | | | | | 0.71 | | 48 | 3 | 176 | 68.94 | 0.00 | 0.00 |
| " | 12 | 2.31 | | | | | | | | 2.27 | 48 | 3 | 160 | 64.34 | 0.01 | 0.08 |
| " | 13 | 0.83 | | | | | 2.72 | | | | 48 | 3 | 153 | 62.31 | 0.03 | 0.12 |
| **Epifaunal SR, Spring** | 1 | 1.28 | 0.22 | 1.35 | 0.43 | 2.31 | 0.33 | | | 0.36 | 13 | 8 | 10.20 | 48.84 | 0.00 | 0.48 |
| " | 2 | 2.50 | 0.29 | 1.54 | 0.07 | 3.14 | | | | | 13 | 6 | 10.83 | 23.62 | 0.01 | 0.59 |
| " | 3 | 0.26 | -0.45 | 2.01 | 0.34 | | | | | | 13 | 5 | 24.14 | 26.62 | 0.00 | 0.18 |
| " | 4 | 0.24 | 1.14 | | | | | | | | 13 | 3 | 34.94 | 21.52 | 0.01 | 0.03 |
| " | 5 | 0.29 | | 2.52 | | | | | | | 13 | 3 | 24.78 | 17.05 | 0.13 | 0.31 |
| " | 6 | 0.21 | | | 0.35 | | | | | | 13 | 3 | 38.66 | 22.84 | 0.01 | 0.00 |
| " | 7 | **2.57** | | | | **3.39** | | | | | **13** | **3** | **19.13** | **13.69** | **0.72** | **0.47** |
| " | 8 | 0.19 | | | | | 0.71 | | | 0.23 | 13 | 4 | 35.26 | 25.97 | 0.00 | 0.00 |
| " | 9 | 0.77 | | | | | | 0.08 | 1.59 | -0.03 | 13 | 5 | 26.90 | 28.02 | 0.00 | 0.08 |
| " | 10 | 1.00 | | | | | | 1.22 | | | 13 | 3 | 34.45 | 21.33 | 0.02 | 0.04 |
| " | 11 | 1.48 | | | | | | | 2.23 | | 13 | 3 | 26.92 | 18.13 | 0.08 | 0.25 |
| " | 12 | 0.79 | | | | | | | | 0.78 | 13 | 3 | 37.03 | 22.27 | 0.01 | 0.00 |
| " | 13 | -0.06 | | | | | 1.06 | | | | 13 | 3 | 35.45 | 21.71 | 0.01 | 0.01 |
| **Epifaunal SR, Summer** | 1 | 0.92 | 0.86 | 1.97 | -0.01 | -1.59 | 1.26 | | | 0.32 | 12 | 8 | 11.27 | 63.24 | 0.00 | 0.24 |
| " | 2 | 1.39 | 0.49 | 1.91 | -0.26 | -1.35 | | | | | 12 | 6 | 19.20 | 34.44 | 0.00 | 0.07 |
| " | 3 | 0.67 | 0.21 | 1.49 | 0.81 | | | | | | 12 | 5 | 24.17 | 28.40 | 0.00 | 0.00 |
| " | 4 | 0.44 | 0.17 | | | | | | | | 12 | 3 | 32.29 | 20.88 | 0.06 | 0.00 |
| " | 5 | **0.78** | | **1.53** | | | | | | | **12** | **3** | **26.24** | **18.39** | **0.22** | **0.11** |
| " | 6 | 0.38 | | | 0.67 | | | | | | 12 | 3 | 31.00 | 20.39 | 0.08 | 0.00 |
| " | 7 | 0.82 | | | | -0.97 | | | | | 12 | 3 | 29.62 | 19.84 | 0.11 | 0.00 |
| " | 8 | -0.01 | | | | | 0.99 | | | 0.18 | 12 | 4 | 27.03 | 23.46 | 0.02 | 0.00 |
| " | 9 | 0.58 | | | | | | -0.47 | -0.35 | 2.00 | 12 | 5 | 19.55 | 25.86 | 0.01 | 0.17 |
| " | 10 | 0.54 | | | | | | -0.92 | | | 12 | 3 | 29.87 | 19.94 | 0.10 | 0.00 |
| " | 11 | 0.27 | | | | | | | -1.02 | | 12 | 3 | 29.32 | 19.72 | 0.11 | 0.00 |
| " | 12 | 0.55 | | | | | | | | 0.89 | 12 | 3 | 29.98 | 19.99 | 0.10 | 0.00 |
| " | 13 | **-0.09** | | | | | **1.39** | | | | **12** | **3** | **27.12** | **18.78** | **0.18** | **0.08** |

Appendix Table 11. Comparison of selected linear regression models of fish abundance, measured as the average number of fish of all species per net sweep per collection date, at Goodwin Islands. Model fit statistics are as described in table 4. The three sets of models (all dates, spring, summer) incorporate all dates in which mesograzers, epiphytic chlorophyll, and predators were sampled, only those dates between day 70-150 of a year, and only those dates between 180 and 260 of year, respectively.

| | | Predictor variables with T values | | | | | | Model Fit Statistics | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Response** | Model # | Constant | Water Temperature | Salinity | Turbidity | Eelgrass density | Mesograzer Total Density | n | K | RSS | $AIC_c$ | $w_i$ | $R^2$ |
| **Total Fish, Post-2004** | 1 | -4.65 | 0.5 | 0.51 | -1.29 | -0.23 | 1.94 | 26 | 7 | 35.29 | 28.16 | 0.00 | 0.03 |
| " | 2 | -5.39 | 0.41 | -0.03 | -1.29 | 0.14 | | 26 | 6 | 41.91 | 28.83 | 0.00 | 0.00 |
| " | 3 | -8.21 | 0.36 | | | 0.05 | | 26 | 4 | 45.29 | 24.33 | 0.02 | 0.00 |
| " | 4 | -6.1 | | -0.04 | | -0.07 | | 26 | 4 | 45.55 | 24.48 | 0.02 | 0.00 |
| " | 5 | -7.88 | | | -1.34 | 0.05 | | 26 | 4 | 42.27 | 22.54 | 0.05 | 0.00 |
| " | 6 | **-7.99** | | | | **-0.77** | **1.93** | **26** | **4** | **39.17** | **20.56** | **0.12** | **0.07** |
| " | 7 | -8.42 | | | | -0.06 | | 26 | 3 | 45.55 | 21.67 | 0.07 | 0.00 |
| " | 8 | -4.96 | 0.54 | 0.65 | -1.36 | | 1.97 | 26 | 6 | 35.38 | 24.43 | 0.02 | 0.08 |
| " | 9 | -6.18 | 0.41 | -0.11 | -1.32 | | | 26 | 5 | 41.95 | 25.43 | 0.01 | 0.00 |
| " | 10 | -8.5 | 0.37 | | | | | 26 | 3 | 45.30 | 21.52 | 0.08 | 0.00 |
| " | 11 | -7.1 | | 0 | | | | 26 | 3 | 45.56 | 21.67 | 0.07 | 0.00 |
| " | 12 | -8.3 | | | -1.37 | | | 26 | 3 | 42.27 | 19.73 | 0.19 | 0.03 |
| **Total Fish, All dates** | 1 | -2.99 | -0.14 | 0.26 | -0.63 | | 1.83 | 48 | 6 | 340.5 | 108.1 | 0.02 | 0.03 |
| " | 2 | -2.49 | -0.31 | 0.52 | -1.26 | | | 48 | 5 | 367.1 | 109.1 | 0.01 | 0.00 |
| " | 3 | -2.58 | -0.32 | | | | | 48 | 3 | 384.3 | 106.4 | 0.05 | 0.00 |
| " | 4 | -2.60 | | 0.49 | | | | 48 | 3 | 383.2 | 106.3 | 0.06 | 0.00 |
| " | 5 | -2.75 | | | -1.40 | | | 48 | 3 | 369.4 | 104.5 | 0.14 | 0.02 |
| " | 6 | **-3.34** | | | | | **2.33** | **48** | **3** | **344.5** | **101.2** | **0.72** | **0.09** |
| **Total Fish, Spring** | 1 | -7.50 | -0.37 | 0.23 | -1.60 | | 1.33 | 13 | 6 | 3.08 | 7.29 | 0.00 | 0.30 |
| " | 2 | -7.14 | -0.34 | 0.44 | -2.28 | | | 13 | 5 | 3.76 | 2.45 | 0.01 | 0.24 |
| " | 3 | -6.14 | -0.80 | | | | | 13 | 3 | 6.25 | -0.86 | 0.03 | 0.00 |
| " | 4 | -5.96 | | 0.03 | | | | 13 | 3 | 6.61 | -0.12 | 0.02 | 0.00 |
| " | 5 | **-7.80** | | | **-2.82** | | | **13** | **3** | **3.84** | **-7.18** | **0.69** | **0.37** |
| " | 6 | **-7.58** | | | | | **2.29** | **13** | **3** | **4.48** | **-5.19** | **0.25** | **0.26** |
| **Total Fish, Summer** | 1 | -1.06 | 1.06 | 0.36 | -0.22 | | 2.02 | 12 | 6 | 150.3 | 59.14 | 0.00 | 0.11 |
| " | 2 | -0.20 | 0.46 | 0.44 | -0.67 | | | 12 | 5 | 237.7 | 55.83 | 0.00 | 0.00 |
| " | 3 | -0.38 | 0.55 | | | | | 12 | 3 | 258.3 | 45.83 | 0.09 | 0.00 |
| " | 4 | -0.27 | | 0.55 | | | | 12 | 3 | 258.4 | 45.84 | 0.09 | 0.00 |
| " | 5 | -0.32 | | | -0.81 | | | 12 | 3 | 250.0 | 45.44 | 0.10 | 0.00 |
| " | 6 | **-1.24** | | | | | **2.16** | **12** | **3** | **181.2** | **41.58** | **0.72** | **0.25** |

Appendix Table 12. Comparison of selected linear regression models of *Syngnathus spp.* pipefish abundance, measured as the average number per net sweep per collection date, at Goodwin Islands. Model fit statistics are as described in table 4. The three sets of models (all dates, spring, summer) incorporate all dates in which mesograzers, epiphytic chlorophyll, and predators were sampled, only those dates between day 70-150 of a year, and only those dates between 180 and 260 of year, respectively.

| | | Predictor variables with T values | | | | | | Model Fit Statistics | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Response** | Model # | Constant | Water Temperature | Salinity | Turbidity | Eelgrass density | Mesograzer Total Density | **n** | **K** | **RSS** | **$AIC_c$** | **$w_i$** | **$R^2$** |
| **Pipefish, Post-2004** | 1 | -0.93 | 1.43 | -0.24 | -1.23 | 1.07 | 0.77 | 26 | 7 | 7.05 | -13.70 | 0.00 | 0.02 |
| " | 2 | -1.31 | 1.42 | -0.48 | -1.28 | 1.25 | | 26 | 6 | 7.26 | -16.75 | 0.01 | 0.04 |
| " | 3 | -1.77 | 1.28 | | | 1.54 | | 26 | 4 | 7.95 | -20.92 | 0.09 | 0.04 |
| " | 4 | -1.26 | | -0.2 | | 0.9 | | 26 | 4 | 8.50 | -19.17 | 0.04 | 0.00 |
| " | 5 | -1.17 | | | -1.26 | 1.3 | | 26 | 4 | 7.96 | -20.87 | 0.09 | 0.04 |
| " | 6 | -1.25 | | | | 0.8 | 0.81 | 26 | 4 | 8.28 | -19.83 | 0.05 | 0.00 |
| " | 7 | -1.55 | | | | 1.19 | | 26 | 3 | 8.51 | -21.94 | 0.16 | 0.02 |
| " | 8 | -1.35 | 1.3 | -1.1 | -0.69 | | 0.99 | 26 | 6 | 7.46 | -16.05 | 0.01 | 0.02 |
| " | 9 | -2.02 | 1.25 | -1.12 | -1.16 | | | 26 | 5 | 7.80 | -18.29 | 0.03 | 0.02 |
| " | 10 | -1.99 | 0.84 | | | | | 26 | 3 | 8.76 | -21.19 | 0.11 | 0.00 |
| " | 11 | -1.95 | | -0.77 | | | | 26 | 3 | 8.80 | -21.09 | 0.10 | 0.00 |
| " | 12 | -1.51 | | | -1.14 | | | 26 | 3 | 8.55 | -21.83 | 0.15 | 0.01 |
| " | 13 | -1.35 | | | | | 1.18 | 26 | 3 | 8.52 | -21.92 | 0.16 | 0.02 |
| **Pipefish, All dates** | 1 | -0.67 | 0.52 | -0.74 | -0.55 | | 1.52 | 48 | 6 | 38.4 | 3.40 | 0.02 | 0.00 |
| " | 2 | -0.21 | 0.36 | -0.52 | -1.09 | | | 48 | 5 | 40.5 | 3.30 | 0.02 | 0.00 |
| " | 3 | -0.06 | -0.02 | | | | | 48 | 3 | 41.7 | -0.16 | 0.11 | 0.00 |
| " | 4 | -0.10 | | -0.34 | | | | 48 | 3 | 41.6 | -0.28 | 0.12 | 0.00 |
| " | 5 | **-0.09** | | | **-1.04** | | | **48** | **3** | **40.8** | **-1.27** | **0.20** | **0.00** |
| " | 6 | **-0.54** | | | | | **1.74** | **48** | **3** | **39.2** | **-3.21** | **0.53** | **0.04** |
| **Pipefish, Spring** | 1 | -0.66 | -0.26 | 0.20 | -1.10 | | -0.57 | 13 | 6 | 1.89 | 0.92 | 0.00 | 0.00 |
| " | 2 | -0.89 | -0.28 | 0.11 | -0.98 | | | 13 | 5 | 1.97 | -5.99 | 0.00 | 0.00 |
| " | 3 | -0.94 | -0.69 | | | | | 13 | 3 | 2.20 | -14.45 | 0.22 | 0.00 |
| " | 4 | -0.92 | | -0.22 | | | | 13 | 3 | 2.28 | -13.96 | 0.17 | 0.00 |
| " | 5 | **-0.98** | | | **-1.30** | | | **13** | **3** | **1.98** | **-15.77** | **0.43** | **0.06** |
| " | 6 | -0.87 | | | | | -0.05 | 13 | 3 | 2.29 | -13.90 | 0.17 | 0.00 |
| **Pipefish, Summer** | 1 | -0.31 | 0.85 | -1.66 | -0.67 | | 2.09 | 12 | 6 | 14.21 | 30.83 | 0.00 | 0.27 |
| " | 2 | 0.52 | 0.26 | -1.26 | -1.06 | | | 12 | 5 | 23.05 | 27.83 | 0.00 | 0.00 |
| " | 3 | 0.69 | 0.30 | | | | | 12 | 3 | 30.27 | 20.10 | 0.09 | 0.00 |
| " | 4 | 0.47 | | -1.22 | | | | 12 | 3 | 26.59 | 18.55 | 0.19 | 0.04 |
| " | 5 | 0.80 | | | -0.99 | | | 12 | 3 | 27.81 | 19.08 | 0.14 | 0.00 |
| " | 6 | **0.01** | | | | | **1.96** | **12** | **3** | **22.06** | **16.30** | **0.58** | **0.21** |

Appendix Table 13. Comparison of selected linear regression models of *Callinectes sapidus* abundance, measured as the average number of blue crabs per net sweep per collection date, at Goodwin Islands. Model fit statistics are as described in table 4. The three sets of models (all dates, spring, summer) incorporate all dates in which mesograzers, epiphytic chlorophyll, and predators were sampled, only those dates between day 70-150 of a year, and only those dates between 180 and 260 of year, respectively.

| | | Predictor variables with T values | | | | | | Model Fit Statistics | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Response** | Model # | Constant | Water Temperature | Salinity | Turbidity | Eelgrass density | Mesograzer Total Density | **n** | **K** | **RSS** | **$AIC_c$** | **$w_i$** | **$R^2$** |
| **Blue Crab, Post-2004** | 1 | -4.69 | -0.31 | 0.8 | -0.45 | 0.3 | 0.94 | 26 | 7 | 44.01 | 33.91 | 0.00 | 0.00 |
| " | 2 | -5.4 | -0.33 | 0.56 | -0.49 | 0.49 | | 26 | 6 | 45.94 | 31.22 | 0.00 | 0.00 |
| " | 3 | -8.84 | -0.21 | | | 0.24 | | 26 | 4 | 47.06 | 25.33 | 0.04 | 0.00 |
| " | 4 | -6.36 | | 0.46 | | 0.5 | | 26 | 4 | 46.73 | 25.15 | 0.04 | 0.00 |
| " | 5 | -8.59 | | | -0.47 | 0.35 | | 26 | 4 | 46.70 | 25.13 | 0.04 | 0.00 |
| " | 6 | -8.54 | | | | -0.01 | 0.83 | 26 | 4 | 45.78 | 24.61 | 0.05 | 0.00 |
| " | 7 | -9.2 | | | | 0.32 | | 26 | 3 | 47.15 | 22.57 | 0.15 | 0.00 |
| " | 8 | -5.18 | -0.35 | 0.75 | -0.43 | | 1.04 | 26 | 6 | 44.21 | 30.22 | 0.00 | 0.00 |
| " | 9 | -6.34 | -0.41 | 0.38 | -0.44 | | | 26 | 5 | 46.47 | 28.10 | 0.01 | 0.00 |
| " | 10 | -9.18 | -0.3 | | | | | 26 | 3 | 47.17 | 22.58 | 0.15 | 0.00 |
| " | 11 | -7.69 | | 0.23 | | | | 26 | 3 | 47.25 | 22.62 | 0.14 | 0.00 |
| " | 12 | -9.1 | | | -0.45 | | | 26 | 3 | 46.95 | 22.46 | 0.16 | 0.00 |
| " | 13 | -8.76 | | | | | 0.91 | 26 | 3 | 45.78 | 21.80 | 0.22 | 0.00 |
| " | 13 | **-8.02** | | | | | **1.79** | **26** | **3** | **40.19** | **18.41** | **0.36** | **0.08** |
| **Blue Crab, All dates** | 1 | -2.48 | -0.82 | 1.87 | -0.48 | | -0.87 | 48 | 6 | 549.8 | 131.1 | 0.03 | 0.00 |
| " | 2 | -2.90 | -0.74 | 1.77 | -0.21 | | | 48 | 5 | 559.5 | 129.3 | 0.06 | 0.01 |
| " | 3 | -3.24 | -0.02 | | | | | 48 | 3 | 602.0 | 127.9 | 0.13 | 0.00 |
| " | 4 | **-3.19** | | **1.65** | | | | **48** | **3** | **568.3** | **125.2** | **0.50** | **0.04** |
| " | 5 | -3.31 | | | -0.46 | | | 48 | 3 | 599.2 | 127.7 | 0.14 | 0.00 |
| " | 6 | -3.06 | | | | | -0.40 | 48 | 3 | 599.8 | 127.8 | 0.14 | 0.00 |
| **Blue Crab, Spring** | 1 | -2.76 | -0.68 | 2.83 | -1.62 | | -0.82 | 13 | 6 | 14.79 | 27.67 | 0.00 | 0.42 |
| " | 2 | -3.19 | -0.70 | 2.78 | -1.43 | | | 13 | 5 | 16.04 | 21.30 | 0.06 | 0.44 |
| " | 3 | -2.34 | 0.68 | | | | | 13 | 3 | 36.72 | 22.16 | 0.04 | 0.00 |
| " | 4 | **-2.96** | | **2.73** | | | | **13** | **3** | **22.79** | **15.96** | **0.81** | **0.35** |
| " | 5 | -2.43 | | | -1.16 | | | 13 | 3 | 34.10 | 21.20 | 0.06 | 0.03 |
| " | 6 | -2.33 | | | | | 0.38 | 13 | 3 | 37.77 | 22.53 | 0.03 | 0.00 |
| **Blue Crab, Summer** | 1 | -0.19 | 0.33 | 0.25 | -0.31 | | -0.41 | 12 | 6 | 420.4 | 71.48 | 0.00 | 0.00 |
| " | 2 | -0.42 | 0.48 | 0.23 | -0.23 | | | 12 | 5 | 430.5 | 62.96 | 0.00 | 0.00 |
| " | 3 | -0.55 | 0.56 | | | | | 12 | 3 | 436.5 | 52.13 | 0.27 | 0.00 |
| " | 4 | -0.48 | | 0.28 | | | | 12 | 3 | 446.4 | 52.40 | 0.23 | 0.00 |
| " | 5 | -0.52 | | | -0.31 | | | 12 | 3 | 445.8 | 52.38 | 0.24 | 0.00 |
| " | 6 | -0.33 | | | | | -0.49 | 12 | 3 | 439.5 | 52.21 | 0.26 | 0.00 |

Appendix Table 14. Comparison of selected linear regression models of *Palaemonetes* spp. shrimp abundance, measured as the average number of shrimp per net sweep per collection date, at Goodwin Islands. Model fit statistics are as described in table 4. The three sets of models (all dates, spring, summer) incorporate all dates in which mesograzers, epiphytic chlorophyll, and predators were sampled, only those dates between day 70-150 of a year, and only those dates between 180 and 260 of year, respectively.

| | | Predictor variables with T values | | | | | | | Model Fit Statistics | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Response | Model # | Constant | Water Temperature | Salinity | Turbidity | Eelgrass density | Epiphytic Chl *a* | Mesograzer Total Density | n | K | RSS | $AIC_c$ | $w_i$ | $R^2$ |
| ***Palaemonetes sp.*, Post-2004** | 1 | -1.46 | 0.12 | 1.62 | -2.9 | 4.33 | -0.55 | 0.58 | 26 | 8 | 882.1 | 116.10 | 0.00 | 0.48 |
| " | 2 | **-1.97** | **0.15** | **1.43** | **-2.97** | **4.71** | | | **26** | **6** | 927.0 | **109.34** | **0.12** | **0.51** |
| " | 3 | -4.37 | 0.37 | | | 3.71 | | | 26 | 4 | 1376 | 113.09 | 0.02 | 0.33 |
| " | 4 | -2.56 | | 1.09 | | 3.87 | | | 26 | 4 | 1316 | 111.93 | 0.03 | 0.36 |
| " | 5 | **-4.11** | | | **-2.8** | **4.56** | | | **26** | **4** | 1032 | **105.60** | **0.76** | **0.50** |
| " | 6 | -3.98 | | | | 3.25 | -0.06 | 0.4 | 26 | 5 | 1371 | 116.09 | 0.00 | 0.30 |
| " | 7 | -4.45 | | | | 3.84 | | | 26 | 3 | 1384 | 110.43 | 0.07 | 0.36 |
| " | 8 | -2.2 | -0.32 | 0.01 | -1.74 | | -0.5 | 0.98 | 26 | 7 | 1754 | 129.72 | 0.00 | 0.02 |
| " | 9 | -3.17 | -0.35 | -0.56 | -1.72 | | | | 26 | 5 | 1906 | 124.66 | 0.00 | 0.03 |
| " | 10 | -4.07 | -0.63 | | | | | | 26 | 3 | 2197 | 122.44 | 0.00 | 0.00 |
| " | 11 | -4.05 | | -0.81 | | | | | 26 | 3 | 2173 | 122.16 | 0.00 | 0.00 |
| ***Palaemonetes sp.*, All dates** | 1 | -2.08 | -1.09 | 1.78 | -1.35 | | | -0.18 | 48 | 6 | 6633 | 250.6 | 0.05 | 0.05 |
| " | 2 | -2.27 | -1.09 | 1.79 | -1.38 | | | | 48 | 5 | 6638 | 248.0 | 0.17 | 0.07 |
| " | 3 | -2.50 | -0.58 | | | | | | 48 | 3 | 7534 | 249.2 | 0.09 | 0.00 |
| " | 4 | **-2.50** | | **1.52** | | | | | **48** | **3** | **7226** | **247.2** | **0.25** | **0.03** |
| " | 5 | **-2.73** | | | **-1.70** | | | | **48** | **3** | **7139** | **246.6** | **0.34** | **0.04** |
| " | 6 | -2.71 | | | | | | 0.66 | 48 | 3 | 7516 | 249.1 | 0.10 | 0.00 |
| ***Palaemonetes sp.*, Spring** | 1 | -5.09 | -0.44 | 2.09 | -3.89 | | | -0.21 | 13 | 6 | 195.2 | 61.22 | 0.01 | 0.65 |
| " | 2 | -5.70 | -0.47 | 2.21 | -4.43 | | | | 13 | 5 | 196.3 | 53.86 | 0.20 | 0.69 |
| " | 3 | -3.09 | -0.19 | | | | | | 13 | 3 | 827.2 | 62.66 | 0.00 | 0.00 |
| " | 4 | -3.26 | | 1.18 | | | | | 13 | 3 | 736.0 | 61.14 | 0.01 | 0.03 |
| " | 5 | **-4.79** | | | **-3.97** | | | | **13** | **3** | **341.3** | **51.15** | **0.78** | **0.55** |
| " | 6 | -3.55 | | | | | | 1.33 | 13 | 3 | 714.5 | 60.75 | 0.01 | 0.06 |
| ***Palaemonetes sp.*, Summer** | 1 | 0.52 | 0.59 | 0.23 | -0.69 | | | -0.40 | 12 | 6 | 3283 | 96.14 | 0.00 | 0.00 |
| " | 2 | 0.41 | 0.77 | 0.21 | -0.64 | | | | 12 | 5 | 3359 | 87.61 | 0.00 | 0.00 |
| " | 3 | 0.34 | 0.89 | | | | | | 12 | 3 | 3562 | 77.32 | 0.31 | 0.00 |
| " | 4 | 0.38 | | 0.30 | | | | | 12 | 3 | 3808 | 78.12 | 0.20 | 0.00 |
| " | 5 | 0.39 | | | -0.77 | | | | 12 | 3 | 3629 | 77.54 | 0.27 | 0.00 |
| " | 6 | 0.46 | | | | | | -0.43 | 12 | 3 | 3773 | 78.01 | 0.22 | 0.00 |

VITA

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