The Ecology of Fear in Estuarine Communities:
Cascading Effects of Multiple Predators

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ABSTRACT

PAMELA LYNN REYNOLDS: The Ecology of Fear in Estuarine Communities:
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(Under the direction of John Bruno)

The role of predation has long influenced our understanding of ecological processes from the individual to the landscape level. Recent interest in the role of nonconsumptive effects of predators, or the consequences of prey defensive behaviors in response to predation risk, has revolutionized how ecologists perceive the role of predators in ecological communities. From focusing on how individual predators affect prey risk taking behaviors and foraging tactics, to the consequences of these behavioral shifts for ecosystem functions and services including primary production, nutrient cycling and energy transfer, we now know that the mere presence of predators can sometimes be more important than their lethal effects on prey density. However, predicting the cascading effects of multiple predator assemblages is often challenging and counterintuitive due to the consequences of behavioral interactions among predators and their prey. I tested the effects of predator presence, identity and richness on prey and basal resources in field and mesocosm experiments based on estuarine communities. By allowing predators to scare but not consume their prey, I examined the generality of nonconsumptive predator effects in these systems across multiple predator species. Predators had varying effects on prey density and the strength of a given prey antipredator behavior, with cascading effects on prey populations and resource dynamics over time. The presence of multiple predators heightened prey antipredator behavior, including reductions in foraging rates, and promoted basal resources despite changes in predation risk. Changes in predator diversity may have profound consequences for marine communities by altering the strength of both consumptive and nonconsumptive predator-prey interactions, with consequences for the strength of a trophic cascade. Complex food web models incorporating both consumptive and nonconsumptive pathways are necessary to understand and predict the effects of ongoing declines in predator abundance and diversity.

To those who turn consumers into producers of knowledge.

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PREFACE

Oh gallant grazer,
there is everything to fear.
Defend yourself - hide.

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INTRODUCTION

The role of predation has long influenced our understanding of ecological processes and their functioning, from the individual to the landscape level (Connell 1980, Carpenter et al. 1985). It is well established that predators can alter prey morphology and physiology, life history characteristics, and behavior as well as prey density, with consequences for the distribution and production of species at adjacent trophic levels (Carpenter et al. 1985, Crowl and Covich 1990, Harvell 1990, Lima and Dill 1990, Strong 1992, Tollrian and Harvell 1999, Werner and Peacor 2003). A recent major advance in our understanding of how predators affect ecological communities is the inclusion of nonconsumptive effects (NCEs, or behaviorally-mediated effects) of predators on their prey. Incorporating NCEs, the consequences of prey defensive behaviors in response to predation risk (Abrams 1984, 1995), has altered how we interpret the magnitude and scale of predator effects in many systems (see reviews by Dill et al. 2003, Werner and Peacor 2003, Preisser et al. 2005, Peckarsky et al. 2008, Schmitz et al. 2008).

The fact that predators shape and alter the behavior of each other and their prey is not novel. Studies over the past three decades demonstrate that the risk of predation can influence almost any aspect of prey decision-making, from foraging patterns and habitat use to mate choice. Indeed, behavioral ecologists may be surprised to realize that community ecologists have largely ignored these interactions up until the last decade. The innovation lies in the marked shift in focus from quantifying individual-based optimality decisions to exploring the consequences of behavioral changes for prey communities, including population growth rates and species interactions, and ecosystem functions such as primary production and nutrient cycling (i.e., ‘the ecology of fear,’ Brown et al. 1999). Indirect effects including trophic cascades, exploitative competition, and keystone predation traditionally thought to be transmitted by changes in species densities (consumptive effects) in food webs (e.g., Paine 1966, Okansen et al. 1981) are now known to also be influenced by changes in behavioral (nonconsumptive) interactions among predators and their prey (Abrams and Matsuda 1997, Grand and Dill 1999). From lynx-hare (Boonstra et al. 1998) to kelp-urchin-sea otter (Estes and Palmisan 1974, Estes et al. 1998, Konar and Estes 2003) and riparian vegetation-moose-wolf dynamics (Post et al. 1999, Wilmers et al. 2007), trait changes in both predators and prey can provide alternate causal mechanisms for interpreting classic studies of the role of predation (see review by Peckarsky et al. 2008).

Thus investigating behavioral interactions is no longer relegated to the field of ethology and pursuits of natural history or species evolution, but is integral for ecologists who aim to quantify and predict the net effects of predators on adjacent trophic levels in natural systems (Werner and Peacor 2003). By determining that predator-induced changes in prey behavior alone can influence plant diversity, productivity, nutrient cycling and energy flux as well as the strength of a trophic cascade, we have begun to appreciate that the mere presence of predators may sometimes be more important than their lethal effects on prey density (see review by Schmitz et al. 2008). Basic models assuming that interactions are an intrinsic feature of the component species, and are thus governed by focal species densities independently of the community at-large (e.g., Lotka-Volterra equations and derivatives) ignore the importance of behavioral changes and are therefore inadequate (Werner and Peacor 2003, Preisser and Bolnick 2008).

However, it is often empirically difficult to distinguish between nonconsumptive and consumptive predator effects, which can differ independently in direction and magnitude and are often context-dependent. For example, NCEs can alter prey resource and habitat use, which may alleviate imminent predation risk but pose potential long-term consequences for growth, fitness, life history characteristics, and susceptibility to alternate predators (Abrams 1990, Peacor and Werner 1997, Lima 1998, McPeek and Peckarsky 1998). The presence of multiple predator species can alter predation risk and the strength of prey antipredator behavior (Soluk and Collins 1988, Morin 1995, Sih et al. 1998, Eklöv and Van Kooten 2001, Duffy 2002, Vance-Chalcraft et al. 2004, Bruno and O'Connor 2005, Byrnes et al. 2006), changing the intensity of both consumptive and nonconsumptive interactions, and make it difficult to predict the community-level consequences of changes in a given predator community. Indeed, attempts to build a general theoretical framework describing or predicting the effects of predator diversity has likely been hampered in part by our almost complete failure to incorporate behavioral ecology, particularly NCEs, into quantitative food web models (but see Preisser and Bolnick 2008). Ecosystem-based management and conservation approaches that ignore these behavioral interactions are unlikely to yield informative predictions on the effects of ongoing predator losses (Dill et al. 2003).

Here I explore several novel pursuits, including integrating multiple predator assemblages and their nonconsumptive effects, to further our understanding of how predators influence estuarine communities. The strongest trophic cascades are often observed in marine benthic communities (Shurin et al. 2002), which thus provide model systems to test the role of NCEs on the strength of a trophic cascade. Using experiments in mesocosms and field cages, we tested the hypothesis that predator presence, identity and species richness can influence prey and basal resources (Chapters 1 and 3). By allowing predators to ‘scare’ but not consume their prey, we examined the generality of nonconsumptive predator effects across multiple predator species in complex food webs and found that predator cues alone could reduce prey density and population growth (Chapter 1), with potential implications for the expression and evolution of plant defenses (Chapter 2). We tested the persistence of predator effects on the strength of a trophic cascade in the field, partitioning nonconsumptive effects from the total predator effect in a complex food web open to natural resource recruitment and alternate prey availability (Chapter 3). Finally, by altering predator and prey species richness we assessed how realistic changes in food web topology (i.e., trophic skewing of richness) can interactively affect prey mortality and overall community dynamics, potentially by altering prey resources in addition to directly altering prey density (Chapter 4).

Chapter 1: Multiple marine predators alter prey behavior, population growth and a trophic cascade in a model estuarine food web

Predators can theoretically influence prey population dynamics and community structure by affecting prey behaviors with strong fitness consequences (Lima 1998). Together with my advisor Dr. John Bruno, I experimentally tested the effects of visual and olfactory cues from three common predators (pinfish, mud crabs, brown shrimp) on the strength of antipredator responses (reductions in grazing rate, dispersal and colonization) of an herbivorous amphipod. To assess population- and community-level impacts of altered prey antipredator behavior, we exposed amphipods to persistent cues from predator monocultures and polycultures for approximately two prey generations. We found that predator cues decreased prey grazing rates, dispersal, colonization, and population growth, with consequences for primary and secondary production. The presence of one intimidator, pinfish, consistently elicited strong antipredator responses and drove the effects of predator richness. In this system, predator richness strengthened both consumptive and nonconsumptive predator effects by increasing amphipod mortality risk and decreasing per capita amphipod consumption of seaweed, thereby strengthening the trophic cascade. Given the persistence of NCEs over time and that trophic cascades are common features of marine systems, changing marine predator diversity may have widespread effects on predator-prey behavioral interactions, with consequences for ecosystem function even in areas of weak predation pressure.

Chapter 2: Nonconsumptive predator effects indirectly influence marine plant biomass and palatability

Predators can reduce herbivory and facilitate plant biomass by consuming herbivores, lowering individual herbivore feeding rates, or both (Hairston et al. 1960, e.g., Trussell et al. 2003, see reviews by Werner and Peacor 2003, Preisser et al. 2005). Although the relative importance of these predator effects (i.e., consumptive and nonconsumptive) is under current debate, predators can have strong effects on grazing intensity (Shurin et al. 2002, Newcombe and Taylor 2010) with consequences for the ecological and evolutionary dynamics of plant phenotypes. With Dr. Erik Sotka, I examined the cascading impacts of predator-induced reductions in herbivorous amphipod grazing on the biomass and phenotype of a common brown alga, *Sargassum filipendula*. By culturing amphipods and algae in the presence or absence of olfactory cues from a major amphipod consumer, pinfish (*Lagodon rhomboides*), we altered amphipod population growth rates and grazing pressure in experimental outdoor mesocosms. The presence of fish cues reduced per-capita rates of amphipod grazing and overall amphipod population growth, which correlated with higher algal biomass. Predator induced reductions in prey grazing also affected the phenotype of the algae, increasing algal tissue palatability and potential susceptibility to future grazing. These results suggest that nonconsumptive effects of predators can regulate herbivore populations, with consequences for the ecological dynamics of plant biomass and chemical defenses.

Chapter 3: Partitioning consumptive and nonconsumptive predator-prey interactions with multiple predators in an oyster reef community

Although the predator-prey behavioral interactions and trophic cascades are common in a variety of ecosystems, ecologists have yet to fully incorporate these studies into an experimental framework that also manipulates predator richness under realistic field settings with natural predator cue diffusion and alternate prey availability. In order to quantify the role of predator richness and the importance of predator-prey behavioral interactions on the strength of a trophic cascade in the field, my advisor and I manipulated the presence of top predators (oyster toadfish, blue and stone crabs) and their ability to access and consume their prey (mud crabs) in experimental oyster reef communities. An amphipod-as-prey system was not pursued in the field due to the constraints of preventing amphipod dispersal and associated caging artifacts that would likely significantly alter natural water flow and the nature of predator-prey interactions.

After two months we found that consumptive and nonconsumptive effects varied with top predator identity. Nonconsumptive effects comprised a strong portion of the total top predator effect on a basal resource (juvenile oysters), further strengthening support for the importance of behavioral pathways in this system. The positive nonconsumptive effects on juvenile oysters weakened with distance from caged top predators, suggesting that NCEs may attenuate sharply with increasing spatial scale in the field and that predator density and distribution may be important for understanding the role of nonconsumptive effects at the landscape level. Surprisingly, the presence of multiple top predators decreased prey mortality but strengthened the trophic cascade. Thus, changes in prey behavior can compensate for a lack of lethal reductions of prey density. Similar to results from Chapter 1, the presence of one predator (oyster toadfish) strongly reduced prey foraging (an antipredator strategy) and likely drove predator richness effects. These results support that nonconsumptive effects can be important in the field and may drive predator richness effects on the strength of a trophic cascade.

Chapter 4: Effects of trophic skew on ecosystem functioning in a model marine community

Widespread overharvesting in coastal and marine ecosystems has “skewed” food webs towards greater domination of species at lower trophic levels (Duffy 2003, Byrnes et al. 2007). However, little is known about how such modifications to food web topology affect ecosystem functioning. Together with my advisor, I experimentally measured the effects of trophic skew on production in an estuarine food web by manipulating species richness across three trophic levels of an estuarine food web in experimental mesocosms. While increasing macroalgal richness promoted plant biomass, this effect disappeared in the presence of grazers. Increasing plant richness may counter intuitively promote both plant and herbivore communities through the increased likelihood of incorporating herbivore resistant plant species in addition to species that provide herbivores with refuge and balanced nutritional requirements. The strongest trophic cascade on macroalgae emerged in bottom-up skewed communities with greater prey to predator richness. These results suggest that predator richness effects may also be contingent upon richness at lower trophic levels, and that trophic skew can influence marine community structure and food web dynamics. This work emphasizes the need for multitrophic approaches to understanding the consequences of changing biodiversity in natural communities.

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CHAPTER 1:
MULTIPLE MARINE PREDATORS ALTER PREY BEHAVIOR, POPULATION GROWTH AND A TROPHIC CASCADE IN A MODEL ESTUARINE FOOD WEB

Abstract

Predators can influence prey population dynamics by affecting prey behaviors with strong fitness consequences. Here, we demonstrate that multiple predator species can nonconsumptively influence prey population growth and the strength of a trophic cascade in a model marine community. We exposed the herbivorous amphipod *Ampithoe longimana* to olfactory and visual cues from common predators (pinfish, mud crabs, brown shrimp) to quantify the nonconsumptive effects (NCEs) of predator identity and richness on individual, population and community level metrics. Predator cues decreased prey grazing rates, dispersal, colonization, and population growth, and influenced primary and secondary production. The presence of one intimidator, pinfish, consistently elicited strong NCEs and drove effects of predator richness. Given the persistence of NCEs over time and the fact that trophic cascades are common features of marine systems, changing marine predator diversity may have widespread effects on predator-prey behavioral interactions, with consequences for ecosystem function even in areas of weak predation pressure.

Key-words*: amphipod, antipredator behavior, diversity, food web, marine, nonconsumptive effects, predator-prey interactions, predator richness, trophic cascade*

Introduction

Predators can control prey populations by reducing their densities via consumption or a variety of nonconsumptive mechanisms (Abrams 1995). Nonconsumptive effects (NCEs, or behavioral interactions) of predators include changes in prey foraging, vigilance, mating and habitat selection (see review by Lima and Dill 1990). While antipredator behavior may impose immediate fitness costs, such behaviors may be beneficial over time and result in a net fitness increase for prey exposed to strong predation pressure (Boeing et al. 2010). Costs of this behavioral plasticity, including decreased births and/or individual size or growth rates, can drive prey population cycles and may influence trophic interactions (Preisser et al. 2005, Peckarsky et al. 2008). NCEs can also affect ecosystem functioning by altering plant diversity, productivity, nutrient cycling, trophic transfer efficiencies, and energy flux (see review by Schmitz *et al.* 2008). NCEs can be as strong or stronger than consumptive effects and qualitatively different (Preisser et al. 2005). They can amplify the impact of rare or less effective predators (Peacor 2002) and operate on larger spatial scales than direct predation (Orrock et al. 2008). Although the importance of predator-prey behavioral interactions is widely recognized, there is a paucity of knowledge regarding how NCEs operate across multiple prey generations (Werner and Peacor 2003).

Although NCEs may be common in marine systems (Raimondi et al. 2000, Dill et al. 2003, Trussell et al. 2003, Heithaus *et al.* 2008), little is known about the long-term influence of these interactions on prey populations and their cascading effects on lower trophic levels in marine food webs. Most previous work on prey behavior and NCEs was conducted on short timescales, often within one prey generation, and measured only a few behavioral responses or fitness components (e.g., McIntosh and Peckarsky 1999, Trussell et al. 2003, Byrnes et al. 2006), although recent work has greatly expanded our appreciation for the persistence and of NCEs over space and time (e.g., Berger et al. 2008, van der Merwe and Brown 2008). To understand the ramifications of NCEs for natural systems, it is necessary to account not only for the benefits of antipredator behavior for prey, but also for the associated population level costs and community level ramifications after multiple prey generations. Over time prey could theoretically habituate and antipredator behavior weaken with chronic exposure to predators in the absence of predation, or prey may compensate for the costs of a specific antipredator behavior by adjusting other behaviors (Lima and Bednekoff 1999). Additionally, it is possible that starving prey or prey facing significantly reduced fitness from a given antipredator behavior may eventually cease responding to predators regardless of predation risk, although this is not generally supported in the literature (see meta-analysis by Bolnick and Preisser 2005). As it can be difficult to isolate the role of NCEs when predation is allowed to occur, experiments partitioning behavioral effects and conducted over ecologically realistic timescales are necessary to address the persistence and ramifications of prey antipredator responses.

It is also necessary to consider NCEs in the context of the broader food web in which prey reside. The presence of multiple predator species can significantly affect behavior and the ramifications of NCEs (Sih et al. 1998, Heithaus et al. 2008). The presence of additional predators may affect specific prey behaviors (i.e., grazing rates) or the efficiency of the predator community at capturing prey (Crowder et al. 1997, Byrnes et al. 2006, Steffan and Snyder 2010). For example, Byrnes et al. (2006) found that although increasing predator richness did not affect herbivore density, it did promote total kelp biomass by reducing short-term herbivore feeding rates in a kelp forest food web.

Here we investigate the NCEs of multiple primary predators on their herbivorous amphipod prey in a model benthic, marine community. We used a series of laboratory, field and mesocosm experiments to address whether the NCEs of multiple predators can influence individual, population and community-level metrics. Specifically, we assessed the strength of NCEs on prey populations and a trophic cascade by manipulating predator identity and richness. We conducted short-term behavioral assays to measure immediate effects on prey behavior (grazing rates, dispersal, colonization), and a longer community experiment to quantify the ramifications of such behavioral modifications at the population and community level.

Methods

STUDY SYSTEM AND EXPERIMENTAL ORGANISMS

The experimental communities were based on a shallow, subtidal food web in Bogue Sound, North Carolina. Macroalgae growing on patches of hard substrate form the basis of this food web as major primary producers and habitat-formers. These algae are grazed intensely by small, mobile invertebrates including amphipods, which in turn are consumed by small invertebrate and vertebrate predators (Nelson 1978, Duffy and Hay 2000, Bruno and O'Connor 2005).

Experimental species chosen in this study are common and generally co-occur in shallow, sub-tidal habitats in North Carolina (Hay and Sutherland 1988). Macroalgae included *Dictyota menstrualis*, *Sargassum filipendula* and *Ulva lactuca* (Fig. 1). These species tend to dominate hard-substratum areas in North Carolina estuaries, and are often found in local marshes and seagrass beds (Hay and Sutherland 1988, Bruno et al. 2005). We selected the herbivorous amphipod *Ampithoe longimana* as our focal prey (e.g., grazer) due to its abundance and strong effects on algal biomass and composition (Duffy and Hay 2000). This tube-building amphipod is relatively sedentary and females produce multiple broods (Nelson 1978). Similar gammaridean amphipods are known to respond to predator cues (Wooster 1998). Predators included the highly mobile pinfish *(Lagodon rhomboides)* and the ambush predators brown shrimp *(Penaeus aztecus)* and mud crabs *(Panopeus herbstii*) (Fig. A1A). Pinfish actively forage in the water column and periphery of macroalgal beds, while brown shrimp and mud crabs hunt primarily within the complex macroalgal habitat or on the substrate, respectively. These predators were chosen due to their local abundance, similar size, and varied foraging strategies. All three predators are known to consume amphipods, including *A. longimana* (see Appendix A2, Bruno and O'Connor 2005).

Mesocosm and lab experiments were stocked with adult female *A. longimana* (5 ± 1 mm), which were exposed to predator cue treatments (no predators, one predator species or three predator species for a total of five treatments) in fully factorial designs. Experimental predators ranged in wet mass from an average of 2.5 *(P. herbstii)* - 4.2 g *(P. aztecus)* and from 1.8 - 5.8 cm in length (n = 32; Table A2). We collected predators within this size range to minimize variability in predator biomass among replicates across treatments. To avoid confounding density with richness, predator density was held constant in a substitutive design of three predators per experimental unit across all treatments, which is within the range of typical field densities for these predators (O'Connor and Bruno 2009). A substitutive design was also used because predator density can affect amphipod behavior (Wooster 1998).

Predators in all experiments were fed crushed amphipods and were replaced as necessary. Macroalgae and predators were used immediately after collection, while grazers were field collected and maintained in cultures for one week prior to experimentation.

EXPERIMENTAL DESIGN

Predators may nonconsumptively alter prey behavior, with consequences for local prey population growth and the strength of a trophic cascade. To examine the NCEs of multiple predators on prey and resource dynamics in this system, we conducted two laboratory behavioral assays (grazing rate, dispersal), a short-term field colonization study, and a five-week community experiment in outdoor mesocosms.

BEHAVIORAL ASSAYS

Laboratory assays were conducted in July 2008 using female *A. longimana* to assess short-term effects of predator cues on prey grazing and dispersal. Temperature, light and salinity in these assays were within the range of ambient conditions experienced throughout the tidal cycle in the field (O'Connor 2009; 24^{o}C, ~400 $\mu M\ m^{-2}\ s^{-1}$ per μA, 32ppt; Reynolds unpublished data).

Grazing Assay: To measure the effects of predator cues on prey grazing rates, one female *A. longimana* was placed in a clear plastic 9 ml cup with 50 mg of freshly collected *S. filipendula*. Cups were weighted with small pebbles to provide additional habitat. Four cups with grazers were paired with four no-grazer control cups and placed in 11.4 L 'predator tubs' provided with an airstone and flow-through filtered seawater (Fig. A1B). Cups were then exposed to ambient filtered seawater or to seawater with visual and chemical cues from the five different predator treatments (n = 8 for a total of 320 cups). Predators could swim freely around the cups. Small holes in the clear plastic cups allowed transmission of both chemical and visual cues while preventing predation. To determine grazing rates of *A. longimana* on *S. filipendula* across predator treatments we first compensated for autogenic changes in the control cups according to Sotka and Hay (Sotka and Hay 2002): $Ti(Cf/Ci)-Tf$ for grazer presence T and absence C, and initial i and final f macroalgal wet mass after 4 days. Cups were excluded if the grazer died (9) or molted (2). To avoid pseudoreplication, we averaged the amount grazed per amphipod within each tub and compared these tub averages across predator treatments.

Dispersal Assay: To measure the effects of predator cues on prey dispersal, we attached one 15 g thalli of each macroalgae species (45 g total) to a 25 x 25 cm Vexar mesh screen secured to the bottom of 12 L tubs such that the algae floated upright in a natural orientation. This macroalgal wet mass was comparable to field densities (Bruno et al. 2005). All tubs received 30 female *A. longimana*. Tubs were then exposed to predator cue treatments (n = 6). Predators were caged in a clear 3 L pitcher weighted with a rock and fixed with 50 μm screens to allow passage of visual and chemical cues while preventing direct predation. Tubs were provided with flow-through seawater; holes allowed water to slowly flow into a 'dispersal' tub (Fig. A1C). We compared the proportion of grazers in the dispersal tub across predator treatments after one week.

Field Colonization Experiment: To assess potential effects of NCEs on prey dynamics in natural field populations with open dispersal, we examined the effects of predator cues on prey colonization of new algal substrate. We stocked cylindrical Vexar cages (20 cm tall with 12 cm diameter; mesh opening of 0.3 cm) with zero, one or three predator species at constant density (n = 10). Two thalli (10 g, ~30 cm long each) of *S. filipendula* were attached to a 12 cm three-strand polypropylene rope fixed to the outside of each cage such that the algae floated upward in a natural orientation (see Figs A1E,F). Predators inside the cage could not access the algae, although it is possible that they may have consumed any amphipods that immigrated into the cage. Cages were attached to rebar fixed in sand adjacent to the jetty at Radio Island, NC on 11 May 2009. Cages were submerged 0.5 m from the surface at low tide and placed 1 m apart in a block design. After 72 hours all *S. filipendula* was removed and the number of grazers on the algae live counted. Replicates were discarded if the algae or cage disappeared (2) or the predators escaped (1).

COMMUNITY EXPERIMENT

If predator-induced changes in prey behavior induce significant fitness costs, NCEs can influence prey population growth in the absence of predation (Nelson et al. 2004). To quantify population- and community-level effects of NCEs, we performed a five-week experiment in 30 L outdoor mesocosms with flow-through filtered seawater (see Bruno and O'Connor 2005). In these experimental communities we tested whether predator NCEs could affect grazer population growth and how this interaction was influenced by predator identity and richness.

We manipulated grazer presence and predator cues in a fully factorial design for a total of seven treatments (n = 8). Treatments with predators but no grazers were included to test whether predator excretions affect algal growth. Mesocosms were first stocked with an ambient macroalgal community; algal screens identical to those in the dispersal assay were secured to the bottom of each mesocosm. The next day mesocosms received no or 30 fecund female *A. longimana* (Fig. A1D). Fecund females (e.g., currently brooding individuals) were added to ensure constant initial population demographics across replicates. The following day mesocosms received visual and olfactory cues from caged predators as in the dispersal experiment. Although predator biomass varied across replicates and shrimp treatments had greatest predator biomass (one-way ANOVA: $F_{3,28}$ =11.41, P < 0.0001, Tukey's HSD), total predator biomass per replicate did not significantly differ between the average single predator species and the three-predator species treatments (*P* = 0.49; LSM planned contrasts).

Mesocosms received gravel-filtered seawater from a dump bucket system to maintain aeration and simulate turbulence of local subtidal habitats. Temperature, nutrients, salinity and light levels in mesocosms were comparable to field conditions at Radio Island and in the nearby Bogue Sound, NC (Bruno et al. 2005, O'Connor and Bruno 2009). Before entering mesocosms, seawater passed through 200-μm mesh filter bags to minimize immigration and fouling. Immigration was minimal, as evidenced by the low number of grazers found in control (no *A. longiman* added) mesocosms at the end of the experiment (see Fig. 1.4A). Mesocosms were randomly assigned to tables in a block design and were rearranged within each table every two days to reduce positioning artifacts. Mesocosm table assignment did not affect final macroalgal biomass (one-factor ANOVA, $F_{1,73}$ = 0.0011, P = 0.97). The experiment began on 6 July 2008 and ran for 35 days, or conservatively for two overlapping generations of *A. longimana* (Cruz-Rivera and Hay 2001). Replicates were excluded if the predators escaped or the mesocosm cracked (6 total).

Throughout the experiment we nondestructively measured a proxy for grazer abundance to assess whether populations were continuously growing. To assess *A. longimana* density, we cable tied an 8 g thalli (c. 25 cm long) of *S. filipendula* to a small patch of Vexar and attached it to the bottom center of each mesocosm. We collected these dispersal patches 12 hours later and counted the number of grazers on the patch. Because *A. longimana* are often more active at night (Sotka pers. comm.), patches were deployed in the evening and collected the following morning (c. 12 hours). All grazers were returned to their respective mesocosms within two hours of removal.

To quantify how predator-induced changes in prey behavior affect prey populations, we quantified final grazer abundance and population size structure, as well as grazer ash free dry weight and size distribution. We quantified final grazer abundance and population size structure by live counting all individuals from each mesocosm and identifying all fecund females. Grazers were preserved in 70% ethanol and size classed using a series of nested sieves. Ash free dry weight and secondary production was then estimated from body-size distributions following Edgar (1990). Assuming generation time (T) was equivalent between predator-cue treatments (mean age of females at offspring birth = 14 days, Sotka and Reynolds in press), we calculated the fundamental net reproductive rate (R or **λ**) using the equation $R = N_1 (N_0)^{-1}$ and the intrinsic rate of natural increase (r) using the equation $r = \ln R\ (T)^{-1}$ (Begon et al. 2006). This calculation was used as we found no evidence for density dependence (see Fig. 1.4), and overlapping generations are implicit in the equation.

We measured final macroalgal biomass per species, and quantified chlorophyll *a* concentration from one 2 x 2 cm tile in each mesocosm as a proxy for microalgal accumulation (for methods, see Appendix A4).

ANALYSIS

Analysis of variance (ANOVA) performed in the Fit Model platform of JMP 8.0 was used to test all hypotheses for the behavioral assays. In the community experiment, we used a repeated measures ANOVA to test the effect of predator treatment on prey abundance over time, and a MANOVA to test the effects of predator treatment on final macroalgal community structure. Least Squares Means (LSM) planned contrasts were used to compare the effects of the mixed predator community with the average of the single predator species to test for predator richness effects. Data were transformed as necessary to meet the assumptions of normality and homoscedacity (Underwood 1997).

Results

BEHAVIORAL ASSAYS

Predator cues affected grazer consumption and dispersal in laboratory assays, and colonization in the field (Table 1.1), and the effects were predator-specific (Figs 1.2A-C). Grazers fed less and dispersed slightly less in treatments with cues from all three predator species compared to cues from the average single predator (*P* = 0.0026 and *P* = 0.047; LSM planned contrasts). All predators reduced grazer feeding in the lab, which was lowest in the presence of pinfish (pinfish and three-predator species treatments did not differ, Fig. 1.2A, Tukey's HSD). Grazer dispersal differed in the lab from the no-predator control only when pinfish were present (Fig. 1.2B, Tukey's HSD). More amphipods were found on algae attached to empty, control cages in the field compared to cages with predators (Fig. 1.2C). Predator identity and richness also affected amphipod colonization (*P* = 0.035; LSM planned contrasts). Observations at low tide did not indicate any bias of natural predator movements between cages, and thus it is not likely that observed differences in grazer abundances across predator treatments were due to differential attraction to the cages and predation by ambient predator communities (personal observation).

COMMUNITY EXPERIMENT

All grazer populations grew throughout this experiment (Fig. 1.3). Predator cues affected grazer population size over time (repeated measures ANOVA: $F_{4,31} = 18.87$, $P < 0.0001$; $P = 0.0009$ based on LSM planned contrasts). Although a likely approximation of total grazer abundance, these data must be interpreted with caution as predator cues may have reduced grazer mobility and patch colonization (see Results: Dispersal and Field Colonization experiments).

After 42 days, grazer abundance increased roughly 10-fold (intrinsic rate of increase, $r = 0.996$). Predator treatment affected final grazer abundance (Table 2), which was lower in the presence of predator cues ($P = 0.0001$; LSM planned contrast) although the effects varied among predator species (Fig. 1.3A). Final grazer populations were smaller in the three-predator treatment compared to the average single predator, indicative of a nonconsumptive predator richness effect (Fig. 1.3A, $P = 0.0070$, LSM planned contrast). Effects of predator presence on estimated final grazer ash free dry mass and secondary production mirrored abundance responses (Table 1.2, Figs 1.4B,C; $P < 0.005$ and $P = 0.0059$, LSM planned contrasts). Predator treatment did not affect the proportion of fecund female grazers (one-factor ANOVA, $F_{4,31} = 0.37$, $P = 0.83$, Fig. 1.3A) nor grazer brood size ($F_{4,31} = 0.48$, $P = 0.75$, n = 4 fecund females per replicate). Compared to the no-predator controls, the frequency of smaller grazer size classes decreased in the presence of predators (Fig. 1.5).

Incidental grazer immigration was minimal (Fig. 1.4, “no grazers” treatment) and thus differences in final grazer densities are likely due to differences in population growth rates across predator treatments.

Grazer and predator treatments interactively affected final macroalgal biomass, community structure, and microalgal chlorophyll *a* (Table 1.2, Figs 1.6 and A4). On average, grazers reduced final macroalgal biomass and microalgal chlorophyll *a* (Fig. 1.6A,B). In the absence of grazers, predator cues did not affect macroalgal biomass or microalgal chlorophyll *a* ($P = 0.31$ and $P = 0.45$, respectively; LSM planned contrasts). In the presence of grazers, only treatments with pinfish cues enhanced algae compared to the no-predator treatment (Fig. 1.6, Tukey’s HSD).

Discussion

Our results indicate that increasing the number of predator species andthe inclusion of strong intimidators can strengthen the nonconsumptive effects of predators, altering prey behavior and population dynamics with broader consequences for other trophic levels. Predator cues decreased prey grazing rates, dispersal, colonization, and population growth and influenced primary and secondary production. Pinfish cues induced the strongest NCEs, indicating strong predator identity effects on predator-prey behavioral interactions in this system.

BEHAVIORAL ASSAYS

On average, grazers consumed approximately 25% less algal biomass in the presence of predator cues in behavioral assays than in their absence (Fig. 1.2A). Such reductions in feeding may reduce the risk of predation by limiting overall activity and gut fullness, which have been proposed to be adaptive in reducing detection by predators (e.g., Giguère and Northcote, 1987). Here, reduced feeding likely led to lower grazer performance, and negatively affected individual fitness and overall population growth (Sotka and Hay 2002). In open systems in the field, NCEs of predators may also influence prey dynamics by altering prey movement (Orrock et al. 2008). We observed decreased grazer dispersal and colonization in the presence of persistent predator cues. On average 38% fewer grazers dispersed from experimental tanks (Fig. 1.2B), and 67% fewer grazers colonized field patches when predator cues were present (Fig. 1.2C). Cues from pinfish had the strongest effects on grazer mobility, reducing dispersal in the lab by 52% and colonization in the field by 74% compared to no-predator controls. These decreases may have unexpected consequences for natural populations, potentially stabilizing source populations while inhibiting sink populations. However, understanding the effects of predator induced changes in prey mobility on local population dynamics may be complicated by predation intensity. If prey mobility is reduced, prey density may increase over the short term in patches with predators due to reduced dispersal, but is predicted to ultimately decline due to active predator consumption and, possibly, reduced immigration (Sih and Wooster 1994, Orrock et al. 2010).

COMMUNITY EXPERIMENT

In the community experiment, persistent predator cues led to lower prey densities after approximately two overlapping generations. At the end of the experiment 57% fewer grazers were recovered in replicates exposed to persistent predator cues (Fig. 1.4A). Several mechanisms could have caused a reduction in prey population growth, including decreased reproduction and/or survivorship.

Predators can affect prey reproductive physiology via changes in body condition from reduced feeding or elevated stress (see review by Peckarsky et al. 2008). In behavioral assays adult female *A. longimana* grazed less in the presence of predator cues (Fig 1.2A), potentially reducing individual growth rates. Such negative effects on body condition can lower birth rates and reduce juvenile survivorship, decreasing fitness and population growth (Peckarsky and McIntosh 1998). For example, offspring from *Daphnia magna* mothers exposed to cues from fish predators are more susceptible to starvation (Stibor and Navarra, 2000). Reduced survivorship or fecundity, as well as delayed reproduction upon exposure to predator cues have been observed for some Daphnia clones (Burks et al. 2000, Hanazato et al. 2001). Similarly, E. Sotka and R. Taylor found that *A. longimana* reproduced several days later when exposed to persistent chemical cues from pinfish compared to controls (unpublished data), supporting the thesis that predator cues may increase the amphipod's generation time and thus reduce the population growth rate. However, cues from predators are also known to promote the fitness of their invertebrate prey; early maturation as well as production of larger clutches resulting in higher fitness has also been observed in other Daphnia clones (e.g., Castro et al. 2007, Boeing et al. 2010). Although we recovered fewer fecund female *A. longimana* in populations exposed to persistent predator cues, predator presence did not affect the proportion of fecund females or their brood size.

Delayed reproduction and/or reduced maternal condition may also affect prey size distributions. The frequency of small, juvenile prey individuals was lower in the presence of predator cues, especially pinfish (Fig. 1.5). However, increases in individual prey growth rates in the presence of predators could have exacerbated our observed differences. For example, Peacor (2002) found that tadpoles grew more quickly in the presence of caged predatory larval dragonflies due to an increase in resource availability caused by an overall decrease in tadpole foraging. Similarly, predators could affect prey morphology by reducing prey activity. Johansson and Andersson (2009) found that carp gained more biomass in the presence of predators due to a decrease in swimming activity and an increase in energetic investment in growth. Regardless of the mechanism, predator cues reduced *A. longimana* population growth with consequences for their resources, algae.

We found that NCEs alone can induce a trophic cascade and affect final algal biomass as well as composition (Figs 1.6, A4). Grazers intensely consumed *Dictyota menstrualis*, a preferred alga of *A. longimana* (Duffy and Hay 1991). Cues from pinfish and the three-predator treatment promoted a six-fold increase in *D. menstrualis* biomass (Tukey's HSD), and drove the interaction effect between grazer and predator cue treatments (Table 1.2). Biomass of the less preferred *Sargassum filipendula* and *Ulva lactuca* was largely unaffected by the presence of predators (Fig. 1.6C), supporting the thesis that observed changes in macroalgal community dynamics were driven by preferential grazing by *A. longimana* on *D. menstrualis*. The presence of predator cues likely did not affect *A. longimana* preference for *D. menstrualis* (see Appendix A5, Sotka and Reynolds in press). While predator excretions may enhance algal growth in some systems by increasing water column nutrient concentrations (e.g., Persson 1997, Layman et al. in press), we saw no direct effect of predator cues on macro- or microalgae in the absence of grazers (Fig. 1.6). It is possible that ambient nutrient levels are high in Bogue Sound, and thus algae are not likely to be nutrient limited in our experimental communities.

EFFECT OF PREDATOR EFFICIENCY

The strength of NCEs may correlate with predator efficiency of prey capture, abundance and evolutionary history with their prey (Sih et al. 1998, Werner and Peacor 2003). The costliness of antipredator behavior suggests prey should modify these behaviors in response to changes in predation risk; more efficient or threatening predators should more strongly intimidate prey (McIntosh and Peckarsky 1999). Although both shrimp *(P. aztecus)* and pinfish (*L. rhomboides)* were equally efficient predators in experimental mesocosms (Appendix 2; Nelson 1978; Bruno and O'Connor 2005), pinfish consistently elicited stronger NCEs. The least efficient predator, mud crabs (*P. herbstii*), elicited the weakest responses.

Recent work suggests that ambush predators such as shrimp or crabs should elicit stronger prey antipredator behavior (Preisser et al. 2007; Schmitz 2008). However, we found the greatest NCEs in the presence of cues from an active predator (pinfish). Heightened NCEs of pinfish on *A. longimana* may be due to increased exposure to this predator in the field with seasonal variation in abundance or encounter rates. Additionally, caging of predators in the community and field experiments restricted their mobility and may have altered our estimates of predator efficiency as well as prey perception by providing prey with persistent, point-source cues of predator presence, thereby enhancing potential NCEs. However, this does not fully explain all results as the pattern of strongest NCEs by pinfish was also observed in grazing assays in which predator mobility was less constrained.

It is also possible that laboratory feeding studies (Appendix A2) overestimated predator efficiencies, which are likely to be lower and may be similar in densely vegetated field habitats (Stoner 1982, Orth et al. 1984). Additionally, it is possible that prey adaptive risk assessment, where prey reduce antipredator behavior when the cost of starvation exceeds that of the risk of predation (see review by Ferrari et al. 2009), varied across predator species, although we cannot directly assess this here. Finally, it is possible that prey intimidation by a given predator and the efficiency of prey capture by that predator may not be correlated in the field, especially if the predator types that capture the most prey are cryptic and able to evade detection by their prey (Brown et al. 1999).

EFFECTS OF PREDATOR RICHNESS

Recent work on plant, grazer and predator diversity highlights the importance of multi-trophic interactions and species richness in governing predator-prey interactions. As the presence of multiple predators can nonadditively influence prey behavior, it is possible that increasing predator richness may alter nonconsumptive predator effects and the strength of a trophic cascade. Because slight increases in predator richness in this system can increase predator efficiency and strengthen a trophic cascade, particularly when predator omnivory is precluded (Fig. A2, Bruno and O'Connor 2005; but see O'Connor and Bruno 2009), more species-rich predator assemblages may be expected to evoke stronger antipredator behavior in their herbivorous prey. Here we add to a growing list of studies demonstrating that increasing the number of predator species may nonconsumptively affect prey populations.

In general, we found an increase in prey antipredator behavior (reduced grazing, dispersal, colonization) and lower prey production with exposure to cues from multiple predator species. Prey behavioral responses followed a hierarchy (McIntosh and Peckarsky 1999) with prey responding maximally to the presence of a single strong intimidator, pinfish. This led to an accumulation of antipredator behavior and an increase in NCEs with elevated predator richness, likely driven by a sampling effect where the likelihood of incorporating a strong intimidator increases in more diverse predator assemblages. Such sampling effects may be especially important if NCEs are not density dependent (i.e., the presence of one individual predator from a species that strongly intimidates its prey evokes the full NCE), as may occur in mesocosms or other systems where cue saturation is possible. However, supplemental work in this system (see Appendix A6) suggests that grazer antipredator behavior (e.g., reduced feeding) is stronger when grazers are exposed to higher predator densities (1 versus 3 pinfish), although this density effect did not persist with subsequent increases in predator density (3 versus 6 pinfish, Fig. A6). Thus, the presence of pinfish may have contributed to the heightened NCEs observed in the three-predator species treatment, but may not completely explain the observed results.

Similar to findings in a terrestrial system with caterpillar prey (Steffan and Snyder 2010) and a kelp forest food web with multiple invertebrate herbivores (Byrnes et al. 2006), we found that increasing the number of predator species elevated prey antipredator behavior. While Steffan and Snyder (2010) found that predator richness effects were mediated by increased foraging by predators in more diverse predator communities, which elevated encounters with prey and induced antipredator responses, here we present evidence that predator richness effects can manifest and persist across multiple prey generations even when predators cannot directly interact with their prey. While Byrnes et al. (2006) found that positive predator richness effects on kelp biomass were transmitted due to complementary prey antipredator responses, here we found that nonconsumptive predator richness effects may also be observed for individual prey populations. Future work should further investigate the role of multiple predator species NCEs on interactions among prey and predator populations.

Interpreting the results of multipredator experiments can be challenging due to emergent predator effects and feedbacks from indirect trophic interactions (Sih et al. 1998). Here we examined how predator richness affects prey behavior, but it may also alter predator behavior with consequences for predator efficiency (Rahel and Stein 1988, Soluk and Collins 1988, Crowder et al. 1997, Steffan and Snyder 2009). In addition, although predators may affect specific prey antipredator behaviors such as dispersal, this may decrease or increase prey vulnerability in the field where predators interact both nonconsumptively and consumptively. Comprehensive examinations of both consumptive and nonconsumptive predator-prey interactions under realistic conditions with multiple predators are necessary for effective predictions of the effects of predators on ecosystem functioning.

Understanding the role of NCEs is recognized as integral to predicting the net effects of predators on the structure and functioning of ecological communities (Stachowicz et al. 2007). Given the persistence of behavioral interactions over time and the fact that that trophic cascades are common features of marine systems (Shurin et al. 2002), changes in predator communities may have widespread effects on prey behavior with cascading impacts on marine communities. If predator cues have large spatial and temporal persistence, predator exclusion experiments in the field may underestimate the total effect of predators on prey dynamics as NCEs from local, natural predator communities may influence prey dynamics within experimental enclosures. Future work on nonconsumptive and other non-additive effects across realistic timescales in a food web context will contribute to our ability to predict the effects of changing marine predator communities.

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Supporting Information

Additional supporting information may be found in **Appendix A**:

Supplemental A1. *Experimental images*

Supplemental A2. *Predator efficiency of prey capture*

Supplemental A3. *Methods for chlorophyll* a *analysis*

Supplemental A4. *Macroalgal composition*

Supplemental A5. *Effects of predator cues on grazer diet preference*

Supplemental A6. *Effects of pinfish density on grazer feeding*

Tables

Table 1.1. Results of ANOVAS on the effects of predator treatment on prey behaviors (grazing, dispersal and colonization).

Treatment effects in each behavioral assay on the change in grazer feeding rate, dispersal, and colonization, analyzed by one-factor ANOVA.

Factor	SS	d.f.	*F*	*P*
Grazing Rate				
Predator treatment	74.50	4	35.57	**< 0.0001**
Error	18.33		35	
Dispersal				
Predator treatment	0.31	4	8.52	**0.0002**
Error	0.23	25		
Field Colonization				
All Gammaridean Amphipods				
Predator treatment	2075.03	4	11.50	**< 0.0001**
Error	1894.29	42		
***Ampithoe longimana* Only**				
Predator treatment	13.31	4	2.87	**0.0344**
Error	49.06		42	

Table 1.2. Results of ANOVAS on the effects of predator treatment on grazers, macro- and microalgae.

Treatment effects in mesocosm experiment on grazer abundance and estimates of ash free dry mass and production (one-factor ANOVA), macroalgal wet mass and microalgal chlorophyll *a* (two-factor ANOVA with both factors fixed), and macroalgal composition (MANOVA).

Factor	SS	d.f.	*F*	*P*
Grazer Abundance				
Predator Treatment	9.01	4	7.95	**0.0002**
Error	8.78	31		
Grazer AFDW				
Predator treatment	13517.45	4	2.95	**0.0356**
Error	35531.63	31		
Grazer Production				
Predator treatment	10.37	4	2.87	**0.0394**
Error	28.01	31		
Macroalgal Biomass				
Grazer presence (G)	1.09	1	13.25	**0.0005**
Predator treatment (P)	1.20	4	3.65	**0.0096**
G X P	1.42	4	4.34	**0.0036**
Error	5.33		65	
***Microalgae chlorophyll* a**				
Grazer presence (G)	275808.54	1	79.86	**< 0.0001**
Predator treatment (P)	54265.77	4	3.93	**0.0064**
G X P	41153.12	4	2.98	**0.0254**
Error	224479.49	65		

Macroalgal Community Structure

Factor	Wilk's λ	d.f.	*F*	*P*
Grazer presence (G)	0.7241	3, 63	15.2055	**< 0.0001**
Predator Treatment (P)	0.7190	12, 167	1.848	**0.0444**
G X P	0.6284	12, 167	2.6711	**0.0026**

Figures

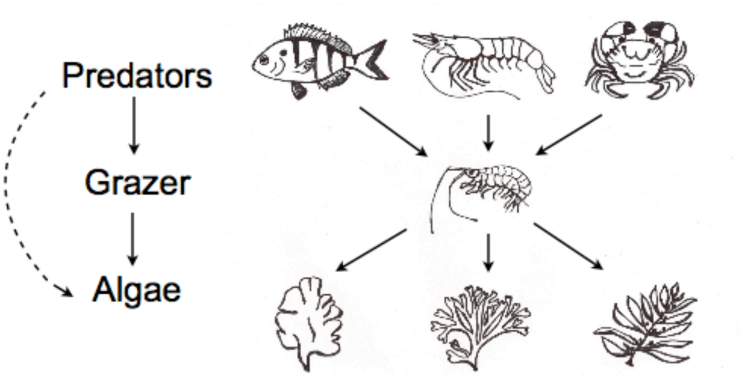

Figure 1.1. Experimental food web and trophic cascade.
Trophic cascade with direct (solid) and indirect (dashed) arrows and experimental food web. Algae and amphipod images redrawn from Schneider et al. (1991) and Bousfield (1973).

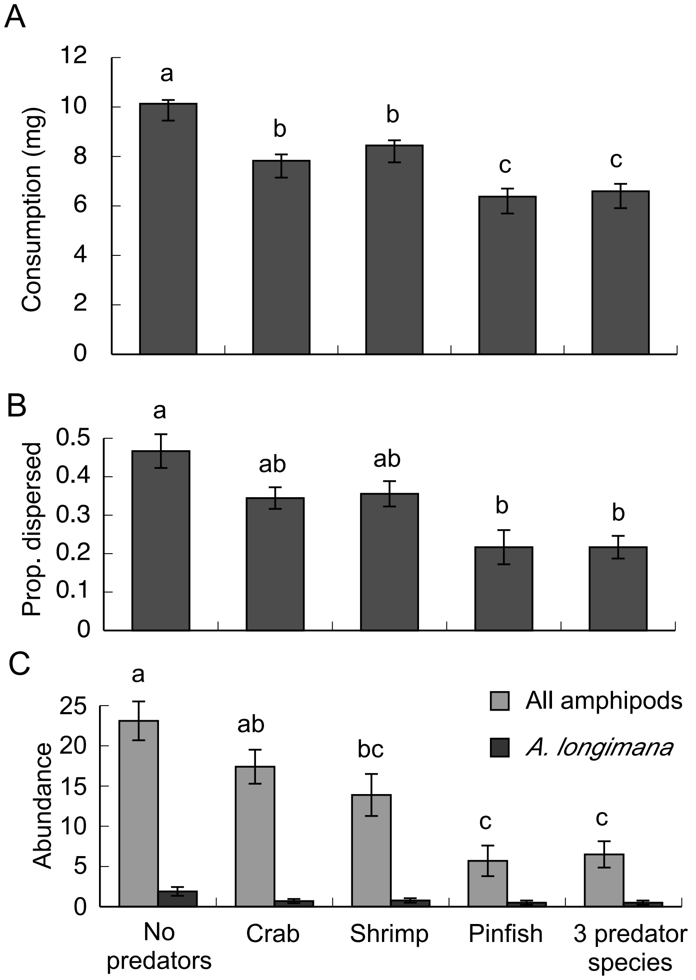

Figure 1.2. Results from behavioral assays.
Effects of predator cues on *A. longimana* A) grazing rate and B) dispersal in the laboratory, and C) colonization of algae in the field. (Values are means ± 1 SE, comparisons are Tukey's HSD.)

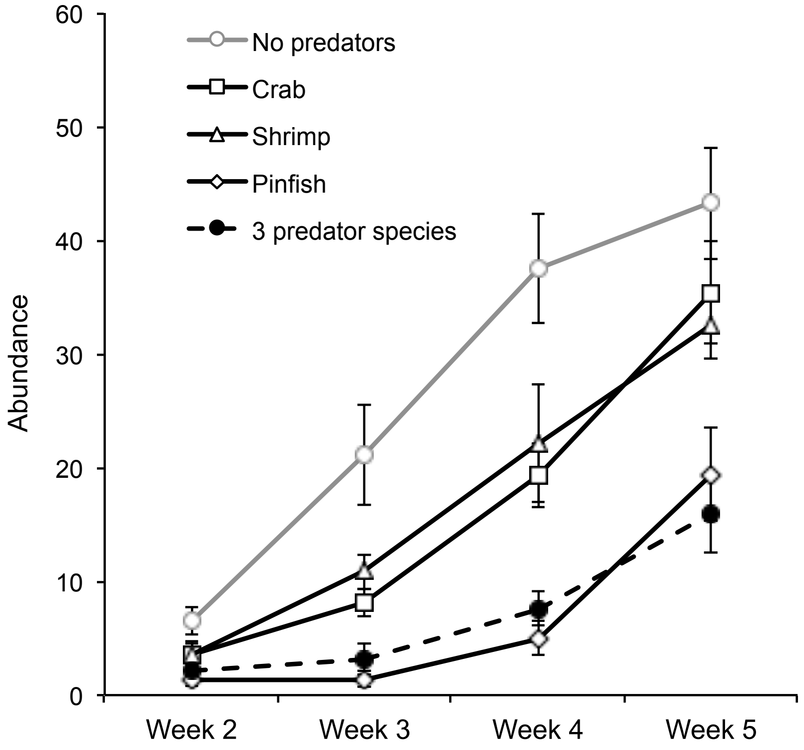

Figure 1.3. Treatment effects on *A. longimana* density over time.
The number of *A. longimana* recovered on dispersal patches during the community experiment. Values are means ± 1 SE.

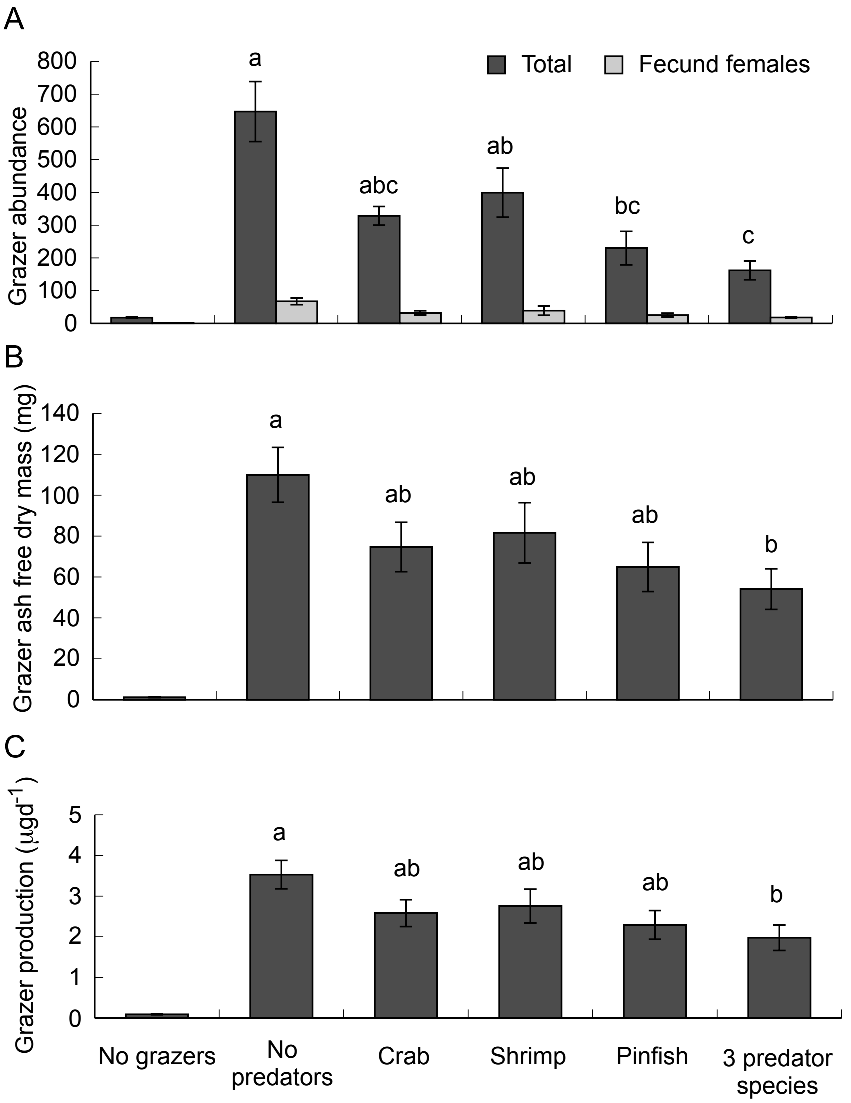

Figure 1.4. Results from community experiment: grazers.
Effects of predator cues on grazer A) abundance, B) estimated biomass (ash free dry mass) and C) production. Incidental amphipod immigration ("no grazers" treatment) was minimal. (Values are means ± 1 SE; comparisons are Tukey's HSD.)

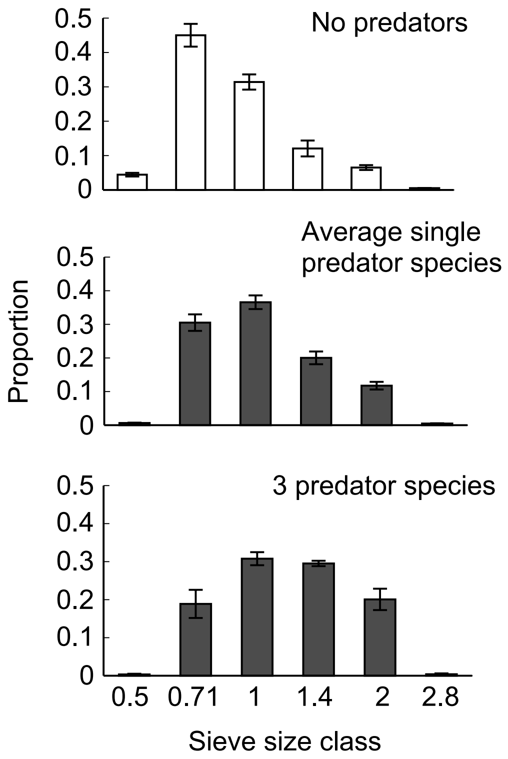

Figure 1.5. Effects of predator cues on grazer size distribution.
Effects of predator cues on grazer size frequency distributions after 5 weeks. Size classes correspond to the mesh size (mm) of the sieve on which the specimens were retained. (Values are means ± 1 SE.)

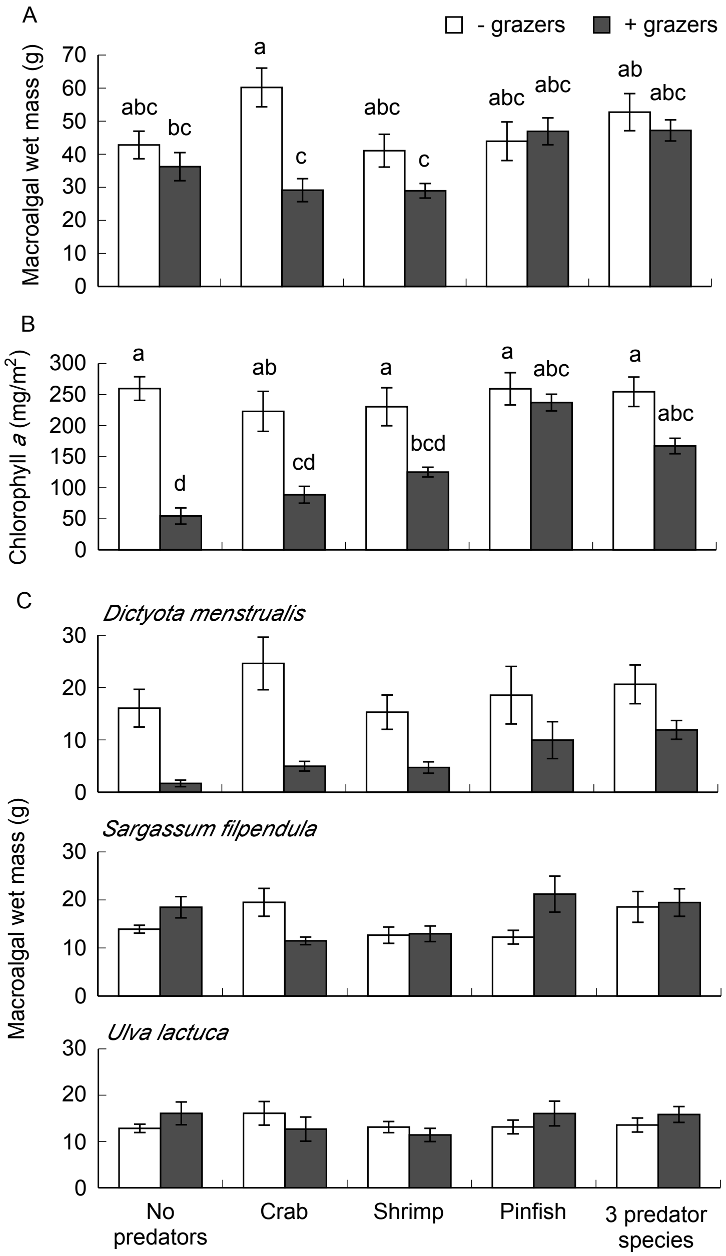

Figure 1.6. Results of community experiment: macro- and microalgae.
Community Experiment: Effects of grazer and predator cue treatments on A) macroalgal biomass, B) microalgal chlorophyll *a*, and C) macroalgal community composition after 5 weeks. (Values are means ± 1 SE, comparisons are Tukey's HSD.)

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CHAPTER 2:
NONCONSUMPTIVE PREDATOR EFFECTS INDIRECTLY INFLUENCE MARINE PLANT BIOMASS AND PALATABILITY

Abstract

Predators can reduce herbivory and increase plant biomass by consuming herbivores, lowering individual herbivore feeding rates, or both. We tested whether the presence of predators increases plant quality by nonconsumptively reducing grazing pressure and thereby weakening the strength of the induced response in plant chemical defences. We performed a 42-day outdoor mesocosm experiment in which the herbivorous amphipod *Ampithoe longimana* was cultured on the brown seaweed *Sargassum filipendula* in the presence and absence of olfactory cues of its principal fish predator, the pinfish *Lagodon rhomboides*. The presence of fish cues reduced per capita rates of amphipod grazing by nearly 50%. Over the span of the mesocosm experiment, this per capita reduction in feeding rate yielded at least a 40% lower growth rate of amphipod populations (i.e. r reduced from 1.01 to 0.61). The lower rates of amphipod grazing (overall or per capita) correlated with higher algal biomass. We then pursued a series of laboratory-based feeding choice assays with naïve amphipods to determine tissue palatability and the plant traits that mediate feeding choices. Tissue from tanks without grazers was more palatable than tissue from tanks with grazers, a pattern of induced plant defences that has been documented previously. Surprisingly, however, plant tissue from tanks with grazers and fish cues was more palatable than tissue from tanks with grazers but without fish cues. All changes in algal palatability were mediated by polar, but not lipophilic metabolites. These results suggest that the nonconsumptive effects of fish predators increases the food quality of *Sargassum* by weakening the strength of its induced chemical defences. The smell of predators has the potential to regulate herbivore populations and affect the ecological dynamics of plant biomass and chemical defences.

Key-words: *algal induction, amphipod, behaviour, herbivory, inducible defence*, *macroalgae, phenotypic plasticity, plant–herbivore interactions, predator-prey interaction*

Introduction

Predators often regulate herbivore densities and thereby facilitate plant growth (Hairston, Smith and Slobodkin 1960). Historically, ecologists assumed that lethal, or consumptive effects of predators largely mediated these tritrophic cascades by reducing herbivore densities. However, more recent evidence suggests that predator-induced changes in prey grazing behaviour (a nonconsumptive effect) may commonly underlie trophic cascades (Peckarsky *et al.* 2008). The presence of predators often lowers per capita grazing rates and alters herbivore host use and feeding preferences of their prey (e.g. Trussell, Ewanchuk and Bertness 2003; see reviews by Lima and Dill 1990; Werner and Peacor 2003; Preisser, Bolnick and Benard 2005). In those instances in which the antipredator behaviours lower herbivore fitness, the predator cues suppress the growth rates of herbivore populations (Nelson, Matthews and Rosenheim 2004). Although the relative importance of these predator effects (i.e. lethal and behaviourally induced) is under current debate, both effects can increase plant biomass (Shurin *et al.* 2002; Newcombe and Taylor 2010).

Predators are also known to alter the ecological and evolutionary dynamics of plant phenotypes, including chemical and morphological defences, via their effects on herbivore grazing intensity. When herbivores escape predation, the subsequent grazing pressure may induce production of secondary metabolites and other plant defensive traits that minimize future grazing. Such induced responses are common to vascular plants and aquatic and marine algae (Hessen and van Donk 1993; Karban and Baldwin 1997; Toth and Pavia 2007), and can themselves have cascading effects on herbivore populations (Agrawal and Rutter 1998) and communities (Denno, McClure and Ott 1995; Hay and Kubanek 2002; Long, Hamilton and Mitchell 2007). When plants across broad spatial and temporal scales are consistently grazed by herbivores that have escaped predation, evolution favours the constitutive production of plant defences to deter this greater herbivore pressure. As an example, marine seaweeds that co-occur in biogeographic areas with predatory sea otters and herbivorous urchins evolved lower levels of water-soluble chemical defences relative to seaweeds from areas that are historically devoid of otters but replete with higher densities of urchins (Estes and Steinberg 1988; Steinberg, Estes and Winter 1995). Virtually all of these studies assume that the effect of predators on plant phenotype is through a consumptive mechanism and relatively few have tested nonconsumptive predator effects on plant quality (but see Griffin and Thaler 2006; Kaplan and Thaler 2010).

Here we examine how predator cues and herbivore grazing intensity interactively influence the growth and food quality of a fucalean brown macroalga, *Sargassum filipendula*. Within an estuarine community in North Carolina (USA), *S. filipendula* is abundant year round and inhabited by a dense and diverse array of amphipod, isopod and gastropod grazers (Hay and Sutherland 1988). Amphipod abundances range from 10 to 145 individuals per gram (wet mass) on *S. filipendula*, although species composition and abundance fluctuates seasonally (Duffy 1989). The common herbivorous amphipod *Ampithoe longimana* (Smith 1873) has strong effects on algal biomass and community structure (Duffy 1989). It is also known to induce grazing resistance in several local algae including *S. filipendula* (Cronin and Hay 1996; Duffy and Hay 2000; Taylor, Sotka and Hay 2002). The pinfish *Lagodon rhomboides* (Linnaeus 1766) is a predominant predator of amphipods in this system (Nelson 1978; Nelson 1979; Stoner 1979).

It has been hypothesized that pinfish and other common fishes in North Carolina estuaries heavily consume *A. longimana* and other mesograzers, yielding cascading effects on macroalgal composition (Duffy and Hay 2000). However, it is also possible that this trophic cascade has a nonconsumptive mechanism when pinfish have strong nonconsumptive effects on *A. longimana* grazing behaviour (P. Reynolds and J. Bruno, unpublished data) in a manner similar to that seen for activity levels of other gammaridean amphipods (Wooster 1998; Wisenden, Cline and Sparkes 1999).

We used this model marine community to address whether predators alter macroalgal biomass and inducible defences via nonconsumptive effects on their herbivore prey. We conducted a 6-week experiment in outdoor mesocosms to test the effects of *L. rhomboides* effluent ('cues') on *A. longimana* grazing rates (Grazing Assay) and population growth, and feeding assays to assess the singular and interactive effects of fish cues and grazing on the tissue palatability of *S. filipendula* (Induction Experiment). Specifically, we asked the following questions: 1) Can pinfish nonconsumptively reduce amphipod grazing intensity by reducing per capita grazing rates, overall population growth, or both? 2) Does lowered grazing pressure facilitate algal biomass, and 3) elevate tissue palatability of *S. filipendula* by weakening the strength of its induction of chemical defences?

Methods

GRAZING ASSAY

To assess a potential mechanism for observed changes in grazer population growth and algal biomass (see Results), assays were conducted to test the short-term effects of predator cues on prey grazing in outdoor water tables on 10 August 2009. Temperature, light and salinity in these assays were within the range of conditions experienced throughout the tidal cycle in the field (24 ˚C, ~400 μM m^{-2} s^{-1} per μA, 35ppt; P. Reynolds and J. Bruno unpublished data). We placed one female *A. longimana* in a plastic 9-mL cup with 50 mg of freshly collected *S. filipendula*. Paired cups without *A. longimana* were placed with *A. longimana* addition cups in a 11.4-L 'predator tub' provided with flow-through filtered seawater (Fig. B1A). Three juvenile pinfish were added to half of the predator tubs (n = 9 for a total of 36 cups). Predators could swim freely around the cups but could not directly consume the amphipods. The opaque cups were weighed with small pebbles to provide additional habitat, and had holes that allowed transfer of olfactory cues from the surrounding water. To determine grazing rates of *A. longimana* on *S. filipendula* across predator and cue treatments, we first compensated for autogenic changes in the control cups according to Sotka *et al.* (2003): $Ti(Cf/Ci)-Tf$ for grazer presence T and absence C, and initial i and final f macroalgal wet mass. The experiment ran for seven days and replicates were excluded if the grazer died. Results were analysed with a two-tailed t-test.

INDUCTION EXPERIMENT

To examine how predator olfactory cues affect algal biomass and quality, we conducted experiments in outdoor mesocosms at the Institute of Marine Sciences in Morehead City, NC, USA (see Appendix S1 in Supporting Information). Replicates were established in two rows of tanks (replicate tank volume = 11.7 L) for a total of 36 top and bottom tanks (see Sotka, Taylor and Hay 2002; Taylor, Sotka and Hay 2002 for description of tank setup). Tanks were covered with window screen and a layer of 0.5-mm diameter Vexar plastic; light, temperature and salinity approximated field conditions (Li-100, measuring 4π irradiance; 23-25 ˚C, 34.5-36 ppt; Taylor, Sotka and Hay 2002). The top tanks received filtered seawater (at 0.08 L sec^{-1}) from the adjacent sound which then flowed into the bottom tanks in one direction. Top tanks were supplied with air stones. Filter bags (200-μm mesh) reduced natural colonization from the water system, and screens prevented emigration from experimental tanks.

Algae and amphipods were collected on 19 June 2009 from the shallow subtidal at Radio Island, NC (34˚ 42'N, 76˚ 41'W) at 1-0.5 m below low tide. All bottom tanks received two 200- to 300-mm-long *S. filipendula* ramets (9.5 ± 0.1g each) culled from two individual plants. Half of the bottom tanks then received 12 fecund female *A. longimana*. The next day, half of the top tanks received four juvenile pinfish *L. rhomboides* (1.53 ± 0.06 g, 37 ± 0.4 mm each fish) per tank. The bottom tanks therefore represent one of four treatments (n = 9): fish cue only (-G+F), grazers only (+G-F), grazers and fish cues (+G+F), or control (-G-F).

All outflow screens were cleaned and the algae lightly disturbed daily to remove sediment buildup. After 21 days, half the water within each tank was exchanged with fresh seawater in order to remove buildup of sediment and potential benthic microalgae at the bottom of the tanks; seawater was drained through a 500-μm mesh to retain all amphipods. Throughout the experiment, fish were fed a slurry of crushed gammaridean amphipods supplemented with frozen brine shrimp. Dead or sick fish were immediately replaced. Fish grew throughout the experiment; fish density was reduced to 3 fish per tank after 12 days and to 2 fish per tank after 24 days to maintain similar initial and final fish biomass.

Potential increases in nitrogen concentration due to bacterial degradation of fish excrement or food were low and likely transient in our flow-through tank system (levels for all tanks: nitrite ~0.25 ppm; nitrate ~10 ppm). Incidental grazer immigration was low in grazer control tanks (-G+F; see Results), and thus, this treatment serves as a proxy for the direct effect of fish cues on algal growth and palatability.

Algae were exposed to treatments for 42 days to allow adequate time for induced resistance to develop (Sotka, Taylor and Hay 2002; Taylor, Sotka and Hay 2002). At the end of the experiment bottom tanks were drained and all algae and grazers removed. Algal wet mass was determined after 60 revolutions in a salad spinner. Final algal wet mass could not be transformed to meet assumptions of normality and was analysed with a two-way nonparametric ANOVA. Significance was evaluated by comparing observed F-ratios with a distribution generated from 1000 permutations of the data set (Anderson 2001) using a custom *R* script (http://cran.r-project.org).

All grazers were live counted. Final grazer abundances were natural log-transformed for normality and analysed with a two-way ANOVA. We pursued log-transformation despite its known limitations (O'Hara and Kotze 2010), which are especially pronounced when means are relatively low and raw data include zeros. In our case, means were relatively high

(> 10 animals per replicate) and no tank had zero animals. We calculated the fundamental net reproductive rate (*R* or *l*) using the equation $R = N_1 (N_0)^{-1}$ and the intrinsic rate of natural increase (*r*) using the equation $r = \ln R\ (T)^{-1}$ (Begon, Townsend and Harper 2006). This calculation was used as we found no evidence for density dependence (see Fig. 2.3), and overlapping generations are implicit in the equation. We assumed generation time was equivalent between fish cue treatments (mean age of females at offspring birth = 14 days; Sotka and Reynolds in press).

An undamaged portion of the uppermost (top stipe) tissue from one plant in each tank was retained for fresh tissue feeding assays. Inducible responses are known to occur primarily in apical tissue in *S. filipendula* (Taylor, Sotka and Hay 2002) and other brown seaweeds (Rohde, Molis and Wahl 2004; Hemmi *et al.* 2005). Meristems in *S. filipendula* are apical, where the youngest and potentially more valuable tissue is found at the tips of the plant, and the oldest tissues at the holdfast. Due to a lack of sufficient tissue per replicate, all remaining healthy top stipe tissue was combined by treatment and immediately frozen for future assays. Frozen tissues were later freeze-dried and ground as in Taylor, Sotka and Hay (2002) to create an algal powder for reconstituted tissue and extract feeding assays, as well as phlorotannin and C:N analysis. Two tanks were excluded from all analyses due to inconsistent water flow.

CHOICE ASSAY—FRESH TISSUE

Feeding choice assays, in which algal tissues from different treatments were simultaneously offered, were conducted as in Sotka, Taylor and Hay (2002) to assess grazer feeding preferences. Grazers were given pairwise choices of fresh *S. filipendula* top stipes from all six combinations of the four treatments. On 2 August 2009 one freshly collected *A. longimana* was added to dishes containing two 30 mg (blotted wet mass) pieces of tissue separated by a plastic rod. Because there was no natural or experimental pairing of replicates across treatments, four pieces of tissue (genets) per replicate were randomly and independently paired with four other genets from different replicates of the compared treatment for a total of 36 pairwise comparisons per choice assay. Tissue without grazers was retained in separate dishes to control for autogenic changes in plant mass. After two days all tissues were reweighed and the amount of algae consumed calculated as described above in Materials and *methods: grazing assay*. In order to assure that grazers made a choice, replicates were dropped if < 3 mg was consumed of either piece of tissue (< 4 replicates per experimental pairing). To reduce potential pseudoreplication from the use of multiple genets per replicate, the proportion consumed of each tissue was averaged without error for each replicate and then compared to a null of 0.5 using two-tailed t-tests.

CHOICE ASSAY—FREEZE-DRIED TISSUE

To examine the influence of structural characteristics on palatability, we conducted feeding choice assays using reconstituted top stipe tissue from the four treatments. Top stipes were lyophilized (i.e. freeze-dried) and ground to a fine powder using a Wiley mill. We created reconstituted foods by adding 1.5 g of ground tissue to 6 mL of water, dissolving 0.36 g of agar in 10 mL of heated water, and combining both mixtures. This cooled mixture was then poured onto window screen, covered on both sides with wax paper, and pressed. We cut several 5×6-cell feeding grids, which were then offered to amphipods as described for the fresh-tissue assays. Choice assay comparisons were conducted on 3 November 2009 using freshly caught amphipods in all of the above pairings except fish cue vs. grazed and fish cue vs. grazed + fish cue because tissue from control and fish cue treatments were equally palatable in the fresh-tissue assay (see *Results*). A small clip was made in the corner of squares made of one tissue type per pairing for identification purposes. The trial ended when at least half of either treatment (>9 cells of one treatment or no more than 45 total) was consumed, typically after 3 days. We calculated consumption per treatment as a percentage of the total number of cells cleared for both squares, where a value of 50% represents no choice (following Bernays and Wedge 1987). Data were assessed statistically using blocked one-way permutation ANOVAs (analogous to a paired t-test) because data were non-normally distributed and could not be transformed to yield normality. Significance from a permuted distribution was generated as described within *Materials and methods: induction experiment.*

CHOICE ASSAY—MACROALGAL EXTRACTS

The palatability of crude water-soluble (polar) and lipophilic (nonpolar) extracts were assayed to assess the potential role of chemical defences in determining feeding preferences observed in the fresh and reconstituted tissue assays. Extractions were modified from Taylor e*t al.* (2003) and Long, Hamilton and Mitchell (2007), and conducted at the College of Charleston's Grice Marine Laboratory. Freeze-dried algal tissue was extracted three times in 2:1 ethyl acetate:methanol, and in 70:30 methanol:water. Organic solvents were removed by rotary evaporation and partitioned between ethyl acetate and water. Extracts were incorporated at natural concentrations by dry mass into 2 g freeze-dried powdered *Ectocarpus sp.* (a highly palatable, filamentous brown alga), which was used to make reconstituted food squares similar to those described in *Materials and methods: choice assays—freeze-dried tissue*. Lipophilic extracts were added to powdered *Ectocarpus* after dissolution in ethyl acetate, and tissue was rotary evaporated to remove the solvent. Water-soluble extracts were dissolved in distilled water and added to the powdered *Ectocarpus* when creating foods. Control foods were treated similarly but did not contain extracts. Choice assays and their analyses were conducted as described in *Materials and methods: choice assays—freeze-dried tissue.* Choice assays began on 1 March 2010 for lipophilic extracts, and on 8 May 2010 for water-soluble extracts.

MACROALGAL TRAITS

We measured several macroalgal traits that may correlate with herbivore feeding choices. Phlorotannins are polyphenolics produced by many brown seaweeds including *S. filipendula* (Cronin *et al.* 1997) that can deter marine grazers and may be induced by prior grazing (van Alstyne 1988; Pavia and Toth 2000; Toth and Pavia 2000; Pavia, Toth and Aberg 2002; Lüder and Clayton 2004; but see Toth and Pavia 2002; Deal *et al.* 2003; Kubanek *et al.* 2004; Long, Hamilton and Mitchell 2007). Phlorotannin analysis was conducted at Grice using the Folin–Ciocalteu method following van Alstyne (1995) and Long, Hamilton and Mitchell (2007). Freeze-dried tissue was extracted with 8:2 $MeOH:H_2O$ and chilled for four hours. Extracted supernatant was then filtered to remove particulates and added to 2M reagent with 1M Na_2CO_3, vortexed and heated. Absorbance (read at 760 nm) was then compared to a standard curve generated with phloroglucinol (Sigma, St. Louis, Missouri, USA) samples. Phlorotannin concentration was calculated as a percentage of dry mass and was analysed with a two-way ANOVA (n = 3) given the data were normally distributed and homoscedastic.

Nitrogen is generally considered to be limiting to primary and secondary productivity (Mann 1979; Mattson 1980) and can influence plant chemical defences and herbivore performance (Yates and Peckol 1993; Koricheva *et al.* 1998; Herms 2002), potentially counteracting induced resistance (Herms 2002; Cruz-Rivera and Hay 2003; Norderhaug, Nygaard and Fredriksen 2006). We measured algal tissue nitrogen content, total nitrogen and total organic carbon (TOC, TN) as well as tissue C:N ratio by gas chromatography–mass spectrometry at UNC-Chapel Hill's Marine Sciences Department (Fry *et al.* 1992; Brand 1996) (n = 3). The C:N and total nitrogen data could not be transformed to meet assumptions of normality, and the sample sizes were too small to pursue nonparametric *post hoc* tests (i.e. Wilcox tests). We thus rank-transformed the data (Conover and Iman 1981) and pursued parametric one-way ANOVAs and *post hoc* tests. Arguably, these three traits are not independent, and so we used a sequential Bonferroni procedure to generate an appropriate alpha (cf. Rice 1989). All three results were significant by this procedure. We combined all samples within a treatment because we had low amounts of top stipe tissue, and thus, statistical estimates of variation in algal traits largely reflect measurement error.

Results

The smell of fish reduced *Ampithoe longimana* grazing by 46% during the 1-week grazing assay (two-tailed t-test, T = -4.18, $p < 0.001$, Fig. 2.1). Fish cues also significantly reduced the population growth rate of *A. longimana* during the 42-day induction experiment (Table 2.1, Fig. 2.2A). Grazer populations exposed to fish cues averaged c. 65 amphipods by the end of the 42-day experiment (r ~ 0.61), while populations lacking fish cues averaged ~245 amphipods (r ~ 1.01). This represents a 40% decline in population growth rate in the presence of fish cues. It is possible that the mean generation time differed among grazer populations across treatments, which could affect our estimates of r. Given that fish cues reduced grazer feeding and likely reduced individual growth rates, our estimated decline in grazer population growth in the presence of fish cues is likely conservative and the true decline is likely greater.

There was incidental immigration of grazers into tanks that were not initially seeded (i.e. '-grazer' treatments), but they averaged fewer than 16 amphipods per tank. It is unlikely that predator-induced reductions are due to emigration because fine-mesh screens at the outflow of all tanks effectively impeded movement of *A. longimana* and because direct assays indicated that *A. longimana* reduces its movement in the presence of pinfish (P. Reynolds and J. Bruno, unpubl. data). Our final densities ranged from < 1 to 10.4 per g algal wet weight, and were within levels measured in the field (Duffy 1989), suggesting that grazing rates were ecologically realistic.

The interactive effect of grazer and fish cue treatments on final biomass of the alga *Sargassum filipendula* was significant (Table 2.1; Fig. 2.2B), indicating that grazers consistently lowered *S. filipendula* biomass, but the effect was greater when fish cues were absent. The negative relationship between algal biomass and grazer density was reflected across treatment means (Fig. 2.2) as well as replicate tanks (Fig. 2.3). Overall, our manipulations effectively created three levels of grazing intensity: lower ('-grazer - fish-cue' and '-grazer + fish-cue' treatments), intermediate ('+ grazer + fish-cue') and higher ('+grazer - fish-cue').

To test whether these levels of prior grazing altered the palatability of algal tissue, we offered fresh tissue to naïve amphipods in a pairwise series of feeding choice assays (Fig. 2.4A). Culturing water with fish cues in the absence of amphipods did not alter the palatability of plant tissues to amphipods (-G -F vs. -G +F), indicating that plant tissue quality did not respond to fish cues directly. Tissue from tanks without grazers was more palatable than those from tanks with grazers (-G -F vs. +G -F), a pattern of induction that was documented previously (Sotka, Taylor and Hay 2002; Taylor, Sotka and Hay 2002). Plant tissue from tanks with amphipods and fish cues was more palatable than tissue from tanks with amphipods but without fish cues (+G +F vs. +G -F). Summarizing all feeding assays, plant palatability grouped into three levels: higher (both treatments without amphipods; i.e., '-grazer'), intermediate ('+ grazer + fish-cue') and lower ('+ grazer - fish-cue') palatability. Thus, grazing intensity, as determined by grazer densities and behavioural responses to fish cues, negatively correlated with plant palatability.

We used several approaches to identify the plant traits mediating observed shifts in palatability. First, feeding choice assays using freeze-dried and reconstituted algal tissue replicated the ranking of palatability seen in fresh-plant assays without exception (compare Figs 2.4A and B). Because freeze-dried tissue removes the effects of morphological differences, these results indicate that patterns of algal palatability are mediated by secondary metabolites, nutritional traits or both. Second, feeding choice assays using the lipophilic extracts of *S. filipendula* revealed no differences in palatability among treatment types (Fig. 2.4C). The feeding responses toward polar extracts (Fig. 2.4D) did replicate the freeze-dried and fresh-tissue assays, suggesting one or more unknown polar compounds was responsible.

Finally, we measured candidate plant traits that have been shown previously to affect herbivore feeding behaviours. Tissues differed significantly in all algal traits (Table 2.1, Fig. 2.5), but these differences were largely explained by the plants that were most intensively grazed (+G-F treatment). Phlorotannin concentration increased from 0.27% to 0.34% (by dry mass) between control (-G-F) and grazed tissues (+G-F; Fig. 2.5A), which represents a 25% increase. In contrast, phlorotannin concentration from control (-G-F), fish cue (-G+F) and '+grazer + fish cue' (+G+F) tissues were similar (0.27-0.28%). Grazed tissues also had 38% less total nitrogen than did control tissues (control vs. grazed: 2.16% vs. 1.35%) and 10% more total carbon (22.8% vs. 25.1%). This yielded a far lower C:N ratio within control than grazed tissues (12.1 vs. 21.7), signifying that grazers would gain nearly twice as much nitrogen (standardized by carbon intake) while consuming control versus grazed tissue. There were much smaller differences in total nitrogen (2.21-2.54%), total carbon (22.8-24.1%) and C:N ratios (10.5-12.1) among control, fish cue and '+grazer +fish cue' treatments (Fig. 2.5B-D).

Discussion

Cascading impacts of nonconsumptive effects appear to be strong within North Carolina fish–epifauna–seaweed interactions. Olfactory cues from the pinfish *Lagodon rhomboides* yielded a 46% reduction in per capita grazing rates in *A. longimana*, 40% reduction in its population growth rate, and a 74% reduction in overall population size after 42 days (or at least 2-3 overlapping generations). Consistent with a trophic cascade, these artificial and predator-induced reductions in amphipod population size correlated with increasing *Sargassum filipendula* biomass (Figs 2.2-3) and palatability (Figs 2.4-5). Thus, non-lethal effects of predators can have cascading effects on both plant quantity and quality in nearshore marine environments.

NONCONSUMPTIVE EFFECT OF FISH PREDATORS ON PLANT BIOMASS

Small herbivorous species that live on seagrasses and macroalgae (termed mesograzers; Brawley 1992) can lower macroalgal growth rates (Norton and Benson 1983) and biomass (Shacklock and Croft 1981; Duffy and Hay 2000; Bruno and O'Connor 2005), as well as alter macroalgal composition (Duffy 1990; Bruno and O'Connor 2005). A negative effect of mesograzers on macrophyte biomass is not ubiquitous (Poore, Campbell and Steinberg 2009) in part because some mesograzers can also reduce epiphytic biomass (Brawley and Fei 1987; Duffy 1990; Mancinelli and Rossi 2001), which could alternatively promote plants via competitive release. In many habitats, fishes facilitate macrophytes by reducing densities of these mesograzers (Kennelly 1983; Dayton *et al.* 1984; Davenport and Anderson 2007; Korpinen, Jormalainen and Honkanen 2007; Newcombe and Taylor 2010). Historically, such trophic cascades were thought to be largely mediated by consumption rates rather than nonconsumptive, behavioural effects (Schmitz 2005; Peckarsky *et al.* 2008; Preisser and Bolnick 2008b). This bias occurs despite the fact that predator-induced reductions in per-capita grazing rates are commonly documented within many predator–prey systems (e.g. McIntosh and Townsend 1996; Trussell, Ewanchuck and Bertness 2003; Byrnes *et al.* 2006; Nelson 2007; Steffan and Snyder 2010). Moreover, reductions in feeding rates that occur with chronic exposure to predator cues over the lifespan of an individual or across generations can generate persistent reductions prey in population size (Fig. 2.2) (Nelson, Matthews and Rosenheim 2004; Pangle, Peacor and Johannsson 2007) and this long-term impact magnifies the nonconsumptive effect on per capita feeding. However, prey state (e.g. body condition) may modify trade-offs between foraging and predation risk, thereby regulating the expression of prey antipredator behaviour (Lima and Bednekoff 1999; Stephens, Brown and Ydenberg 2007). Starving prey are more likely to engage in risky activities, thereby limiting the degree of NCEs in systems where prey experience persistent predation risk and/or reduced resource abundance and quality.

As a consequence of the growing recognition of nonconsumptive predator effects, published studies that demonstrated trophic cascades are being re-assessed (Peckarsky *et al.* 2008). As an example, Duffy and Hay (2000) found relatively weak evidence for a trophic cascade involving spottail pinfish (*Diplodus holbrooki*) and *A. longimana* in experimental algal communities. In their 22-week experiment, outdoor mesocosms stocked with algae and amphipods were divided in half with a mesh barrier and predators were added to one side. Surprisingly, spottail pinfish did not significantly reduce *A. longimana* densities nor set in motion a trophic cascade effect on algal biomass. Assuming amphipods are able to detect the presence of fish predators, our data suggest that the lack of a trophic cascade in Duffy and Hay (2000) may have reflected the presence of fish cues, which would have nonconsumptively inhibited *A longimana* grazing and population growth rates on both sides of the mesocosm including in the 'no fish' treatments. Thus, the real effect of *A. longimana* on seaweed communities may be even greater than quantified by Duffy and Hay. In a similar vein, field studies that use cages (e.g. Davenport and Anderson 2007) or chemical means (Poore, Campbell and Steinberg 2009) to remove predatory fishes and increase mesograzer densities may underestimate the true effect of mesograzers on algal communities because olfactory cues from fish will continue to lower grazing rates. For these scenarios to be true, the spatial scale of the experimental manipulation must be similarly sized or smaller relative to the spatial scale across which fish cues are effective. If the spatial scale of manipulation is greater than the scale at which fish cues are effective, then the ensuing heterogeneity will complicate interpretations of observed responses by plants and mesograzers.

NONCONSUMPTIVE EFFECT OF FISH PREDATORS ON PLANT QUALITY

As with numerous plant and algal species (Toth, Karlsson and Pavia 2007; Toth and Pavia 2007), *S. filipendula* responds to grazing by *Ampithoe longimana* by becoming less palatable (Taylor, Sotka and Hay 2002). We demonstrate that the strength of this induced resistance is graded with the degree of mesograzer herbivory (Fig. 2.4). In particular, the nonconsumptive effect of pinfish moderated *A. longimana* grazing pressure and increased *S. filipendula* food quality by lowering its induced response. Although induced resistance after prior grazing in *S. filipendula* was previously documented by Taylor *et al.* (2002) and Sotka *et al.* (2002), the seaweed traits responsible have yet to be explored. We show here that shifts in seaweed palatability with grazing pressure were mediated by polar, and not lipophilic, algal tissue extracts (Fig. 2.4) and likely reflect an increase in the production of secondary metabolites, a decrease in nutritional content, or both. We do not believe that water-soluble phlorotannins explain observed tissue palatability patterns because the concentration of phlorotannins was very low (< 0.5%) relative to other brown seaweeds (van Alstyne, Duggins and Dethier 2001), but is consistent with previous observations from other tropical regions (e.g. Steinberg and Paul 1990; Pereira and Yoneshigue-Valentin 1999), and *A. longimana* appears to readily consume these and greater levels of phlorotannins (Kubanek *et al.* 2004). Moreover, definitive proof that phlorotannins play a role requires that herbivores are offered the isolated compounds in a feeding choice assay (e.g. Boettcher and Targett 1993). Regardless, it seems likely that a non-phenolic water-soluble deterrent was induced by amphipod grazing, as has been suggested for other brown seaweed–mesograzer interactions (Deal *et al.* 2003; Long, Hamilton and Mitchell 2007).

Nutritional traits or the interaction of nutritional traits with secondary metabolites may be responsible for shifts in tissue palatability. Plant nutritional traits can shift with herbivory pressure and may be adaptive in some cases (Ritchie, Tilman and Knops 1998; Norderhaug, Nygaard and Fredriksen 2006; Bracken and Stachowicz 2007). We found that *S. filpendula* tissues that were least preferred (i.e. exposed to prior grazing) exhibited lower nitrogen, greater carbon and a substantially greater C:N ratio compared to more preferred tissues exposed to less grazing pressure (Fig. 2.5). In theory, herbivores are nitrogen-limited (Mann 1979; Mattson 1980), and many herbivores attempt to maximize nitrogen relative to carbon intake (or protein to carbohydrate, Raubenheimer and Simpson 2009). However, when in the presence of predators, stressed grazers may selectively seek carbohydrate-rich foods and consume higher C:N plant tissue (Hawlena and Schmitz 2010a), with consequences for plant tissue composition, grazer nutrient assimilation and nutrient cycling (Trussell, Ewanchuck and Bertness 2006, Hawlena and Schmitz 2010b). Unfortunately, we did not generate ash-free dry mass values relative to wet mass, which is known to negatively correlate with no-choice feeding rates exhibited by *A. longimana* across algal species, although strong variation in this parameter was unlikely within our single, focal algal species (Cruz-Rivera and Hay 2001).

We do not believe that predator cues alone or direct grazer excretions strongly affected algal traits. Fish cues in the absence of herbivores (-G +F) had no effect on algal palatability (Fig. 2.4) nor algal biomass (Fig. 2.3). Although pinfish effluent may have increased ammonia concentration in the water, promoting N uptake and leading to decreased algal tissue C:N in the absence of grazers, the magnitude of these changes was relatively slight (Fig. 2.5). Similarly, although it is possible that increased herbivore density can lead to elevated local deposits of nutrient-rich herbivore excretions (Taylor and Rees 1998), we found lower nitrogen within tissues that were exposed to greater grazer densities (Fig. 2.5).

The nonconsumptive effect of predators on plant biomass is positive, but counter-intuitively, predator effects on plant quality may result in higher grazing rates over time. This is because when predators weaken algal-induced responses and increase food quality, the growth rates and fecundity of herbivores can increase. Indeed, grazer-induced defences of *Ascophyllum nodosum* decrease the fecundity of gastropod grazers (Toth, Langhamer and Pavia 2005). Similarly, Haavisto, Välikangas and Jormalinen (2010) found that the isopod *Idotea baltica* exhibited decreased egg production when fed defended *Fucus vesiculosus* compared to ungrazed algae. On the other hand, recent evidence suggests that waterborne cues of grazed macroalgae attract predators (Coleman *et al.* 2007), which raises the possibility that grazer-induced tissue will lower herbivore fitness by both increasing predator pressure and lowering food quality. Clearly, the chemical mediation of tritrophic interactions between predators, mesograzers and macrophytes deserve increased attention.

SUMMARY

Marine herbivores can alter algal community dynamics and their phenotype, including morphology and defensive chemistry (Cronin and Hay 1996). Here we demonstrate that predator cues can ameliorate the effects of grazers on algal growth and improve algal food quality by weakening the strength of induction. The behaviourally mediated trophic cascade among pinfish, amphipods and *Sargassum* raises the untested possibility that nonconsumptive effects of predators are as large as their consumptive effects, especially in regions of low predation pressure where predators scare more herbivores than they consume.

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Supporting Information

Additional supporting information may be found in **Appendix B,** *Supplemental figures:*

Figure B1 *Experimental images.*

Figure B2 *Design of algal induction experiment.*

Tables

Table 2.1. ANOVA of treatment effects on amphipods, algae and phlorotannins.

Results of two-factor ANOVAs on final amphipod abundance, algal wet mass and tissue phlorotannin concentration.

Factor	**SS**	**d.f.**	***F***	***P***
Grazer Abundance				
+ Grazers (G)	46.83	1	104.62	**< 0.001**
+ Fish-Cue (F)	6.39	1	14.26	**< 0.001**
GXF	2.00	1	4.47	**0.043**
Error	13.43	30		
Algal Wet Mass				
+ Grazers	13437.10	1	118.72	**< 0.001**
+ Fish Cue	923.67	1	8.16	**0.008**
GXF	499.95	1	4.417	**0.044**
Error	3395.38	30		
Algal Phlorotannins				
+ Grazers (G)	3.03e-7	1	7.54	**0.025**
+ Fish-cue (F)	4.62e-7	1	11.49	**0.010**
GXF	4.40e-7	1	10.95	**0.011**
Error	3.22e-7	8		

Figures

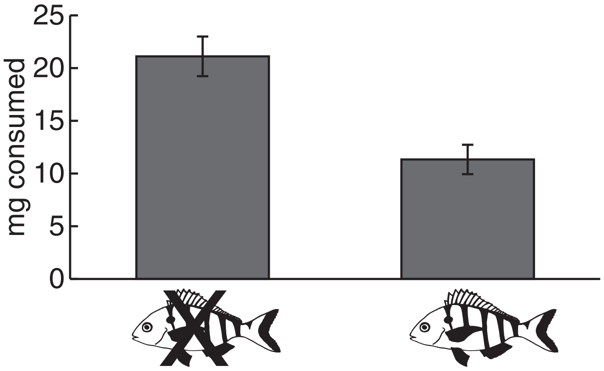

Figure 2.1. Effects of fish cues on amphipod grazing rates
A. longimana grazing rates over 7 days in the presence (+) and absence (-) of olfactory cues from fish (fish icon). (T = -4.18, $p < 0.001$) Mean consumption (mg) ±1 SE are shown.

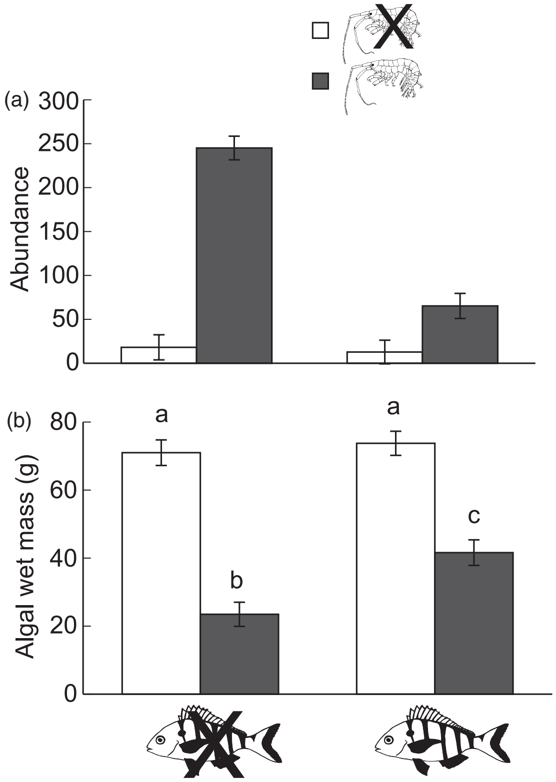

Figure 2.2. Effects of grazer and fish cue treatments on final grazer and algal populations
Effects of grazer (dark bars) and fish cue (fish icon) treatments on final A) grazer population size and B) algal wet mass after 42 days. Letters represent treatments that are significantly different by Tukey's HSD. Mean ± SE values are shown.

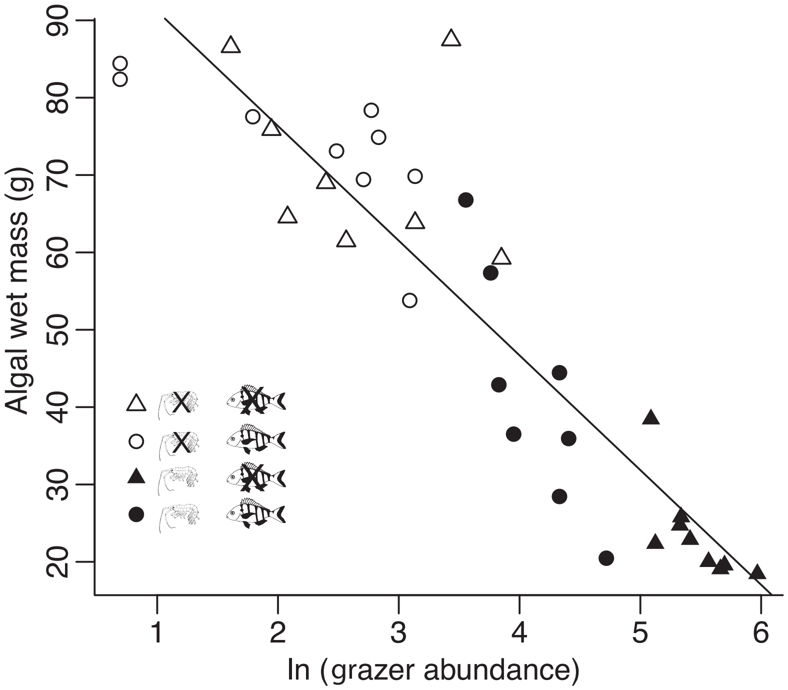

Figure 2.3. Exponential decay of final algal biomass with grazer density
(Biomass X ln(grazer abundance): $r^2 = 0.827$; $p < 0.001$). Filled shapes represent replicates initially stocked with grazers, and triangles and circles represent replicates without and with olfactory cues from fish, respectively.

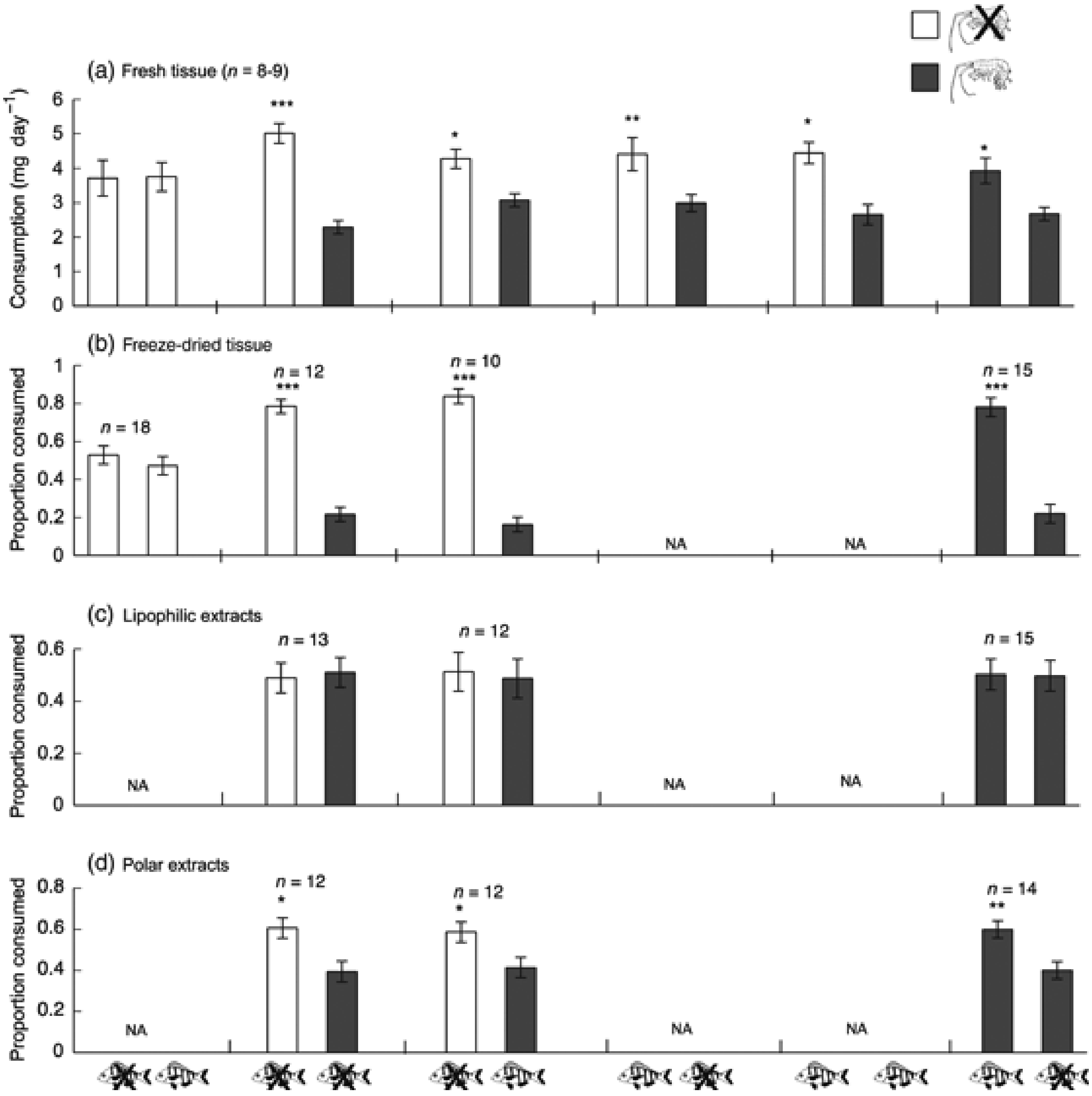

Figure 2.4. Grazer and fish cue treatment effects on algal palatability

Effects of grazer (dark bars) and fish cue (fish icon) treatments on algal palatability in A) fresh tissue (statistics are for two-tailed *t*-tests of first tissue choice with null = 50% consumption; n = 8-9); and pooled B) reconstituted tissue, C) lipophilic extracts and D) polar extract assays (non-parametric ANOVA permutation with cup as block). Mean ± 1 SE are shown. **P* < 0.05, ***P* < 0.01, ****P* < 0.001. NA refers to treatments that were not conducted due to a lack of a direct fish cue effect found in A).

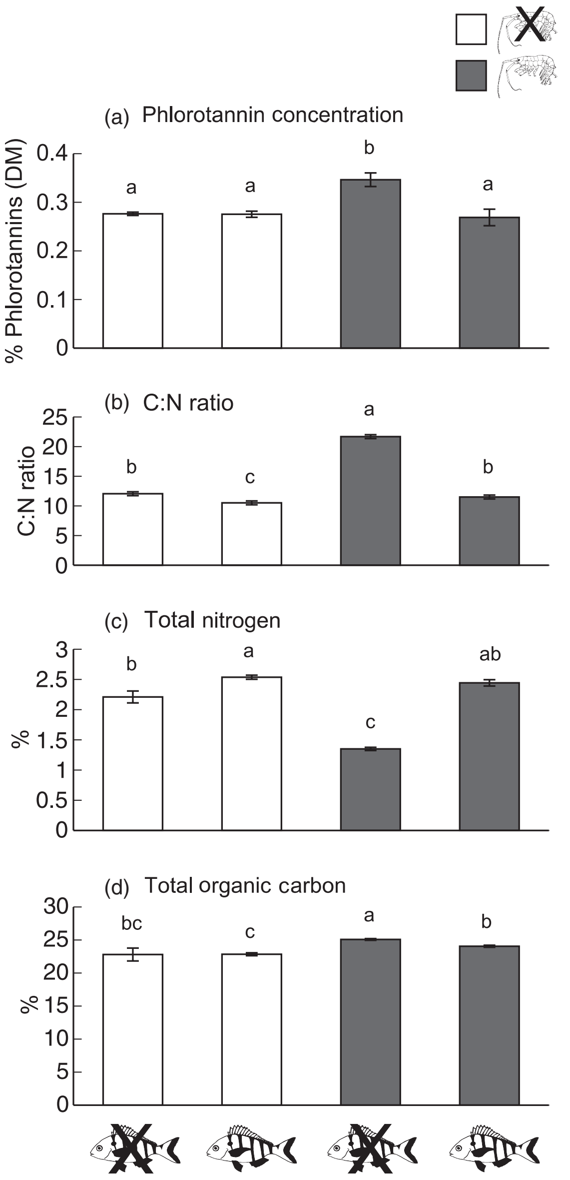

Figure 2.5. Grazer and fish cue treatment effects on macroalgal traits
Effects of grazer (dark bars) and fish cue (fish icon) treatments on pooled algal tissue A) phlorotannin concentration, B) C:N ratio ($F_{3,8} = 254.6$, $p < 0.001$), C) total nitrogen ($F_{3,8} = 81.952$, $p < 0.001$) and D) total organic carbon ($F_{3,8} = 4.570$, $p = 0.030$). $\pm$1 SE. Letters in represent treatments that are significantly different by Tukey's HSD. Refer to Table 2.1 for overall test for phlorotannins.

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CHAPTER 3:
PARTITIONING CONSUMPTIVE AND NONCONSUMPTIVE PREDATOR-PREY INTERACTIONS WITH MULTIPLE PREDATORS IN AN OYSTER REEF COMMUNITY

Abstract

Predators can affect prey populations via lethal changes in prey density (a consumptive effect) or by altering prey behavior (a nonconsumptive effect), both of which can influence the strength of trophic cascades. The role of predator richness and composition in modifying consumptive predator effects is becoming more clear, if often system specific. However, very little is known about how predator community composition modifies nonconsumptive predator effects, particularly in a field setting. We experimentally assessed the role of predator richness and identity in controlling prey density and the strength of the trophic cascade via both consumptive and nonconsumptive effects. We manipulated the presence, composition and richness of predators (oyster toadfish, blue and stone crabs) and their ability to access and consume their prey (mud crabs) in experimental oyster reef communities in the field. After two months, we found that top predators consumed mud crabs and nonconsumptively reduced their foraging (an antipredator response) on juvenile oysters. These two effects varied among the three top predators, indicating that predator identity can have independent effects on the strength of consumptive and nonconsumptive predator-prey interactions. Trophic cascades of top predators on juvenile oysters were almost exclusively driven by nonconsumptive interactions. Surprisingly, predator richness promoted juvenile oysters despite reducing mortality risk of their consumers. Thus, predator richness weakened the consumptive effects of predators but strengthened their nonconsumptive effects on basal resources. Interspecific interference among top predators, as well as strong mud crab antipredator responses induced by the presence of one top predator (oyster toadfish), are likely responsible for this counterintuitive result. However, positive cascading NCEs of toadfish on juvenile oysters attenuated at small spatial scales, indicating that while these effects can persist over time in the field in the presence of multiple predators and alternate prey resources, predator density and distribution may play a large role in shaping the overall magnitude and direction of NCEs a given ecosystem property.

Key-words: *nonconsumptive effect, multiple predators, species richness, trophic cascade oyster reef*

Introduction

Predators can have strong direct and indirect effects in food webs (Connell 1980, Carpenter et al. 1985), influencing productivity, nutrient cycling, and community structure (Duffy 2003, Cardinale et al. 2006, Fukami et al. 2006, Greig and McIntosh 2006, Schmitz 2006, Stief and Holker 2006, Trussell et al. 2006). Cascading effects on lower trophic levels can occur when predators consume (a lethal density reduction, CE) or alter the behavior (a nonconsumptive effect, NCE) of consumers at intermediate trophic levels (Carpenter et al. 1985, Strong 1992, Werner and Peacor 2003). Predator-induced changes in prey activity, habitat, and diet preferences can affect prey growth and survivorship, with consequences for the prey and resource dynamics (Trussell et al. 2002, Dill et al. 2003, Werner and Peacor

2003, Grabowski 2004). Understanding the role of such nonconsumptive predator-prey interactions is now recognized as integral to predicting the overall effect of predators in natural food webs (Preisser et al. 2005, Peckarsky et al. 2008, Schmitz et al. 2008).

Although there is a long history of studying predator-prey behavioral interactions, predicting the role of predator communities and their cascading nonconsumptive effects on a given ecosystem property is challenging. To date most research has examined the effects of single predators, but there is growing evidence that interactions among multiple predators can have important and often counterintuitive effects on prey and community dynamics (Soluk and Collins 1988, Morin 1995, Sih et al. 1998, Eklöv and Van Kooten 2001, Duffy 2002, Vance-Chalcraft et al. 2004, Bruno and O'Connor 2005, Byrnes et al. 2006, Steffan and Snyder 2009). Multiple predators can exhibit emergent effects on prey density via risk reduction or enhancement, in which fewe or more prey are consumed, respectively, than expected based on the effects of a single predator (Soluk and Collins 1988, Sih et al. 1998). Antagonistic interactions and/or interference among multiple predators can reduce prey mortality risk (Sih and Krupa 1996, Crowder et al. 1997, Finke and Denno 2004, Vance-Chalcraft et al. 2004, Griffen and Byers 2006), whereas predator complementarity or facilitation can increase prey risk (Losey and Denno 1998, Fodrie and Kenworthy 2008).

The presence of multiple predators can also heighten prey antipredator responses such as suppressed foraging activity, reducing consumption of basal resources and further strengthening a trophic cascade (Byrnes et al. 2006, Steffan and Snyder 2010). However, if prey exhibit risk-sensitive behavior (Lima and Bednekoff 1999) and can accurately assess predation risk, predator assemblages that reduce prey mortality may actually suppress prey antipredator responses and potentially increase prey foraging (Botham et al. 2008, Martin et al. 2009), thereby weakening a trophic cascade. However, if prey utilize simple cues for predator detection and cannot perceive changes in risk, a change in predator assemblage may have no effect or the opposite effect on the strength of a trophic cascade. Thus, changes in the number of predator types (e.g., species richness) can strongly influence the strength of a trophic cascade via both consumptive and nonconsumptive pathways (see reviews by Duffy et al. 2007, Stachowicz et al. 2007, Bruno and Cardinale 2008). Given that the presence of multiple predators is a common feature of most ecological systems (Cohen and Briand 1984, Schoener 1989, Polis 1991), understanding how predator richness influences predation risk and its effect on prey behavior is critical to our ability to predict and model the consequences of trophic interactions on food web dynamics.

Despite a growing emphasis on multiple predator effects and the role of behavior, how prey antipredator responses contribute to the total predator effect and how they are modified by predator richness in natural field settings is poorly understood. Most behavioral studies in aquatic systems have been conducted in mesocosms in which cue saturation is likely (e.g., Byrnes et al. 2006, Grabowski et al. 2008, but see Trussell et al. 2002) and may therefore represent a 'maximum' estimation of the role of nonconsumptive effects. Under field conditions, more natural cue diffusion may influence our ability to detect NCEs and their relative contribution to the total effect of a predator community on a given ecosystem property. For example, high flow regimes in aquatic systems can provide prey with hydrodynamic refuges from olfactory-mediated predation, but may also interfere with their ability to detect and properly respond to predators in the field (Weissburg and Zimmer-Faust 1993). The presence of alternate resources may also mask predator richness effects in diverse, open systems where both predators and their prey can exhibit diet switching behaviors (O'Connor and Bruno 2009). Thus, complicated predator-prey behavioral interactions and alternate prey availability in open systems make it difficult to predict the effects of changing predator communities in natural food webs.

To examine how multiple predators affect the strength of a trophic cascade by altering both predation risk (a consumptive effect) and prey antipredator behavior (a nonconsumptive effect), we manipulated model oyster reef communities in experimental field cages. To test multiple predator effects on predation risk, we manipulated the richness of top predators (0, 1 or 3 species). To partition the cascading nonconsumptive (behavioral) effects of multiple predators from the total predator effect (lethal + behavioral), we manipulated predator access to their prey (see supplemental Fig. C1 for images of the experimental design).

Oyster reefs support diverse and dense communities of invertebrate and fish predators and their associated prey including bivalves, decapods, gastropods, and other resident invertebrates (Wells 1961, Ulanowicz and Tuttle 1992, Peterson et al. 2003, Grabowski et al. 2005). Interactions among top predators and a common consumer of juvenile oysters, the mud crab (*Panopeus herbstii*), are well studied in this system (Grabowski 2004, Grabowski and Powers 2004, Grabowski and Kimbro 2005, Grabowski et al. 2008). Mud crabs are important consumers of bivalves, although mud crab density alone is not necessarily a good predictor of juvenile oyster survivorship (McDermott 1960, Bisker and Castagna 1987, Abbe and Breitburg 1992). The presence of predatory oyster toadfish (*Opsanus tau*) is known to reduce mud crab mobility and consumption of juvenile oysters, thereby promoting oysters even in complex reef habitats where fish predation is low and mud crab densities are high (Grabowski 2004). Another common mud crab predator, adult blue crabs (*Callinectes* *sapidus*), may also be negatively affected by toadfish and reduce its overall predation pressure on mud crabs on reefs with resident toadfish (Bisker et al. 1989). Adult stone crabs (*Menippe mercenaria*) also utilize oyster reefs (Irlandi and Peterson 1991, Nakaoka 2000), and are known to interfere with predation activities of other predators (Hughes and Grabowski 2006). Interactions among these top predators may influence mud crab predation risk and subsequently the strength of their antipredator behavior, with consequences for their consumption of basal resources such as juvenile oysters (see Fig. 3.1 for experimental food web and hypothetical predator-prey interactions).

Based on previous work in this system (Grabowski 2004, Grabowski et al. 2005, Hughes and Grabowski 2006, O'Connor et al. 2008) and in other multiple predator studies (Sih et al. 1998, Byrnes et al. 2006, Steffan and Snyder 2010), we predicted that: (1) Top predators nonconsumptively increase oyster abundances by reducing mud crab foraging; (2) Increased top predator richness reduces mud crab mortality risk; and therefore (3) Increased top predator richness weakens a trophic cascade if mud crabs exhibit risk-sensitive behavior, or strengthen the cascade if they are risk-insensitive (Table 3.1).

Methods

The experiment was conducted at Hoop Pole, a sheltered estuarine wildlife refuge in Bogue Sound, near the University of North Carolina’s Institute of Marine Sciences in Morehead City, NC. Field plots were established in July and were maintained until October of 2009, incorporating a period of peak oyster spawning and recruitment (Southworth and Mann 2004). Experimental plots were established in a channel bordered by intertidal mud flats adjacent to native and restored bivalve beds, including oysters (*Cassostrea virginica*) and clams (*Mercenaria mercenaria*), and salt marsh (primarily *Spartina alterniflora*) and seagrass (*Zostera marina*) habitats. Juvenile and adult mud, stone and blue crabs, as well as oyster toadfish, are common consumers in this system (Grabowski 2004, O'Connor et al. 2008).

EXPERIMENTAL DESIGN

The experimental design was a split-split-plot in a randomized design with two overlapping temporal blocks (n = 9). Experimental cages (plot-level) were stocked with a given top predator community and divided into sub-plots such that the top predators could or could not access their mud crab prey (total effect or NCE-only treatments). Sub-plots were visually divided into two zones (near or far the central divider) to quantify spatial biases in oyster survivorship and recruitment within the cages.

We manipulated top predator presence and identity to create five different top predator community types: MC (mud crab) only, MC + stone crabs (SC), MC + blue crabs (BC), MC + oyster toadfish (TF), and MC + SC + BC + TF for a total of 10 experimental treatments (Fig. C1). With this design, we could directly compare the strength of nonconsumptive effects to the total predator effect without confounding changes in prey density. Specific treatment comparisons were used to test hypotheses (Table 3.1). Top predators were manipulated in an additive design such that predator density increased with species richness. Although this potentially confounds the effects of species richness with density (Connolly 1988, Sih et al. 1998, Benedetti-Cecchi 2004), this design maintained natural field densities per species and may more accurately reflect field distributions given the highly intraspecific territorial nature of some of our species (O'Connor et al. 2008).

Experimental animals were collected with crab traps or by hand at Hoop Pole and surrounding field sites. Units were stocked with species at densities based on previously collected data including field surveys conducted on shellfish reefs at this and nearby sites (e.g., McDonald 1982, Zimmerman et al. 1989, Lenihan et al. 2001, Silliman et al. 2004, Grabowski and Kimbro 2005, O'Connor and Crowe 2005). All species were observed together in the field in areas of similar size to our experimental units. Treatments contained 0 or 1 stone crab, blue crab or oyster toadfish, or one individual of all three species, and 0 or 15 mud crabs. Top predators were collected within a similar size range representative of the native assemblage at this site. The average initial individual mass of each species (n = 10) was 103 (± 5.1) g for toadfish, 70 (± 5.4) g for blue crabs, and 88 (± 6.0) g for stone crabs (values are means ± 1 SE). Mud crabs were collected within their most common size range at the field site (15 - 30 mm CW) and stocked at densities within the range observed on local reefs (10 - 49 mud crabs/m^2, McDonald 1982). Experimental plots without cages were included to control for caging artifacts but are not analyzed here. Supplemental laboratory trials verified consumption of juvenile oysters by mud crabs and examined any potential direct effects of the top predators on juvenile oyster survivorship, which were minimal (Reynolds and Bruno, unpublished data). Although blue and stone crabs are known to consume oysters, laboratory feeding trials indicated that the size class of these predators used in this study did not have a significant effect on juvenile oyster mortality compared to mud crabs (Reynolds and Bruno, unpublished data; Bisker and Castagna 1987). Thus, we attributed differences in oyster survivorship to changes in mud crab consumption. Given the predator sizes used in this study, prior work supports that all top predators were capable of consuming mud crabs, while top predator losses due to intraguild predation by toadfish on blue and stone crabs was unlikely (Bisker et al. 1989).

FIELD SETUP

Field plots were randomly assigned to treatments and were installed in an area of 500 m^2 in water 1 m deep at mean low tide. Species were stocked in cages within experimental plots to maintain treatments. Cages were rectangular, 100 x 50 x 50 cm made of plastic Vexar mesh (5 mm aperture) attached to a 20 mm diameter PVC pipe frame. Cages included lids fastened with re-sealable cable ties. Cages were separated by least 1.5 m and buried 10 cm into the sediment; reinforcing rebar were attached at two opposite corners of each cage. Cages were filled with ~70 L mud and 40 L defaunated oyster shells to provide a natural substrate. Mud crabs were added to the cages a week after installation to allow time for natural infaunal recruitment, and top predators were added one week later. Crushed mussels (food) were added to the cages at the time of mud crab addition to reduce transportation stress and promote initial mud crab survivorship. Cages were installed in two blocks established and broken down two weeks apart.

Field cages were divided in the center with 5 mm Vexar to create two compartments (50 x 50 x 50 cm). Top predators were added to only one compartment, in which they could directly access their mud crab prey (CE + NCE = Total Effect treatment). No predators were added to the adjoining compartment; top predators could only nonconsumptively influence prey in this compartment (NCE treatment). Thus mud crabs in the NCE compartments received kairomes (olfactory cues from their predators) as well as pheromones (cues from conspecifics, including alarm and death cues). With this nested design we could directly compare nonconsumptive effects on a given response variable to that of the total predator effect. Replicates were excluded from the analysis if a top predator was missing (one set of replicates) or the cage was damaged.

RESPONSE VARIABLES

Mud crab mortality juvenile oyster abundance: To assess changes in mud crab mortality across treatments, we collected all mud crabs from each experimental compartment after two months. All oyster shells were removed from the cages and the mud sieved on a 2 mm screen to capture any burrowed crabs. All fauna within the oyster shell and mud matrix were preserved in 70% EtOH and later identified. To assess treatment impacts on the abundance of juvenile oysters that settled and successfully grew in field cages (e.g., oyster recruits), we collected 20 oyster shells from each compartment (10 shells from each of the two zones; 40 total per cage) at the end of the experiment. Shell collection was standardized as in O'Connor et al. (2008), selecting horizontally positioned shells of the same size class. We counted all live juvenile oysters that had recruited on each sampled shell; the abundance of recruits was averaged per shell per zone.

Juvenile oyster survivorship: Juvenile oysters (< 20 mm) were purchased from a local oyster hatchery and maintained in flow through seawater tables. Five individual oysters were glued onto a dead adult oyster shell to mimic natural settlement and accessibility (as in Grabowski 2004), which was tethered with nylon monofilament and attached within the experimental unit for ease of recovery. Each cage received four feeding shells, tethered haphazardly in each of two areas: close (A) and far (B) from the center unit divider (e.g., one feeding shell per zone). The assay was deployed immediately after top predator addition (initial feeding assay), and immediately prior to experimental breakdown (final feeding assay). The number of juvenile oysters surviving in each zone for every compartment was recorded after 24 hours and the feeding shells were removed. We examined cascading predator richness effects on juvenile oyster mortality from this assay using the multiplicative risk model above.

ANALYSIS

Top predator and access treatments: To assess the presence of a trophic cascade, we first tested the effect of top predator presence on mud crab mortality, and mud crab presence on oyster survivorship and recruitment for Total Effect and NCE treatments separately using a two factor ANOVA with time block as the second factor. Analyses were performed using the fit model platform in JMP version 8.0.1 (SAS Institute, Cary, North Carolina, USA). Time block was not significant for either analysis and was subsequently dropped and the data re-analyzed (Underwood 1981).

To assess the effects of top predator community type (BC, SC, TF, BC+SC+TF) and access (TE, NCE) to their mud crab prey on mud crab mortality, we conducted a four-way ANOVA with top predator community, access (total or nonconsumptive) and block as fixed factors, and experimental unit (cage) as a random effect nested within predator community treatment. Although ANOVA is robust to deviations from normality (Underwood 1981), mud crab mortality data could not be transformed to meet test assumptions and results must be interpreted cautiously. Thus, we set the alpha for this test at 0.01 to avoid type II error (Underwood 1981). We also used a four-way ANOVA on the final abundance of oyster recruits with top predator community type, access, and block as fixed factors and cage as a random factor nested within predator community treatment. Block and cage effects were not significant for either analysis and were consequently removed from the analysis and the data were re-analyzed with a two-way ANOVA (Underwood 1981). Zone biases for juvenile oyster survivorship in the feeding assay and final oyster recruit abundance were assessed with two-tailed matched paired t-tests. Additional analyses utilizing a split-split plot design mixed-effects model to incorporate zone within the model testing predator identity effects on oyster survivorship and recruitment was also pursued and yielded similar results (see Appendix C, Supplemental C2).

Top predator richness effects: To test for a predator richness effect on final mud crab survivorship in the total and nonconsumptive predator effect treatments, we compared expected proportions derived from a multiplicative risk model (Soluk 1993, Sih et al. 1998) to the observed experimental values. The multiplicative risk model is preferred for additive experimental designs with fixed initial prey abundances as it adjusts expected prey mortalities such that prey cannot be consumed twice (Soluk 1993, Sih et al. 1998). The model employed here was adapted from Nilsson et al. (2006), accommodating three top predator species:

$$E_{SC+BC+TF} = p_{SC} + p_{BC} + p_{TF} - (p_{SC} \cdot p_{BC}) - (p_{SC} \cdot p_{TF}) - (p_{BC} \cdot p_{TF}) + (p_{SC} \cdot p_{BC} \cdot p_{TF}).$$

Here, $E_{BC+SC+TF}$ is the expected proportion of mud crab mortality in the three-predator treatments and p_{SC}, p_{BC}, and p_{TF} are the respective mean observations in the one-predator treatments. The term $p_{SC} \cdot p_{BC} \cdot p_{TF}$ is included to remove the first correction from occurring twice for a specific prey individual. The expected and observed values were compared with two-tailed t-tests (Quinn and Keough 1993), where no deviation from zero implies that the results follow model predictions and significance indicates an emergent multipredator effect.

A similar analysis was used to examine emergent multiple predator effects on oyster mortality risk for the final feeding assay.

Because top predator identity and not diversity per se influenced mud crab foraging (see Results), and because a multiplicative risk model is inappropriate for analyzing resource abundance in an open system, we assessed predator richness effects on the abundance of oyster recruits using post hoc tests.

Mud crab foraging: We assumed that no mud crab mortality occurred during the initial feeding assay. Because juvenile oyster losses on feeding shells in control (mud crabs absent) cages were minimal (< 12%), we calculated changes in mud crab foraging rates by dividing juvenile oyster losses by either initial or final mud crab abundance as per the respective feeding assay, and comparing this to the average of the mud-crab only control: $[M_O/A_{MC}]_{+p} / [M_O/A_{MC}]_{-p}$ for oyster mortality $[M_O]$ and mud crab abundance $[A_{MC}]$ in the presence (+) and absence (-) of top predators, p). Deviations from 1 therefore indicate a change in foraging rate from baseline. These data were then analyzed with a two-way ANOVA as block and cage effects were not significant.

Results

MUD CRAB MORTALITY AND OYSTER RECRUIT ABUNDANCE

We found very low abundances of mud crabs in control cages (only 2 of 9 control cages had any mud crabs in them), indicating that our cages successfully prevented natural immigration into the no mud crab (“none”) treatment replicates. Mud crab mortality was observed in all treatments (30% in mud crab only controls, i.e., without top predators), and many have occurred due to both predation and cannibalism (Fig. 3.2A). Final mud crab abundances in NCE treatments (i.e., top predators were present without access) did not vary from the mud crab only controls, indicating that NCEs alone had little effect on mud crab mortality (Fig. 3.2A). Top predator access to mud crabs, but not predator community type, affected mud crab mortality (Table 3.2A), further supporting that changes in mud crab survivorship were due to consumption by top predators and not predator cues alone. On average, when top predators could access their prey (Total Effect treatment), mud crabs experienced a 58% increase in mortality relative to the mud crab only controls. Top predators did not affect mud crab mortality when they could not access their prey (NCE treatment). Stone crabs with access to their prey (Total Effect treatment) caused the highest mud crab mortality (60%, Fig. 3.2A).

Mud crabs decreased the final abundance of juvenile oyster recruits (Fig. 3.2B), indicating the potential for a trophic cascade. Predator community type (e.g., top predator identity and richness), but not access to their mud crab prey, increased the final abundance of juvenile oysters that recruited into field cages (Table 3.2B), indicating the presence of a trophic cascade driven by both consumptive and nonconsumptive predator-prey interactions. The greatest abundance of oyster recruits was observed in the presence of toadfish (Fig. 3.2B). On average, juvenile oyster abundance and survivorship was greater in the zones closest to an adjoining compartment housing a toadfish (Fig. 3.3 “near”), indicating that the strength of nonconsumptive effects of some predators may attenuate over small spatial scales in the field.

TOP PREDATOR RICHNESS EFFECTS

Predator richness influenced mud crab mortality in both total and nonconsumptive predator treatments (multiplicative risk model, df = 8, $P < 0.0001$ and $P = 0.0183$, respectively), indicating that emergent predator effects may be governed by nonconsumptive mechanisms. Assuming that predator density influenced mud crab mortality, the presence of three predator species reduced mud crab mortality risk regardless of whether the predators could directly access their prey (Fig. 3.4). Predator richness also affected juvenile oyster survivorship in the final feeding assay in both total and nonconsumptive predator treatments (multiplicative risk model, df = 8, $P = 0.035$ and $P = 0.004$, respectively), indicating that top predator richness strengthened the trophic cascade and reduced the risk of juvenile oyster mortality (Fig. 3.5).

MUD CRAB FORAGING

Reduced foraging is a common antipredator strategy by which prey increase their likelihood of surviving an encounter with predators (Werner and Peacor 2003, Grabowski 2004). In control (mud crab only) cages, mud crabs on average consumed just under one juvenile oyster per crab per day. Predator community, and not access, influenced per capita mud crab foraging in both the initial and final the 24-hr feeding assays relative to no predator (mud crab only) controls (Table 3.2C,D, Fig. 3.5). On average, foraging was lowest in the presence of toadfish (MC + TC and MC + SC + BC + TF treatments).

Discussion

Here we show that nonconsumptive effects can be strong in the field when predators are held at natural densities. Nonconsumptive effects comprised a large portion of the total predator effect on juvenile oysters after two months in experimental field cages, further supporting the importance of behavioral pathways on the strength of the trophic cascade in this system (Fig. 3.2). Top predators generally promoted juvenile oysters, even when they could not lethally reduce the density of mud crabs. These findings support the results of laboratory studies in similar and other systems (e.g., Grabowski 2008) and demonstrate that predator cue saturation, as may occur in laboratory and mesocosm studies, may not drive the strength of NCEs. However, our data provide a cautionary note to the emerging conclusion of the widespread pre-eminence of NCEs. Specifically, we found that the cascading positive NCEs of toadfish on juvenile oysters declined with only minimal distance from the cue source in the field. This suggests that NCEs may be far more dependent on predator density than previously appreciated, and this issue warrants future attention.

ROLE OF PREDATOR IDENTITY

Predators can have species-specific differences in the efficiency of prey capture and the overall intensity of a predator-prey interaction (Polis 1988). Thus it is no surprise that the type and strength of prey antipredator responses often vary with predator identity (e.g., McIntosh and Peckarsky 1999, Botham et al. 2008, Reynolds and Bruno, submitted), with potential consequences for the strength of a trophic cascade (Byrnes et al. 2006). Although predator density may also influence the strength of prey antipredator responses (Sih 1992), here we found that predator identity, and not necessarily density, influenced the strength of an antipredator response (reduced per capita mud crab foraging on juvenile oysters). Although we found that stone crabs were the strongest consumers of mud crabs (Fig. 3.2A) and posed the greatest predation risk in our experimental enclosures, they had little effect on mud crab foraging (Fig. 3.5). Oyster toadfish, despite their weaker effects on mud crab mortality, evoked the strongest reductions in mud crab foraging and strengthened the trophic cascade. Here we provide evidence that the predator species that causes the highest prey mortality does not necessarily have the greatest positive effect on the strength of a trophic cascade and the abundance of basal resources.

Several factors, including predator species differences in cue dispersal and detectability, as well as shared natural history with their prey, may contribute to shape prey perception and the subsequent strength of their antipredator responses to a given predator type (Sih et al. 1998, Werner and Peacor 2003). Predator species-specific activities, such as foraging mode and microhabitat utilization (e.g., burrowing), may influence the dispersion of olfactory cues and detection by their prey in underwater environments. As distribution and concentration of complex chemical signals can be an important part of prey risk assessment (Tomba et al. 2001), species-specific biases in the type and strength of predator odor plumes may have consequences for prey antipredator behavior (e.g., Smee and Weissburg 2006). Predators with specific hunting modes, such as sit-and-pursue species including oyster toadfish, may provide prey with more accurate and consistent information on their location and allow prey to more accurately assess and respond to predation risk (Preisser et al. 2007, Schmitz 2008). In aquatic systems, sedentary predators such as toadfish may exude stronger chemical signatures, providing prey with more robust cues to localize predation threat. This, coupled with the release of predator-specific chemical compounds, may also influence prey antipredator behavior. Oyster toadfish, unlike adult crabs, produce highly concentrated urea, which they can excrete in pulses when out of their shelters (e.g., when foraging, Walsh 1997). This may further enhance prey detection of oyster toadfish presence, and may further elevate prey antipredator behaviors.

Observed spatial biases in oyster survivorship and recruitment within experimental compartments exposed to toadfish cues provide some evidence for the ability of mud crabs to accurately localize and respond to predation risk from this predator (MC + TF NCE treatment, Fig. 3.3). However, as we were unable to assess mud crab habitat utilization within the experimental cages, we cannot determine definitively whether these differences in oyster survivorship by zone are due strictly to differences in mud crab per capita foraging rates overall, or to mud crab migration within the experimental cages and their concentration in to regions further away from the predator (Orrock et al. 2008). Regardless of the mechanism, we see evidence that the positive effects of top predators on basal resources can attenuate over a small spatial scale.

Variability in predation risk and the shared history of predators and their prey in the field can further alter prey antipredator responses (Sih et al. 1998, Werner and Peacor 2003). For example, in our oyster reef community adult mud crabs are strong intraguild predators and will consume juvenile stone and blue crabs. Thus, cues from adult stone and blue crabs may not be strongly informative of predation risk unless mud crabs can infer size-specific information from cues emitted by larger, predaceous crabs. Additionally, oyster toadfish are likely the dominant consumer of mud cab populations at the study site (Grabowski 2004), and thus heightened antipredator responses to this predator may not be surprising (Botham et al. 2008, Martin et al. 2009).

RICHNESS EFFECT MEDIATED BY CHANGES IN PREY FORAGING RATE

Predator richness can have strong yet counterintuitive effects in aquatic and terrestrial systems, with consequences for basal resources (Bruno and Cardinale 2008). Difficulty in predicting predator richness effects may arise when the presence of multiple predators independently affects the strength of consumptive and nonconsumptive predator-prey interactions. Here, increasing top predator richness decreased mud crab mortality risk (consumptive pathway, Fig. 3.4), potentially by reducing top predator efficiency and/or negative intraspecific interactions among individual mud crabs (Sih and Krupa 1996, Crowder et al. 1997, Finke and Denno 2004). As was documented by Grabowski et al. (2008) and has been found in many other multiple predator studies (see review by Sih et al. 1998), the presence of one predator (e.g., toadfish) may have suppressed foraging by other predators thereby releasing the shared prey (mud crabs) from predation. This contrasts with the work by Steffan et al. (2010), who found that increasing predator diversity in an old field grassland system reduced negative intraguild interactions among predators and increased their foraging activity. The presence of multiple predators may have further reduced prey mortality risk in our system by reducing mud crab mobility (Grabowski 2004), conspecific encounters, and negative intraspecific interactions such as cannibalism, which are common among brachyurian crabs (e.g., Wolcott 1996).

Similar to the findings of Steffan et al. (2010) and Byrnes et al. (2006), predator richness strengthened a trophic cascade in our oyster reef system via heightened nonconsumptive interactions. However, in those studies predators had little effect on prey density, and cascading richness effects on plant biomass were mediated exclusively by nonconsumptive predator-prey interactions. Predator richness in our experimental oyster reefs increased the abundance of lower trophic level consumers, and perhaps counterintuitively, strengthened the trophic cascade (Fig. 3.4). Classically, predator release should *decrease* the strength of a trophic cascade (Paine 1980). This conflict may be explained by a failure of mud crabs to exhibit risk sensitive antipredator behavior (Lima and Bednekoff 1999), or to modulate foraging behavior with actual predation risk. Despite reduced risk with increased predator richness, mud crabs exhibited reduced foraging rates to the multiple predator species assemblage (Fig. 3.5).

Mud crabs are strong consumers of bivalves and feed on a wide size range of oysters (McDermott and Flower 1952, Bisker and Castagna 1987). McDermott (1960) determined that individual mud crabs could eat up to two juvenile oysters (spat) per crab per day. Mud crabs in our field cages consumed juvenile oysters at a considerably slower rate of less than one oyster per crab per day, which was likely influenced by ambient predator-induced reductions in overall foraging activity (Grabowski 2004) as well as the availability of alternate prey in the field cages. Foraging on juvenile oysters versus other reef fauna such as polychaetes or gastropods, may be risky in the field as it forces the crabs to the top of the reef matrix where they may be more obvious to cruising predators and/or attract nearby burrowed predators.

Understanding the cascading effects of predators and their prey on basal resources may be further complicated by the ability of many plants and lower trophic level organisms to exhibit defensive trait changes in the presence of their consumers (Tollrian and Harvell 1999). For example, decreased mud crab risk in the toadfish and three top predator species communities may have had a negative feedback on mud crab foraging efficiency on juvenile oysters via induced oyster defenses. Nonlethal exposure to predatory crabs can increase oyster shell strength (Newell et al. 2007), which may reduce oyster vulnerability to predation. It is unknown whether inducible changes in oyster shell morphology are due to enemy-avoidance kariomes (emitted directly by their consumers) or an alarm pheromone released by damaged conspecifics (Newell et al. 2007), although both types of cues are known to increase bivalve shell thickness and resistance to predatory crabs (Leonard et al. 1999) and may have operated in our experimental enclosures. Elevated juvenile oyster recruit survivorship in enclosures with toadfish, whether via reduced mud crab foraging or increased oyster defenses, may have also positively affected oyster recruitment over time as larval oysters are attracted to and settle on or near the shells of living conspecifics (Newell et al. 2007), further exacerbating differences among experimental treatments.

In general, the strength of NCEs, relative to density or lethal effects, has been observed to increase as the abundance of resources increases (Luttbeg et al. 2003). Negative interspecific interactions among top predators may have further reduced mud crab foraging on juvenile oysters by promoting the abundance and availability of alternate shared resources, such as solitary tunicates (*Styela plicata* and *Mogula manhattensis*) and other infauna (see supplemental Fig. C2). Reduced alternate resources (e.g., tunicates) in treatments lacking toadfish may have also elevated mud crab consumption of oysters via an inherent tradeoff to balance predation and starvation risks. Prey will often modulate the strength of their antipredator responses with body condition, where starving prey are less likely to fear predators compared to satiated prey individuals (e.g., Luttbeg et al. 2003, Moir and Weissburg 2009). Thus, NCEs of diverse predator assemblages may promote the persistence of intermediate trophic levels while mediating their effects on basal resources.

Similar to studies with invertebrates in kelp forest mesocosms (Byrnes et al. 2006) and in a model grassland system (Steffan and Snyder 2010), we found that the presence of multiple top predator species in a model oyster reef community heightened prey antipredator responses, thereby reducing prey consumption of resources and strengthening the trophic cascade. Strong, emergent species richness effects can be transmitted through behavioral predator-prey interactions, and lethal reductions in prey density may not be necessary to maintain the strength of a trophic cascade. Although mechanisms influencing the effects of predator diversity may operate similarly in both consumptive and nonconsumptive pathways, predicting the total effect of changing predator richness on a given ecosystem property requires an understanding of synergistic interactions among these factors. Interactions among predators with changing predator richness, whether antagonistic or facultative, may strongly affect trophic cascades depending on the ability of prey to assess and accurately respond to changes in predation risk.

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Supporting Information

Additional supporting information may be found in **Appendix C***:*

Supplemental C1: *Image of experimental cages.*

SupplementalC2: *Mixed-effects modeling.*

Supplemental C3: *Treatment effects on tunicate recruitment.*

Tables

Table 3.1. Theoretical consumptive and nonconsumptive effects of multiple predators.

Predicted cascading effects of multiple predator interactions on the strength of a trophic cascade, categorized as consumptive and nonconsumptive mechanisms that increase (+) or decrease (-) resources (juvenile oysters) by altering prey (mud crab) foraging rates. Sensitive prey modulate their antipredator responses (e.g., foraging rate) with variations in predation risk, whereas insensitive prey do not.

Predator interactions	Consumptive effect	Prey perception	Nonconsumptive effect	Resource abundance	Trophic Cascade
Decreased negative *intraspecific* interactions; Increased predator facilitation	Increased risk	Risk insensitive	- foraging	+ resources	Stronger
		Risk sensitive	- foraging	+ resources	
Increased negative *interspecific* interactions (predator interference)	Decreased risk	Risk insensitive	- foraging	+ resources	
		Risk sensitive	0/+ foraging	0/- resources	Weaker

Table 3.2. Results of two-factor ANOVAS on the effects of predator community and access to prey on mud crab and juvenile oyster abundance and mud crab foraging rate.

Two-factor ANOVAs for A) final mud crab mortality, B) final juvenile oyster abundance, and C) change in mud crab foraging rate across two fixed factors: predator community type (four levels: MC + SC; MC + BC; MC + TF; MC + SC + BC + TF) and access (two levels: total or nonconsumptive effect). *p* < 0.05 in bold.

A. Mud crab mortality

Factor	d.f.	SS	*F*	*P*
Community	3	39.00	1.96	0.1296
Access	1	156.06	23.47	**< 0.0001**
Community*Access	3	43.39	2.18	0.0996
Error			64	425.56

B. Final juvenile oyster recruit abundance

Factor	d.f.	SS	*F*	*P*
Community	3	5.36	4.08	**0.0103**
Access	1	1.07	2.44	0.1229
Community*Access	3	2.88	2.19	0.0976
Error	64	28.01		

C. Change in initial per capita mud crab feeding rate (from 24-hr feeding assay)

Factor	d.f.	SS	*F*	*P*
Community	3	1.21	28.70	**< 0.0001**
Access	1	0.11	7.74	**0.0071**
Community*Access	3	0.00	0.02	0.9967
Error	64	0.90		

D. Changes in final per capita mud crab feeding rate (from 24-hr feeding assay)

Factor	d.f.	SS	*F*	*P*
Community	3	3.00	29.04	**< 0.0001**
Access	1	0.00	0.06	0.8012
Community*Access	3	0.20	1.92	0.1348
Error	64	2.20		

Figures

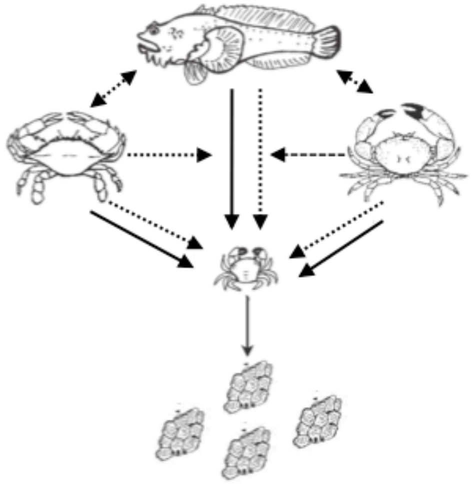

Figure 3.1. Experimental food web.
Experimental food web with multiple top predators (blue crab, oyster toadfish, stone crab) and their hypothetical consumptive (line) and nonconsumptive (dashed) effects on prey (mud crabs) and basal resources (juvenile oysters).

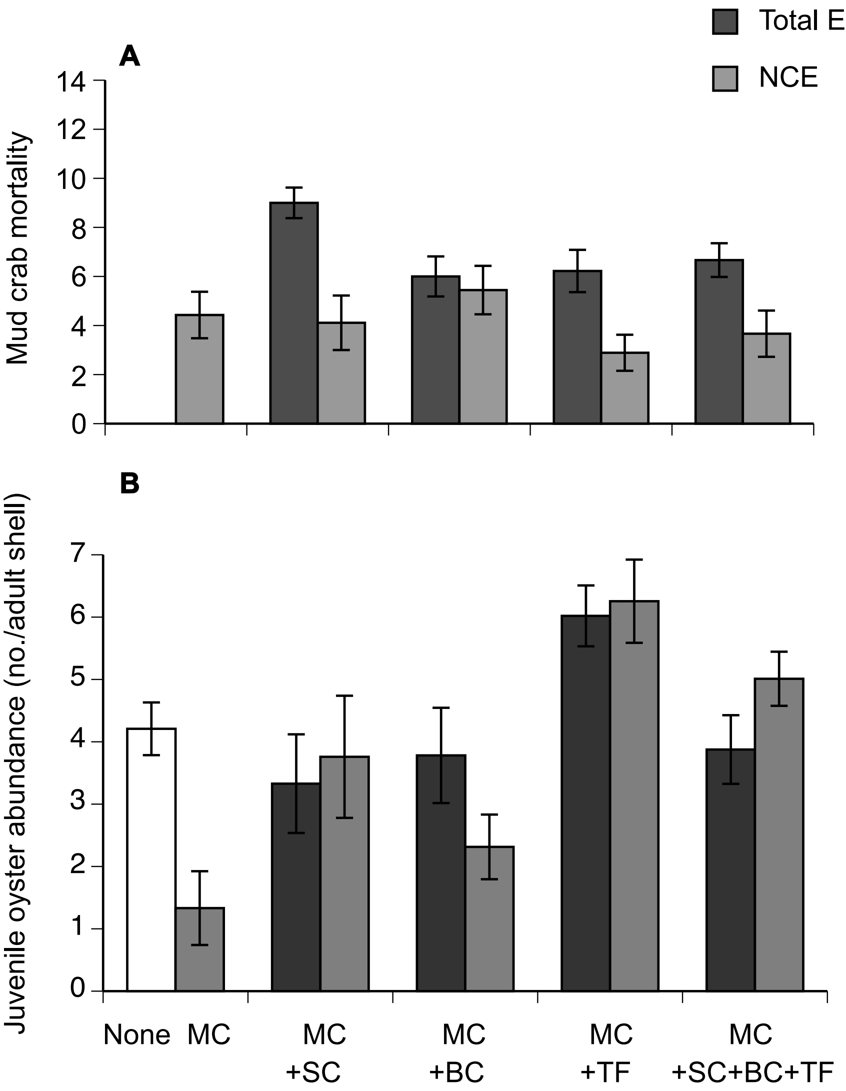

Figure 3.2. Effects of predators on mud crab and juvenile oyster abundance. Number of lost mud crabs (A) and final abundance of juvenile oyster recruits (B) in the presence of lethal (Total Effect) and nonlethal (NCE) top predator communities in field cages after two months. Treatment codes: None (no consumers), MC mud crabs only, SC stone crab, BC blue crab, TF oyster toadfish. Means ± SE.

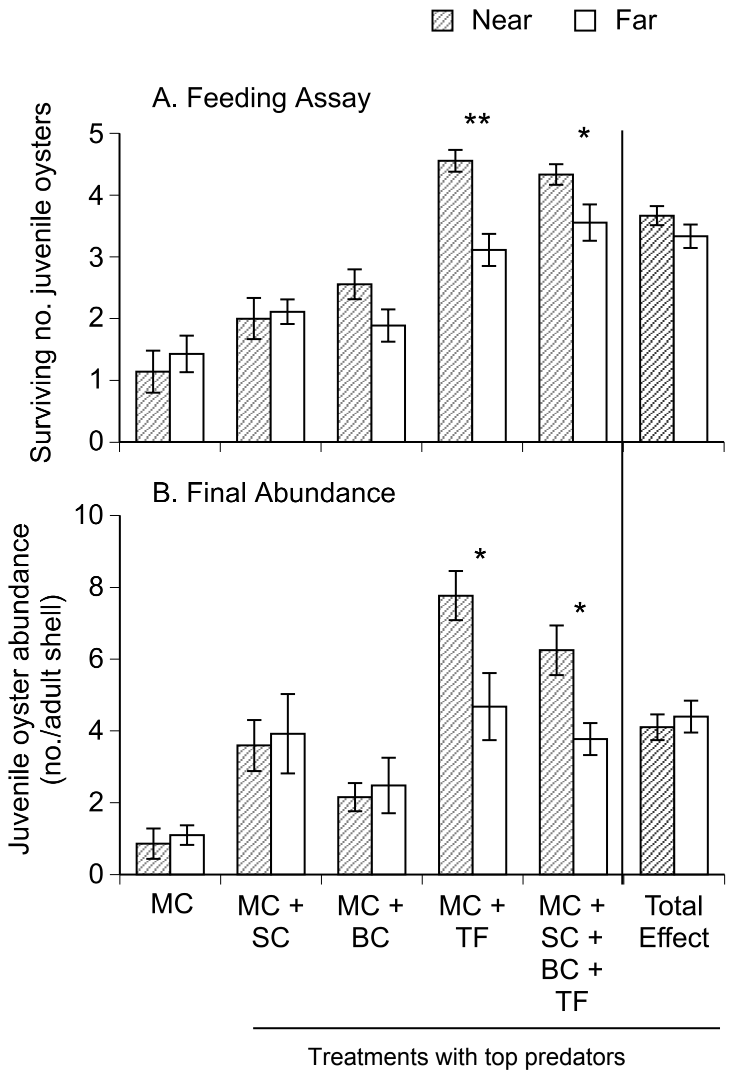

Figure 3.3. Spatial effect of predators on oysters.
Number of surviving juvenile oysters in the final 24-hr feeding assay (A) and final juvenile oyster abundance (B) by zone across top predator communities for nonconsumptive (NCE) treatments. No zone biases were found for total effect treatments in which predators could access and consume mud crabs; averages for the total effect treatment are included for comparison. The "near" zone (striped bars) was closer to the adjoining cage compared to the "far" zone (white bars). Treatment codes: None (no consumers), MC mud crabs only, SC stone crab, BC blue crab, TF oyster toadfish. Means ± SE. (* $p < 0.05$, ** $p < 0.001$).

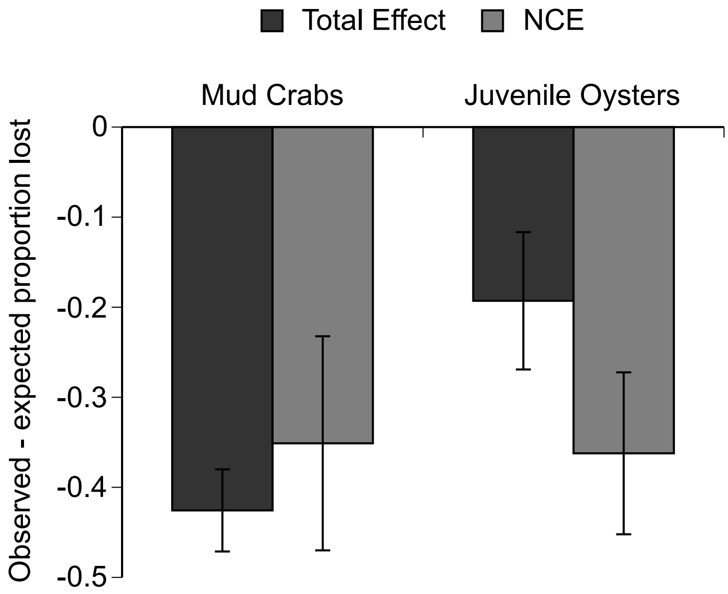

Figure 3.4. Multiple predator effects on mud crab and oyster mortality.
Mean deviations from expected proportions (observed - expected ± 1 SE) of lost mud crabs (after 2 months) and juvenile oysters (from 24-hr feeding assay) for the total and nonconsumptive treatments with all three top predator types (MC + SC + BC + TF). Expected proportions were calculated using the multiplicative risk model (Sih et al. 1998, Nilsson et al. 2006). Deviations were compared to zero in a two-tailed t-test (i.e., no difference is the null hypothesis of no richness effect). Significant deviations (risk reduction) for both consumptive and nonconsumptive treatments indicated emergent top predator effects for both response variables.

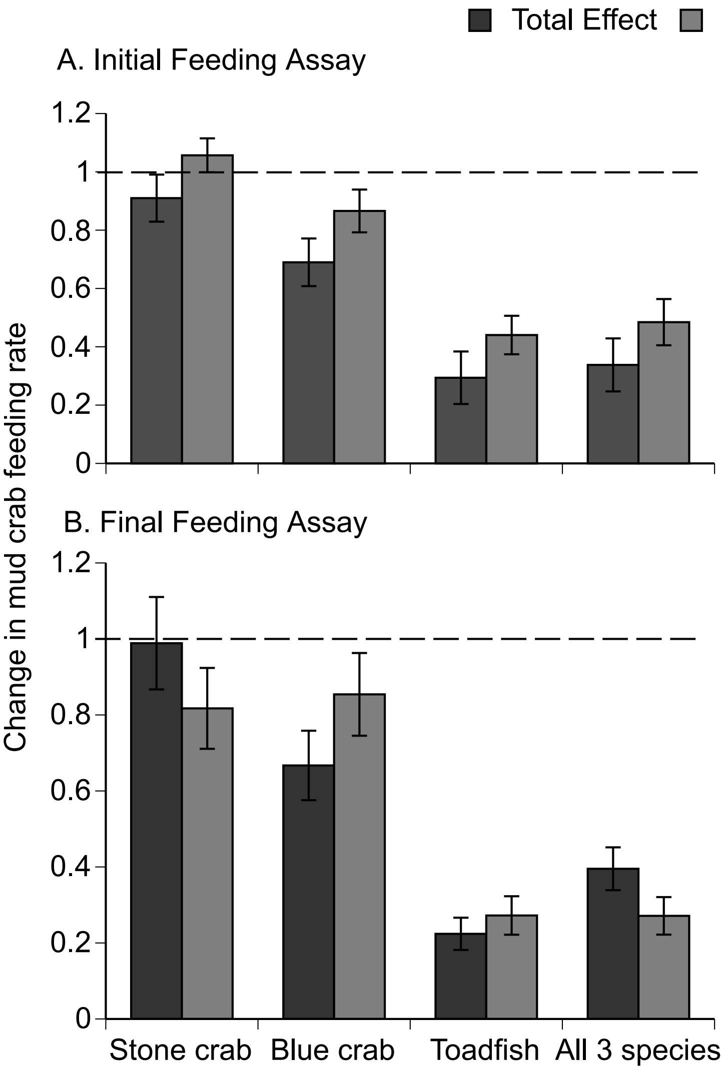

Figure 3.5. Predator effects on mud crab foraging.
Change in mud crab feeding rate compared to mud crab only controls (dashed line) in the initial (A) and final (B) 24-hr feeding assays when top predators could (Total Effect, dark bars) and could not (NCE, light bars) access their prey. All top predators except stone crabs reduced mud crab foraging (e.g., values < 1). Means ± SE.

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CHAPTER 4:
EFFECTS OF TROPHIC SKEW ON ECOSYSTEM FUNCTIONING IN A MARINE COMMUNITY

Abstract

Widespread overharvesting of top consumers of the world's ecosystems has "skewed" food webs, in terms of biomass and richness, towards greater domination at lower trophic levels. This skewing is exacerbated in locations where exotic species are predominantly low-trophic level consumers, e.g., benthic macrophytes, detritivores and filter feeders. However, in some systems where numerous exotic predators have been added, sometimes purposefully, e.g., many freshwater systems, food webs are skewed in the opposite direction, i.e., towards consumer dominance. Little is known about how such modifications to food web topology, e.g., changes in predator to prey species richness, affect ecosystem functioning. We experimentally measured the effects of trophic skew on production in an estuarine food web by manipulating species richness across three trophic levels in experimental mesocosms. After 24 days, increasing macroalgal richness promoted plant biomass, although this effect disappeared in the presence of grazers. The strongest trophic cascade on macroalgae emerged in bottom-up skewed communities (with a greater ratio of prey to predator richness), while the strongest cascade on microalgal accumulation emerged in the top-down skewed communities (where predator richness was high relative to that of grazers and plants). These results suggest that trophic skew can influence marine community structure and food web dynamics, and emphasize the need for multitrophic approaches to understanding the consequences of marine extinctions and invasions.

Key-words: *trophic skew, food web topology, species richness, marine*

Introduction

Species losses from habitat destruction and overharvesting, and species gains from accidental and intentional introductions, are changing the topology of aquatic food webs (Duffy 2003, Byrnes et al. 2007). Although the rate of species gain from introductions often outpaces those lost to extinctions with potentially little effect on overall community diversity at local scales (Sax and Gaines 2003), inherent species biases in extinction and invasion processes are altering the *distribution* of diversity in marine food webs (Byrnes et al. 2007). While natural food webs are thought to be slightly weighted toward greater species richness at lower trophic levels (Petchey et al. 2004), biases in which species are more likely to be lost and gained can result in food webs skewed toward greater or lower predator to prey richness.

Generally, large consumers, such as top predators, are more likely to go extinct due to their characteristic small population sizes, small geographic ranges, slow population growth, low initial diversities, and high susceptibility to over-harvesting and habitat loss (Tracy and George 1992, McKinney 1997, Terborgh et al. 2001). Conversely, in some estuarine systems species at lower trophic levels, such as macroplanktivores and plants (particularly species found in ballast water), are more likely to be transported and gained (Byrnes et al. 2007). In streams and lakes modified for human recreation, however, intentional or accidental releases of predatory game fish are increasing predator richness (Gido and Brown 1999, Eby et al.

2006). This trophic skew, or re-organization of trophic structure due to a change in the ratio of predator to prey richness (Duffy 2003, Dobson et al. 2006), is changing the structural biodiversity of aquatic food webs with unknown consequences for ecosystem processes (Lotze and Milewski 2004, Petchey et al. 2004, Byrnes et al. 2007).

A wealth of research over the past decade indicates that changes in the biodiversity of aquatic food webs alter their functioning and services (Stachowicz et al. 2007). Generally, biodiversity is thought to have cascading, positive effects where increasing species richness results in elevated abundance or biomass at a given trophic level with consequences for other ecosystem processes including stability and invasibility (see review by Cardinale et al. 2006, Stachowicz et al. 2007). However, most work to date in aquatic systems has focused on manipulating richness at one trophic level (e.g., Bruno et al. 2005, Zhang and Zhang 2006, but see Dyer and Letourneau 2003, Bruno et al. 2008, Douglass et al. 2008), or elevated richness across all trophic levels simultaneously to detect biodiversity effects (e.g., Downing and Leibold 2002).

Understanding the consequences of trophic skew, or shifts in species richness across multiple trophic levels, is difficult because diversity effects are often contingent upon the presence and diversity of adjacent trophic levels (Gamfeldt et al. 2005, Thebault and Loreau 2006). For example, terrestrial studies indicate that capture and consumption of prey from within a given trophic level are influenced by richness at lower and higher trophic levels (Aquilino et al. 2005). In addition, diversity can affect production through fundamentally different mechanisms across trophic levels (Long et al. 2007). Recent studies manipulating richness across multiple trophic levels simultaneously have largely featured designs in which overall community richness varied across treatments, and/or featured limited species pools where results were largely ascribed to identity effects and changes in community composition (e.g., Bruno et al. 2008, Douglass et al. 2008). Thus, empirical studies are necessary to predict how trophic skew will affect marine systems.

We measured the effects of trophic skew on primary and secondary production in outdoor mesocosms by manipulating predator to prey species richness in a diverse tritrophic estuarine food web. We created four food web structures that reflected real or predicted skewing scenarios for natural systems: 1) top-down skewed (inverted triangle) with greater predator to prey richness, consistent with predictions based on accidental and intentional predator additions (Ruzycki et al. 2003), 2) neutral (rectangle) with constant predator to prey richness, and 3-4) two degrees of bottom-up (triangular) skewing with greater prey to predator richness, as may be typical for impacted estuarine food webs (Byrnes et al. 2007). We tested the direct and indirect effects of predator (top-down) and plant (bottom-up) diversity (Hairston et al. 1960, Hunter and Price 1992, Dyer and Letourneau 2003) by comparing production across these trophic skewing scenarios (Fig. 4.2).

Previous studies in this and similar systems have found that: 1) Increasing plant richness increases plant biomass (Bruno et al. 2005); 2) The presence of grazers reduces plant biomass (Bruno et al. 2008); and 3) Increasing predator richness can suppress grazers, thereby increasing plant biomass (Bruno and O'Connor 2005, Douglass et al. 2008). With concurrent and opposite changes in prey and consumer richness, it is possible that 1) one type of richness effect, bottom-up or top-down, may dominate and dictate final primary biomass, 2) these effects may cancel out, resulting in constant primary production across different food web structures, or 3) the effects may interact additively or nonadditively (Aquilino et al. 2005, Douglass et al. 2008).

Methods

The experiment was performed in outdoor, flow through mesocosms at the University of North Carolina at Chapel Hill's Institute of Marine Science (IMS) in Morehead City, NC in July 2007. We independently manipulated secondary consumer (hereafter referred to as predator) and algal richness to create three different food web structures with constant total community richness (Fig. 4.1). Experimental food webs were top-skewed (2 macroalgal: 4 grazer: 6 predator species), neutral (4:4:4), slightly bottom-up skewed (5:4:3), or bottom-up skewed (6:4:2) (*n* = 8). We also included no-predator and algae only controls to compare changes in trophic cascade strength across experimental communities.

We used a substitutive design, manipulating macroalgal and predator richness and identity while holding biomass and density constant per mesocosm (35 g algae and 6 predator individuals, *c*. 18 g) at densities comparable with natural levels in North Carolina subtidal communities (Nelson 1979a, Bruno and O'Connor 2005, Powers et al. 2005, O'Connor and Bruno 2009). Algal and predator species composition per replicate were chosen randomly from a larger pool of nine algae and eight predator species (Table 4.1). Selection of species identity from these larger species pools allowed for a conservative test of richness effects in our system by varying community composition, but not richness, for replicates within a given treatment. This controlled for species identity and composition effects (Tilman 1997). Initial grazer richness, composition and abundance were held constant such that each treatment received a mixture of four grazer species.

We chose a substitutive design because it is effective for multiple predator effects experiments and does not confound diversity with density, as do additive designs (Sih et al. 1998, Finke and Denno 2004). Although replacement designs can potentially diminish intraspecific interactions by decreasing species-specific densities with increasing species richness (Jolliffe 2000, Griffin et al. 2008), this design is appropriate for our system because high predator diversity in the field may increase negative interspecific interactions among predator types, thereby limiting the densities of the different predator populations and reducereducing intraspecific interactions (see Bruno and O'Connor 2005, O'Connor and Bruno 2009).

Replicates were maintained in 30 L clear plastic aquaria provided with gravel-filtered seawater from Bogue Sound (see supplemental Fig. D1 for images of experimental mesocosms). Seawater flowed through 100 μm nylon mesh filter bags to limit outside grazer recruitment, and was delivered through a dump bucket system that maintained aeration and approximated natural subtidal turbulence (Duffy and Hay 2000, Bruno et al. 2005, Bruno and O'Connor 2005). Mesocosms were covered with 5 cm opening Vexar mesh lids to reduce light levels to natural field conditions, and were placed in water tables in a blocked design to maintain constant temperature. Light, temperature and salinity within the mesocosms closely approximated field conditions in the nearby Bogue Sound during the course of the experiments (J.F. Bruno, unpublished data). Mesocosms were rotated every 5 days to reduce positioning artifacts.

After 24 days we quantified treatment effects on grazer abundance, macroalgal biomass, and microalgal accumulation. This endpoint was based on observable changes in algal growth among treatments and represented approximately two or more overlapping grazer generations (Sotka and Reynolds, in press). Grazers were preserved in 70% EtOH and later identified and counted. To assess microalgal production, we measured the chlorophyll *a* concentration from standardized 2 cm^2 samples scraped from the side of each mesocosm. We extracted and quantified chlorophyll *a* concentration as in Bruno and O’Connor (Bruno and O'Connor 2005) to quantify microalgal accumulation. We used a two-factor ANOVA (performed in the Fit Model platform of JMP) to test for food chain length and food web structure treatment effects on all response variables. Results were log transformed as necessary to meet the assumptions of normality and heteroscedacity (Underwood 1997).

STUDY SYSTEM AND ORGANISMS

The South Atlantic Bight hard-substratum communities are highly diverse, composed of tropical and temperate species of algae, invertebrates and fishes (Hay and Sutherland 1988). Macro- and epiphytic algae, the main primary producers in this system, are intensely grazed by a diverse macroinvertebrate community composed largely of amphipods and isopods (Jernakoff et al. 1996, Duffy and Hay 2000), which are in turn consumed by an array of invertebrate and vertebrate predators including shrimp, crabs and fishes (Nelson 1979a, b). Experimental communities featured local algal, grazer, and predator species that commonly co-occur and typically dominate hard-substratum sites of North Carolina estuaries. Organisms were collected and cultured or maintained in outdoor water tables at IMS prior to experimentation.

Chosen macroalgal species (Table 4.1) are common in NC estuaries, although their abundances fluctuate seasonally (Hay and Sutherland 1988, Powers and Kittinger 2002, Bruno et al. 2005). We attached seven algal thalli haphazardly to 25 x 25 cm Vexar mesh screens (with 5mm openings), which were secured to the bottom of each 30 L polypropylene mesocosm such that algae floated upright in natural orientation. Initial total algal biomass per mesocosm was held constant at 35 g with approximately 5 g per individual thalli attachment.

Initial algal biomass was purposefully lower than field densities (Bruno et al. 2005) in order to allow room for growth. Algal biomass was determined after first immersing the algae in seawater for at least 15 minutes and then spinning it 15 revolutions in a salad spinner to remove excess water. We dipped algae in a diluted pesticide (Sevin, as in Carpenter and Lodge 1986, Duffy and Hay 2000, Bruno et al. 2005) and rinsed it with fresh seawater to remove existing invertebrates and trace pesticides before placement in mesocosms.

Mesocosms received an initial equal volume of grazers from a mixture of three amphipods (*Dulichiella appendiculata*, *Gammarus mucronatus*, and *Elasmopus levis)* and one isopod (*Paracerceis caudata*) prior to predator additions. These mesograzers are common in NC estuaries, achieving densities of 10-145 individuals g^{-1} wet mass of the alga *Sargassum filipendula* (Duffy 1989). They also have short generation times, respond quickly to changes in habitat and predation, and consume various types of macro- and microalgae (Nelson 1979a, Virnstein and Curran 1986, Edgar 1992). Each subsequent week an additional equal volume of grazers was added to each mesocosm to mimic natural recruitment and to remove the possibility of predator overexploitation (for a total of *c*. 120 herbivores per mesocosm overall). Volume additions were subsampled (*n* = 20) and composed mostly of *E. levis* for the initial additions, and *D. appendiculata* and *P. caudata* for the recruitment additions. The initial volume addition was supplemented with five individuals of each grazer species to ensure that all replicates received the same grazer richness. Grazers were added within the lower range of natural field densities to allow for natural reproduction and population growth throughout the experiment (Duffy et al. 2003, Bruno and O'Connor 2005, Duffy et al. 2005).

Predator assignments were chosen randomly from a pool of functionally distinct invertebrates and fishes including omnivorous and strictly carnivorous species (Table 4.1). Due to low field abundances it was impossible to collect enough of any one of the mud and swimming crab species. To resolve this issue without risking elevating richness, each replicate assigned to either of these crab groups received individuals of only one species for that group throughout the duration of the experiment. Each mesocosm received six individual predators, which is within natural field densities (O'Connor and Bruno 2009). Predators were collected within their respective average juvenile size classes. This was most important for *L. rhomboides*, which ontogenetically switches from a strict predator to an omnivore at 3.5 cm (Stoner 1979, Muncy 1984), or around 2.5 g (feeding trial pilot study, *n* = 8). Total predator mass per mesocosm varied (0.29-3.71 g), but was not significantly different across treatments. Mesocosms were checked daily and dead or stressed predators were replaced throughout the experiments; the absolute number of predator replacements did not vary across treatments (one-way ANOVA, $F_{2,23} = 0.90$, $p = 0.42$). This predator press design maintained the potential for species interactions (e.g., intraguild predation), although it precluded direct, long-term effects of such encounters on lower trophic levels.

Results

Trophic skew (the presence and richness of adjacent trophic levels) influenced final algal biomass and final grazer abundance (Table 4.2). Generally, grazers reduced final macroalgal wet mass by 33% compared to grazer-free controls, but generally this effect disappeared when their predators were present indicating a trophic cascade (Fig. 4.2A; see Appendix Fig. D2 for images of final macroalgal biomass). Predators generally increased macroalgal biomass when in the presence of grazers (LSM contrast, $F_{1,83} = 2718.93$, P <

0.0001). The distribution of species richness across trophic levels also influenced final macroalgal biomass, where the strongest trophic cascade on macroalgae appears in the bottom-up skewed food web structures (e.g., triangular shaped, Fig. 4.2A).

Microalgae, including early-successional *Cladophora sp*. and *Ulva linza* as well as the chain-forming diatom *Tabellaria sp.*, colonized and grew in all mesocosms. Chlorophyll *a* concentration, a proxy for microalgal growth, was affected by the presence of upper trophic levels, but not changes in predator to prey richness (Table 4.2B). On average grazers reduced chlorophyll *a* concentration by 83% in the absence of predators, and 66% in their presence (Fig. 4.2B). Predators generally promoted microalgal accumulation (LSM contrast, $F_{1,83} = 7.15$, $P = 0.009$). In contrast with the macroalgal results, the strongest trophic cascade on microalgal accumulation emerged in the top-skewed food web structure (inverted triangle) with greater predator to prey richness.

Incidental grazer immigration was minimal across all food web structures (~39 individuals per algae-only control mesocosm). Both the presence of predators and changes in predator to prey richness influenced final grazer abundance (Table 4.2C). On average, predators reduced grazer abundance by 91%. Grazer abundance was greatest in the bottom-up skewed food web structures regardless of the presence of predators (LSM contrast $F_{1,83} = 50.79$, $P < 0.0001$, Fig. 4.2C) indicating that algal richness promoted grazer population growth. The presence of predators, but not the distribution of species richness across trophic levels, influenced grazer community composition (Fig. 4.3).

Discussion

The results of our experiment suggest that the distribution of species richness across trophic levels, or trophic skew, can affect the strength of a trophic cascade in a diverse estuarine food web. As evidenced by changes in primary producers and grazer communities across experimental food web structures, increasing prey to predator richness can strengthen or weaken a trophic cascade depending on the primary producers of interest.

Similar to results from other aquatic and terrestrial studies, we found that increasing plant richness increased primary production in experimental mesocosms after 24 days (Tilman et al. 2001, Bruno et al. 2005, Hooper et al. 2005, Stachowicz et al. 2008). These positive diversity effects are thought to be largely due to complementarity and sampling effects, whereby the likelihood of incorporating facilitators, resource partitioning, and the most productive species increases with elevated richness (Loreau et al. 2001). Although here we do not have macroalgal monocultures and cannot test specific mechanisms underlying observed richness effects, it is likely that increasing algal richness played a strong role in governing the effects of trophic skew on primary biomass by increasing the likelihood of incorporating palatable and highly productive algal species.

Although increasing plant richness may increase biomass accumulation, these effects are often weak or undetectable in the presence of consumers (Naeem et al. 2000, Fox 2004, Gamfeldt et al. 2005, Bruno and Cardinale 2008), suggesting that consumers can decouple the relationship between plant diversity and productivity (Mulder et al. 1999). Here we found that grazers generally reduced macroalgal biomass and microalgal accumulation (Figs 4.2AB), mitigating the effects of macroalgal richness. Additionally, plant richness was positively correlated with grazer abundances (Fig. 4.2C). Elevating plant richness and the number of plant functional groups is known to promote herbivore diversity and overall herbivory (Haddad et al. 2001, Scherber et al. 2006) directly through resource availability and indirectly by influencing the interactions between herbivores and their predators (Siemann et al. 1998, Moran et al. 2010). Although result in reduced plant biomass over time, here we do not see suppression of consumer effects on plant with increasing plant diversity. It is possible that this experiment was not of sufficient duration to allow unpalatable algal species to replace the biomass of the more palatable species lost to herbivory. Alternatively, as several of the algal species in our species pool are known to be less preferred by one or more of our herbivores, it is possible that increasing plant richness increased overall plant community palatability. Additionally, trophic skewing may affect different functional groups of primary producers (e.g., macro- vs. microalgae), making it difficult to predict the overall effects of concurrent species gains and losses on primary production.

Increasing predator richness can increase prey capture or antipredator behaviors, due to diet complementarity and niche differences (Burkepile and Hay 2008), thereby releasing plants from herbivory and strengthening a trophic cascade (Bruno and O'Connor 2005, Byrnes et al. 2006). Unlike results here, work in detrital food webs found that “top-down” diversity effects of consumer richness had strong effects on functioning (e.g., decomposition, Srivastava et al. 2009). Increasing predator richness in our experimental communities was likely correlated with increased likelihood and potential promotion of omnivory (both a sampling and nonadditive richness effect), thereby increasing overall consumption of plants and appearing to weaken the trophic cascade (Finke and Denno 2004, Bruno and O'Connor 2005).

Community composition and identity effects may drive observed differences among experimentally skewed food webs. Elevated macroalgal richness, coupled with decreased predator richness, may have promoted grazer survivorship and population growth through increased refuge and food quality, and/or decreased predator efficiency of prey capture (Bruno and O'Connor 2005, Moran et al. 2010). An increased likelihood of incorporating unpalatable algae due to higher algal richness, and a decreased likelihood of omnivory due to lower predator richness, could promote algal biomass in bottom-up skewed communities. Reduced predator richness in these food webs could also reduce predator efficiency if intraspecific competition among predators is stronger than interspecific interactions. As food webs become skewed, the identity of the species being gained or lost (e.g., whether they are an omnivorous predator or a palatable algae) may become increasingly important.

Biodiversity can significantly affect primary production, nutrient cycling and community composition. Control of algal blooms, yield of important commercial and recreational fisheries, and other ecosystem services may depend not only on the maintenance of biodiversity, but of its distribution throughout a given food web. Understanding the effects of trophic skew on ecosystem functioning may be an important tool in predicting the potentially synergistic effects of species extinctions and invasions on ecosystem functioning and will be an important challenge for empirical and applied endeavors across systems.

Supporting Information

Additional supporting information may be found in **Appendix D**:

Supplemental D1. *Images of experimental mesocosms*

Supplemental D2. *Images of experimental algal communities*

Tables

Table 4.1. Experimental species list.

Macroalgae
Codium fragile
Ulva lactuca
Dictyota menstrualis
Gracilaria tikvahiae
G. verrucosa
Padina gymnospora
Sargassum filipendula
Ceramium sp.
Hypnea musciformis

Grazers
Dulichiella appendiculata
Elasmopus levis
Gammarus mucronatus
Paracerceis caudata

Predators
Hypleurochilus geminatus
Monacanthus hispidus
Fundulus heteroclitus
Lagodon rhomboids
Palaemonetes vulgaris
Penaeus aztecus
Mud crabs (*Panopeus herbstii, Eurypanopeus depressus* or *Neopanope sayi*)
Swimming crabs (*Callinectes sapidus* or *C. similis*)

Table 4.2. Results of two-way ANOVA on the effects of trophic skew.
Degree of trophic skewing encompassed the presence of upper trophic levels (e.g., food chain length: algae only, + grazers, + grazers + predators) and the distribution of species richness (top-down, neutral, slightly bottom-up, bottom-up skewed).

Response Factor	d.f.	SS	*F*	*P*
A. Macroalgal biomass				
Food chain length (FCL)	2	3875.99	26.59	**< 0.001**
Richness distribution (RD)	3	2589.50	11.84	**0.002**
FCL * RD	6	555.12	1.27	0.281
Error	83	6050.01		
B. Microalgal accumulation				
Food chain length	2	10.17	40.08	**< 0.001**
Richness distribution	2	0.11	0.28	0.841
FCL * RD	4	1.44	1.89	0.092
Error	83	10.53		
C. Grazer abundance				
Food chain length	2	16718.14	275.54	**< 0.001**
Richness distribution	3	611.64	11.60	**< 0.001**
FCL * RD	6	495.43	4.70	**< 0.001**
Error	83	1458.96		

Figures

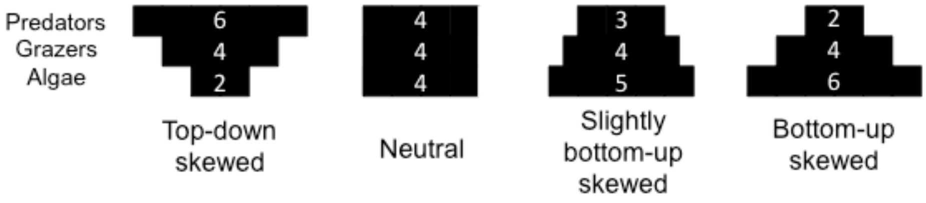

Figure 4.1. Experimental food web structures with varying species richness per trophic level.

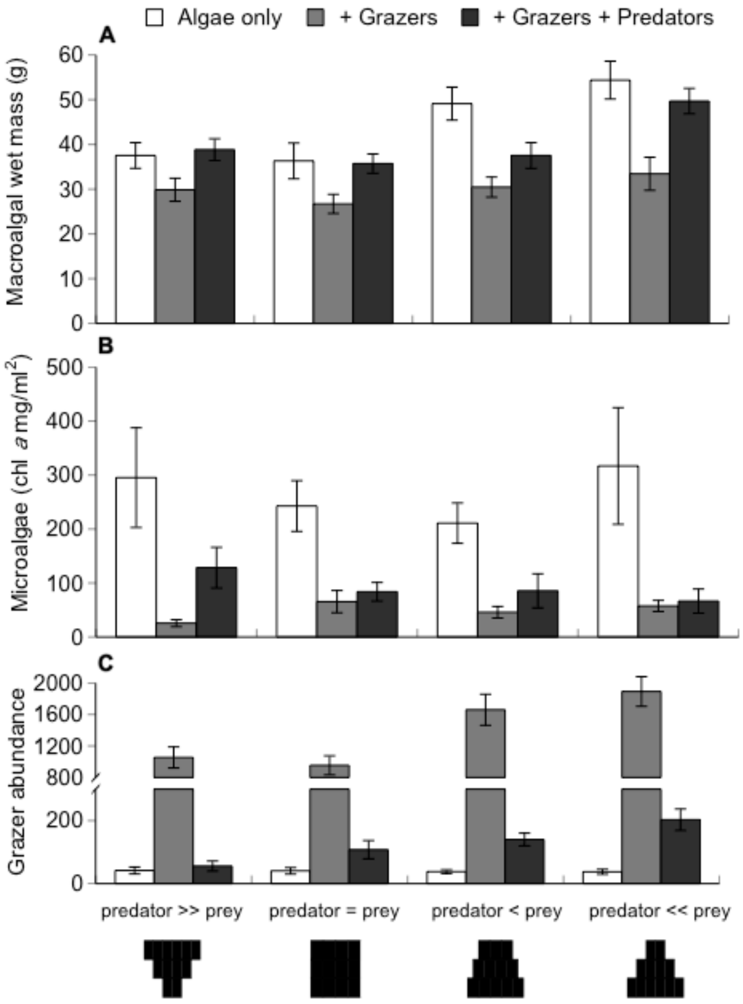

Figure 4.2. Effects of trophic skew on macroalgae, microalgae and mesograzers. Final A) macroalgal biomass, B) microalgal chlorophyll *a* concentration, and C) mesograzer abundance in experimental mesocosms across trophic skewing treatments after 24 days. Values are means ± SE.

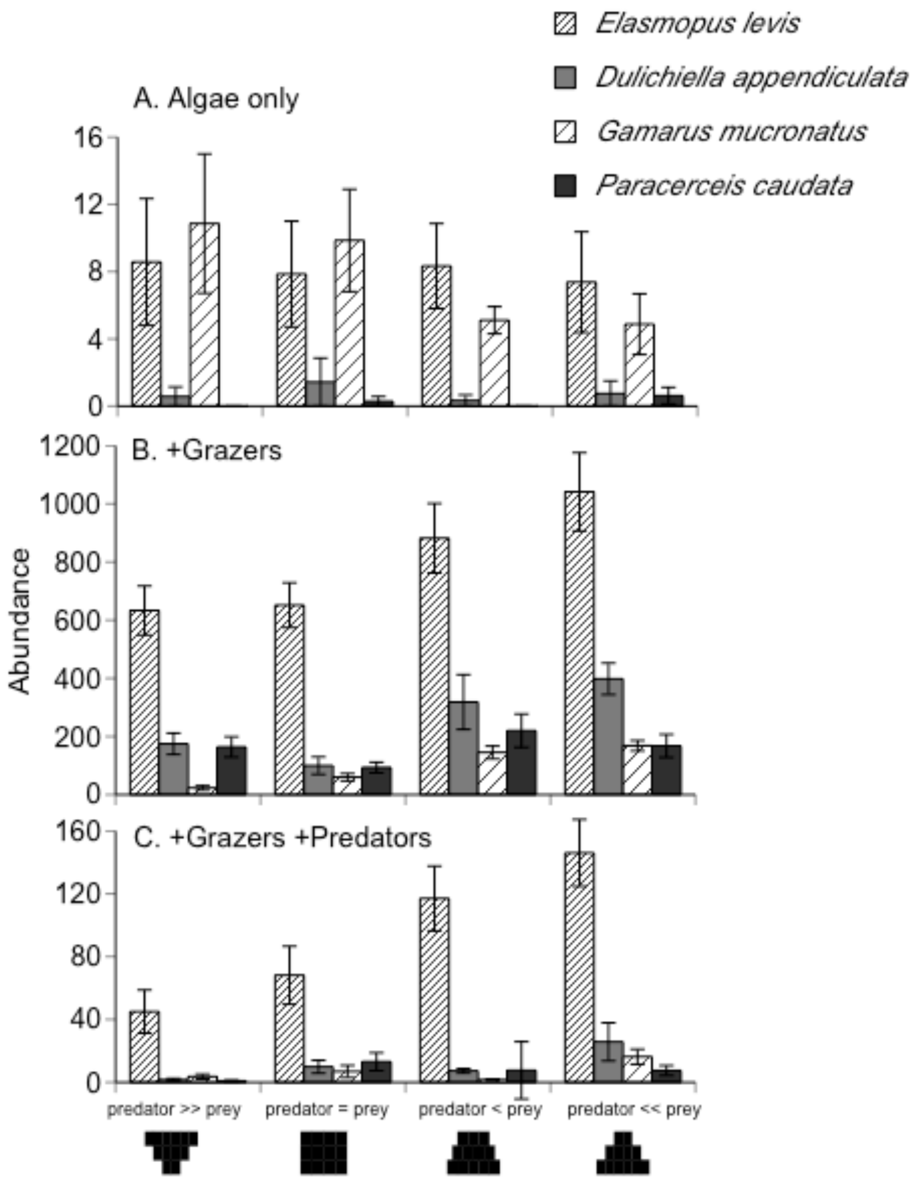

Figure 4.3. Final grazer abundance per species across skewing treatments. (Means ± 1SE).

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CONCLUSIONS

The role of predators has long been acknowledged as important for prey populations and ecosystem functioning. Predator effects on prey density and behavior have been well studied across many taxa and systems, yet many questions concerning the causes and consequences of the relative strength of these consumptive and nonconsumptive effects remain unanswered. The research I have presented in this thesis addresses several of these questions and suggests avenues of future research that may further our ability to understand the role of predators, and not simply predation, in ecological communities. Specifically, we found that 1) multiple predators influenced prey behavior, 2) changes in individual prey behavior had implications for population- and community-level dynamics, 3) multiple predators (e.g., predator species richness) enhanced a trophic cascade via strengthened nonconsumptive predator-prey interactions, and 4) nonconsumptive effects persisted over time and in a diverse food web.

First, we found that multiple predators can affect both predation risk (a consumptive interaction) and prey antipredator responses (a nonconsumptive interaction). Predators consistently reduced prey foraging (an antipredator response; Chapters 1-3), although the degree of this antipredator response varied with predator identity. Effective antipredator behaviors to a given predator reduce direct predation (consumptive effect), but often result in costly changes in prey behavior, morphology or life history characteristics (Sih et al. 1985, Lima 1998, Peacor 2003, Creel and Christianson 2008). Thus prey should modulate the intensity of their response with the associated predation risk posed by a given predator (e.g., McIntosh and Peckarsky 1999). However, we did not observe a clear correlation between the intensity of the antipredator response and the predator species that posed the greatest predation risk (e.g., consumed the most prey individuals) in either of our experimental estuarine food webs. Amphipods responded maximally to pinfish despite findings that brown shrimp were equally as efficient predators as pinfish (Chapter 1). Mud crabs responded maximally to oyster toadfish, whereas stone crabs caused the greatest mud crab mortality in experimental enclosures (Chapter 3). Other factors, such as cue dispersal and reception, as well as life history characteristics and evolutionary history among predators and their prey may contribute to these counterintuitive results.

Second, we provide evidence that predators can affect prey density and population dynamics without accessing or consuming their prey (Chapters 1-2). As noted above, while antipredator behaviors reduce the likelihood of predation, they often depress fitness when predation does not occur (Magurran 1999). Reduced prey activity levels including foraging rates, for example, may reduce detection by predators but result in slowed growth and maturation as well as reduced fecundity and maternal provisioning. In addition, predator-induced stress can alter prey physiology; stress hormones can lower fecundity and alter prey resource use (Boonstra et al. 1998, Hawlena and Schmitz 2010a). Over time, predators may affect prey densities via nonconsumptive reductions in prey population growth rates (Chapters 1-2, Nelson et al. 2004). In turn, nonconsumptive predator reductions in prey grazing at the scale of the individual (Chapters 1-3) and the population (Chapters 1-2) can strengthen a trophic cascade by promoting basal resources. Thus we provide support that nonconsumptive predator-prey interactions can influence individual prey, as well as population, community and ecosystem level dynamics (Peacor and Werner 2001, Werner and Peacor 2003, Preisser et al. 2005, Trussell et al. 2006, Pangle et al. 2007, Creel and Christianson 2008, Schmitz et al. 2008, Hawlena and Schmitz 2010b).

Third, we found that the presence of multiple predators (e.g., increasing predator richness) influenced prey populations with cascading, positive effects on basal resources. The presence of multiple predators reduced prey grazing, although in one instance this increase in predator richness heightened predation risk (Chapter 1) and in another reduced predation risk (Chapter 3). Universal increases in antipredator responses with predator richness may occur when prey responses are graded toward the most threatening predator in a given assemblage (e.g., McIntosh and Peckarsky 1999), and/or when prey are unable to detect changes in predation risk due to interspecific interactions among their predators. Investigating the causes and consequences of prey sensitivity and their ability to detect changes in predation risk will improve our understanding of how multiple predator assemblages can affect the strength of a trophic cascade in areas of weak predation pressure.

Finally, we observed that nonconsumptive effects persisted over time in both experimental mesocosms and field enclosures. Controversy over the relative importance of consumptive and nonconsumptive interactions often contrasts differences in spatial and temporal scales of these two effects (Preisser and Bolnick 2008b). For example, starving prey will reduce their responses or fail to respond altogether to the presence of predators (Sih 1992), or they may compensate for lost feeding opportunities by increasing consumption after the threat of predation has passed. If predators promote dispersal, they can reduce immediate prey abundance with positive effects on prey resources in that patch and negative impacts at the destination sites (Power et al. 1985), with little overall change in prey density and consumer pressure on the landscape scale. Thus adaptive prey responses may cancel out any positive nonconsumptive effects of predators on basal resources over time.

However, NCEs that affect prey fitness or dispersal may have important implications for metapopulation dynamics (Orrock et al. 2008) over both short and long timescales. NCEs that reduce fitness (e.g., depressed growth, fecundity due to stress or reduced resource intake) may influence prey populations more slowly than direct predation but may also 'spill over' and indirectly impact other members of the community at large (Huang and Sih 1990, Abrams et al. 1996, Peacor and Werner 1997). Nonconsumptive effects that promote prey dispersal, as has been commonly observed for many stream invertebrates, may reduce prey abundances faster than direct predator consumption (Wooster and Sih 1995), thereby positively influencing basal resources in local patches. If instead predators inhibit prey activities including dispersal (e.g., Chapter 1), any immigrating prey may be retained and overall prey abundances elevated in areas with more predators (Sih and Wooster 1994, Wooster and Sih 1995). Such NCEs that promote prey retention and/or colonization may operate faster than within-patch reproduction thereby reducing resources in local patches (Sih et al. 2010), with consequences for prey population stability (Luttbeg and Schmitz 2000).

Additionally, while consumptive effects can only occur in the immediate area of the predator, predators with wide ranging cues that trigger antipredator behavior in their prey (e.g., olfactory cues) can scare prey that are far from the predator (Chapter 3)(Sih et al. 1992). Further investigations into the relationships between temporal and spatial scales and the strength of both consumptive and nonconsumptive predator effects in a community context are necessary to elucidate the importance of predators in natural systems.

This research raises several avenues for future work and potential applications of predator-prey interactions for understanding ecological processes. To better understand variations among systems in the strength of both consumptive and nonconsumptive predator effects, it is necessary to integrate sensory/cognitive and behavioral ecology with traditional community ecology approaches. In particular, it will be useful to compare prey populations and prey species to detect whether differences in the type of predator cue (e.g., olfactory, visual, auditory) explain differences in antipredator responses. Quantifying how the local environment shapes the dispersal of predator cues, as well as how prey sensory biases influence their detection, will also elucidate observed system and prey-type biases in the strength of NCEs on ecosystem functioning.

Just as different predators have varying consumptive and nonconsumptive effects on a given prey population or species, different prey types will likely experience differences in CEs and NCEs to a given predator or predator community (Byrnes et al. 2006). Recent reviews suggest that variations in prey behavior, rather than predator diet selection, affect the relative impact of predators on different prey species (Sih and Christensen 2001). Prey with effective antipredator behaviors (e.g., are able to hide or escape) suffer lower consumptive effects and stronger nonconsumptive effects, while species with ineffective antipredator behaviors experience stronger lethal consumption by predators. This suggests that NCEs may drive trophic cascades in habitats with greater structural complexity where predation intensity is low and refuge abundance high (e.g., oyster reefs), while CEs may drive effects in open habitats where prey experience high predation rates (e.g., sand flats). Additionally, native prey should experience stronger NCEs than CEs from predators compared to introduced prey which have little to no prior exposure to the given predator. Isolating attributes of prey and habitats that make prey more likely to be affected by NCEs compared to CEs may further aid our ability to predict when and where we find the strongest nonconsumptive trophic cascades.

Placing NCEs in the context of invasion ecology may also aid in assessing and predicting the abilities of prey to cope with native and non-native predators (Sih et al. 2010). Native and non-native predators may differ in their consumptive and nonconsumptive effects on prey, which may partially explain invasion outcomes and the establishment of exotic predators. For example, prey that respond to general predator cues may be more likely to detect and respond to novel predators with effective antipredator behaviors thereby limiting the success of that predator, while prey that respond to specific predator cues may not respond appropriately and thereby facilitate the predator. Furthering investigations on how prey respond and adapt to changes in predation risk, whether from predator extinctions, novel predator invasions or native predator re-introductions, will improve our ability to predict the effects of trophic skew (Chapter 4) and our ability to conserve and manage natural systems.

In closing, understanding the role of predators is critical as habitat loss and harvesting for sustenance, profit, and sport have led to significant declines in the abundance and species richness of predators in a wide variety of habitats (Pimm et al. 1995, Ricciardi and Rasmussen 1999, Terborgh et al. 2001). This is particularly true in the ocean where decades, and in some cases centuries, of overfishing have greatly reduced the diversity and abundance of top predators (Jackson *et al.* 2001, Wing and Wing 2001, Myers and Worm 2003). By determining how changes in predator communities influence natural food webs, we may better understand and predict how ongoing predator losses will affect the processes shaping natural communities. While most standard ecological theory implicitly assumes that the role of nonconsumptive effects of predators on their prey does not deviate from their consumptive effects (see review by Peckarsky et al. 2008), recent studies have begun to incorporate density-independent effects of predators on prey population dynamics (Abrams 1995, Bolker et al. 2003, Preisser and Bolnick 2008a). Further inquiry and the development of models accounting for independent effects of both consumptive and nonconsumptive predator-prey interactions will improve our ability to detect and predict changes in ecosystem function with ongoing changes in predator communities. Indeed, it may be time to amend Tennyson's (1849) "Nature, red in tooth and claw" with "Fearing tooth and claw, Nature is green" as a more accurate reflection of our modern understanding of the importance of predation and fear for ecological and evolutionary processes, including the persistence of trophic cascades.

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APPENDICES

Appendix A: Supplementary Materials for Chapter 1

Supplemental A1: Experimental images

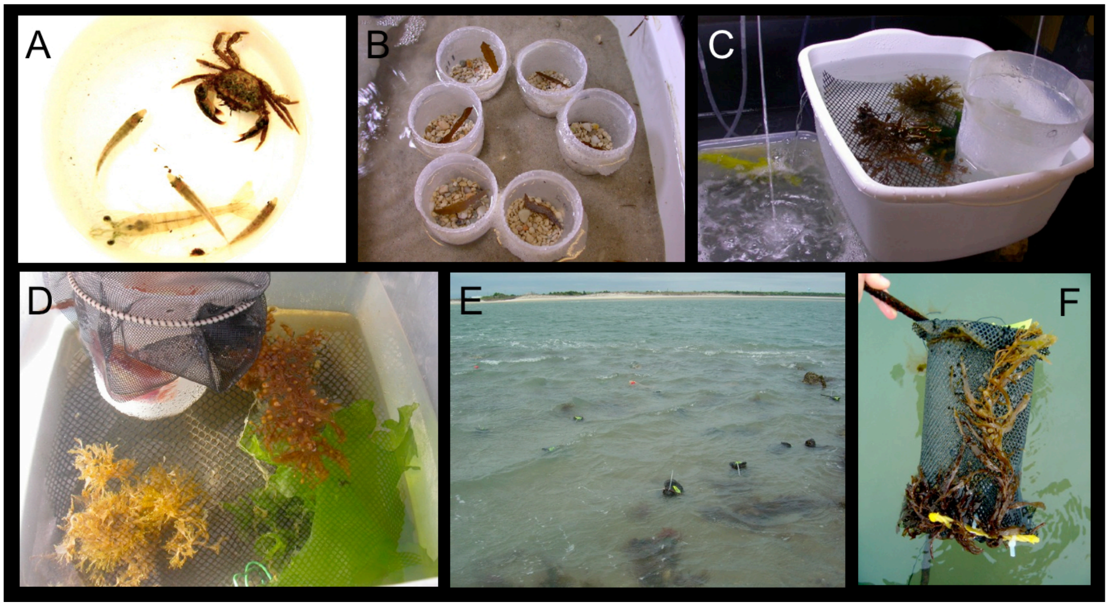

Figure A1. Images of experimental predators and setup.
(A) Experimental predators: pinfish, mud crab, brown shrimp. Setup for behavioral grazing (B) and dispersal (C) assays, mesocosm community experiment (D), and colonization experiment field site (E) and cage (F).

Supplemental A2: Predator efficiency of prey capture

To assess how efficiently our predators consumed *A. longimana*, we conducted a feeding trial in September of 2008 at the University of North Carolina at Chapel Hill's Institute of Marine Science (IMS) in Morehead City. The experimental setup was the same as in the community experiment except predators were not caged (n = 6). After 6 hours we removed all predators and counted the remaining *A. longimana*. Lower recovery indicated greater predator efficiency. We compared the number of amphipods recovered across predator treatments using a one-factor ANOVA with LSM planned comparison of the 3 predator polyculture to the average predator monoculture in JMP.

Predator identity affected *A. longimana* survival (one-factor ANOVA: $F_{3,20} = 7.896$, $P = 0.0011$, Fig. A2). Fewer *A. longimana* were recovered in predator polycultures compared to the average monocultures (P = 0.016, LSM planned comparison). Almost all amphipods were recovered in the no-predator replicates, which on average lost 2.8% of prey. Fish and shrimp were the most efficient predators in monoculture, consuming on average 53.8 and 46.7% of prey respectively during the feeding trial, while crabs only consumed 24.4%. These results are similar to those of Bruno (2005) and Nelson (1979). The fewest prey were recovered in predator polycultures, which consumed on average 57.8% of prey during the trial. These conclusions should be interpreted with caution as predator efficiency and richness effects may change under more natural conditions with open prey populations (O'Connor & Bruno 2009).

Table A2. Sizes of predators used in the community experiment.
Lengths are true length for fish and shrimp, carapace width for crabs. Values are means ± 1 SE. n = 32.

Predator	Wet Mass (g)	Length (cm)
Panopeus herbstii (mud crab)	2.5 ± 0.1	1.8 ± 0.1
*Penaeus aztec*us (brown shrimp)	4.2 ± 0.2	5.8 ± 0.1
Lagodon rhomboides (pinfish)	2.6 ± 0.1	4.3 ± 0.1

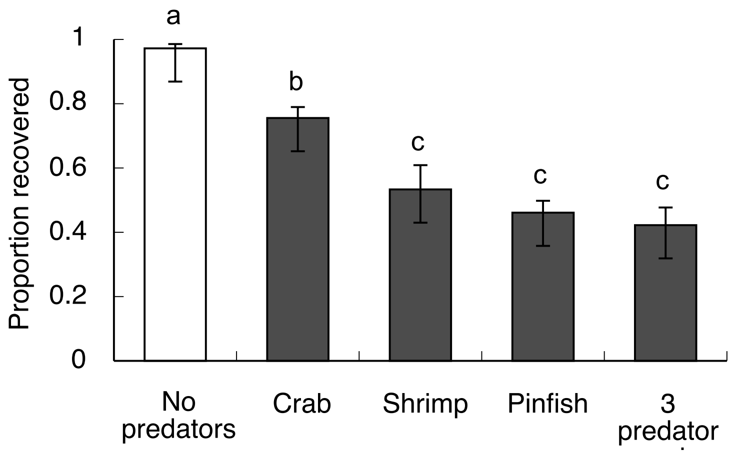

Figure A2. Effects of predator identity and richness on predator community efficiency of prey capture.
Lower recovery indicates greater predator efficiency. (Values are means ± 1 SE; comparisons are Tukey's HSD.)

Supplemental A3: Methods for chlorophyll *a* analysis

Microalgae colonized all mesocosms during the community experiment. Microalgal communities included the early-successional alga *Cladophora sp*., *Ulva intestinalis* and the diatom *Tabellaria*. We measured chlorophyll *a* concentration as a proxy for microalgal production. A 4 cm^2 square of vinyl tiling was attached to plastic coated copper wires and fixed in the center of each mesocosm at approximately 10 cm from the surface and at least 6 cm from the bottom. Tiles were collected at the end of the experiment, stored in light-blocking containers, and frozen until processed. Chlorophyll *a* was extracted using a sonicator and a 10 mL solvent of 45:45:10 acetone, ethanol and DI water solution for 24 hours, and then quantified using a TD-700 fluorometer or tri-chromatic spectrometer (US EPA Method 446.0). Water samples filtered from a random subsample of mesocosms prior to macroalgal and animal additions exhibited similar initial chlorophyll *a* levels across tables (one-factor ANOVA, $F_{1, 38} = 0.0915$, $P = 0.7639$; n = 5) and thus final chlorophyll *a* differences are likely due to amphipod grazing.

Supplemental A4: Macroalgal composition

To graphically compare species composition between experimental communities, we generated a nonmetric multidimensional scaling analysis (NMS) on a distance matrix of Bray–Curtis similarities generated from macroalgal wet mass in PC-ORD (McCune & Grace 2002). A NMS step-down procedure was performed and the NMS scree plot and results of a Monte Carlo test were evaluated to select a 2D configuration for generation of the NMS ordination. A 2D NMS ordination was then performed with a maximum of 250 iterations, 20 real data runs, and 20 randomized data runs. The 2D NMS ordination plot was graphed with R statistical software v2.11.0 (R Foundation for Statistical Computing, Vienna, Austria).

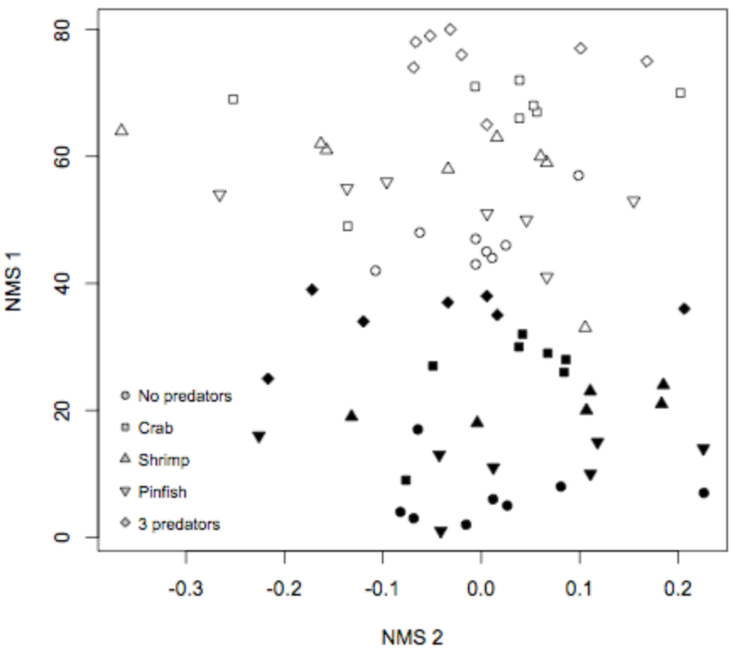

Figure A4. NMS plot of macroalgal community composition across predator and grazer treatments in the five-week community experiment.
(r^2 NMS 1 = 0.85, NMS 2 = 0.14; stress level 3.24). Grazers were absent in light symbols, and present in dark symbols.

Supplemental A5: Effects of predator cues on grazer diet preference

To assess whether predator presence and richness affect preference for the chemically defended *D. menstrualis*, amphipods were exposed to chemical cues from predators in an identical setup to the grazing rate experiment (see Chapter 1, methods) except the assay was run in a laboratory and each cup received feeding screens rather than fresh algal tissue. Feeding screens were prepared using either freeze-dried, ground *D. menstrualis* or a control alga, the highly palatable *Ulva linza,* mixed with agar and spread over windowscreen (for detailed methods and recipe see Reynolds and Sotka in press). Each cup received one *D. menstrualis* screen and one *U. linza* screen. Cups were checked daily and the assay ended per replicate when the amphipod consumed >30% of one screen, or 40% total across both screens. As there was no loss of algae in the control cups (grazers absent), we directly compared the average proportion of *D. menstrualis* consumed per amphipod for each tub across predator treatments using a one-factor ANOVA in JMP. Data were transformed as needed to meet assumptions of normality and homoscedacity. Predator presence, identity and richness did not significantly affect grazer preference for the chemically defended alga (one-factor ANOVA: $F_{4,\,40} = 1.30$, $P = 0.29$; Fig. A5).

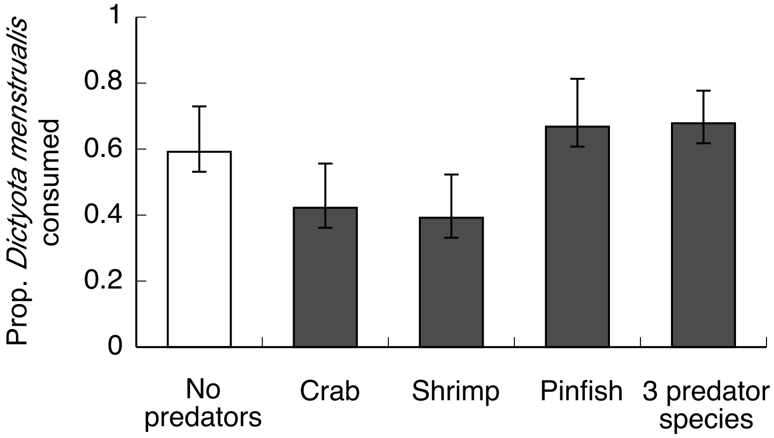

Figure A5. Effect of predator cues on grazer preference for the chemically rich alga, *Dictyota menstrualis*.
(Values are means ± 1SE.)

Supplemental A6: Effects of pinfish density on grazer feeding

To assess whether predator density influences the strength of predator NCEs on prey grazing, on 22 August 2009 we placed female *A. longimana* in clear plastic cups with 50 mg *Sargassum filipendula* and exposed them to olfactory and visual cues from 0, 1, 3 or 6 pinfish (see Methods: Grazing Assay for description of replicate setup; n = 8) for 7 days. Pinfish density affected grazer feeding ($F_{3,28} = 22.22$, $P < 0.0001$). Increasing pinfish density from 1 to 3 individuals per tub nonconsumptively reduced grazer feeding, although there was no difference in grazing rate in the 3 and 6 pinfish treatments (Fig. A6).

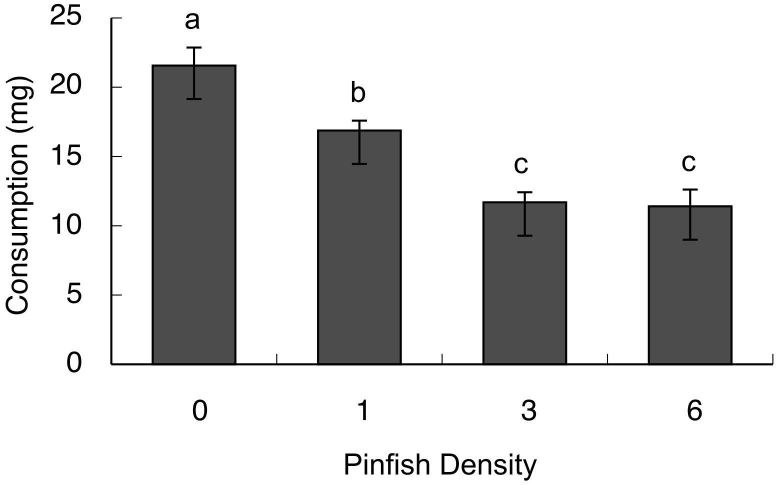

Figure A6. Effects of predator density on prey grazing rate.
(Values are means ± 1 SE; comparisons are Tukey's HSD.)

Appendix B: Supplementary Materials for Chapter 2

Supplemental B: Experimental images.

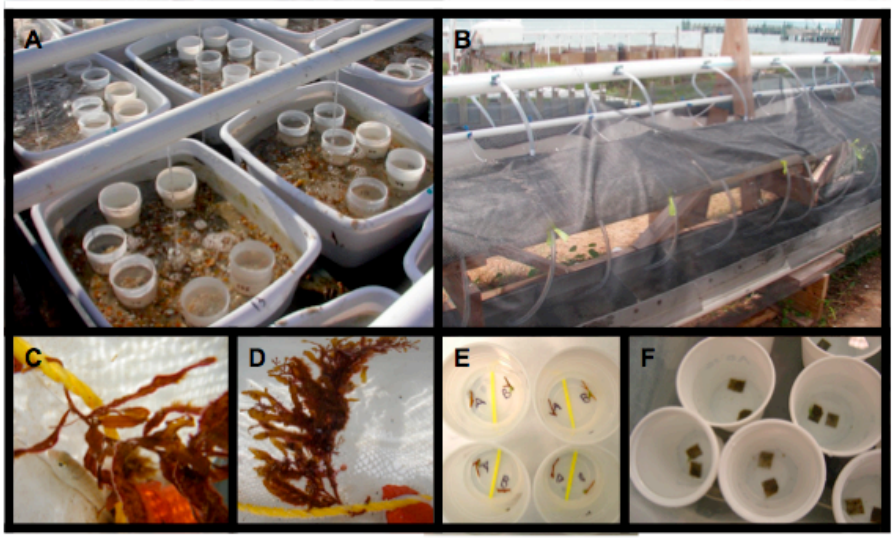

Figure B1. Experimental images.
A) Grazing rate assay and algal induction; B) Outdoor mesocosms; C) Top tank with pinfish; D) Bottom tank; E) Fresh tissue choice assay experiment; F) Freeze-dried tissue choice assay experiments.

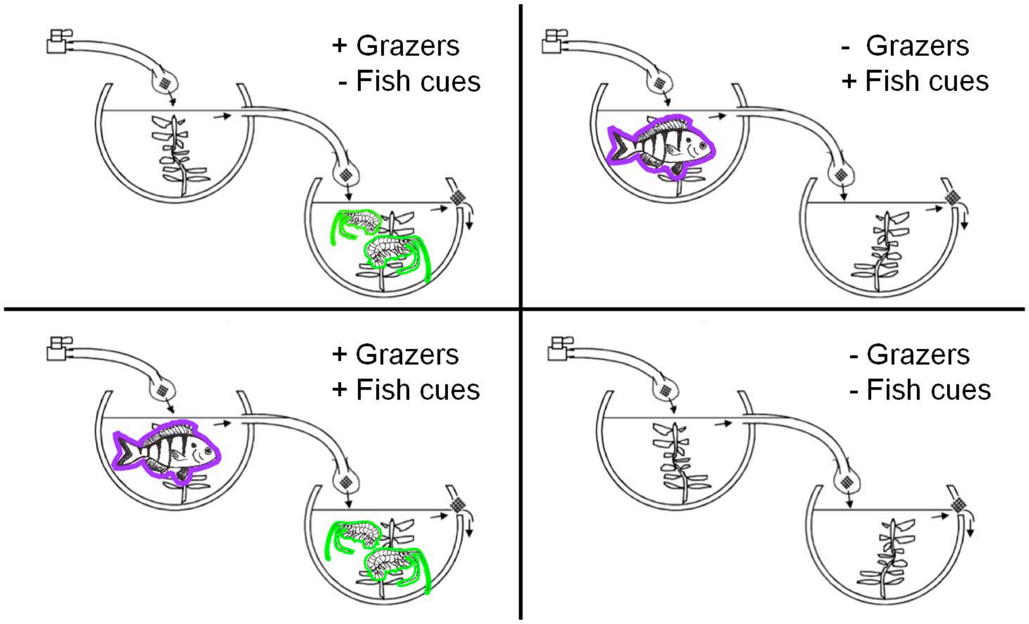

Figure B2. Setup of tanks for the algal induction experiment.
Algal tissue was quantified and assayed for all bottom tanks. Figure adapted from Sotka *et al.* (2002).

Appendix C: Supplementary Materials for Chapter 3

Supplemental C1. Image of experimental cages

Figure C1. Field site at Hoop Pole, NC, with experimental cages (top) and cage design with access treatments (Total Effect and NCE) and zone (Near and Far) placement (bottom).

Supplemental C2: Mixed-effects modeling

To directly incorporate zone (position within sub-plot) into our examination of top predator identity effects on oyster survivorship in the feeding assays, we used a mixed-effects model incorporating time block (two levels: block 1 or 2) and predator identity (three levels: stone crab, blue crab, toadfish) as fixed effects and predator access (two levels, Total Effect or NCE), cage (27 levels), and zone (two levels, near or far) as random effects. Here, random effects take into account correlations of subplots within the cages; the mixed effect model allows one to account for any heterocedacity or correlation structure present in the data. Model comparisons were conducted to select the best model. Cages without a top predator and cages with all three top predators were excluded in this analysis. The outcome of interest was the number of oysters remaining in each sub-subplot (e.g., zone). As oyster survivorship per zone was bounded (0-5, respectively), we tested the main effects and interactions using a linear regression model with a binomial distribution fit using lmer (nlme4 package) in R (version 2.11.0).

Table C2. Comparisons of multiple mixed-effects models.

A. Model comparison for oyster survivorship per zone in the initial feeding assay.

Component removed from model	LR std.	df	P	AIC
Access*Zone	5.042	1	**0.02474**	194.59
Predator identity*Zone	10.619	2	**0.004945**	198.16
Time	0.2765	1	0.599	191.55
Predator identity*Access	0.0305	2	0.9849	193.27
Predator*Access*Zone	5.5594	2	0.06206	197.24

B. Model comparisons for oyster survivorship per zone in the final feeding assay.

Component removed from model	LR std.	df	P	AIC
Predator identity*Zone	8.0848	2	**0.01756**	95.903
Access*Zone	2.0872	1	0.1485	91.818
Time	0.2082	1	0.6482	91.731
Predator identity*Access	0.0577	2	0.9716	93.523
Predator identity*Access*Zone	5.2975	2	0.07074	97.465

Supplemental C3. Treatment effects on tunicate recruitment.

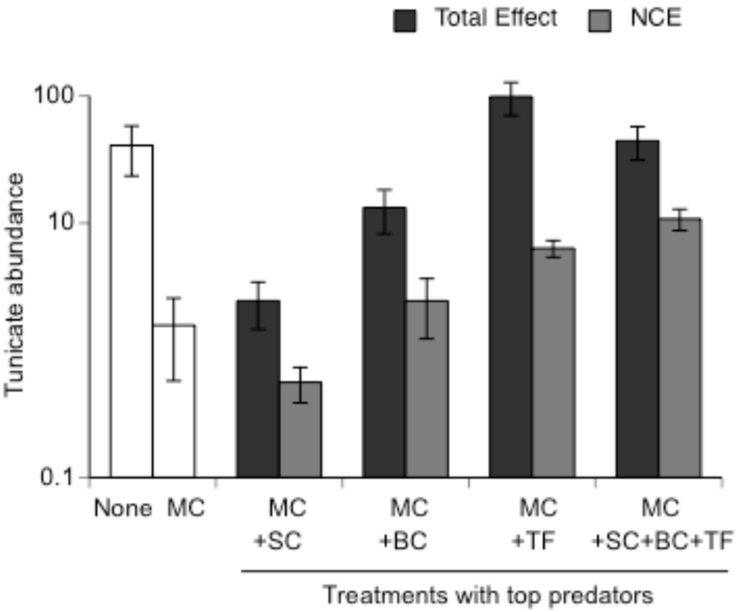

Figure C3. Final abundance of solitary tunicates that recruited into experimental field cages.

Means ± SE tunicates after two months in the presence of lethal (“Total”) and nonlethal (“NCE”) top predator communities. Treatment codes: None (no consumers), MC mud crabs, SC stone crab, BC blue crab, TF oyster toadfish. The presence of toadfish strongly promoted tunicate recruitment.

Appendix D: Supplementary Materials for Chapter 4

Supplemental D1. Setup of experimental mesocosms

Table D1. Species composition of A) macroalgal and B) predator community in each mesocosm.

Shaded algae are known to be chemically defended and least preferred by most of our experimental mesograzers. Light algae are highly palatable. Shaded predators are omnivorous, while light predators are strict carnivores.

A		Macroalgal Species					
	Replicate	1	2	3	4	5	6
Top-down skewed	1	*Ceramium sp.*	*Gracilaria verrucosa*				
	2	*Dictyota menstrualis*	*Gracilaria verrucosa*				
	3	*Dictyota menstrualis*	*Hypnea musciformis*				
	4	*Gracilaria tikvahiae*	*Codium fragile*				
	5	*Gracilaria tikvahiae*	*Sargassum filipendula*				
	6	*Gracilaria verrucosa*	*Sargassum filipendula*				
	7	*Hypnea musciformis*	*Ulva lactuca*				
	8	*Padina gymnospora*	*Ulva lactuca*				
	9	*Padina gymnospora*	*Ulva lactuca*				
Neutral	1	*Ceramium sp.*	*Dictyota menstrualis*	*Gracilaria verrucosa*	*Hypnea musciformis*		
	2	*Ceramium sp.*	*Codium fragile*	*Gracilaria tikvahiae*	*Hypnea musciformis*		
	3	*Codium fragile*	*Dictyota menstrualis*	*Gracilaria verrucosa*	*Ulva lactuca*		
	4	*Dictyota menstrualis*	*Hypnea musciformis*	*Padina gymnospora*	*Ulva lactuca*		
	5	*Codium fragile*	*Dictyota menstrualis*	*Padina gymnospora*	*Ulva lactuca*		
	6	*Gracilaria tikvahiae*	*Gracilaria verrucosa*	*Padina gymnospora*	*Sargassum filipendula*		
	7	*Gracilaria tikvahiae*	*Gracilaria verrucosa*	*Hypnea musciformis*	*Ulva lactuca*		
	8	*Gracilaria tikvahiae*	*Padina gymnospora*	*Sargassum filipendula*	*Ulva lactuca*		
	9	*Codium fragile*	*Gracilaria verrucosa*	*Sargassum filipendula*	*Ulva lactuca*		
Slightly bottom-up skewed	1	*Codium fragile*	*Dictyota menstrualis*	*Padina gymnospora*	*Sargassum filipendula*	*Ulva lactuca*	
	2	*Ceramium sp.*	*Dictyota menstrualis*	*Gracilaria verrucosa*	*Sargassum filipendula*	*Ulva lactuca*	
	3	*Ceramium sp.*	*Gracilaria verrucosa*	*Hypnea musciformis*	*Padina gymnospora*	*Sargassum filipendula*	
	4	*Dictyota menstrualis*	*Gracilaria tikvahiae*	*Hypnea musciformis*	*Padina gymnospora*	*Ulva lactuca*	
	5	*Codium fragile*	*Dictyota menstrualis*	*Gracilaria tikvahiae*	*Gracilaria verrucosa*	*Hypnea musciformis*	
	6	*Dictyota menstrualis*	*Gracilaria tikvahiae*	*Gracilaria verrucosa*	*Hypnea musciformis*	*Padina gymnospora*	
	7	*Codium fragile*	*Gracilaria tikvahiae*	*Padina gymnospora*	*Sargassum filipendula*	*Ulva lactuca*	
	8	*Codium fragile*	*Gracilaria verrucosa*	*Gracilaria tikvahiae*	*Hypnea musciformis*	*Sargassum filipendula*	
	9	*Codium fragile*	*Gracilaria verrucosa*	*Padina gymnospora*	*Sargassum filipendula*	*Ulva lactuca*	
Bottom-up skewed	1	*Ceramium sp.*	*Codium fragile*	*Gracilaria tikvahiae*	*Gracilaria verrucosa*	*Sargassum filipendula*	*Ulva lactuca*
	2	*Codium fragile*	*Dictyota menstrualis*	*Gracilaria tikvahiae*	*Gracilaria verrucosa*	*Padina gymnospora*	*Ulva lactuca*
	3	*Ceramium sp.*	*Codium fragile*	*Dictyota menstrualis*	*Gracilaria tikvahiae*	*Sargassum filipendula*	*Ulva lactuca*
	4	*Ceramium sp.*	*Dictyota menstrualis*	*Gracilaria verrucosa*	*Hypnea musciformis*	*Padina gymnospora*	*Sargassum filipendula*
	5	*Codium fragile*	*Dictyota menstrualis*	*Gracilaria tikvahiae*	*Hypnea musciformis*	*Padina gymnospora*	*Sargassum filipendula*
	6	*Dictyota menstrualis*	*Gracilaria tikvahiae*	*Gracilaria verrucosa*	*Hypnea musciformis*	*Padina gymnospora*	*Ulva lactuca*
	7	*Codium fragile*	*Dictyota menstrualis*	*Gracilaria verrucosa*	*Hypnea musciformis*	*Padina gymnospora*	*Sargassum filipendula*
	8	*Dictyota menstrualis*	*Gracilaria verrucosa*	*Hypnea musciformis*	*Padina gymnospora*	*Sargassum filipendula*	*Ulva lactuca*
	9	*Gracilaria tikvahiae*	*Gracilaria verrucosa*	*Hypnea musciformis*	*Padina gymnospora*	*Sargassum filipendula*	*Ulva lactuca*

B		**Predator Species**					
	Replicate	1	2	3	4	5	6
Top-down skewed	1	Blenny	Blue crab	Brown shrimp	Grass shrimp	Mud crab	Mummichog
	2	Blue crab	Brown shrimp	Grass shrimp	Mud crab	Mummichog	Pinfish
	3	Blenny	Blue crab	File fish	Mud crab	Mummichog	Pinfish
	4	Blue crab	Brown shrimp	File fish	Grass shrimp	Mummichog	Pinfish
	5	Blue crab	File fish	Grass shrimp	Mud crab	Mummichog	Pinfish
	6	Blenny	File fish	Grass shrimp	Mud crab	Mummichog	Pinfish
	7	Brown shrimp	File fish	Grass shrimp	Mud crab	Mummichog	Pinfish
	8	Blenny	Brown shrimp	File fish	Grass shrimp	Mummichog	Pinfish
	9	Blenny	Brown shrimp	File fish	Grass shrimp	Mud crab	Pinfish
Neutral	1	Blenny	Brown shrimp	Brown shrimp	File fish	Pinfish	Pinfish
	2	Blenny	File fish	Mummichog	Mummichog	Pinfish	Pinfish
	3	Blenny	File fish	File fish	Grass shrimp	Grass shrimp	Mud crab
	4	Blenny	Blue crab	Brown shrimp	Grass shrimp	Grass shrimp	Mud crab
	5	Blue crab	Blue crab	File fish	File fish	Mummichog	Pinfish
	6	Blue crab	Brown shrimp	Mummichog	Mummichog	Pinfish	Pinfish
	7	Blue crab	Blue crab	File fish	Grass shrimp	Mummichog	Mummichog
	8	Brown shrimp	Brown shrimp	Mud crab	Mud crab	Mummichog	Pinfish
	9	File fish	File fish	Grass shrimp	Grass shrimp	Mud crab	Pinfish
Slightly bottom-up skewed	1	File fish	File fish	Grass shrimp	Grass shrimp	Mummichog	Mummichog
	2	Blenny	Blenny	Brown shrimp	Brown shrimp	Mummichog	Mummichog
	3	Blenny	Blenny	File fish	File fish	Mud crab	Mud crab
	4	Brown shrimp	Brown shrimp	Grass shrimp	Grass shrimp	Mud crab	Mud crab
	5	Blue crab	Blue crab	Brown shrimp	Brown shrimp	Mud crab	Mud crab
	6	File fish	File fish	Mummichog	Mummichog	Pinfish	Pinfish
	7	File fish	File fish	Grass shrimp	Grass shrimp	Pinfish	Pinfish
	8	Blue crab	Blue crab	Mummichog	Mummichog	Pinfish	Pinfish
	9	Brown shrimp	Brown shrimp	Grass shrimp	Grass shrimp	Pinfish	Pinfish
Bottom-up skewed	1	Blenny	Blenny	Blenny	File fish	File fish	File fish
	2	Blenny	Blenny	Blenny	Mud crab	Mud crab	Mud crab
	3	Blue crab	Blue crab	Blue crab	Mud crab	Mud crab	Mud crab
	4	Blue crab	Blue crab	Blue crab	Mummichog	Mummichog	Mummichog
	5	File fish	File fish	File fish	Grass shrimp	Grass shrimp	Grass shrimp
	6	File fish	File fish	File fish	Pinfish	Pinfish	Pinfish
	7	Brown shrimp	Brown shrimp	Brown shrimp	Grass shrimp	Grass shrimp	Grass shrimp
	8	Brown shrimp	Brown shrimp	Brown shrimp	Mummichog	Mummichog	Mummichog
	9	Mummichog	Mummichog	Mummichog	Pinfish	Pinfish	Pinfish

Figure D1. Experimental mesocosm setup.
Clockwise: mesocosms receiving filtered seawater, macroalgal community, and mesocosm side view (courtesy of M. O'Connor).

Supplemental D2. Images of final experimental algal communities.

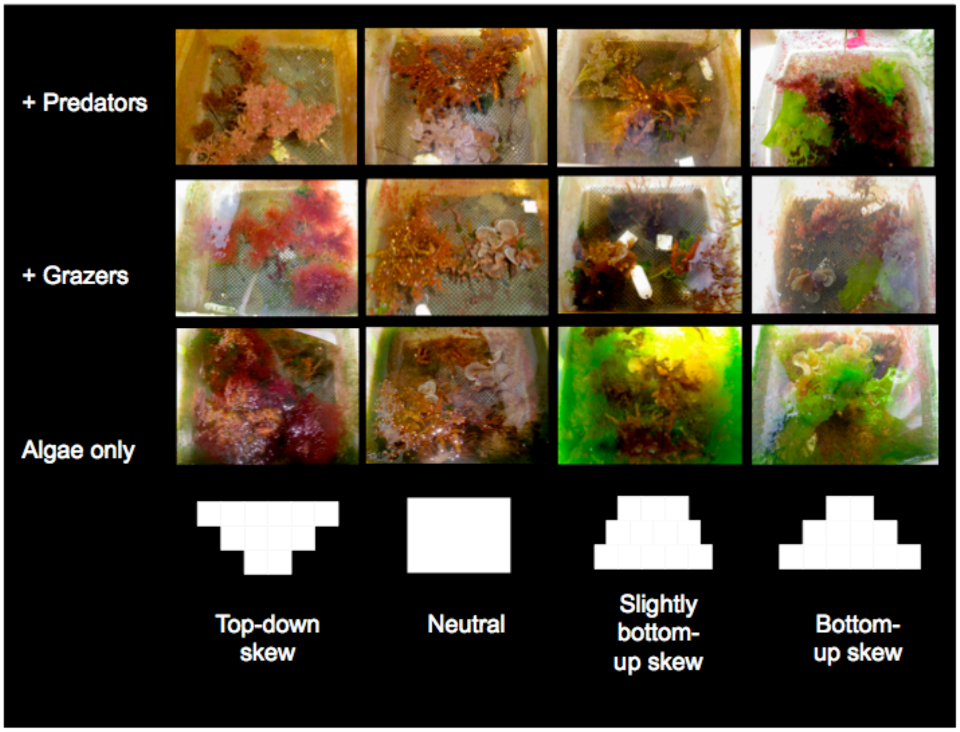

Figure D2. Example algal communities after 24 days in experimental mesocosms exposed to different types and degrees of trophic skew.

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