

## LETTER

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

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## Abstract

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

## Keywords

Additive design, coexistence, ecosystem functioning, herbivore diversity, macroalgae, mesograzer, predator diversity, replacement design.

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## INTRODUCTION

There is now considerable evidence that species diversity influences a variety of ecosystem processes including productivity, decomposition, and nutrient cycling (reviewed by Hooper *et al.* 2005; Balvanera *et al.* 2006; Cardinale *et al.* 2006a). Experiments with plants and herbivores suggest that in general, increasing diversity within a given trophic level tends to increase the total abundance or production at that level, and to decrease the abundance of organisms or resources at the level below (Balvanera *et al.* 2006; Cardinale *et al.* 2006a). However, most experiments testing effects of biodiversity on ecosystem function (BEF) have focused on diversity within a single, lower trophic level (Hooper *et al.* 2005). In contrast, effects of diversity changes at higher trophic levels, and the interactive effects of changes at multiple trophic levels, are less well understood. Predator diversity effects are especially difficult to generalize because there is intriguing variation in how multiple predators interact to affect prey populations and ecosystem processes (Duffy

*et al.* 2007; Bruno and Cardinale 2008, Schmitz 2007). Increasing predator diversity may strengthen total impact on prey through complementary prey choices or emergent multi-predator effects such as risk-enhancement (Losey & Denno 1998; Sih *et al.* 1998; Byrnes *et al.* 2006), but it may also reduce impacts on prey due to competition and intraguild predation among predators, and/or omnivory (Hart 2002; Finke & Denno 2004; Bruno & O'Connor 2005; Jonsson *et al.* 2007). How changes in diversity at multiple trophic levels interact is also poorly understood, though theory predicts that such interactions can have additive, synergistic, or antagonistic effects on ecosystem processes (Thebault & Loreau 2003; Worm & Duffy 2003; Fox 2004b).

Biodiversity may be thought of as having a horizontal component, which constitutes diversity within a trophic level, and a vertical component, which constitutes the number and distinctness of trophic levels (Duffy *et al.* 2007). Changes in number of trophic levels tend to have strong effects on ecosystems in the form of trophic cascades (Shurin *et al.* 2002). Some have argued that community-wide cascades,

which affect abundance and biomass across whole trophic levels, are rare in diverse systems, implying an interaction between effects of changing vertical and horizontal diversity (Strong 1992; Polis 1999; but see Bruno & O'Connor 2005; Borer *et al.* 2006). Although a growing number of BEF studies have included multiple trophic levels, few have been designed to rigorously evaluate the effects of changing diversity within a level on adjacent levels, or the interactive effects of simultaneous diversity change at multiple levels (reviewed by Duffy *et al.* 2007; Bruno and Cardinale 2008). With the exception of experiments performed in plant-pollinator systems (Fontaine *et al.* 2006), the only factorial manipulations of diversity of adjacent trophic levels have been done in microbial systems in the laboratory (Naeem *et al.* 2000; Fox 2004a; Gamfeldt *et al.* 2005). Naeem *et al.* (2000) manipulated decomposers and algae, and found that increasing decomposer diversity and algal diversity acted synergistically to increase algal production. Manipulations of ciliates (primary consumers) and microalgae have produced mixed results. Gamfeldt *et al.* (2005) found that increasing ciliate diversity decreased algal biomass and increased consumer production, and that algal diversity increased consumer production only at the highest diversity of consumers. In contrast, Fox (2004a) found that algal diversity increased algal biomass but did not affect consumer production, and that consumer diversity had no effects. These divergent results call for deeper investigation of how changing diversity at multiple trophic levels affects ecosystem properties, especially in systems with macroscopic animals and plants.

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

Here we report an experimental test of the independent and interactive effects of herbivore and predator species

composition and richness on algal growth, herbivore abundance, and predator growth and survival in a benthic marine community. Small, invertebrate grazers (mostly amphipod and isopod crustaceans hereafter referred to as 'grazers') are the most abundant herbivores in this system, as they are in many benthic communities worldwide. Some species have generation times of less than three weeks in warm water (Fredette & Diaz 1986; Duffy *et al.* 2003). Grazer species differ in their impacts on macroalgae (Duffy 1990; Duffy & Hay 2000), and increasing grazer diversity decreases macroalgal biomass in seagrass mesocosms (Duffy *et al.* 2003, 2005). Grazers also consume epiphytic microalgae, however, and may thus have a positive influence on some species of macroalgae by reducing competition with microalgae for light and nutrients (Duffy 1990). Grazers are a primary food source for many larger crustaceans and small fishes, including the prey of commercially harvested species, and are an important link from benthic algal production to fisheries yield (Edgar & Shaw 1995; Williams & Heck 2001). Commercial harvest of large fishes and crustaceans could alter vertical diversity (i.e., effective food chain length) in marine macroalgal systems. This in turn could increase densities of smaller predators, leading to reduced abundance of grazers and increased abundance of algae via a trophic cascade (e.g. Heck *et al.* 2000; Worm & Myers 2003; Frank *et al.* 2005).

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

## METHODS

### Mesocosm system

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

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

### Experimental design

All organisms were collected from lagoons, inlets, and tidal creeks in the immediate vicinity of Morehead City (34.43 N, 76.43 W). Grazer and predator diversity treatments were crossed in a factorial design. A simplified gradient of diversity was used for both grazers and predators: monocultures of each of three species, and 'diverse' treatments with all three species. Grazers were stocked using a replacement design (Jolliffe 2000), with 60 of one species inoculated in monocultures, or 20 of each species in three-spp. treatments. Predators were stocked in a similar manner, with a total of three individuals per mesocosm. However, we also included a high-density predator treatment based on an additive design, which had three individuals of each predator species. We included both types of three-spp. predator treatments to simulate two potential relationships of predator abundance and predator species richness (see introduction). We did not incorporate an additive, high-density grazer treatment, because we assumed that rapid population growth would bring grazer communities to near carrying capacity for each species by the end of the experiment, reducing effects of differently sized inocula, as found previously (Duffy & Harvilicz 2001). We included a no-predator treatment but not a no-grazer treatment. Each of the 24 unique treatments was replicated five times, for a total of 120 mesocosms.

### Community assembly

All mesocosms were stocked with the same three macroalgal species: *Sargassum filipendula*, a fleshy, brown alga; *Gracilaria tikvahiae*, a coarse, branched red alga; and *Ulva sp.*, a leafy, green alga. These algae were selected because they tend to dominate local hard substratum benthic communities and

represent distinct taxonomic groups that have been shown in previous mesocosm experiments to be differently susceptible to consumption by grazers (Duffy & Hay 2000). The macroalgae were added in equal proportions (14 g wet mass each) to each mesocosm by attaching algal thalli to Vexar™ screens at the bottom of the mesocosms (Bruno *et al.* 2005). Algae and screens were defaunated before installation by soaking in a dilute solution of Sevin™ insecticide (43% 1-naphthyl-N-methylcarbamate) in seawater (0.1 g Sevin™ L<sup>-1</sup>) for 30 min, followed by rinsing in flowing seawater.

The three species of grazers used are generally very common in the system and represented the numerically dominant species in the field at the time of the experiment. All are pericaridean crustaceans with rapid life cycles and direct development of young too large to escape through the outflow screens on the mesocosms. Adults of all species reach a maximum size of about 1 cm length. *Paracerceis caudata* is an isopod that consumes algae and detritus. *Elasmopus levis* and *Dulichieilla appendiculata* are gammaridean amphipods known to consume both micro- and macroalgae (Duffy & Hay 2000).

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

### Statistical analyses

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

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

hypothesized effects of grazer and predator diversity (see Supplemental Table S1). To distinguish nontransgressive and transgressive effects, we compared the performance of polycultures to the average performance of monocultures, and to the performance of the highest performing monoculture (Hector *et al.* 2002). By necessity, these contrasts were not orthogonal. For responses that allowed, we included two, additional contrasts within the interaction term of the ANOVA (Table S1). The 'grazer richness + predator richness' contrast compared treatments with monocultures at both trophic levels to the treatment with polycultures at both trophic levels (e.g. Fontaine *et al.* 2006). The 'grazer richness \* predator richness' contrast assessed whether grazer richness effects were altered by predator richness and / or predator richness effects were altered by grazer richness. We considered predator polycultures that used additive density vs. replacement density in separate contrasts, but contrasts in the interaction term were conducted only for the replacement-density predator polyculture. Effects of predator density and diversity on predator survival were tested with a Kruskal-Wallis comparison among predator monocultures, low-density polycultures, and high-density polycultures. Correlations among final predator biomass, final grazer abundance, and algal mass change were assessed with multiple linear regression.

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

## RESULTS

### Treatment efficacy

Algae, grazers, and predators generally survived and grew or multiplied over the course of the experiment. Contamination

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

### Macroalgae responses

Top-down control by grazers and predators had little influence on macroalgal biomass relative to the inadvertent variation caused by mesocosm position. However, the green seaweed *Enteromorpha intestinalis*, which recruited into the mesocosms, varied significantly among predator treatments in ANOVA (Supplemental Table S2), and multiple regression suggested that this was because *E. intestinalis* was slightly reduced by the omnivorous grass shrimp (Table S3). Grazer treatment had no effect on algae in ANOVA, but multiple regression found that total grazer abundance slightly reduced the biomass of *Ulva sp.* and *Sargassum filipendula*. Predator and grazer diversity had weak and idiosyncratic effects on macroalgae, never explaining more than 8% of the variance (Table S2).

### Grazer responses

Total grazer abundance increased roughly 10-fold by the end of the experiment, representing an intrinsic rate of growth  $r$  of about  $0.1 \text{ d}^{-1}$ . There was no main effect of grazer treatment on total grazer numbers or individual species' population growth rates. However, grazer and predator treatments interacted to affect total grazer numbers, indicating that grazer species composition and richness influenced total grazer abundance under some conditions (Table 1, Fig. 1). Specifically, a planned contrast ('grazer richness \* predator richness') suggested that herbivore richness had a stronger effect on herbivore abundance in the presence of multiple predators than in predator

**Table 1** Results of 2-way, fully crossed ANOVAs examining effects of grazer and predator species composition and diversity on a) grazer populations and b) predator proportional mass change.

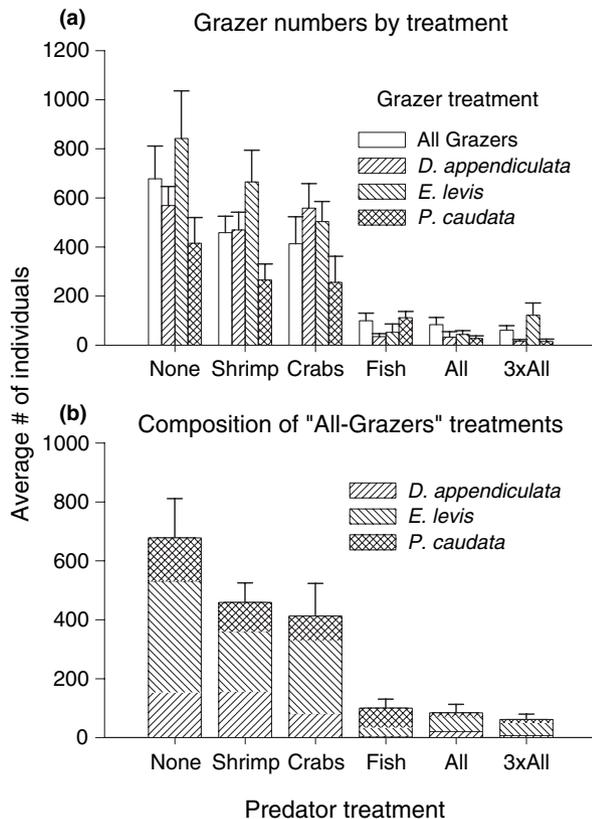
a. Effects	Response															
	ln(total grazer numbers)				<i>Paracerceis caudata</i> PGR				<i>Elasmopus levis</i> PGR				<i>Dulichieilla appendiculata</i> PGR			
	d.f.	F	$\omega^2$	P	d.f.	F	$\omega^2$	P	d.f.	F	$\omega^2$	P	d.f.	F	$\omega^2$	P
<b>Grazer treatment</b>	3	2.14	0.01	0.102	1	0.36	0.000	0.551	1	4.03	0.026	0.052	1	0.09	0.000	0.772
> Monos. vs. polyculture	1	1.97	0.00	0.168												
> Best mono. vs. polyculture	1	0.22	–	0.638												
<b>Predator treatment</b>	4	34.91	0.55	<b>&lt;0.0001</b>	4	10.10	0.433	<b>&lt;0.0001</b>	4	16.70	0.549	<b>&lt;0.0001</b>	4	16.70	0.717	<b>&lt;0.0001</b>
> Monos. vs. low density poly.	1	39.05	0.22	<b>&lt;0.0001</b>	1	19.24	0.217	<b>&lt;0.0001</b>	1	14.86	0.028	<b>0.000</b>	1	12.16	0.063	<b>0.001</b>
> Monos. vs. high density poly.	1	54.27	0.15	<b>&lt;0.0001</b>	1	28.52	0.328	<b>&lt;0.0001</b>	1	7.64	0.058	<b>0.009</b>	1	50.19	0.279	<b>&lt;0.0001</b>
> Best mono. vs. low density poly.	1	0.84	–	0.149	1	8.75	–	<b>0.005</b>	1	0.54	–	0.467	1	3.07	–	0.088
> Best mono. vs. high density poly.	1	2.13	–	0.363	1	13.96	–	<b>0.001</b>	1	3.21	–	0.081	1	0.50	–	0.483
<b>Grazer trt. * predator trt.</b>	12	1.95	0.05	<b>0.041</b>	5	0.56	0.000	0.695	5	0.90	0.000	0.473	5	1.70	0.016	0.170
> Grazer richness + pred. richness	1	0.73	0.00	0.396												
> Grazer richness * pred. richness	1	10.53	0.05	<b>0.002</b>												
b. Effects	Total predator PMC				Shrimp PMC				Crab PMC				Fish PMC			
	d.f.	F	$\omega^2$	P	d.f.	F	$\omega^2$	P	d.f.	F	$\omega^2$	P	d.f.	F	$\omega^2$	P
	d.f.	F	$\omega^2$	P	d.f.	F	$\omega^2$	P	d.f.	F	$\omega^2$	P	d.f.	F	$\omega^2$	P
<b>Grazer treatment</b>	3	1.06	0.002	0.370	3	0.25	0.000	0.863	3	3.15	0.073	<b>0.034</b>	3	0.67	0.000	0.576
> Monos. vs. polyculture	1	3.44	0.021	0.068	1	–	–	–	1	5.00	0.041	<b>0.030</b>	1	1.17	0.003	0.285
> Best mono. vs. polyculture	1	0.08	–	0.776	1	0.62	–	0.440	1	0.00	–	0.961	1	1.78	–	0.189
<b>Predator treatment</b>	4	1.24	0.005	0.295	2	0.16	0.000	0.851	2	5.62	0.105	<b>0.006</b>	2	0.30	0.000	0.740
> Monos. vs. low density poly.	1	2.79	0.015	0.100	1	–	–	–	1	7.00	0.061	<b>0.011</b>	1	0.80	0.000	0.376
> Monos. vs. high density poly.	1	0.43	0.000	0.514	1	0.07	0.000	0.798	1	3.17	0.022	0.082	1	0.00	0.000	0.996
> Best mono. vs. low density poly.	1	2.26	–	0.137												
> Best mono. vs. high density poly.	1	12.32	–	<b>0.001</b>												
<b>Grazer trt. * predator trt.</b>	12	0.63	0.000	0.708	5	4.02	0.054	0.373	6	3.46	0.179	<b>0.007</b>	6	1.82	0.098	0.117
> Grazer richness + pred. richness	1	0.98	0.000	0.325												
> Grazer richness * pred. richness	1	0.21	0.000	0.650												

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

monocultures (Table 1, Fig. 1). This effect was small, however, explaining only 5% of the variance.

Comparisons of individual grazer species population growth rates in monoculture vs. in the all-grazer treatments directly tested whether horizontal diversity influenced grazer populations (Table 1). For *P. caudata* and *D. appendiculata*, final abundances were higher in monocultures than in polycultures (Fig. 1), and population growth rates (PGR) were not affected by grazer treatment (Table 1). Conversely, for *E. levis*, final abundance was similar in monocultures and polycultures (Fig. 1) and PGR was marginally higher in polycultures (Table 1). This suggests that *E. levis* was released from intraspecific competition in the presence of other grazers, whereas *P. caudata* and *D. appendiculata* experienced intra- and interspecific competitors equally. Accordingly, grazer richness tended to increase total grazer abundance relative to the average of monocultures, although this effect was not statistically significant ( $P = 0.102$ , Table 1).

In contrast to the generally weak effects of grazer composition and richness, predator treatment and the



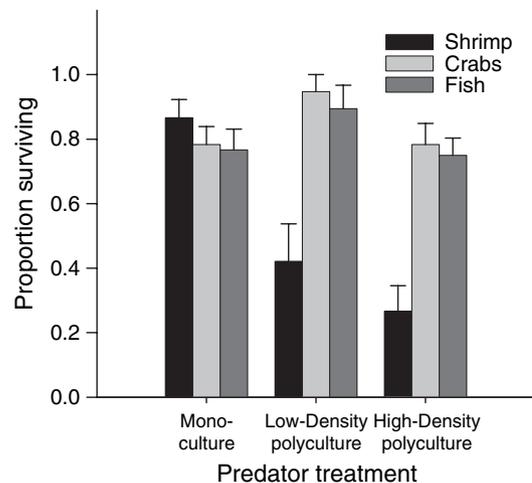
**Figure 1** (a) Final mean ( $\pm 1$  SEM) total grazer abundance as a function of grazer and predator treatment. (b) Final, relative abundance of grazer species in treatments initially stocked with equal numbers of each grazer species. Error bars are SEM of total grazer abundance.

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

**Predator responses**

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

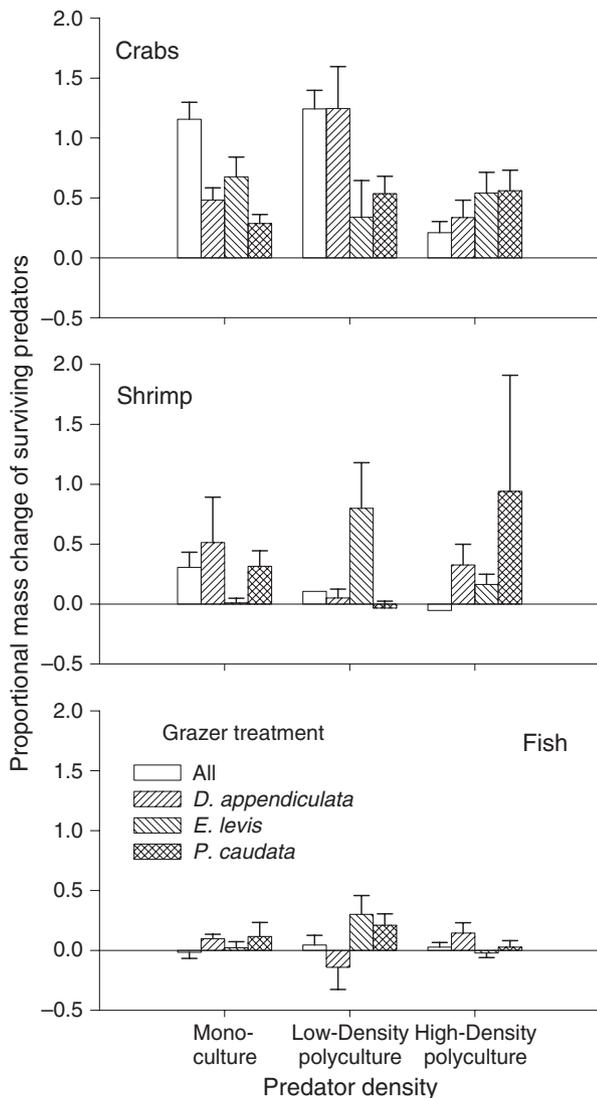
Proportional mass changes (PMC) of surviving fish and shrimp were unaffected by treatment, but crab growth was significantly influenced by composition and richness of both



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

**Table 2** Kruskal–Wallis comparisons of predator survival vs. predator diversity treatment (high density polyculture, low density polyculture, or monoculture) d.f. = 2. Bold values indicate nominal  $P < 0.05$ 

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

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

predators and grazers (Fig. 3, Table 1). Grazer richness increased crab growth in crab monocultures, and slightly increased crab growth in low-density predator polycultures,

but surprisingly decreased crab growth in high-density predator polycultures (Fig. 3).

### Prevalence and magnitude of predator and grazer effects

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

### DISCUSSION

By definition, species differ functionally between trophic levels, such that adding or removing a complete trophic level—altering ‘vertical diversity’ (Duffy *et al.* 2007)—often has strong ecosystem effects (Shurin *et al.* 2002; Borer *et al.* 2006). Within a trophic level, relationships between diversity and ecosystem function may be weaker because of greater similarity among species (Strong 1992; Walker 1992). However, species’ traits can vary widely even within a trophic level, and changing this horizontal diversity has often been shown to influence ecosystem properties (reviewed by Hooper *et al.* 2005), especially when diversity is initially low (e.g., Jonsson *et al.* 2002). In our experimental communities the number of trophic levels varied and species differed functionally within both grazer and predator trophic levels, leading to significant effects of both horizontal and vertical diversity, and their interaction, on ecosystem properties.

In the horizontal dimension, we found weak effects of herbivore diversity (defined broadly to include both species richness and composition) but much stronger effects of predator diversity. One possible contributor to this disparity

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

Only two effects of grazer diversity in our experiment approached statistical significance. First, as indicated by a significant predator richness by grazer richness interaction (Table 1), grazer richness increased predation resistance when all three predators were present at low density, but not when only a single predator species was present (Fig. 1a). The contingency of this grazer diversity effect on the predator community resembles a previous report that grazer diversity enhanced grazer biomass only in the presence of a predator (Duffy *et al.* 2005). The difference in our result, however, is that the grazer diversity effect did not arise in the presence of a single predator species, but only at high predator diversity. Second, grazer richness interacted with predator richness by increasing crab growth when crabs were the sole predators, but reducing crab growth when other predators were present at high density (Fig. 3, Table 1). These interactions between grazer diversity and predation may reflect the differing vulnerability of grazer species to predation. In the absence of fish, *Elasmopus levis* and *Dulichella appendiculata* reproduced rapidly. In the presence of fish, however, *Paracercis caudata* tended to do better, perhaps because its tough cuticle provided resistance to predation, resulting in higher grazer abundance in grazer polycultures than in monocultures without *P. caudata* (Fig. 1). A similar switch in prey species dominance under predation has been observed in previous experiments with marine grazers (Duffy *et al.* 2005). The importance of different grazers' functional traits to total grazer production under different conditions of predation might be considered an example of 'response diversity' (Elmqvist *et al.* 2003), in which different species maintain ecosystem function under different environmental conditions.

Changes in horizontal diversity at the level of predators had important community-level impacts in our experiment, i.e. predator polycultures kept grazers at a lower abundance than did predator monocultures, on average. This appeared

to be largely a sampling effect driven by the inclusion of the strongest predator (fish) in all three-species predator treatments. Thus it is similar to Straub & Snyder's (2006) finding that predator identity but not predator richness *per se*, affected prey abundance in an agroecosystem. However, we also detected an element of predator complementarity or facilitation in the reduction of grazers; the combined effects of crabs, fish, and shrimp reduced *P. caudata* to a lower level in predator polycultures than in any single-predator treatment (Fig. 1, Table 1), an analog of 'overyielding' in plant studies.

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

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

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

While we found strong interactions between predators and herbivores, we observed little influence of predator and grazer treatments on macroalgal mass change. This result probably reflects the fact that our grazer assemblages did not include amphitoid amphipods, which were scarce in the field at the time of the experiment but are known to feed heavily on macroalgae (Duffy & Hay 2000). Top-down effects of grazers and trophic cascades from predators to macroalgae have been documented in experiments that included amphitoids (Duffy & Hay 2000; Bruno & O'Connor 2005). It would be useful to perform joint predator and grazer diversity manipulations with a selection of grazer species with stronger effects on plant biomass, to investigate the hypothesized role of intermediate trophic level diversity in attenuating trophic cascades (Duffy 2002; Hillebrand & Cardinale 2004; Ives *et al.* 2005; Duffy *et al.* 2007).

Perhaps the most intriguing result we observed involved the interactive effects of herbivore and predator treatment on performance of mud crabs. When crabs were the only predator species they grew better in the presence of diverse grazer species than with any grazer monoculture (Fig. 3). This supports the balanced diet hypothesis (DeMott 1998; Gamfeldt *et al.* 2005), which appears to hold in many marine consumers (Worm *et al.* 2006; Stachowicz *et al.* 2007). But in high-density polycultures of predators, crabs actually grew more, on average, with monocultures of grazers. We were not able to determine exactly why this happened, but we suspect a trait-mediated indirect interaction (TMII) (Werner & Peacor 2003). For example, crabs may have restricted

their foraging behaviors in the presence of high densities of other predators, such that they were unable to fully exploit all prey in three-spp. grazer treatments. The presence of predators and competitors has been shown to change feeding behavior of organisms in other systems, and such TMII's appear to be common (Lima 1998; Werner & Peacor 2003).

Complex, natural communities with their reticulate food webs can be difficult to understand with pairwise predator-prey models and experiments manipulating just one or a few species (Polis & Strong 1996; Ives *et al.* 2005; Duffy *et al.* 2007; but see Schmitz & Sokol-Hessner 2002; Schmitz 2007), and it is virtually impossible to experimentally study or accurately model every trophic link in a community and its indirect effects on ecosystem state. Nevertheless, by factorially manipulating a moderate number of grazer and predator species, we were able to observe interactions within and between trophic levels that were important in structuring our experimental communities and may be relevant to real ecological systems. As in some previous studies that have manipulated consumer diversity, we found that grazer abundance can be increased by grazer richness (Duffy *et al.* 2005) and decreased by predator richness (Snyder *et al.* 2006). More importantly, by simultaneously manipulating both trophic levels we were able to observe the net effect of increasing diversity at both levels. In this case, as in Gamfeldt *et al.* (2005), the result was consistent, top-down control by the highest trophic level, with ecosystem properties most strongly affected by changing diversity at that level.

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#### SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article:

**Table S1** Experimental design and explanation of planned contrasts.

**Table S2** Results of 2-way, fully crossed ANOVAs examining effects of grazer and predator species composition and diversity on macroalgae mass change.

**Table S3** Results of multiple linear regression analyses of top-down effects on grazers and algae.

This material is available as part of the online article from: <http://www.blackwell-synergy.com/doi/full/10.1111/j.1461-0248.2008.01175.x>

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## TECHNICAL COMMENT

# Correction of statistical miscalculation slightly alters conclusions about diversity effects for Douglass *et al.* (2008)

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### Abstract

While most of the conclusions about diversity effects in Douglass, J.G., Duffy, J.E. & Bruno, J.F. [*Ecol. Lett.*, 11, 2008, 1] are upheld, correction of a statistical miscalculation indicates that grazer diversity and predator diversity had combined effects on responses, but did not have interactive effects as initially reported.

### Keywords

Analysis of variance, contrasts, herbivore diversity, interaction, predator diversity.

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We are grateful to Dr Murtaugh for discovering the statistical miscalculation that affected a portion of the results of our manuscript (Douglass *et al.* 2008), and to the editors of *Ecology Letters* for giving us the opportunity to rectify that regrettable mistake. However, we emphasize that only the results of our contrasts testing the combined and interactive effects of grazer and predator richness were affected by the miscalculation. Our results for the main effects of grazer and predator treatment, their interaction, and the main effects of grazer and predator richness are correct as originally presented.

We traced the error to the way we wrote and applied the R program (R Development Core Team 2007) that specified the 'grazer richness + predator richness' and 'grazer richness × predator richness' contrasts in ANOVA. We have since rewritten the code for those contrasts and achieved results for log(total grazer numbers + 1) matching Murtaugh's results in his Table 1. We have also recalculated our contrast results for the other response variables that were affected by the miscalculation. The results of the new contrasts for total predator proportional mass change (Table 1b in our original manuscript) are presented in Table 1 of this note. The grazer richness + predator richness effect for that response is now significant, while the grazer richness × predator richness contrast is non-significant as in the original results. Our

contrast results for algae growth responses (Supplemental Table S2 in our original manuscript) were also changed by the correction, but remained non-significant, and are not presented in this note because they do not alter our conclusions.

In addition to pointing out the miscalculation described above, Murtaugh mentioned some minor points of confusion regarding our manuscript, which are addressed in this paragraph. First, the missing replicate from the all-predator/*Elasmopus* treatment combination was a sample that was physically lost before processing. Second, our exclusion of no-predator treatments from most statistical

**Table 1** Corrected contrast results for combined and interactive grazer and predator richness effects in the ANOVA of log(predator proportional mass change + 0.2) from Douglass *et al.* (2008)

Effect	d.f.	<i>F</i>	$\omega^2$	<i>P</i>
Grazer richness + predator richness	1	7.42 (0.98)	0.048 (0.000)	0.008 (0.325)
Grazer richness × predator richness	1	0.31 (0.21)	0.000 (0.000)	0.581 (0.650)

The *F*,  $\omega^2$  and *P*-values in parentheses are the incorrect values originally reported by Douglass *et al.* (2008).

analyses was a conservative measure intended to insure that significant predator-treatment effects in ANOVA could be interpreted as effects of predator species composition or richness, and not merely as effects of predator presence.

To summarize our findings in light of the corrected results, grazer and predator species composition, predator richness and predator density all significantly affected one or more responses, as originally reported. However, contrary to the original results, there were small but significant combined effects of grazer and predator richness on total grazer abundance and total predator proportional mass change, and there was no significant interaction between predator and grazer richness *per se* on any measured response. Ironically, while these corrected statistics only impact a small part of our conclusions, they do belie our

title, which advertises *interactive* effects of herbivore and predator diversity that are no longer supported by our results.

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