Changes in biodiversity and environmental stressors influence community structure of an experimental eelgrass *Zostera marina* system

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ABSTRACT: Changes in biodiversity can result in decreased ecosystem functioning and loss of ecosystem services, but altered biodiversity is only one of many stressors impacting ecosystems. In many estuaries, environmental stressors such as warming water temperatures and eutrophication are increasing and negatively impacting biological communities, particularly seagrasses such as the important habitat-forming species *Zostera marina* (eelgrass). These negative impacts may change the diversity, composition, and functioning of this important ecosystem, but the interactions of stressors with changes in biodiversity are poorly understood. We manipulated eelgrass communities in a factorial experiment to test how changes in crustacean grazer diversity, warmer water temperatures, and nutrient enrichment interact to affect ecosystem biomass, stability, and community composition. We found that the presence and richness of crustacean grazers had a larger effect on grazer, algal, and sessile invertebrate biomass than experimental warming or nutrient enrichment. Diverse grazer assemblages stabilized epiphytic algal biomass in the face of stressors, and counteracted the promotion of epiphytic microalgae by stressors. Nutrient enrichment and warming both promoted epiphytic microalgae, while reducing macroalgae and eelgrass. A more diverse grazer assemblage stabilized epiphytic algal biomass, but we did not detect interactions among environmental stressors and grazer diversity. These results emphasize that loss of herbivore diversity can exacerbate the impacts of environmental stressors on grazing, relative dominance of microalgae versus macrophytes, producer biomass, and stability.

KEY WORDS: Diversity · Stressors · Eelgrass · Grazers

INTRODUCTION

There is increasing evidence that biodiversity, the variety of life, supports ecosystem processes, properties, and functions that are ecologically and economically important, such as water and nutrient cycling and fisheries production (Cardinale et al. 2012). Higher diversity among and within trophic levels can positively affect resource use and production, and increase stability (Tilman 1996, Tilman et al. 1998, Griffiths et al. 2000, Duffy et al. 2003, Allison 2004, Caldeira et al. 2005, Balvanera et al. 2006, Blake & Duffy 2010). Increased biomass within a functionally diverse assemblage has the potential to enhance important ecosystem processes, such as grazing, that play critical roles in marine systems (Worm et al. 1999, Nyström et al. 2000, Duffy et al. 2003, Valentine & Duffy 2006, Stachowicz et al. 2007). However, biodiversity on both global and local scales is threatened, largely by human activities that are rapidly changing ecosystems. These changes in biodiversity are of concern because they can result in losses of important ecosystem functions (Tilman 1999). Change in biodiversity also acts as a stressor in ecological systems, and may interact with and be as important as, or more important than, other stressors

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in marine systems (Stachowicz et al. 2007, Hooper et al. 2012). The impacts of biodiversity change and other stressors in marine systems are an increasing concern because we know little about their interactions, especially in the context of global climate change.

Climate change is a primary threat affecting marine systems around the globe, and the impacts are predicted to increase as more CO_2 is dissolved in the oceans, global temperatures continue to warm, and ocean circulation patterns change (Doney et al. 2012). Climate warming, an important stressor, is predicted to increase both extreme temperatures as well as mean temperatures (Najjar et al. 2010, Doney et al. 2012). Warming temperatures can lead to changes in oceanic physical processes and chemical properties such as ocean circulation, sea level, and dissolved oxygen concentrations that can impact other components of marine communities. Increased water temperatures can alter ecosystem properties, services, and functions indirectly through effects on the diversity of organisms in a community, or directly through effects on biomass and primary production. Water temperatures in most shallow temperate estuaries along the US east coast closely follow changes in air temperatures (Najjar et al. 2000). In one such estuary, Chesapeake Bay, average temperatures are predicted to increase by 2 to 6°C by the end of the 21st century (Najjar et al. 2010). Warmer waters may influence biogeochemical cycling, life histories and ranges of resident and transient organisms, fisheries production, and important habitats such as salt marshes, oyster reefs, and seagrass beds (Short & Neckles 1999, Hoegh-Guldberg & Bruno 2010, Doney et al. 2012).

Increasing pressure is also being put on coastal habitats as human populations in the coastal zone continue to rise (McGranahan et al. 2007). Anthropogenic impacts along coasts include shoreline hardening and development in coastal zones for residential, commercial, and recreational use. This development leads to decreased filtration of runoff through loss of vegetation buffers, and increased direct inputs of sewage, fertilizer, and pollutant runoff to coastal waters (Vitousek et al. 1997, Kemp et al. 2005). These impacts have led to the eutrophication of estuaries, such as Chesapeake Bay, which are now characterized by increased occurrence of bloom forming micro- and macroalgae (Hauxwell et al. 2001, Burkholder et al. 2007, Najjar et al. 2010). The conditions that favor blooms of ephemeral algae are, at the same time, detrimental to important habitats such as seagrass meadows.

Seagrass systems are important habitats in Chesapeake Bay that provide shelter for juvenile fish and crabs, stabilize sediments and coastlines, and sequester carbon (Duarte 2000, Heck et al. 2003, Duarte et al. 2005, Polte et al. 2005, Canuel et al. 2007). Historically the dominant seagrass in Chesapeake Bay, eelgrass *Zostera marina* is declining at an accelerated pace due largely to recent record summer temperatures and continued eutrophication (Orth & Moore 1983, 1988, Orth et al. 2002, 2006, Moore & Jarvis 2008). Elevated temperatures, especially in the summer months, can lead to eelgrass dieoffs (Bintz et al. 2003, Oviatt 2004). These declines are attributed to higher respiration than production, internal carbon stress, and reduced growth rates in eelgrass (hereafter referred to as *Zostera*) under higher temperature conditions (Short & Neckles 1999, Touchette & Burkholder 2000, Bintz et al. 2003, Touchette et al. 2003).

Eutrophication has negative consequences for *Zostera*, both direct and indirect. Nutrient enrichment impacts eelgrass physiology similarly through carbon limitation and ammonium toxicity, both of which lead to reduced shoot production and overall growth (Touchette & Burkholder 2000, Touchette et al. 2003). Indirect impacts of nutrients on *Zostera* include overgrowth by micro- and macroalgae, and associated light limitation (Hauxwell et al. 2001, Burkholder et al. 2007). However, extensive previous work shows that grazing by invertebrates such as amphipods and isopods in temperate seagrass beds can counteract algal overgrowth, thus fostering dominance by seagrasses, and that grazing has effects of similar or greater magnitude to water column nutrient enrichment (Neckles et al. 1993, Hughes et al. 2004). While grazing has the potential to counter the effects of water column nutrient enrichment in some circumstances (Neckles et al. 1993, Hays 2005, Jaschinski & Sommer 2011), *Zostera* habitats in Chesapeake Bay are increasingly faced with multiple simultaneous stressors whose interactions are poorly understood and difficult to predict (Orth et al. 2006).

In the present study, we examined the effects of crustacean grazer diversity, warming, and nutrient enrichment on the ecosystem properties of experimental seagrass communities. We wanted to investigate indirect impacts of stressors on ecosystem function through changes in grazer diversity, as well as direct impacts of stressors on ecosystem function. We planted outdoor mesocosms with *Zostera marina*, stocked the crustacean grazers species *Elasmopus levis*, *Erichsonella attenuata*, and *Gammarus mucro-*

natus alone and in mixture, and assessed their impacts on abundance and biomass of primary producers, grazers, and fouling invertebrates. Specifically, we tested whether (1) grazing effects were of similar magnitude as stressor effects, (2) stressors affected primary producer community composition and relative dominance, and (3) change in biodiversity interacted with stressors to exacerbate or buffer against stressor impacts. We show that stressors facilitate microalgae at the expense of macrophytes, that grazing effects are large and counteract these stressor effects, but that there were no non-additive interactions between loss of species richness and stressor impacts.

MATERIALS AND METHODS

Experimental design

We manipulated crustacean grazer species richness, water temperature, and water column nutrients in a factorial mesocosm experiment during the summer of 2007. Located outdoors at the Virginia Institute of Marine Science, Gloucester Point, Virginia, USA (37.25° N, 76.50° W) in a flow-through tank system, experimental mesocosms (one hundred 19 l buckets consisting of 5 replicates of 20 unique treatments) were supplied with filtered estuarine water from the adjacent York River. In order to exclude crustacean grazer recruits but allow algal and sessile invertebrate propagules to recruit to the mesocosms, estuarine water was filtered by a coarse sand filter and then through 150 μm mesh. Mesocosms were surrounded by a 25 cm deep water bath to buffer diurnal temperature changes, and were covered by shade screens to reduce downwelling insolation by about 60% and to approximate light conditions found in local seagrass beds. Each mesocosm contained a substrate mixture of approximately 2.5 l of sand and 0.5 l of mud, and was planted with 30 shoots of defaunated *Zostera marina* to approximate field shoot densities.

We used 2 amphipod species (*Gammarus mucronatus* and *Elasmopus levis*) and 1 isopod species (*Erichsonella attenuata*) to establish 5 grazer treatments: each species as a monoculture (1 species), a multi-species assemblage (3 species), and a grazer-free control. These grazer species (hereafter referenced by genus) are among the 5 most abundant crustacean grazers present in local eelgrass beds in the summer season, and consume a variety of epiphytic macro- and microalgae (Cruz-Rivera & Hay 2000, Duffy &

Hay 2000, Duffy & Harvilicz 2001, Douglass et al. 2010). Grazers were added to mesocosms in a replacement design, with monocultures receiving 30 individuals of 1 species and multi-species assemblages receiving 10 individuals of each species. Mesocosms were either left at ambient water temperature, or were heated approximately 3°C above ambient water temperature using a 200 W aquarium heater (warmed treatment). This warming simulated a realistic climate warming scenario projected for Chesapeake Bay (Najjar et al. 2010). Slow-release pelletized fertilizer (Osmocote®, N:P:K = 3:1:2) was used to enrich nutrient levels to approximately 5 times ambient levels in the nutrient-addition treatments. The fertilizer (15 to 20 g) was contained in fine mesh inside a perforated PVC tube and suspended in the nutrient-enriched mesocosms. Fertilizer was replaced every 4 days to maintain elevated nutrient levels (Spivak et al. 2009). Water column nutrients in each mesocosm were tested for the amount of ammonium once during the experiment to assess the enrichment treatment. Both the temperature and nutrient treatments were applied continuously, and grazers had sufficient time to reach carrying capacity, approximately 2 overlapping generations (Sainte-Marie 1991), during the 6 wk duration of the experiment.

During the experiment, epiphytic chlorophyll *a* was sampled from standardized artificial seagrass units (ASUs) placed in each mesocosm at the start of the experiment (4 ASUs of 2 polypropylene ribbons each), as a proxy for epiphytic algal biomass (Parsons et al. 1984). One ASU was removed during Weeks 2, 4, and 6, and processed using standard procedures (Parsons et al. 1984). Grazer density was estimated non-destructively at those same time points by catching grazers in 3 standardized dip-net sweeps. All captured grazers were counted, identified, and returned to their respective original mesocosms.

At the conclusion of the experiment, all biomass was harvested from each mesocosm and frozen until samples could be processed. Thawed biomass retained on a 500 μm sieve was sorted to the lowest taxonomic level possible, dried in a 60°C oven until the mass was stable, and then combusted at 450°C to obtain ash-free dry mass (AFDM). Grazers were sorted by size (using sieves with mesh sizes of 8.0, 5.6, 4.0, 2.8, 2.0, 1.4, 1.0, 0.71, and 0.05 mm), identified to species, and enumerated. Biomass of grazers was calculated using the empirical equation, $\log B = -1.01 + 2.64 \times \log S$, where B is AFDM (mg), and S is the sieve size (mm) on which the animal was retained (Edgar 1990).

Statistical analysis

We used 3-way factorial ANOVAs to test for main and interactive effects of warming, nutrients, and crustacean grazer species richness on *Zostera* shoot biomass, epiphytic algal biomass, macroalgal biomass, and sessile invertebrate biomass (SAS Enterprise Guide 4.3). All data were untransformed with the exception of the epiphytic chlorophyll *a* data, which were log transformed to improve normality. We also used *a priori* orthogonal contrasts to test for the effects of grazer presence (all treatments with grazers vs. no-grazer controls) and grazer species richness (grazer monocultures vs. 3-species assemblage treatments). To estimate the size of effects in the ANOVA analyses, we calculated the ω^2 values for each main effect and interaction using the generalized equation of Olejnik & Algina (2003):

$$\omega^2 = [SS_{effect} - df_{effect} \times MS_{error}] / [SS_{effect} + (N - df_{effect}) \times MS_{error}]$$

To examine whether crustacean grazer species richness had a stabilizing effect on ecosystem properties in the face of environmental stressors, we used the Brown-Forsythe test (Brown & Forsythe 1974, Blake & Duffy 2010). This variation of Levene's test (Levene 1960) employs the median rather than the mean in testing for equality of variance. We tested both community resistance to stressors (variation among stressor treatments), as well as stochastic variation within communities (variation among replicates within stressor treatments) (see Blake & Duffy 2010 for further details and equations).

We also used multivariate non-metric multidimensional scaling (NMDS) to examine which factors most influenced the similarity and composition of communities among all mesocosms in the experiment. We conducted the analysis in R 2.13.0 (R Development Core Team 2011), using the metaMDS function in the vegan package (Oksanen et al. 2011) and a Bray-Curtis dissimilarity matrix. Instead of using untransformed data as suggested by the default settings in metaMDS, we square-root transformed the data to reduce the influence of very abundant species. We plotted the results in 2 dimensions, and used the envfit procedure in vegan (Oksanen et al. 2011) to overlay species vectors (determined by principal components analysis) on the final NMDS plot. To statistically test the results of the NMDS ordination, we conducted PERMANOVA using the adonis function in the vegan package. This routine carries out an analysis of variances test using a dissimilarity matrix, and a

permutation test to determine significance. It is an especially appropriate test for ecological data because it uses a non-Euclidian distance matrix, is robust to multivariate non-normality, and handles datasets with more variables than degrees of freedom (McArdle & Anderson 2001). We also examined the multivariate homogeneity of the treatments using the function betadisper in the vegan package, which is analogous to Levene's test for equality of variances (Levene 1960).

To examine the direct and indirect effects of warming, nutrients, and crustacean grazer species richness on primary producer biomass and sessile invertebrate biomass, we used maximum-likelihood estimated structural equation models created with Amos 18.0.0 (Arbuckle 2011). Structural equation modeling (SEM) is a multivariate framework for graphical modeling and analysis that draws from both path and factor analysis techniques (Grace 2006). SEM allows for the exploration of direct and indirect causal relationships between observed and hypothesized (latent and composite) variables through paths relations, and is well suited to studying systems with multiple simultaneous processes (Grace et al. 2010).

Models were evaluated based on criteria described in Grace (2006). It is important to note that we used SEM in the 'model generating' mode. We first specified an initial model based on theory and previous knowledge of our experimental system, after which we modified and re-tested this model until the model fit the data well from both statistical and ecological points of view.

Grazer treatments in 4 mesocosms were contaminated, and thus removed from all statistical analysis. All treatments retained a minimum of 4 replicates.

Background environmental conditions

Global surface temperatures in the summer of 2007 were the seventh warmest on record. In the continental USA, 2007 was the sixth warmest summer since records began in 1895, and the average temperature was 1°C above the 20th century mean (NOAA 2007). In the York River, Virginia, water temperatures averaged 26°C, and the average daily maximum temperature was 27°C in the period from June through July (VECOS 2012). Experimental mesocosms at ambient temperature had an average daily maximum of 29°C, while warmed mesocosms averaged 31°C (Fig. 1).

Precipitation in the summer of 2007 was below average both nation-wide and in Virginia. Much of the Chesapeake Bay watershed experienced abnor-
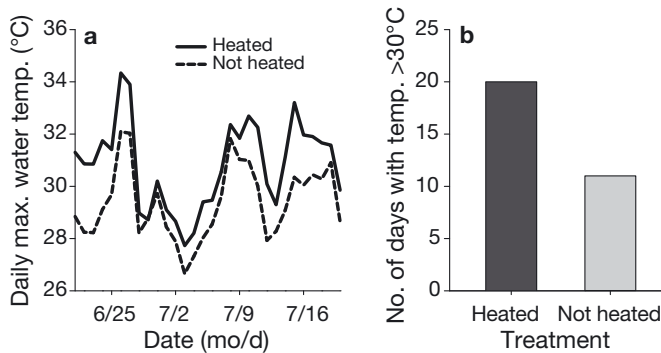
Fig. 1. (a) Average maximum daily water temperatures in heated (solid line) and unheated (dashed line) treatments over the duration of the experiment. (b) Number of days during the experiment with maximum water temperatures over 30°C in heated and unheated treatments

mally dry to moderate drought conditions during these months (NOAA 2007). Salinity averaged 20 ppt both in the York River and in the mesocosms during this experiment.

Ambient nutrient levels in the York River, measured as concentration of NH_4^+, averaged 2 µM during this experiment. In nutrient enriched treatments, NH_4^+ averaged 10 µM, or 5 times ambient nutrient levels. This level of nutrient enrichment is at or above the levels found at sites that no longer support *Zostera*, and is comparable to the levels used in previous studies simulating eutrophication in this system (Moore & Wetzel 2000, Spivak et al. 2009).

RESULTS

Species richness and multiple stressors

Crustacean grazer treatments, including grazer presence, had the largest effect of any experimental treatment in this study (Table 1). Crustacean grazer presence significantly reduced the biomass of epiphytic microalgae, macroalgae (*Ceramium* spp., *Polysiphonia* spp., *Enteromorpha* spp., and *Cladophora* spp.), and sessile invertebrates (*Molgula manhattensis*, *Botryllus schlosseri*, and *Balanus improvisus*) in experimental communities (p < 0.0001, p = 0.0052, and p = 0.0002 respectively; Fig. 2, Table 1). Species-rich grazer treatments also reduced macroalgal biomass more strongly than the average single species treatment (p = 0.0255; Fig. 2c, Table 1). Although all grazer treatments began with the same abundance, final biomass of crustacean grazers was significantly greater in species-rich grazer treatments compared to the average single-species grazer

Table 1. Results from 3-way factorial ANOVAs, and estimates of effect size (ω^2) for each response variable. Any negative ω^2 values are reported as zero. Calculation of the MS used the Type-III SS. *A priori* contrasts for the effects of grazer presence (no-grazer treatments vs. pooled grazer treatments) and grazer richness (3-species treatments vs. pooled 1-species treatments) are included. –: not applicable. Values significant at p = 0.05 are in **bold**

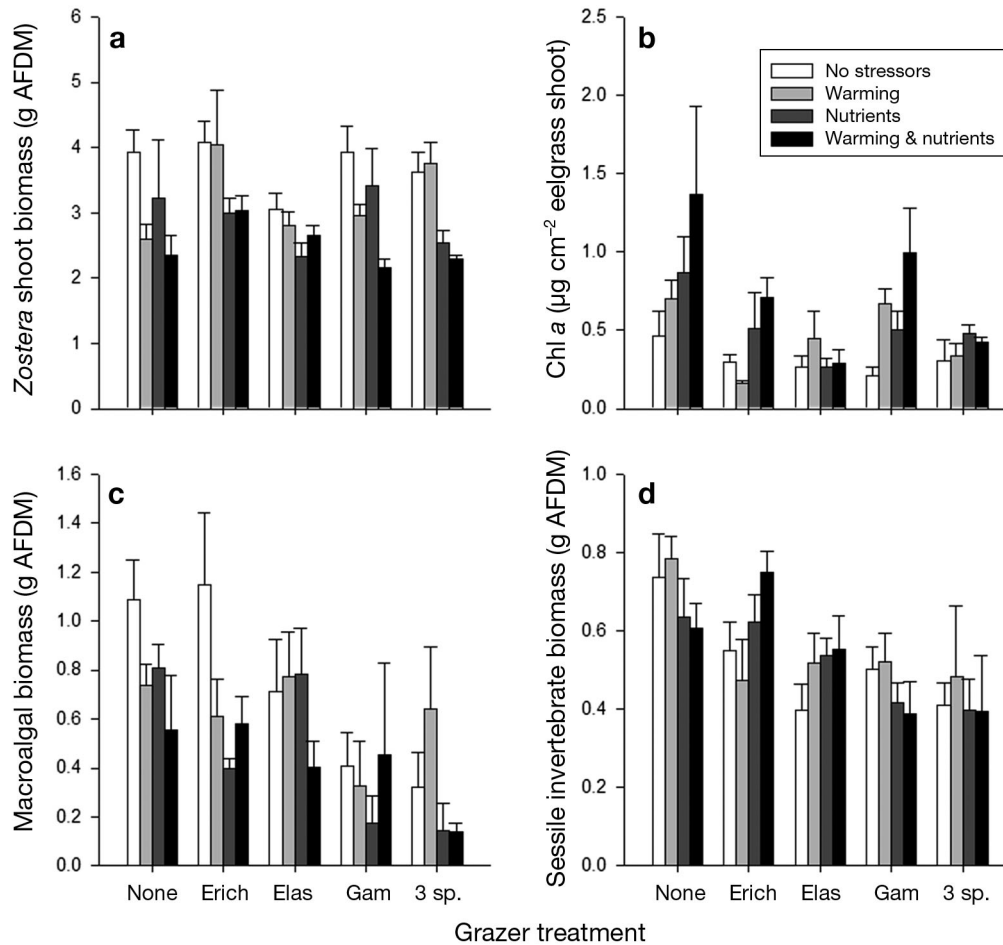| Factor	Grazer biomass			Zostera shoot biomass			Epiphytic algal biomass (log chl *a*)			Macroalgal biomass			Sessile invertebrate biomass		
	MS	p	ω^2	MS	p	ω^2	MS	p	ω^2	MS	p	ω^2	MS	p	ω^2
Grazer species (Sp.)	333741.72	**0.0007**	0.166	1.663	0.0718	0.049	0.0490	**0.0002**	0.177	0.966	**0.0004**	0.168	0.229	**0.0003**	0.172
Sp. presence contrast	–	–	–	0.107	0.7045	–	0.1400	**<0.0001**	–	1.365	**0.0052**	–	0.579	**0.0002**	–
Sp. richness contrast	580662.52	**0.0014**	–	0.064	0.7691	–	0.0020	0.6559	–	0.855	**0.0255**	–	0.135	0.0642	–
Warming (Warm.)	84917.46	0.2050	0.008	4.785	**0.0130**	0.054	0.0530	**0.0116**	0.056	0.113	0.4094	0.000	0.018	0.4988	0.000
Nutrients (Nutr.)	60068.63	0.2855	0.000	14.357	**<0.0001**	0.161	0.0900	**0.0011**	0.098	1.196	**0.0087**	0.061	0.001	0.8484	0.000
Sp. × Warm.	32343.70	0.6015	0.000	1.747	0.0608	0.054	0.0130	0.1564	0.029	0.202	0.3061	0.009	0.004	0.9821	0.000
Sp. × Nutr.	42856.36	0.4834	0.000	0.636	0.4925	0.000	0.0130	0.1699	0.026	0.110	0.6149	0.000	0.085	0.0747	0.048
Warm. × Nutr.	112549.66	0.1454	0.015	0.046	0.8036	0.000	<0.0001	0.9367	0.000	0.027	0.6891	0.000	0.003	0.7936	0.000
Sp. × Warm. × Nutr.	23518.48	0.7150	0.000	0.214	0.8842	0.000	0.0050	0.6767	0.000	0.261	0.1871	0.024	0.019	0.7445	0.000
Error	51737.82			0.740			0.0080			0.165			0.038		

Fig. 2. Final biomass (±1 SE) of (a) *Zostera* shoots (aboveground biomass), (b) epiphytic algae (as chlorophyll *a*), (c) macroalgae (primarily *Cladophora* sp., *Ulva* sp., *Ceramium* sp., *Polysiphonia* sp.), (d) sessile invertebrates (*Botryllus schlosseri*, *Molgula manhattensis*, and *Balanus improvisus*). None: no grazers; Erich: *Erichsonella attenuata* alone; Elas: *Elasmopus levis* alone; Gam: *Gammarus mucronatus* alone; 3 sp.: 3-species assemblage. Statistical analysis in Table 1

treatment (p = 0.0014; Fig. 3a, Table 1), and the composition of the grazer community was dominated by *Gammarus* (Fig. 3b). However, grazer diversity did not decrease stochastic variation in grazer biomass or increase grazer community resistance to stressors (Table 2). Overall, grazer presence and species richness had strong and significant effects in this experiment.

Abiotic stressors also significantly influenced all primary producers. Elevated temperatures significantly decreased *Zostera* biomass (p = 0.013; Fig. 2a, Table 1), but significantly increased epiphytic algal biomass (p = 0.0116; Fig. 2b, Table 1). There was no effect of warming on grazers, macroalgae, or sessile invertebrates. Nutrient enrichment, which elevated nutrients to approximately 5 times ambient levels, had the strongest effect on *Zostera* and significantly reduced its biomass in all enriched treatments (p <

0.0001; Fig. 2a, Table 1). Macroalgal biomass was also lower in the nutrient enriched treatments (p = 0.0087; Fig. 2c, Table 1). Conversely, epiphytic algal biomass was greater in the presence of nutrient enrichment (p = 0.0011; Fig. 2b, Table 1). Thus, both nutrient enrichment and warming increased microalgae but decreased macrophytes. Epiphytic algal biomass was generally lower and more stable in the face of stressors in treatments with multiple grazer species relative to the average of treatments with a single grazer species (p = 0.04519; Fig. 2b, Table 2). This was not the case for macroalgal or *Zostera* biomass, which showed no difference in either stochastic or resistance stability with grazer species richness (Table 2). In this experiment, warming and nutrient enrichment had similarly strong effects on primary producers, but the direction of the effect differed among producers.
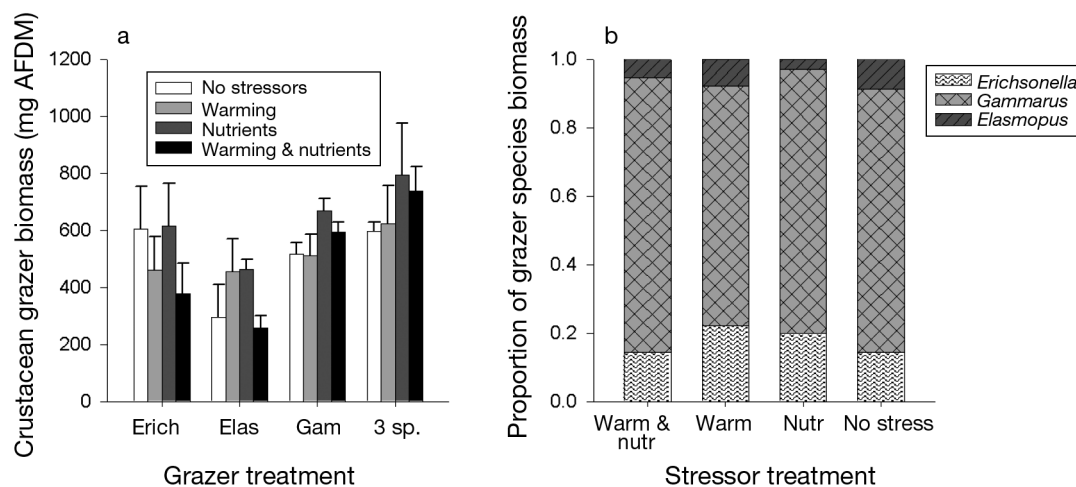
Fig. 3. (a) Final crustacean grazer biomass (±1 SE) for all experimental treatments. Grazer treatment abbreviations as in Fig. 2. Statistical analysis is in Table 1. (b) Final proportion of crustacean grazer species biomass (g AFDM) in the 3-species assemblage treatments. Warm & nutr: warming and nutrient enrichment; Warm: warming; Nutr: nutrient enrichment; No stress: no stressors

Table 2. Results of 2-sample Welch t-tests examining the effects of species richness on stochastic (within treatment) and resistance (among treatment) stability. Tests were performed on deviations from the median (Brown-Forsythe test), and were conducted using R. Values significant at 0.05 are in **bold.** Ab. dev.: absolute deviation; Rel. dev.: relative deviation

Response	Stochastic stability		Resistance stability	
	Ab. dev.	Rel. dev.	Ab. dev.	Rel. dev.
Grazer biomass	0.3432	0.2964	0.9528	0.4565
Zostera shoot biomass	0.1394	0.7743	0.1062	0.0736
Epiphytic algal biomass (log chl *a*)	0.1862	0.4333	0.06579	**0.04519**
Macroalgal biomass	0.9529	0.6675	0.8005	0.5598
Sessile invertebrate biomass	0.0854	0.6297	0.4483	0.9227

Community structure

NMDS analysis showed that experimental communities were significantly structured by all 3 treatments (grazer species, warming, nutrient enrichment). Crustacean grazer species treatments structured the experimental communities not only by their presence, but also by their identity (p = 0.0009; Fig. 4c, Table 3). Species-rich and *Gammarus*-only treatments were similar (Fig. 4c), reflecting the dominance of this species in multi-species grazer assemblages, and contained more red algae (*Ceramium* spp. and *Polysiphonia* spp.) but less green algae (*Enteromorpha* spp. and *Cladophora* spp.) than other treatments (Fig. 4a). Alternatively, no-grazer and *Elasmopus*-only treatments were similar (Fig. 4c), and contained more green algae and sessile invertebrates but less red

algae than other treatments (Fig. 4a). *Erichsonella*-only treatments were intermediate, and contained approximately equal amounts of red and green algae, and moderate biomass of sessile invertebrates (Fig. 4a,c). However, variation among these communities was minimal, with similar variance within all grazer treatments (p = 0.50, betadisper test).

Communities were also structured by nutrient enrichment (p = 0.001; Fig. 4b, Table 3), which reduced biomass of *Zostera* (see Fig. 4a,b). But variation among nutrient-enriched communities was less than variation among non-enriched communities, meaning nutrient enrichment made communities more similar (p = 0.028, betadisper test). Warming also significantly influenced experimental community structure (p = 0.004; Fig. 4d, Table 3), but to a slightly lesser degree than nutrients or grazers, and did not change community similarity (p = 0.61, betadisper test). Warmed communities generally had lower *Zostera* biomass, a pattern similar to nutrient-enriched communities.

Stressors and species identity

The SEM model (Fig. 5a), used to explore complex interactions at the species level, showed that final grazer species abundances were influenced by both initial grazer assemblage composition and by stres-
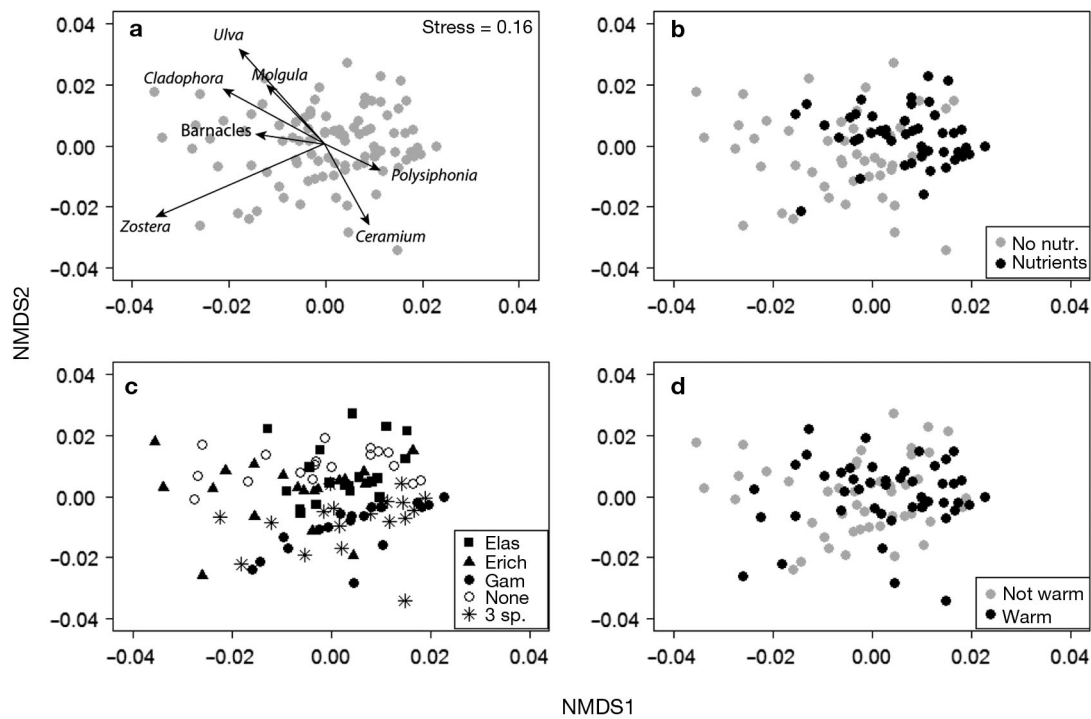
Fig. 4. Non-metric multi-dimensional scaling (NMDS) ordination of all invertebrate and algal species (stress = 0.16); each point represents the community in a single mesocosm. (a) Plot of all mesocosm communities (points), and species with the most influence (vectors), (b) same plot as (a) color-coded by nutrient addition treatment (c) same plot as (a) with shapes representing the crustacean grazer treatments (see Fig. 2 for abbreviations) (d) same plot as (a) color-coded by warming treatment

Table 3. Results from PERMANOVA analysis, testing for differences in community structure with experimental treatment. This analysis used square-root transformed data and a Bray-Curtis dissimilarity matrix, as used for the NMDS ordination in Fig. 4

Factor	df	SS	MS	F	p
Grazer species (Sp.)	4	0.0034	0.0008	8.5577	**0.001**
Warming (Warm.)	1	0.0004	0.0004	4.5097	**0.004**
Nutrients (Nuts.)	1	0.0018	0.0018	18.0309	**0.001**
Sp. × Warm.	4	0.0003	0.0001	0.8806	0.604
Sp. × Nuts.	4	0.0003	0.0001	0.8722	0.588
Warm. × Nuts.	1	0.0000	0.0000	0.1726	0.904
Sp. × Warm. × Nuts.	4	0.0002	0.0001	0.5363	0.901
Error	76	0.0075	0.0001		

sors. Presence of both *Elasmopus* and *Gammarus* reduced *Erichsonella* abundance in 3-species treatments, and together with the negative impacts of warming, explained 70% of the variation in *Erichsonella* abundance (Fig. 5b, Table 4). Our model also suggests that *Gammarus* and *Elasmopus* had direct positive effects on each other and explained about 70% of the variation in abundance of each species (Fig. 5b, Table 4), but this is likely an artifact of the experimental design (see 'Discussion'). *Gammarus* was

the only grazer species whose final abundance had a direct, negative impact on primary producers, specifically macroalgal biomass. As shown in the analysis of community structure, warming and nutrient enrichment increased epiphytic algal biomass ($R^2 = 0.21$; Fig. 5b), while nutrients reduced *Zostera* and macroalgal biomass ($R^2 = 0.29$ and $R^2 = 0.21$ respectively; Fig. 5b).

DISCUSSION

The effects of multiple stressors are frequently assumed to be additive (Crain et al. 2008). However, changes in biodiversity occurring simultaneously with multiple stressors may have additive or synergistic effects, because diversity is important in providing temporal, resistance, and resilience stability in ecosystems (Tilman 1996, 1999, Griffin et al. 2009). Less diverse communities have fewer ecological redundancies, leading to less stable ecosystem properties in the face of stressors (Breitburg et al. 1998, Hooper et al. 2005). Our results demonstrate additive effects among diversity change,
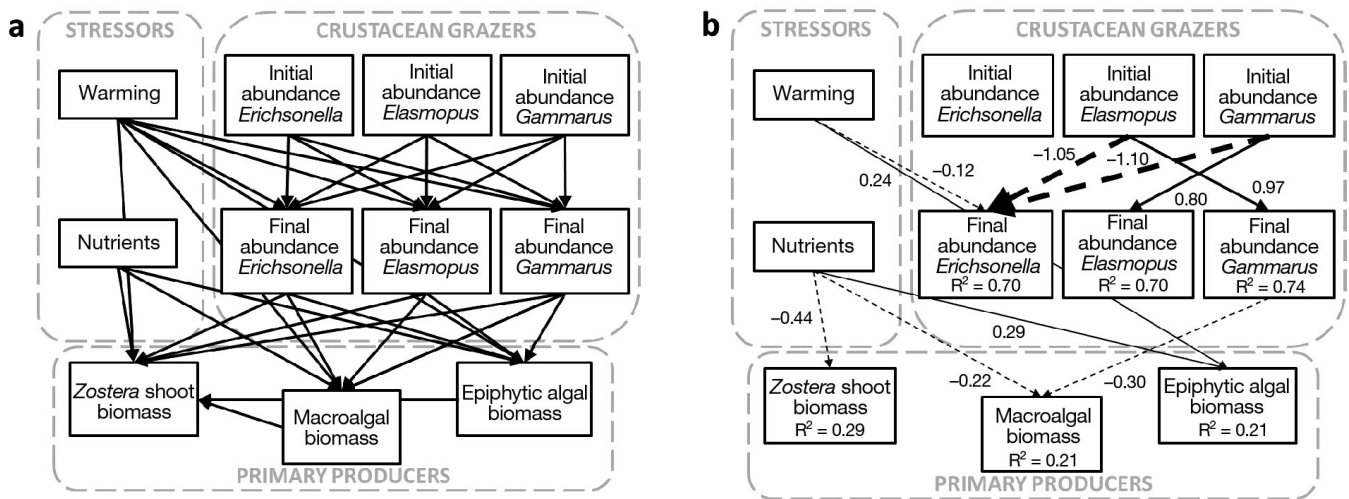
Fig. 5. (a) Full structural equation model (SEM) that was estimated for this experiment, with warming and nutrients as stressors. (b) Graphical results from analysis of model shown in (a). Only paths significant at p = 0.05 are shown; all path coefficients are standardized. Solid and dashed lines represent positive and negative relationships respectively; line thickness indicates the explanatory power of a path. Results in Table 4

Table 4. Results from SEM examining the experimental effects of warming, nutrients, and grazer species richness (see Fig. 5). Each line indicates one path in the model, with the arrow showing the direction of the effect. Significant path coefficients (p = 0.05) are in **bold**. Whole-model chi-square = 22.404, df = 21, p = 0.377. Stand. est.: standardized estimate; unstand. est.: unstandardized estimate; CR: critical ratio

Model path			Stand. est.	Unstand. est.	SE	CR	p
Final *Elasmopus* abundance	←	Warming	−0.022	−24.45	71.07	−0.344	0.731
Final *Gammarus* abundance	←	Warming	−0.032	−36.191	66.047	−0.548	0.584
Final *Erichsonella* abundance	←	Warming	−0.124	−27.007	13.764	−1.962	**0.05**
Final *Elasmopus* abundance	←	Initial *Erichsonella* abundance	−0.043	−4.008	21.306	−0.188	0.851
Final *Gammarus* abundance	←	Initial *Erichsonella* abundance	0.116	10.796	19.8	0.545	0.586
Final *Erichsonella* abundance	←	Initial *Erichsonella* abundance	−0.256	−4.63	3.536	−1.309	0.19
Final *Elasmopus* abundance	←	Initial *Gammarus* abundance	0.797	73.965	21.282	3.476	**<0.0001**
Final *Gammarus* abundance	←	Initial *Gammarus* abundance	0.11	10.101	19.777	0.511	0.61
Final *Erichsonella* abundance	←	Initial *Gammarus* abundance	−1.096	−19.526	3.617	−5.399	**<0.0001**
Final *Elasmopus* abundance	←	Initial *Elasmopus* abundance	−0.039	−3.607	21.259	−0.17	0.865
Final *Gammarus* abundance	←	Initial *Elasmopus* abundance	0.969	87.523	19.757	4.43	**<0.0001**
Final *Erichsonella* abundance	←	Initial *Elasmopus* abundance	−1.053	−18.527	3.615	−5.125	**<0.0001**
Epiphytic algal biomass	←	Final *Elasmopus* abundance	−0.231	0	0	−1.596	0.111
Macroalgal biomass	←	Final *Elasmopus* abundance	0.147	0	0	1.02	0.308
Epiphytic algal biomass	←	Final *Gammarus* abundance	0.067	0	0	0.47	0.638
Macroalgal biomass	←	Final *Gammarus* abundance	−0.304	0	0	−2.148	**0.032**
Epiphytic algal biomass	←	Final *Erichsonella* abundance	−0.059	0	0	−0.43	0.668
Macroalgal biomass	←	Final *Erichsonella* abundance	0.056	0	0	0.407	0.684
Epiphytic algal biomass	←	Nutrients	0.287	0.012	0.004	2.812	**0.005**
Macroalgal biomass	←	Nutrients	−0.223	−0.014	0.006	−2.186	**0.029**
Epiphytic algal biomass	←	Warming	0.237	0.076	0.033	2.274	**0.023**
Macroalgal biomass	←	Warming	−0.016	−0.007	0.048	−0.152	0.879
Zostera biomass	←	Final *Elasmopus* abundance	−0.217	0	0	−1.48	0.139
Zostera biomass	←	Final *Gammarus* abundance	−0.128	0	0	−0.885	0.376
Zostera biomass	←	Final *Erichsonella* abundance	0.177	0.001	0.001	0.993	0.321
Zostera biomass	←	Macroalgal biomass	−0.041	−0.082	0.188	−0.438	0.662
Zostera biomass	←	Epiphytic algal biomass	−0.155	−0.447	0.269	−1.661	0.097
Zostera biomass	←	Nutrients	−0.439	−0.054	0.011	−4.912	**<0.0001**
Zostera biomass	←	Warming	−0.112	−0.104	0.095	−1.088	0.277

warming, and nutrient enrichment stressors in this mesocosm experiment because we did not observe any interactions. The absence of non-additive interactions among environmental stressors and changes in diversity may indicate that these factors act independently in this system. However, we did observe increased resistance stability of epiphytic algae in the face of stressors in higher diversity grazer assemblages, demonstrating that diversity can buffer certain ecosystem processes against stressor impacts. If the additive effects we observed are general, ecological consequences of these stressors will still need to be evaluated for each ecosystem process of interest, but interpretation and prediction of ecosystem changes may be more straightforward.

Grazer presence and changing species richness had larger overall effects than warming or nutrient enrichment on all response variables except *Zostera* biomass in these experimental mesocosm communities (Table 1), in which we attempted to use realistic levels of variation in each stressor. Biomass of grazers in the mesocosms, standardized to biomass of seagrass, was comparable (within 1 SD) to the biomass of grazers in local field collections (Douglass et al. 2010). As found previously, grazers reduced biomass not only of epiphytic microalgae and macroalgae, but also of sessile invertebrates (Fig. 2, Table 1) (Bell 1991, Duffy & Hay 2000, Duffy & Harvilicz 2001, Moksnes et al. 2008). This reduction in sessile invertebrate biomass with grazing is likely due to nonconsumptive removal of newly-settled barnacles and tunicates from the *Zostera* blades and tank surfaces by grazing amphipods and isopods (Osman & Whitlatch 1995, 2004).

Reductions in epiphytes via grazing are often assumed to lead to increases in *Zostera* biomass due to alleviation of light limitation. We did not observe this, likely because light levels in the mesocosms were high enough, despite shading screens, to effectively eliminate the competitive impacts of epiphytes. In addition, the length of this experiment (6 wk) may not have been long enough to observe strong competitive effects of epiphytes on *Zostera*. However, recent field experiments in this same system show that when grazers are removed, epiphytic algal overgrowth can greatly reduce *Zostera* biomass (Whalen et al. in press). Reductions in epiphytes via grazing are likely important for seagrasses over longer time frames than this experiment encompassed, and under lower light conditions when competition may play a greater role.

We found that more diverse grazer assemblages produced higher grazer biomass, as suggested by ecological theory (Carlander 1955, Mulder et al. 2001, Gamfeldt & Hillebrand 2008) and demonstrated by prior research in this system (Duffy et al. 2003). Perhaps this is because the experimental stressors did not impact grazer biomass directly (Table 1), or interact with species richness mechanisms (facilitation, niche complementarity, sampling effect) (Mulder et al. 2001).

Species-rich grazer assemblages also potentially provide resistance stability by maintaining ecosystem processes and properties in the face of stressors. We found that increasing grazer species richness buffered epiphytic algae against stressor impacts by increasing the resistance stability of epiphytic algal biomass (Fig. 2, Table 2) (Griffin et al. 2009), as shown previously with warming and salinity stressors in this system (Blake & Duffy 2010). We saw this stabilizing effect of grazer species richness despite strong stressor effects on primary producers. This may be because interaction strengths between grazers and epiphytic algae remain high in the 3-grazer-species communities due to the dominance of the strong consumer *Gammarus* (Zimmerman et al. 1979, Duffy & Harvilicz 2001, Thébault & Loreau 2005, Douglass et al. 2010). In the future, it will be important to test diverse grazer assemblages of differing compositions in order to isolate diversity and composition effects and determine the generality of these results.

Finally, grazer identity and composition structured the mesocosm communities through differing preferential consumption of macroalgal species (Fig. 4a,c). Green algae were consumed by all grazers, but red algae were not consumed by *Gammarus* (Duffy & Harvilicz 2001). Overall, the dominant effect of grazer presence and species richness confirm that grazing is an important ecosystem process, and that changes in mesograzer species composition and diversity can have impacts on primary producers and epifaunal and epiphytic community composition comparable to or greater than those of environmental stressors.

Warming and nutrient enrichment had larger effects on *Zostera* biomass than variation in grazer assemblages (Fig. 2, Table 1). The experimental addition of nutrients to the water column reduced *Zostera* biomass by 22 % on average (Table 1), as shown in many previous studies (see review by Hughes et al. 2004). This direct, negative, physiological effect of nutrients is likely due to increased nitrogen uptake and resulting carbon limitation (Touchette & Burkholder 2000), but may have co-occurred with shading from epiphytic algal over-growth in no-grazer treatments,

exacerbating the negative effects of nutrient addition. Nutrient additions also had a smaller but significantly negative effect on macroalgal biomass (Figs. 2 & 5, Tables 1 & 4). This may be because grazers consumed almost all standing algal biomass, primarily palatable green algae, in nutrient addition treatments (Burkepile & Hay 2006).

Warming treatments, which increased temperatures approximately 2 to 3°C and doubled the number of days with temperatures above 30°C (Fig. 1), significantly increased epiphytic algal biomass but reduced *Zostera* biomass (Table 1). This 30°C threshold appears to be near the physiological limit of *Zostera*, and likely increases the compensating light requirements above available light levels in Chesapeake Bay (Moore et al. 1997), leading to summer-time *Zostera* die-offs (Moore & Jarvis 2008). Overall, stressors produced conditions favoring higher standing biomass of 'nuisance' microalgae at the expense of macroalgae or eelgrass. If this holds true at an ecosystem scale, the impacts of climate warming in eutrophied systems such as Chesapeake Bay may be especially large.

SEM corroborated many results from the other analyses, but also provided additional insights into the structure of, and effects within, these experimental communities. In this analysis, we modeled grazer species individually in order to examine stressor effects on each species. However, modeling grazer species this way made detection of grazer presence and richness effects unlikely. The positive effects of warming and nutrient enrichment on epiphytic algae, and the negative effects on *Zostera* and macroalgae seen in ANOVA results were again evident. The isopod *Erichsonella* was significantly less abundant under warmed conditions, which follows a trend of reduced biomass in the warmest month (August) in York River, Virginia, field collections over several years (Douglass et al. 2010). The presence of other grazer species also appeared to reduce the abundance of *Erichsonella*, likely because *Erichsonella* is a poor competitor, especially when grazer populations have had sufficient time to near carrying capacity in the mesocosms and competition for algal food resources is at a maximum. The apparent positive effects of the other 2 grazer species, *Elasmopus* and *Gammarus*, on each other in this SEM are likely an artifact of the substitutive design of this experiment: since initial numbers of all species were lowest in the 3-species treatments, similar final densities of species across treatments would appear as higher population growth rates in the 3-species treatment. Finally, *Gammarus* had a strong negative effect on macroalgal biomass through consumption. This is also evident in Fig. 2, but not in the ANOVA results (Table 1) because grazer species were analyzed together. This consumptive effect is significant despite *Gammarus'* documented promotion of red algae, which can lead to overall higher macroalgal biomass (Duffy & Harvilicz 2001). However, *Gammarus* remains a strong consumer of most other macro- and microalgae (Zimmerman et al. 1979). Thus, SEM provides information complementary to the ANOVA results that helps tease apart the effects of individual species and interactions among factors.

The present study shows that grazer presence and diversity can have comparable or larger effects on community structure than warming and nutrient enrichment stressors in an experimental temperate seagrass system. Diversity is important for maintaining critical ecosystem processes, such as grazing, in the face of multiple stressors (Elmqvist et al. 2003, Larsen et al. 2005, Valentine & Duffy 2006, Blake & Duffy 2010), and grazing in seagrass systems has effects similar in magnitude to water column nutrient enrichment (Neckles et al. 1993, Hughes et al. 2004). However, we see that nutrients and warming favor ephemeral and epiphytic algae over eelgrass, potentially leading to a shift in basal primary producer composition as coastal nutrient enrichment remains unabated and the climate continues to warm. These results might suggest a bleak future for temperate estuarine seagrass systems, but also emphasize that these processes depend strongly on stressor identity and the composition of the grazer assemblage. Experimental grazer richness manipulations such as ours are often logistically constrained and cannot capture the range of species richness in the field, and experimental manipulations of warming and nutrient additions can only simulate actual conditions for a short duration. However, recent experiments suggest that grazer control of epiphytic algae is similarly important in field settings (Cook et al. 2011, Whalen et al. in press). Whether these patterns of diversity and stressor effects hold true for other larger experimental and natural systems remains a topic for further investigation. This work highlights the importance of evaluating the impacts of climate warming on individual species as well as the entire system, and reexamining the effects of potential interactions between environmental stressors and the structure of consumer assemblages as climate change continues.

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