Grazer diversity effects on ecosystem functioning in seagrass beds

Abstract
High plant species richness can enhance primary production, animal diversity, and invasion resistance. Yet theory predicts that plant and herbivore diversity, which often covary in nature, should have countervailing effects on ecosystem properties. Supporting this, we show in a seagrass system that increasing grazer diversity reduced both algal biomass and total community diversity, and facilitated dominance of a grazer-resistant invertebrate. In parallel with previous plant results, however, grazer diversity enhanced secondary production, a critical determinant of fish yield. Although sampling explained some diversity effects, only the most diverse grazer assemblage maximized multiple ecosystem properties simultaneously, producing a distinct ecosystem state. Importantly, ecosystem responses at high grazer diversity often differed in magnitude and sign from those predicted from summed impacts of individual species. Thus, complex interactions, often opposing plant diversity effects, arose as emergent consequences of changing consumer diversity, advising caution in extrapolating conclusions from plant diversity experiments to food webs.

Keywords
Biodiversity, ecosystem functioning, grazing, marine, seagrass, secondary productivity.

INTRODUCTION
Accelerating loss and homogenization of biodiversity worldwide have stimulated vigorous research to evaluate how changing diversity influences functional properties of ecosystems. It has long been appreciated that plant species composition influences ecosystem properties. But a growing body of theory and empirical data indicates that several ecosystem properties can also be enhanced by plant species richness through the mechanisms of complementary resource use, and more complete sampling of species functional traits, in more diverse assemblages (reviewed by Loreau et al. 2001). Empirical demonstrations of such plant diversity effects, primarily from ungrazed grasslands, have been cited in support of practical rationales for conserving biodiversity (Chapin et al. 2000; Lawler et al. 2002). Compared with the rich literature on plant diversity effects on ecosystem functioning, however, the potential consequences of declining animal diversity are poorly known, both because of the added complexity of theory for multi-trophic level systems (Holt & Loreau 2002) and because of sparse empirical evidence (Raffaelli et al. 2002; Schmid et al. 2002). Yet understanding the impacts of consumer diversity on ecosystem functioning is critically important because animals often face more severe extinction threats than plants (Chapin et al. 2000; Jackson et al. 2001), and because animals have strong direct and indirect impacts on ecosystem processes in many systems (Naiman 1988; Jones et al. 1994; Jackson et al. 2001). Indeed, consumer impacts on ecosystem processes were comparable with, or greater than, those of changing plant diversity in the very few studies where both factors were examined (Mulder et al. 1999; Naeem et al. 2000; Paine 2002). Thus, there is general recognition that incorporating consumers represents a frontier in understanding linkages between biodiversity and ecosystem function (Duffy 2002; Naeem 2002; Raffaelli et al. 2002).

Theory predicts that increasing herbivore diversity should reduce plant community biomass as the most efficient grazers come to dominate a system, leading to ‘overgrazing’ (Holt & Loreau 2002), which can also depress plant diversity and facilitate invasion of grazing-resistant species (Leibold et al. 1997). Thus, in a system with two trophic levels, changing diversities at plant and herbivore levels are expected to influence ecosystem processes in opposite directions, such that herbivores may counteract ecosystem responses predicted from plant processes alone. In
laboratory experiments, the predicted decline in producer biomass with increasing consumer diversity was indeed observed when a single algal species supported the food web (Naeem & Li 1998), but not with multiple algae because of compensatory growth of resistant species under heavy grazing (Norberg 2000). Countervailing effects of autotroph and heterotroph diversity are also supported by experiments with aquatic microbes (Naeem et al. 2000) and herbivorous insects (Mulder et al. 1999); in both of these studies, addition of heterotrophs erased the positive relationship between diversity and production found in autotroph-only treatments. Whether consumer diversity per se influences ecosystem properties in more natural, open systems (Leibold et al. 1997) remains untested.

Here we report results of a mesocosm experiment that tested effects of grazer diversity on community and ecosystem properties using beds of eelgrass (Zostera marina) as a model system. Eelgrass is the northern hemisphere’s most widespread and abundant marine macrophyte and the base of an economically important ecosystem (Hemminga & Duarte 2000). In addition to eelgrass itself, the major functional groups of benthic primary producers in eelgrass beds include macroalgae and microalgae, which grow both as epiphytes on eelgrass blades and directly on the sediment surface. Invertebrate grazers, primarily small crustaceans and gastropods, play a pivotal organizing role in temperate-sea grass ecosystems worldwide, facilitating seagrass dominance by consuming their competitively superior epiphytic algae (Jernakoff et al. 1996). Whereas seagrasses provide the major physical structure in these beds, the fast-growing, edible algae supply most of the primary production that supports higher trophic levels, including commercially exploited species (Klumpp et al. 1989). If unchecked by grazers, however, epiphytic algae can overgrow and suppress seagrasses, dramatically altering ecosystem structure and functioning. As grazer species differ considerably in their qualitative and quantitative impacts on the plant assemblage (Duffy & Hay 2000; Duffy & Harvilicz 2001; Duffy et al. 2001), grazer diversity may be predicted to have important consequences for eelgrass ecosystem structure and functioning. Our experimental results confirm that grazer species composition, richness, and interactions have pervasive impacts on eelgrass-bed ecosystem properties.

**METHODS**

**Experimental design**

We manipulated grazer species composition and diversity in an array of 45 outdoor tanks, 0.6 m diameter × 0.6 m deep, supplied with flowing water from the adjacent York River estuary (Virginia, USA). The water supply was filtered with 0.15-mm mesh to minimize grazer invasion, but allowed passage of microscopic propagules of algae and sessile invertebrates, allowing their recruitment into the mesocosms. We established nine grazer treatments (n = 5 mesocosms each), including each of six grazer species stocked alone, three species (random combinations), all six species together, and a grazer-free control. The six grazer species all are common in local eelgrass beds, and include the gastropod *Bittium varium*; amphipods *Cymadusa compta*, *Dulichiella appendiculata* and *Ganymorus mucronatus*, and isopods *Erichsonella attenuata* and *Idotea baltica*. Given inherent logistical constraints and trade-offs in interpretive power of alternative experimental designs for detecting biodiversity effects (Allison 1999), we opted to include all six grazer species in monoculture. Such a design allows unambiguous tests of overyielding (Huston 1997; Loreau 1998), and partitioning of diversity effects into complementarity and sampling components (Loreau & Hector 2001). As a trade-off, we could only include three levels of species richness (1,3,6). Thus, we focused on the basic question of whether grazer diversity effects exist in this system, rather than on the form of such relationships, which are likely to differ considerably with context in any case (Cardinale et al. 2000). In August 2001, we planted 75 defaunated eelgrass shoots in clean sand, and added 120 grazers, in each mesocosm. Three- and six-species treatments received 40 and 20 individuals, respectively, of appropriate species in a replacement-series design.

The experimental duration was 6 weeks (~2–3 grazer generations), long enough for grazer relative abundances to adjust to natural levels and for all treatments to reach carrying capacity (Duffy & Harvilicz 2001), thus negating potential artefacts of initial differences in conspecific density (Huston et al. 2000). After 6 weeks, all organisms ≥0.5-mm were harvested, identified, and ash-free dry mass (AFDM) of each taxon was measured. AFDM of each grazer species present was estimated from body-size distributions (following Edgar 1990) in a sorted subsample (usually 1/8) from each mesocosm. Total grazer AFDM, pooled across all species present, was measured directly in the remainder of each sample by combustion. Surficial sediment was sampled with a 45-mm-diameter core, dried, acidified, and sediment organic carbon was measured using standard methods.

**Statistical analysis**

Because preponderance of single-species treatments and exclusion of eight contaminated mesocosms (see Results) resulted in an unbalanced design, we tested grazer diversity effects on a given response variable by resampling the data matrix with replacement 10 000 times and comparing observed statistics to the distribution of resampled values. For each response variable we tested two statistics: the difference between largest and smallest one-species
treatments (i.e., effect of species composition), and slope of
the regression on number of stocked grazer species,
excluding grazer-free controls (i.e., effect of species richness).
Richness tests were one-tailed when they tested a priori
predictions (Table 1). As an additional, conservative estimate
of diversity effects on each variable, we calculated $D_{max}$, the
overyielding criterion, which measures the difference
between the observed total response (yield) in mixture and
the maximal monoculture response, as a proportion of the
maximal monoculture response (Loreau 1998).

We tested effects of grazer species interactions on
ecosystem responses as deviations from additive expecta-
tions based on per-capita grazer effects measured in
monoculture (Norberg 2000). Per capita effect of each
grazer on an ecosystem property was estimated as the
regression coefficient on its log(10) abundance in a multiple
regression including only grazer-free and single-species
treatments. The regression equation was then used to
predict responses in mixed-grazer mesocosms based on
observed final abundances of each grazer species therein.
Deviations of observed from predicted responses in the six-
species treatment were assessed by resampling.

Table 1 Effects of grazer diversity on ecosystem properties. Composition tests variance among individual species in mono-
cultures; richness tests the average change in
response across levels of grazer species
richness; and species interaction tests deviation
of the response in the six-species
treatment from that predicted by summing
estimated impacts of individual species present

<table>
<thead>
<tr>
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<th>$P$-value</th>
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<tbody>
<tr>
<td></td>
<td>Composition</td>
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<tr>
<td>Grazer biomass</td>
<td>0.017</td>
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<tr>
<td>Algal biomass</td>
<td>0.081</td>
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<tr>
<td>Eelgrass change in biomass</td>
<td>0.014</td>
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<tr>
<td>Botryllus biomass</td>
<td>0.962</td>
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<tr>
<td>Benthic diversity</td>
<td>0.087</td>
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<td>Dominance (whole)</td>
<td>0.007</td>
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<tr>
<td>Dominance (fouling)</td>
<td>0.007</td>
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<tr>
<td>Sediment organic C</td>
<td>0.242</td>
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</tbody>
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$P$-values are from resampling tests (see text). Tests of composition and species interaction
effects are two-tailed; richness tests are one-tailed, reflecting a priori expectations, except for
sediment organic C (two-tailed). $D_{max}$ cannot meaningfully be calculated for eelgrass mass
change or dominance (fouling) as effects of different treatments differed in sign.

RESULTS

We excluded eight of 45 mesocosms from analysis because of
contamination because final ash-free dry biomass of invading
unstocked grazer species exceeded 500 mg. Despite such
invasions, treatments maintained a gradient in grazer diversity:
the Shannon–Wiener index, based on grazer biomass at the
end of the experiment, averaged (±1 SE) 0.57 ± 0.08,
1.12 ± 0.10, and 1.35 ± 0.09 for one, three, and six-species
treatments, respectively ($P < 0.0001$, resampling test).

Ecosystem properties at both consumer and prey
levels responded strongly to grazer biodiversity. Paralleling
previous terrestrial results at the plant level, diversity
significantly enhanced (secondary) production at the grazer
level. Populations of all but one grazer species grew at
comparable or faster rates in mixture than in monoculture
(Fig. 1). A regression of estimated biomass production per
stocked individual in mixture vs. in monoculture had a
slope of 2.2 (Fig. 1a), suggesting enhanced average
production of individual grazer species at high diversity.
This slope was not significantly different than 1.0,
although sample size (6) and, thus, power of the test
was low. However, total grazer biomass, which was
measured directly, was significantly enhanced at high
diversity ($P = 0.008$, Fig. 2a), averaging 56% higher than
in the average monoculture. The positive, albeit small,
value of $D_{max}$ also supports overyielding of grazer biomass
at high diversity (Table 1).

High grazer secondary production reflected more com-
plete use of the algal resource in multi-species assemblages.
Intense consumption at high grazer diversity essentially
eliminated algae, reducing algal biomass ($P = 0.017$) by 87%
compared with the average of single-species treatments and
by 96% relative to grazer-free controls (Fig. 2b). In contrast,
change in eelgrass biomass was unaffected by grazer
diversity ($P = 0.139$), but strongly affected by grazer species
composition ($P = 0.014$, Fig. 2c). Overall diversity of the
eelgrass community was also significantly reduced at high
grazier species richness ($P = 0.035$, Fig. 2c); the number of
taxa recruiting naturally to six-grazer mesocosms was 14%
lower than in the average single-grazer treatment, and 38%
lower than in grazer-free controls, as diverse grazers
suppressed establishment of edible algae, which dominated
ungrazed mesocosms.

In contrast to the negative effect of grazers on algal
establishment and total community diversity, grazer species
richness strongly facilitated dominance by the tunicate
Botryllus schlosseri ($P = 0.003$), a sessile invertebrate that is resistant to several consumers (e.g. Teo & Ryland 1994). $B. \text{schlosseri}$ reached nearly 3X higher biomass in the six-grazer treatment, where it was second in abundance only to eelgrass, than in the average monoculture (Fig. 2d), resulting in a strongly positive value of the overyielding criterion $D_{\text{max}}$ (Table 1). In summary, increasing grazer diversity directionally altered eelgrass ecosystem composition, suppressing algae and shifting ecosystem biomass dominance from edible autotrophs towards resistant heterotrophs such as tunicates and sponges ($P = 0.032$, Fig. 3a). Indeed, excluding eelgrass, which serves primarily as habitat for these grazers, the remaining fouling assemblage shifted from 76% algae in the absence of grazers to 84% sessile invertebrates at high grazer diversity (Fig. 3b), a significant ($P = 0.013$) effect of grazer richness.

In addition to their direct effects on the eelgrass community, grazers indirectly changed organic carbon accumulation in underlying sediments (Fig. 2f), a fundamental controller of biogeochemical processes, nutrient remineralization, and benthic food availability in marine ecosystems, as in soils. Whereas grazing reduced sediment carbon overall compared with grazer-free controls, increasing grazer diversity enhanced sediment carbon accumulation above the average of single-species treatments ($P = 0.032$). This sediment C was apparently stored, in part, in benthic microalgal biomass as sediment chl $a$ showed a similar but non-significant increase with grazer diversity (not shown), probably reflecting greater light penetration to sediments under heavy grazing.

Grazer diversity effects on ecosystem properties were clearly related in part to grazing intensity, which was highest in the multi-species treatments (Fig. 4). Nevertheless, grazing intensity alone explained a relatively small proportion of the variance in ecosystem responses, and both Botryllus and sediment carbon were greater at highest diversity than expected from grazing intensity alone (i.e. residuals in the six-species treatment were positive, Fig. 4), suggesting that indirect interactions also influenced these responses. Benthic diversity was unimodally related to grazing intensity (Fig. 4), presumably reflecting the common phenomenon of maximal diversity under intermediate disturbance (in this case, grazing).

**DISCUSSION**

Experimental studies in grasslands (reviewed in Tilman et al. 2002), aquatic algal microcosms (Naeem et al. 2000), salt marshes (Zedler et al. 2001), and freshwater wetlands (Engelhardt & Ritchie 2001) have documented higher primary production in high-diversity plant assemblages. More diverse sessile invertebrate assemblages similarly show more complete resource (space) use (Stachowicz et al. 1999). Our results provide the first experimental evidence of a parallel phenomenon at the level of mobile consumers, that is, enhanced secondary production and efficiency of resource use in more diverse grazer assemblages (see Naeem et al. 2000 for a similar phenomenon in decomposers). These findings from organisms as disparate as terrestrial plants, sessile invertebrates, marine herbivores, and bacteria suggest that enhancement of resource use and

![Figure 1](image-url)
Production at high diversity is a robust ecological trend with considerable generality across taxa and ecosystems. Enhanced secondary production at high grazer diversity is also of potential practical importance. Small crustaceans such as the grazers we studied dominate diets of shallow-water fishes, and crustacean production is the best single predictor of fish production in vegetated marine ecosystems (Edgar & Shaw 1995; Taylor 1998). Thus, our results suggest that high biodiversity at this intermediate trophic level may enhance trophic transfer up the food chain, and potentially fishery yields.

Our finding that grazer diversity influences several ecosystem properties may appear to contradict a previous experiment in this system, which used only three of the grazer species and found no effect of grazer richness on any response variable (Duffy et al. 2001). It seems clear, however, that the richness effects we found (Fig. 2) are driven largely by the highest diversity treatment, with six species. The contrast between these two studies underscores the point that diversity effects may only become apparent when examining a relatively wide range in species richness. They also present an interesting contrast with the commonly suggested saturation of ecosystem functional properties at low levels of diversity (Schwartz et al. 2000). In this system, loss of a few species from current high levels of biodiversity may significantly influence ecosystem properties, a point with implications for conservation.

Mechanistically, enhanced secondary production at high grazer diversity could be mediated either by increased efficiency of grazing (more complete resource utilization), by stimulating increased primary production, or by some combination of these two mechanisms. The first possibility is consistent with documented differences among these grazers in the propensity to consume macroalgae, microalgae, and detritus (Duffy & Hay 2000; Duffy & Harvilicz 2001; Duffy et al. 2001), and in microhabitat use. For example, the clinging isopod *Erichsonella* rarely ventured away from eelgrass blades in our experiment, whereas the highly mobile amphipod *Gammarus* was frequently observed on the sediment surface, probably grazing on benthic microalgae and detritus there. The second possible mechanism, enhancement of primary production by diverse grazer assemblages, is consistent with the compensatory responses to grazing documented in other marine algae (Carpenter 1986) as well as in grasslands (McNaughton 1985). Enhancement by grazers of specific primary production, together with detritus feeding by some species, might also explain how secondary production could be maximal at high grazer diversity despite low standing biomass of algae.
The negative effects of increasing grazer diversity on algal biomass and total community diversity in our experiment are contrary to previously documented effects of increasing plant diversity. In field studies, higher plant diversity was associated with higher plant biomass (Terrados et al. 1998) and higher diversity of associated animals (Parker et al. 2001) in seagrass beds, as in terrestrial grasslands (Siemann et al. 1998; Tilman et al. 2002). Thus, our results support theoretical predictions (Holt & Loreau 2002) that changing diversity at adjacent trophic levels has countervailing impacts on ecosystem properties. We also found that increasing grazer diversity produced a marked shift in prey species composition, from algal to sessile invertebrate dominance. This shift between resistant and edible taxa with changing grazing pressure has parallels in coral reefs (Hughes 1994), lake plankton (Leibold et al. 1997), and terrestrial vegetation (Naiman 1988), and suggests that well-known ecosystem phase shifts in these systems may be mediated partially by declining consumer diversity.

Within trophic levels, biodiversity can affect ecosystem processes via complementarity (including facilitation), whereby multi-species assemblages use a greater fraction of available resources, and/or by sampling, i.e. higher probability that diverse assemblages contain a species of strong effect. Both mechanisms are important in experimental plant assemblages (Loreau & Hector 2001; Loreau et al. 2001; Tilman et al. 2002). Analogous processes can explain diversity effects we observed at the grazer level. Enhancement of grazer biomass production and of Botryllus...
dominance at high grazer richness (Fig. 2) evidently involve complementarity, as indicated by positive values of the conservative (Loreau 1998) overyielding criterion, $D_{\text{max}}$ (Table 1). For other ecosystem properties, responses at high grazer diversity did not exceed the most extreme monoculture response (Fig. 2), indicating that grazer richness effects resulted partly from sampling. Although the ecological relevance of the sampling effect has been questioned (Huston 1997; Wardle 1997; Huston et al. 2000), we consider it an important biodiversity effect for three reasons. First, in our system sampling simulates realistic compositional variance as most monocultures and mixtures approximate field assemblages observed at certain sites or seasons. Second, sampling and complementarity can jointly influence ecosystem function (Loreau & Hector 2001), an interaction obscured by the stringent overyielding criterion $D_{\text{max}}$. Specifically, grazer biomass production exceeded monoculture expectations for three of six species and was below expectation for only one (Fig. 1a). Summing these production differentials across grazer species, and using Loreau & Hector’s (2001) equation, 74% of the excess grazer production at high diversity was attributable to complementarity, whereas 26% was because of sampling.

The third and most general argument for sampling as an important biodiversity effect is that, even when sampling can explain individual ecosystem responses, only more diverse assemblages maximized multiple ecosystem responses simultaneously. This is because different grazers maximized different properties. For example, Dulichiella, Cymadusa and Erichsonella most strongly influenced grazer biomass, grazing impact, and sediment organic carbon, respectively, yet the six-species mixture achieved similarly extreme values for each of these response variables (Fig. 2) and thus resulted in an ecosystem state noticeably different than in any of the grazer monocultures. We suggest the term multivariate dominance effect for this phenomenon, in which diverse assemblages produce extreme values of multiple ecosystem properties simultaneously. To our knowledge, it has not been recognized before, presumably because much previous research has focused narrowly on the single response variable of plant biomass production. Yet it is likely to be a common diversity effect in the more general case where multiple ecosystem responses are of interest. A similar point was made by Petchey & Gaston (2002), who showed that as more variables were incorporated into their multivariate index of functional diversity, species were recognized as increasingly unique, and the relationship between species richness and (multivariate) functional diversity became more linear.

In multi-level food webs, emergent effects of biodiversity can also arise from interactions among trophic levels (e.g. Naeem & Li 1998; Downing & Leibold 2002). Such interactions often involve complex, indirect impacts (Menge 1995; Naeem et al. 2000), which can be detected as deviations from expected additive effects of consumer species present (Norberg 2000). These species-combination effects significantly influenced several ecosystem properties in our experiment (Fig. 2, Table 1), suggesting pervasive complex interactions even in this relatively simple system. The most dramatic of these involved Botryllus biomass and sediment organic carbon. For both of these variables, responses at high grazer diversity differed not only in magnitude, but also in sign from those predicted from single-species responses (Fig. 2). A likely explanation for these deviations, and the lower than predicted grazer biomass in mixture (Fig. 2a), is interference competition among grazers, and perhaps intra-guild predation. Similarly, the absence of a consistent grazer diversity effect on eelgrass biomass presumably reflects the shift from competitive suppression of eelgrass by dense algae, to facilitation by epiphyte grazing, to suppression by direct grazing of eelgrass, as grazer diversity increased.

In summary, as grazer diversity and impact increased, the eelgrass fouling community shifted from high biomass, high diversity, and dominance by edible algae to low biomass, low diversity, and dominance by a resistant invertebrate. These grazer-mediated changes are opposite in direction to those associated with increasing plant diversity in seagrass beds (Terrados et al. 1998; Parker et al. 2001) and grasslands (Siemann et al. 1998; Tilman et al. 2002), suggesting that effects of covarying plant and herbivore diversity on ecosystem functioning may counteract one another, on average, as diversity is lost in nature. Despite the near elimination of algae at high grazer diversity in our experiment, however, grazer overyielding analogous to that demonstrated in diverse plant assemblages resulted in higher secondary production of the small crustaceans that sustain many marine fish populations. This multifaceted shift in structure and function of the eelgrass ecosystem depended directly on a diverse grazer assemblage, via a multivariate dominance effect, as no single grazer species could produce all of the component changes in the system. The trophic processes involved are common in a variety of ecosystems, emphasizing that successfully predicting ecosystem-level consequences of eroding biodiversity, particularly in aquatic systems with strong top-down forcing (Cyr & Pace 1993; Shurin et al. 2002), will require close attention to food-web interactions.

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