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Decoupling of nutrient and grazer impacts on a benthic estuarine diatom assemblage

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Abstract

Strong interactions between top-down (consumptive) and bottom-up (resource supply) trophic factors occur in many aquatic communities, but these forces can act independently in some microphytobenthic communities. Within benthic estuarine diatom assemblages, the dynamics of these interactions and how they vary with abiotic environmental conditions are not well understood. We conducted a field experiment at two sites with varying habitat characteristics to investigate the interactive effects of grazers and nutrients on benthic estuarine diatoms. We crossed snail (*Cerithidea californica*) and nutrient (nitrogen and phosphorus) addition treatments in enclosures on a restored tidal sandflat and a reference tidal mudflat in Mugu Lagoon, southern California. We repeated the study in summer 2000 and spring 2001 to assess temporal variation in the interactions. Snails caused a large decrease in diatom relative abundance and biomass (estimated as surface area); nutrients increased diatom abundance but did not alter diatom biomass. Snails and nutrients both reduced average diatom length, although the nutrient effect was weaker and temporally variable, occurring in the reference mudflat in the spring. There were few interactions between snail and nutrient addition treatments, suggesting that links between top-down and bottom-up forces on the diatom community were weak. There were no consistent differences in diatom assemblage characteristics between the two study sites, despite marked differences in sediment grain size and other abiotic characteristics between the sites. The strong diatom response to herbivores and weaker responses to enrichment differed from the previous studies where cyanobacteria increased in response to nutrient enrichment, further dissolving the “black box” perception of microphytobenthic communities.

Keywords

Cerithidea californica; benthic microalgae; diatoms; restoration; eutrophication; herbivores

Regional index terms

USA; California; Mugu Lagoon

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1. Introduction

Aquatic habitats often exhibit strong interactions between top-down (consumptive) and bottom-up (resource supply) forces (McQueen et al., 1986; Worm et al., 2002). Interactions between grazers and nutrients are particularly common in aquatic systems (Burkpile and Hay, 2006). In some cases, consumers selectively graze enriched plant tissue (Boyer et al., 2004) and in others, grazer impacts on producers are reduced when nutrients are added (Liess and Kahlert, 2007). Studies that have evaluated interactive top-down and bottom-up effects on whole benthic microalgal communities (Lever and Valiela, 2005) and diatom communities in particular (Hagerthey et al., 2002) reveal that estuarine microphytobenthos, including diatoms, do not always follow that paradigm. Previous works on interactive effects within diatom communities suggest that diatom assemblages are independently controlled by either top-down (Neckles et al., 1993; Pan and Lowe, 1994; Hagerthey et al., 2002) or bottom-up (Elser et al., 2005) forces. However, most of the previous work has examined epiphytic or freshwater diatom assemblages, and very little is known about the interactive roles of grazers and nutrients on benthic estuarine diatom assemblages.

Benthic microalgal communities make substantial contributions to many shallow estuaries (Zedler, 1980; Sullivan and Currin, 2000). Microphytobenthos produce oxygen (Dalsgaard, 2003), fix large amounts of carbon (Gerbersdorf et al., 2005), drive benthic–pelagic nutrient exchanges (Ferguson et al., 2004), and provide trophic support (Sullivan and Moncreiff, 1990). Microphytobenthic communities are diverse at the species and group levels (Sullivan, 1978; Cahoon, 1999), but are often treated as a “black box,” where benthic microalgae are considered as a whole and the characteristics of individual key groups such as diatoms, green algae, and cyanobacteria are not evaluated individually. In fact, different taxonomic groups may have considerably different roles in nutrient cycling (Pinckney et al., 1995) and trophic support (Armitage and Fong, 2004b), but there are still substantial knowledge gaps in this expanding field, as the roles of individual algal groups are only partially understood.

In many estuaries, diatoms comprise the dominant component of the benthic microalgal community (Sullivan and Moncreiff, 1988; Stolz, 1990; Cariou-Le Gall and Blanchard, 1995; Brotas and Plante-Cuny, 1998; Buffan-Dubau and Carman, 2000; Armitage and Fong, 2004b). Diatoms are critical for stabilizing substrate and reducing erosion through the production of extracellular polymeric substances (Austen et al., 1999). Diatoms have important roles in increasing oxygen production and nutrient fluxes at the sediment surface (Sundbäck et al., 1991; Bartoli et al., 2003). Diatom assemblages are also an essential food resource for a variety of invertebrate (Whitlatch and Obrebski, 1980; Buffan-Dubau et al., 1996; Byers, 2000; Goldfinch and Carman, 2000) and vertebrate (James-Pirri et al., 2001) species.

Grazers can strongly influence diatom community structure. Selective grazing on diatoms can decrease diatom biomass relative to other microalgal groups (Buffan-Dubau et al., 1996; Goldfinch and Carman, 2000). Within the diatom assemblage, some benthic grazers select specific diatom species or smaller size classes (Whitlatch and Obrebski, 1980; Cuker, 1983;

Byers, 2000). In other cases, benthic grazers are generalist consumers, reducing diatom species in proportion to their abundance in the community (Hagerthey et al., 2002).

Diatom communities are also influenced by many bottom-up processes, including the supply of major limiting nutrients such as nitrogen and phosphorus. On organically rich, fine-grained sediments, diatoms do not typically exhibit nutrient limitation (Admiraal et al., 1982; Underwood and Kromkamp, 1999), but on sandy sediments or in anthropogenically impacted coastal estuaries, nutrient input can alter diatom communities. Elevated nutrient input, particularly nitrogen and silica, has been shown to increase benthic diatom biomass in freshwater and marine habitats (Carrick and Lowe, 1988; Peterson et al., 1993; Agatz et al., 1999; Camacho and de Wit, 2003). Nutrient enrichment can sometimes decrease diatom abundance relative to other microalgal groups such as cyanobacteria, but diatoms typically remain the dominant taxonomic group (Armitage and Fong, 2004b; Liboriussen and Jeppesen, 2006). Enrichment may also increase diatom division rates, resulting in smaller individuals but little change in species composition. Changes in diatom species composition and sizes can alter trophic support for benthic grazers (Whitlatch and Obrebski, 1980; Byers, 2000).

Microalgal communities are often markedly different between natural and restored marshes (Piehler et al., 1998; Janousek et al., 2007). Often, sandy dredge spoil is used for coastal wetland restoration projects (Lindau and Hossner, 1981; Currin et al., 1996; Boyer and Zedler, 1998; Campbell et al., 2002; Armitage and Fong, 2004a), despite generally higher levels of ecological functions such as increased primary production, nutrient retention, and nutrient cycling, as well as lower nutrient limitation on muddy sediments relative to sandy soils (Lindau and Hossner, 1981; Admiraal et al., 1982; Sundbäck et al., 1991; Boyer and Zedler, 1998; Underwood and Kromkamp, 1999; Watermann et al., 1999). Given that both diatom (Montagna et al., 1983; Zheng et al., 2004) and grazer (Armitage and Fong, 2004a) biomass can differ between restored and reference marshes, it is likely that interactions between grazers and nutrients on benthic diatom communities differ from those between habitat types. Manipulative field studies are necessary to examine the development of these interactive relationships within grazer–diatom assemblages in restored tidal marshes.

Our objective was to investigate the interactive effects of nutrients and grazers on benthic estuarine diatom assemblages. Because top-down and bottom-up trophic interactions are closely linked to abiotic environmental conditions (Armitage and Fong, 2006) and may vary among seasons (Armitage and Fong, 2004b), we conducted our studies at two field sites at two different times of year. Based on the previous work in the system that revealed strong impacts of grazers and nutrient enrichment on the benthic microalgal community as a whole (Armitage and Fong, 2004b), we hypothesized that nutrient enrichment and grazing pressure would alter the biomass and size frequency distribution of an estuarine benthic diatom assemblage. Based on the previous work on epiphytic and freshwater diatoms (Neckles et al., 1993; Pan and Lowe, 1994; Hagerthey et al., 2002; Elser et al., 2005), we also expected that nutrient and grazer impacts would independently affect the diatom community.

2. Methods

2.1. Study site

We performed our studies on two tidal flats in Mugu Lagoon, southern California, USA (34.11°N, 119.12°W). One site had been open lagoon and salt marsh until about 1950, when it was filled to upland elevation with offshore dredge spoil. The site was subsequently dredged back to intertidal elevation in the fall of 1997 to create an area of mudflat and tidal creeks with graded banks around the perimeter. Sediments were about 92% sand, so this site was termed the “restored sandflat.” Immediately adjacent to the restored area was a reference marsh that has not been dredged or filled in known history. The reference marsh featured dense vegetation dominated by pickleweed *Salicornia virginica*, sinuous tidal creeks, and smaller mudflat areas. Sediments in this site were about 71% sand, so this site was designated as the “reference mudflat.” A series of narrow roads encircled the entire study area. Tidal flow entered the sites through multiple culverts underneath the roads.

2.2. Experimental design

To evaluate the effects of site, nutrient supply, and herbivory on diatom communities, we conducted three-factor experiments varying site, grazer presence, and nutrient supply in summer 2000 and spring 2001. In both the reference mudflat and the restored sandflat, we installed twenty 0.5 m × 0.5 m open-topped enclosures constructed from fiberglass window screening (1.6 mm mesh). Walls of the enclosures extended 30 cm above and 2 cm below the sediment to minimize animal immigration and emigration. Weekly maintenance removed all algal growth and other fouling organisms. Earlier studies determined that hydrology alterations and shading artifacts from this cage design are minimal (Armitage and Fong, 2006). All benthic macrofauna (e.g., California horn snails *Cerithidea californica*) present in the enclosures following installation were removed.

The 20 plots in each site were randomly assigned to one of the four treatments: (1) snails removed & ambient nutrients; (2) snails removed & nutrients added; (3) snails added & ambient nutrients; and (4) snails added & nutrients added. We added 10 g of slow release Osmocote® fertilizer (18% N, 6% K, and 12% P) to all enriched plots in a 2-mm mesh bag (4 × 4 cm²) that was secured to the center of the plot with a small bamboo stake; empty bags were placed in ambient nutrient plots. Fertilizer was replenished after 6 weeks. Osmocote® is formulated to release nutrients for 4 months, but to ensure continuous nutrient enrichment, we topically applied 2 g of granulated urea fertilizer (46% N) to the enriched plots every 2 weeks. Loading rates were approximately 0.088 g N day⁻¹ plot⁻¹ and 0.024 g P day⁻¹ plot⁻¹, and were established based on fertilization protocols at other regional salt marsh restoration projects (Boyer and Zedler, 1999). Earlier analyses of this nutrient addition protocol revealed that sediment nitrogen content did not increase in enriched treatments, but substantial microalgal biomass responses demonstrated that our enrichment technique was effective in altering the benthic microalgal community (Armitage and Fong, 2004b). After a 4-week acclimation period, we added 350–380 snails (*Cerithidea californica*) to each enclosure in densities and size frequency distributions that approximated natural populations at the same site (Armitage and Fong, 2004a). Earlier studies determined that the snails did not display any avoidance behavior in the presence of nutrients (Armitage and Fong, 2004b).

The snails remained in the cages for an experimental period of 8 weeks. The experiment was performed in summer 2000 and spring 2001; between iterations, the enclosures were removed. For the spring 2001 iteration, enclosures were installed at different locations within the same tidal flats as in summer 2000. In summer 2000, three plots were lost during the course of the experiment due to erosion and disturbance: one each from enriched without snails in the reference mudflat, enriched with snails in the reference mudflat, and ambient nutrients with snails in the restored sandflat. In spring 2001, one enriched with snails plot in the reference mudflat was lost during the experiment.

To quantify diatom abundance and size structure in surficial sediments, sediment cores (1.5 cm diameter and 3 mm deep) were collected at the end of each experimental time period. Previous work indicates that most benthic microalgal biomass is confined to the top 0.5 mm in muddy sediments but can extend deeper than 3 cm in sandy sediments (Cartaxana et al., 2006). Benthic microalgal migration, however, occurs primarily within the upper 3 mm of sediment (Pinckney et al., 1994). Therefore, we limited our cores to a depth of 3 mm in both sediment types in order to capture the portion of the diatom assemblage that is most likely to be accessible to epibenthic gastropod grazers at any given time of day. Three cores were collected and combined from each plot for a total sampled surface area of 5.3 cm². The core locations were equidistant from the mesh bag in the center of the plot and the cage walls (~15 cm from each). Cores were immediately preserved in 6% Lugol's solution in filtered seawater, and the samples were stored in the dark until analysis.

Methods for determining diatom abundance and size structure were adapted from Byers (2000). Slides were prepared by vortexing the sample vial for 1 min and immediately transferring 300 µl of the sample into a micro-centrifuge tube containing 1 ml of double-distilled H₂O. The subsample was vortexed again for 30 s, and 200 µl was immediately transferred to a microscope slide. The slides were dried uncovered in the dark for 12 h. Immediately prior to analysis, two drops of Lugol's solution were added to the slide to stain chlorophyll *a*, and a cover slip was added. Using a microscope at 200× magnification, cells were enumerated along a 10 µm × 2000 µm transect with a haphazardly located starting point and oriented through the center of the slide. Transect length and diatom length and width were quantified with a calibrated eyepiece reticle. Diatoms were not identified to species, but virtually all of the diatoms we observed in all treatments were pennate in shape. We calculated one-sided diatom surface area using a symmetrical ellipsoid formula. One-sided surface areas were summed in each plot to estimate relative diatom biomass. Based on previous work that determined *Cerithidea* mainly consumes diatoms less than 25 µm in length (Whitlatch and Obrebski, 1980), we calculated the number of diatoms in each plot in 25 µm length size classes.

2.3. Data analysis

The variances of all data were tested for homoscedasticity using the $F_{\max}$ test and, if necessary, were log transformed to conform to the assumptions of ANOVA. Relative diatom abundance, total diatom surface area per plot, and average diatom length per plot were analyzed with three-way ANOVA, where the independent factors were site (reference mudflat vs. restored sandflat), snail addition, and nutrient addition. These analyses were

performed separately for the summer 2000 and spring 2001 iterations. We report partial η^2 values to assess the effect size of each factor.

3. Results

Diatom abundance was significantly higher (29% increase) in plots with nutrients added than in ambient plots in summer 2000 (Table 1a, Fig. 1a). A significant snail $\times$ site interaction in summer 2000 was driven by a stronger negative snail effect on diatom abundance in the restored sandflat (59% decrease in diatom abundance) relative to the reference mudflat (31% decrease). In spring 2001, diatom abundance was 65% higher in plots with nutrients than in ambient plots and was decreased by 42% when snails were added, with no interactions between factors (Table 1b, Fig. 1b). Partial η^2 scores suggest that snail effects were stronger than nutrient effects in summer 2000 but snails and nutrient impacts were similar in spring 2001 (Table 1). The strengths of site effects were small in both the experimental iterations.

Total one-sided diatom surface area per plot was significantly lower in plots with snails (70% decrease in summer 2000 and 66% decrease in spring 2001) regardless of site, enrichment treatment, or season (Table 2, Fig. 2). Nutrient addition and site did not affect total diatom surface area, and there were no interactions between factors. Partial η^2 scores suggest that snail effects were much stronger than nutrient or site effects in summer 2000 and spring 2001 (Table 2).

Diatom length was significantly less (24% shorter) in the restored area than in the reference mudflat in summer 2000 (Table 3a, Fig. 3a). A marginally significant interaction among site, snails, and nutrients was largely driven by the occurrence of numerous long individuals ($>50 \mu\text{m}$) in control plots (no snails and ambient nutrients) in the reference mudflat (Fig. 4). In spring 2001, diatom length was significantly less (52% shorter) in plots with snails than in plots without (Table 3b, Fig. 3b). A significant interaction between site and nutrients was driven by the occurrence of longer individuals in ambient nutrient than in enriched plots, particularly in the reference mudflat (Fig. 4). Partial η^2 scores suggest that snail and site effects were much stronger than nutrient effects in summer 2000 and that snail effects were stronger than site and nutrient effects in spring 2001.

4. Discussion

The benthic diatom community was consistently and strongly impacted by the top-down effects of herbivores, while the bottom-up effects of nutrient enrichment and site were weaker and more variable. Each of these factors was largely independent of the others. The paradigm of strong interactions between top-down and bottom-up forces in aquatic habitats (McQueen et al., 1986) frequently fails in diatom communities, where previous studies suggest that assemblages are independently controlled by grazers (Neckles et al., 1993; Pan and Lowe, 1994; Hagerthey et al., 2002) or nutrients (Elser et al., 2005), though most of these studies examined epiphytic or freshwater diatom assemblages. Few studies have focused specifically on diatom communities within marine tidal mudflats, but manipulative studies that investigate whole microphytobenthic assemblages suggest similar decoupling of grazer and nutrient effects (Lever and Valiela, 2005). We observed strong effects of grazers,

and to a lesser degree, nutrients and site on diatom abundance and biomass, but in agreement with Lever and Valiela's (2005) work on whole microphytobenthic communities, top-down and bottom-up impacts often acted separately. Since our snail and nutrient treatments were at the upper end of potential natural ranges (Boyer and Zedler, 1999; Armitage and Fong, 2004a), our study design maximized the potential to observe grazer–nutrient interactions, and we are thus confident that top-down and bottom-up impacts acted independently on this diatom assemblage.

We detected large decreases in diatom abundance and biomass in the presence of grazers, supporting other studies that demonstrated strong grazer impacts on diatom biomass (Hagerthey et al., 2002; Sahan et al., 2007). We also detected decreases in average diatom length in snail addition treatments, suggesting that snails are capable of consuming larger (>25 μm long) diatoms. Previous gut content analyses have suggested that *Cerithidea californica* exhibits size-dependent grazing on diatoms, primarily consuming diatoms less than 25 μm (Whitlatch and Obrebski, 1980). Although we did not examine *C. californica* gut contents to verify their diet, our study showed that these snails can also consume diatoms in larger size classes.

Nutrient effects were generally weaker than herbivore effects, concurring with previous work showing that diatoms on organically rich mudflats can be limited by carbon instead of nitrogen or phosphorus (Admiraal et al., 1982; Underwood and Kromkamp, 1999). Furthermore, previous studies at this site revealed that background nutrient levels in the sediment are high (Armitage and Fong, 2004b) relative to other estuaries in the region (Boyle et al., 2004), which may have muted the effects of our enrichment treatment on the diatoms.

Although nutrient enrichment had a small impact on the diatom community relative to grazer effects, nutrients increased total abundance without altering biomass, suggesting that enrichment caused a shift towards more and smaller diatoms. This shift was particularly apparent in the reference mudflat. Enrichment can stimulate diatom cell division (Saburova and Polikarpov, 2003), subsequently decreasing cell size and producing a population of smaller individuals. Alternatively, a larger number of smaller diatoms in enriched plots may have been a species-specific response. In mesocosms, enrichment has caused increases in small epiphytic diatom species (Coleman and Burkholder, 1994), though other studies have shown substantial increases in larger benthic diatoms (Sundbäck and Snoeijs, 1991). In the field, many studies reveal that nutrient enrichment increases diatom biomass but causes little change in species composition (Peterson et al., 1993; Camacho and de Wit, 2003; Frankovich et al., in press). We did not observe any nutrient impact on diatom biomass, contrasting with biomass increases documented in other enriched aquatic systems (Peterson et al., 1993; Camacho and de Wit, 2003). Biomass responses are not consistent across systems, and enrichment studies in marine habitats often show shifts between microalgal groups, especially increases in the abundance of cyanobacteria relative to diatoms (Pinckney et al., 1995; Miller et al., 1999). The lack of a nutrient impact on diatom biomass may have been attributable to the proliferation of cyanobacterial mats in these study plots (Armitage and Fong, 2004b).

A key physical difference between the two study sites was sediment grain size: the restored site was substantially sandier than the reference site. Sandy and muddy substrata can have distinct benthic diatom species composition (Zheng et al., 2004), and diatom biomass and size structure may also be linked to sediment grain size (Montagna et al., 1983; de Jong and de Jonge, 1995), but patterns are not consistent across spatial or temporal scales (Cammen, 1982; Cartaxana et al., 2006). Relative to mudflats, sandy habitats tend to have fewer and smaller diatoms (Watermann et al., 1999; Woelfel et al., 2007), possibly because diatoms may be more susceptible to resuspension and export on sandier substrata (Barranguet et al., 1998). At our study site, we detected differences in benthic microalgal communities between the sandflat and mudflat habitats at the division level (Armitage and Fong, 2004b).

However, closer inspection of the diatom assemblages in the present study revealed few consistent differences in size structure between habitats, although diatoms were somewhat longer in the reference mudflat than in the restored sandflat in summer 2000. This suggests that diatom species composition may have been similar, although further species-level work is necessary to confirm this conclusion. Diatom size structure and biomass are not typically independent of sediment grain size (Cartaxana et al., 2006; Facca and Sfriso, 2007), but sediment grain size was one of several physical differences between our two study sites; soils in the reference mudflat had higher organic content, moisture content, and nitrogen concentration than in the restored area (Armitage and Fong, 2004a,b). These physical differences between sites did not, however, diminish the strong impact of snails or alter the relatively small impacts of nutrient enrichment on the diatom communities.

5. Conclusions

Strong interactions between consumers and nutrients within benthic microalgal communities are most likely when nutrient enrichment dramatically alters primary producer composition (Armitage and Fong, 2004b), or when consumers directly alter nutrient input to the primary producer community (McCormick and Stevenson, 1991). Other biotic and abiotic factors, such as salinity (Sahan et al., 2007), sediment grain size (Watermann et al., 1999), or the non-trophic impacts of higher consumers (Armitage and Fong, 2006) can disrupt these interactions and decouple grazing and enrichment effects on benthic microalgal communities. We detected few interactions between snail and nutrient addition treatments, possibly because of high background nutrient levels or strong herbivore pressure. Interactive effects may be more common at the division level, where shifts between diatoms and more opportunistic groups such as cyanobacteria can occur in enriched and grazed conditions (Pinckney et al., 1995; Miller et al., 1999; Armitage and Fong, 2004b). Within the diatom community, however, our study demonstrated that benthic diatoms responded more strongly to grazing than to enrichment or site. Other benthic microalgal groups in this system, such as cyanobacteria, showed much stronger responses to enrichment than to grazers (Armitage and Fong, 2004b). These contrasting responses among different microalgal groups further dissolve the black box perception of microphytobenthic responses to natural and anthropogenic habitat alterations.

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Fig. 1. Impacts of snail and nutrient addition on the relative abundance of diatoms from a reference mudflat and a restored sandflat in (a) summer 2000 and (b) spring 2001. Error bars represent SE.

Fig. 2.

Impacts of snail and nutrient addition on diatom biomass, as represented by total one-sided diatom surface area in each plot, from a reference mudflat and a restored sandflat in (a) summer 2000 and (b) spring 2001. Error bars represent SE.

Fig. 3. Impacts of snail and nutrient addition on average diatom length per plot from a reference mudflat and a restored sandflat in (a) summer 2000 and (b) spring 2001. Error bars represent SE.

Fig. 4.

Size frequency distribution of diatom lengths in response to snail and nutrient addition in a reference mudflat and a restored sandflat in (a, b) summer 2000 and (c, d) spring 2001. Grey bars: ambient nutrient treatment; white bars: enriched nutrient treatment. Error bars represent SE.

Table 1

Summary of three-way ANOVA of site, snails, and nutrients on relative diatom abundance (log transformed) in (a) summer 2000 and (b) spring 2001.

	df	MS	F	p	Partial η^2
<i>(a)</i>					
Site	1	0.04	1.25	0.272	0.04
Snails	1	0.72	22.79	<0.001	0.44
Nutrients	1	0.14	4.57	0.041	0.14
Site $\times$ snails	1	0.17	5.47	0.027	0.16
Site $\times$ nutrients	1	<0.01	0.09	0.770	<0.01
Snails $\times$ nutrients	1	0.01	0.33	0.571	0.01
Site $\times$ snails $\times$ nutrients	1	0.01	0.42	0.521	0.01
Residual	29	0.03			
<i>(b)</i>					
Site	1	0.17	2.45	0.128	0.07
Snails	1	0.38	5.35	0.028	0.15
Nutrients	1	0.36	5.11	0.031	0.14
Site $\times$ snails	1	<0.01	0.05	0.822	<0.01
Site $\times$ nutrients	1	<0.01	0.05	0.817	<0.01
Snails $\times$ nutrients	1	<0.01	0.01	0.941	<0.01
Site $\times$ snails $\times$ nutrients	1	0.12	1.75	0.196	0.05
Residual	31	0.07			

Summary of three-way ANOVA of site, snails, and nutrients on total diatom surface area/plot (log transformed) in (a) summer 2000 and (b) spring 2001.

Table 2

	df	MS	F	p	Partial η^2
(a)					
Site	1	0.11	1.13	0.296	0.04
Snails	1	2.03	20.93	<0.001	0.42
Nutrients	1	0.01	0.12	0.729	<0.01
Site $\times$ snails	1	0.05	0.54	0.467	0.02
Site $\times$ nutrients	1	0.04	0.37	0.545	0.01
Snails $\times$ nutrients	1	0.10	0.97	0.332	0.03
Site $\times$ snails $\times$ nutrients	1	0.32	3.31	0.079	0.10
Residual	29	0.10			
(b)					
Site	1	<0.01	<0.01	0.984	<0.01
Snails	1	1.96	10.43	0.003	0.25
Nutrients	1	0.02	0.08	0.776	<0.01
Site $\times$ snails	1	0.07	0.35	0.559	0.01
Site $\times$ nutrients	1	0.25	1.33	0.257	0.04
Snails $\times$ nutrients	1	0.36	1.93	0.174	0.06
Site $\times$ snails $\times$ nutrients	1	0.08	0.45	0.509	0.01
Residual	31	0.19			

Table 3

Summary of three-way ANOVA of site, snails, and nutrients on average diatom length (log transformed) in (a) summer 2000 and (b) spring 2001.

	df	MS	F	p	Partial η^2
<i>(a)</i>					
Site	1	0.13	4.57	0.041	0.14
Snails	1	0.09	3.35	0.078	0.10
Nutrients	1	0.04	1.61	0.215	0.05
Site $\times$ snails	1	<0.01	0.11	0.742	<0.01
Site $\times$ nutrients	1	0.01	0.34	0.562	0.01
Snails $\times$ nutrients	1	<0.01	0.07	0.785	<0.01
Site $\times$ snails $\times$ nutrients	1	0.11	4.07	0.053	0.12
Residual	29	0.03			
<i>(b)</i>					
Site	1	0.03	1.22	0.278	0.04
Snails	1	0.31	12.02	0.002	0.28
Nutrients	1	0.09	3.67	0.065	0.11
Site $\times$ snails	1	<0.01	0.01	0.905	<0.01
Site $\times$ nutrients	1	0.13	4.89	0.034	0.14
Snails $\times$ nutrients	1	0.09	3.63	0.066	0.11
Site $\times$ snails $\times$ nutrients	1	<0.01	0.09	0.760	<0.01
Residual	31	0.03			