

**Effects of the nuisance diatom *Didymosphenia*
geminata on invertebrates in a Rocky Mountain
stream**

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EFFECTS OF THE NUISANCE DIATOM *DIDYMOSPHENIA GEMINATA* ON INVERTEBRATES IN A ROCKY MOUNTAIN STREAM

Abstract

Nuisance blooms of the stalked diatom *Didymosphenia geminata* have become an increasing concern worldwide, yet little is known about their effect on stream food webs. We investigated the effects of *D. geminata* blooms on stream invertebrates of the East River in the western Rocky Mountains. Areas with natural blooming levels of *D. geminata* had greater total invertebrate density but similar invertebrate biomass when compared to areas of stream bottom where *D. geminata* was experimentally removed. Most notable was a dramatic increase in the density and biomass of Chironomidae larvae, which were 6.5 and 4.2 times greater, respectively, in areas with *D. geminata* blooms relative to areas where it was removed. The density and biomass of Perlidae stoneflies, historically rare in the East River, were 1.9 and 3.1 times greater, respectively, in areas containing *D. geminata* blooms. In contrast, Heptageniidae mayfly biomass decreased by 50% in areas with *D. geminata* blooms, but their density was unchanged. There was no significant change in either density or biomass of the numerically abundant and highly mobile mayfly, Baetidae. *D. geminata* did not significantly alter the growth rate of *Baetis* or *Epeorus* mayflies in experimental growth chambers. The thick mats of stalks created by *D. geminata* blooms likely alter stream food webs, particularly due to dramatic increases in the density of Chironomidae larvae.

Keywords: *Didymosphenia geminata*; algae; diatom; stalks; invertebrates; East River; bloom; benthic; mayflies; chironomidae; stoneflies..

Introduction

In aquatic ecosystems such as lakes and streams, algae are important primary producers even when macrophytes or aquatic vascular plants are abundant or terrestrial plant litter inputs are high (Forsberg et al. 1993; Lewis et al. 2000; McCutchan and Lewis 2002). Stream algae can influence many characteristics (e.g. taxa diversity, density, and movements) of herbivore communities (Allan 1995), but the effects of stream algae on invertebrates are not limited to its role as a food source, as algae can also modify the chemical and physical features of aquatic environments (Dudley 1986; Mulholland et al. 1994; Sabater et al. 2000). Stream algae play an important role in food webs by affecting invertebrate communities. For example, they may alter an important food source for trout, birds, spiders and other predators (Allan 1981, 1982; Nakano and Murakami 2001; Sabo and Power 2002; Sanzone et al. 2003).

The freshwater diatom *Didymosphenia geminata* has drawn global attention in the past two decades as either an invasive or native species with the propensity to form nuisance blooms that extend over several kilometers in streams and persist for several months (Spaulding and Elwell 2007). During nuisance blooms, the unique polysaccharide stalks produced by *D. geminata* form dense brown mats that can cover the bottom of streams and alter the habitat of benthic invertebrates. Unlike the stalks of many diatoms (Hoagland et al. 1993), the stalks of *D. geminata* are large, carbon-rich, and may contain secondary compounds that make them resistant to decay and, perhaps, herbivory (Spaulding and Elwell 2007). For unknown reasons, *D.*

geminata has reached excessive biomass, mostly due to an increase in stalks (Spalding and Elwell 2007), and is being found in a wider range of environments than previously documented (Kawecka and Sanecki 2003).

Interactions between *D. geminata* and stream invertebrates may be similar to those of other species of stalked and filamentous algae. Stalked diatoms or filamentous algae can affect stream invertebrates by: (1) outcompeting sessile invertebrates for space on the stream bottom, (2) providing a beneficial structural habitat, (3) interfering with the mobility of invertebrates specialized for foraging on smooth substrates, or (4) providing a more edible food source by increasing the availability of algal epiphytes (Dudley et al. 1986). Algal stalks and filaments can also alter invertebrate habitat by trapping particulate matter, blocking light, changing water flow near the streambed, or decreasing dissolved oxygen concentrations when they decompose (Dudley et al. 1986).

Although *D. geminata* has gained notoriety as a nuisance blooming alga, we still lack basic biological and ecological knowledge about this organism (May 2006 symposium on *D. geminata* in Bozeman, Montana, co-sponsored by EPA Region 8 and the Federation of Fly Fishers). In this study, we looked at secondary producers to assess the effect of *D. geminata* on stream food webs. We focused on two questions relating to the effect of *D. geminata* and its stalks on benthic invertebrates: (1) does *Didymosphenia geminata* alter the composition and abundance of stream invertebrates? and (2) does *Didymosphenia geminata* affect the growth rate of stream invertebrates?

Studies in New Zealand have found greater total invertebrate density in *D. geminata*-affected areas, but lower proportions and biomass of invertebrate taxa associated with good water quality (i.e. mayflies, stoneflies and caddisflies) (Kilroy et al. 2005). The same study also found no effect of *D. geminata* blooms on taxa richness (Kilroy et al. 2005). The effects of *D. geminata* are likely to differ among invertebrate taxa, depending on their morphology and feeding habits. The 1-3 cm thick mat formed by *D. geminata* stalks may interfere with the mobility of invertebrates that are adapted to foraging on smooth substrates. Dorsoventrally-flattened heptageniid mayflies, for example, may not be able to move or forage easily among *D. geminata* stalks. We predicted that the heptageniid *Epeorus spp.* would decrease in density and grow at a slower rate in the presence of *D. geminata*. Baetid mayflies, on the other hand, are more mobile and may be able to grasp onto the stalks and forage on *D. geminata* or its associated epiphyte algae. We predicted that *Baetis spp.* would increase in density and grow more quickly, or be unaffected by the presence of *D. geminata*.

Methods

Response of invertebrates to removal of D. geminata

We manipulated invertebrate habitats in the East River by removing *D. geminata* from selected areas of the stream bottom. Sixteen square (0.5 x 0.5 m) plots were placed haphazardly throughout a 200 m section of the river, but paired adjacent to one another. Each pair had similar water depth and velocity, and consisted of one plot containing natural abundances of *D. geminata* that we did not manipulate (*D. geminata* present) and one plot in which we manually removed visible tufts of *D. geminata* from the substrata (*D. geminata* removed) using a glass bottom viewing box and forceps. This technique has been used to remove other stalked diatoms from stream bottoms (Dudley et al. 1986). During each removal we delineated each plot by placing a 0.5 x 0.5 m grid (divided into 25 - 10 x 10 cm squares) over two steel bars inserted into

the stream bed upstream of the plot. To control for the disturbance caused by the forceps and grid, we also placed the grid over the plots with *D. geminata* present and touched the substrata with forceps, but did not remove *D. geminata*. The first removal was on June 20th 2007, and we removed *D. geminata* every 3-6 days until the end of the experiment on July 30th 2007.

We estimated the abundance of *D. geminata* in each plot using a visual *D. geminata* volume index method similar to that described by Kilroy et al. (2005). Briefly, this index is calculated by estimating the percentage of the stream bottom covered with *D. geminata* and multiplying it by the depth of the *D. geminata* mat. We estimated the *D. geminata* volume index for each of the 25 - 10 × 10 cm squares in each plot beginning on June 20th 2007 before removing any *D. geminata* and approximately every 10 days during the experiment until July 31st 2007, for a total of four estimates over 41 days. On July 9th and 30th, we sampled benthic invertebrates from the center of each of the 16 plots using a modified 31 × 31 cm Hess sampler and electrobug sampling methods (Taylor et al. 2001). We identified and counted invertebrates in the lab using a dissecting microscope. Taxa were identified to genus or species using Ward et al. (2002) and Peckarsky et al. (1985), except for chironomidae larvae, which we identified to family.

To visualize the spatial distribution of *D. geminata*, we created krigged variate maps of the *D. geminata* volume index for each plot. Using the geostatistical software GS +, version 5.3.2 (Robertson 2001), we produced variate maps by performing semivariograms and using block kriging as the interpolation procedure with a block size of 5 cm² (Isaaks and Srivastava 1989). Maps were generated by computing the mean *D. geminata* volume index for each 10 × 10 cm square in the 0.5 m² grid and pooling among the 3 dates when *D. geminata* was removed. To test for differences in the *D. geminata* volume index between the *D. geminata* present and *D. geminata* removed plots among the four sampling dates, we performed a repeated measures ANOVA using PROC MIXED in Version 8.2 of SAS statistical software (SAS Institute Inc. 1999). In addition to testing for differences in the volume index between treatments (*D. geminata* present and *D. geminata* removed), we used a post hoc multiple comparison test to test for differences in *D. geminata* among sampling dates. The *D. geminata* volume index was log₁₀ transformed to reduce heteroscedasticity prior to ANOVA. No transformations were performed on the data used to generate the variate maps.

To investigate changes in the composition and abundance of stream invertebrates in *D. geminata* blooms, we tested for differences in density, biomass and rarefied taxa richness between patches with *D. geminata* and patches where it was manually removed. Taxa were lumped into nine families or groups of families as follows: Baetidae, Chironomidae, Ephemerellidae, Heptageniidae, Leptophlebiidae, Perlidae, other Plecoptera, other Trichoptera and other taxa. To protect against type II errors, we performed a Hotelling's T² test, a multivariate paired T-test, to test for overall differences between the treatments for all groups. Individual differences between treatments were then tested for each group of taxa with separate T-tests. We also tested for differences in total density between treatments with a T-test. Equivalent tests were performed on biomass data. To control for bias in taxonomic richness caused by differences in number of invertebrates among plots, we used the program EcoSim to generate rarefied estimates of taxa richness, which standardizes for differences in number of individuals sampled (Gotelli 2006). Total invertebrate density was standardized to 2000 individuals, slightly less than the total invertebrate density of the plots where *D. geminata* was removed (2118 individuals/m²), which was lower than plots with *D. geminata* (3082 individuals/m²). Rarefied richness was compared between treatments by calculating 95 percent

confidence intervals. In addition, we calculated the percent similarity of species between plots with and without *D. geminata*.

Invertebrate growth rates

We measured the growth rates of two invertebrate taxa from the East River by rearing them *in situ* in flow-through chambers with substrata completely covered with *D. geminata* or with substrata from an adjacent stream without any visible tufts of *D. geminata*. The two species selected were *Baetis* spp. and *Epeorus longimanus*, based on their ecological significance in the East River and their potential response to *D. geminata* given their different behaviors and morphologies. Both are abundant algivores and important food for trout (Allan 1981, 1982), but have different body shapes and mobility.

D. geminata-covered rocks were collected from the East River, and rocks visibly free of *D. geminata* tufts were collected from nearby Copper Creek, which has some patches of *D. geminata* but no nuisance blooms. The chambers were constructed from clear plastic cylinders (9.5 diameter x 15 length cm) with 100 or 500 μm mesh secured on each end with a stainless steel ring clamp. We secured the chambers on the stream bottom by attaching them to steel bars inserted into the stream bed. Initial body mass was determined by one of two methods: measuring head capsule width (Huryn and Wallace 1986) of each individual specimen and estimating body mass with power model regressions (Benke et al. 1999), or taking a subsample of each size class to be massed back at the lab. Final body mass was measured after 6-13 days. Individuals massed in the lab were placed in pre-massed aluminum foil trays, dried in a drying oven at 60 $^{\circ}\text{C}$ for at least 24 h, placed in a dessicator, and massed at ambient temperature on a Cahn C-31 microbalance. *Baetis* spp. were massed to the nearest 100th of a mg and *E. longimanus* to the nearest 10th of a mg. We measured growth of *Baetis* spp. in two trials (July 2-11 and 14-20, 2007), and *E. longimanus* in three trials (July 2-12, 14-20, and 17-28, 2007).

We estimated the mean instantaneous growth rate of the individuals in each chamber by calculating the difference between mean final body mass and initial body mass and dividing by the number of days of the growth trial (Huryn and Wallace 1986). Initial body mass and instantaneous growth rate were $\log_{10}$ transformed to reduce heteroscedasticity. We tested for effects of *D. geminata* on instantaneous growth rate using an analysis of covariance (ANCOVA), but tested for homogeneity of slopes before interpreting the treatment effect using JMP statistical software (SAS Institute Inc. 2001).

Results

Response of invertebrates to removal of D. geminata

Manual removal of *D. geminata* was visually effective (Fig. 1). There was 3.7 times as much *D. geminata* in unmanipulated plots as in the removal plots (an average of 16 cm^3 in unmanipulated plots and 4.3 cm^3 in removal plots). Removal plots were similar to plots with *D. geminata* present before any *D. geminata* was removed ($P = 0.6963$), but were different following the first experimental removal and diverged significantly over time ($P < 0.0001$), with the volume of *D. geminata* decreasing and remaining low in the removal plots, and increasing in the *D. geminata* present plots as the season progressed (Fig. 2).

Total invertebrate density was greater in plots with *D. geminata* compared to plots where it was removed (3082 and 2118 individuals/ m^2 respectively; $P = .037$; Fig 3), though total biomass was unchanged (552 and 550 mg/m^2 respectively; $P = .989$; Fig 3). Despite

insignificant P values for both density and biomass in the Hotelling's T^2 tests ($P = .187$ and $.528$, respectively), we chose to interpret the results of T-tests performed on individual taxa groups. Chironomidae larvae appeared in very high densities (830 insects/m²) in *D. geminata* plots and low densities in removal plots (110 insects/m², $P = .004$; Fig. 4). Stoneflies of the Perlidae family displayed a similar pattern, though not significant, of greater density in *D. geminata* plots (31 insects/m² compared to 11 insects/m² in removal plots, $P = .068$; Fig. 4). The biomasses of both Chironomidae and Perlidae were also greater in *D. geminata* plots, though not significantly in Perlidae (30 mg/m² and 24 mg/m², respectively, in *D. geminata* plots and 6 mg/m² and 6 mg/m², respectively, in removal plots, $P = .018$ and $.366$, respectively; Fig. 5). Heptageniidae mayflies did not differ in density between treatments (331 insects/m² in *D. geminata* plots and 334 insects/m² in removal plots, $P = .489$; Fig. 4), but had lower biomass in plots with *D. geminata* (61 mg/m² in *D. geminata* plots and 121 mg/m² in removal plots, $P = .037$; Fig. 5). The dominant grazers, Baetidae mayflies, did not differ in density (1466 insects/m² in *D. geminata* plots and 1272 insects/m² in removal plots, $P = .479$; Fig. 4) or biomass (103 mg/m² in *D. geminata* plots and 102 mg/m² in removal plots, $P = .998$; Fig. 5) between treatments.

The rarefied taxa richness was significantly lower in *D. geminata* plots than in plots where *D. geminata* was removed (Fig. 6). The percent similarity between species found in *D. geminata* and removal plot treatments was 76.4%.

Invertebrate growth rates

D. geminata did not affect the growth rate of *Baetis* spp. (0.0652 mg/mg/day on rocks without visible *D. geminata* tufts and 0.0726 mg/mg/day on *D. geminata*-covered rocks, $P = 0.8898$; Fig. 7). *E. longimanus* showed a trend towards slower growth in the *D. geminata* treatment, but it was not statistically significant (0.0238 mg/mg/day on rocks without visible *D. geminata* tufts and 0.0075 mg/mg/day on *D. geminata*-covered rocks, $P = 0.5853$; Fig 8).

Discussion

Our study investigated two questions related to the effects of *Didymosphenia geminata* on benthic invertebrates: (1) does *Didymosphenia geminata* alter the composition and abundance of stream invertebrates? and (2) does *Didymosphenia geminata* affect the growth rate of stream invertebrates? We found that total invertebrate density was greater in areas with *D. geminata*, as seen in the study by Kilroy et al. (2006), while total invertebrate biomass was unchanged. The response of invertebrates to the removal of *D. geminata* differed among taxa groups, and the effect of *D. geminata* on invertebrate growth rate was in accordance with these patterns. Unlike Kilroy et al. (2006), we found decreased taxa richness in areas with *D. geminata*.

In our experimental removal of *D. geminata*, we opened up sections of substrate in a stream reach that was otherwise covered with stalks. We were effective in removing the *D. geminata*, and there was greater overall invertebrate density in plots where *D. geminata* was present but no difference in invertebrate biomass. This suggests that, while there were more invertebrates in the *D. geminata* plots, they were of smaller size. Accordingly, the most notable increase in density in *D. geminata* plots was seen in the tiny worm-like midge larvae of the Chironomidae family. Predatory stoneflies of the Perlidae family, historically rare in the East River, also displayed higher densities with *D. geminata*. Both Chironomidae and Perlidae also displayed higher biomass in *D. geminata* plots. Heptageniidae mayflies, an important grazer by

biomass, did not differ in density but had decreased biomass in *D. geminata* plots, indicating a negative effect of the alga on the size of these invertebrates. Baetidae mayflies, an important grazer by density, did not differ in density or biomass between habitats with and without *D. geminata*. The growth effects of *D. geminata* on *Epeorus longimanus* (Heptageniidae) and *Baetis spp.* (Baetidae) were consistent with these biomass patterns, but highly variable. *Epeorus longimanus* grew more slowly in *D. geminata*, while the growth rate of *Baetis spp.* was not affected.

We predicted that insects that do poorly in *D. geminata* blooms will be more abundant in habitats where the stalks are removed, whereas insects that benefit from *D. geminata* blooms will be more abundant in habitats where stalks are present. The morphology of an insect may be important in their interaction with *D. geminata* blooms and could explain the patterns we saw in the density, biomass and growth rate of these invertebrates. Baetidae mayflies, which are good swimmers, and Chironomidae larvae, which are thin and worm-like, may be able to navigate the stalks and use them as a beneficial structural habitat and refuge from predators. Other body types, like the ventrally-flattened Heptageniidae mayflies, may find the stalks too cumbersome.

In addition to altering the structural habitat, *D. geminata* may provide an attractive food source to some invertebrates. Though their head capsule width is probably too small to allow them to ingest the large and nutritious *D. geminata* cell, Chironomidae larvae may benefit by grazing epiphytic diatoms growing on *D. geminata* stalks or by eating particulate organic matter trapped within the mats. The larvae themselves may also be an attractive food source for predators such as Perlidae stoneflies, which were more prevalent in *D. geminata* patches. The high density of the Chironomidae larvae (>800 individuals/m²) in the plots with *D. geminata* present is striking compared to relatively low (122 individuals/m² in 1999, B.W. Taylor *unpublished data*) historical densities in the East River (Peckarsky 1991; Taylor et al. 2002), and may prove to be the largest contributor to *D. geminata*'s effect on stream food webs.

Our experimental removal of *D. geminata* revealed changes in the density and biomass of certain invertebrate taxa with the presence of *D. geminata* stalks. These patterns suggest changes in the composition of invertebrate communities in streams with *D. geminata* blooms. Streams that acquire *D. geminata* blooms may become dominated by Chironomidae midges, show increased numbers of Perlidae stoneflies and have decreased numbers of Heptageniidae mayflies. To look at the effects of *D. geminata* blooms at the community level, we plan to perform an observational study of multiple streams with varying intensities of *D. geminata* and compare their invertebrate assemblages. This may reveal patterns that were not visible in our experimental manipulation, such as the exclusion or introduction of an invertebrate species that was present prior to the arrival of *D. geminata* blooms.

As *D. geminata* blooms are discovered in new streams around the world, it will become increasingly important to understand the effects this diatom will have on stream ecosystems. Nuisance algal blooms are uncommon in pristine, low-nutrient streams like those in many parts of the Rocky Mountains. The unusual circumstances under which *D. geminata* blooms and the unique composition of its stalks may result in ecosystem effects that do not mimic those of typical algae blooms. Understanding the impact of *D. geminata* on benthic invertebrates may provide insight into the potential consequences for stream food webs, and help to predict the ways in which *D. geminata* blooms will alter stream ecosystems.

Acknowledgements

Thank you to Brad W. Taylor for assistance with experimental design, analysis and field work,

and for his support throughout the summer. Thank you to Laura Aldrich-Wolfe, Bobbi Peckarsky, and Todd Wellnitz for their suggestions and encouragement. Funding was provided by a NSF Research Experience for Undergraduates (REU) grant to RMBL and Dartmouth College funds to BWT. This is a contribution to the REU program at the Rocky Mountain Biological Laboratory.

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Figure Legends

Figure 1. Variate maps of the experimental plots with *D. geminata* present (left) and manually removed (right).

Figure 2. The *D. geminata* volume index was different between plots where *D. geminata* was naturally present and where it was removed, and *D. geminata* increased in abundance as the season progressed in the plots where it was present. Different letters and case (upper or lowercase) indicate points that are significantly different based on a repeated measures ANOVA followed by a multiple comparisons test. Each point is the mean of 8 plots for a treatment on a given date. Error bars are 1 standard error of the mean. Note: the error bars are too small to be seen on the removal points.

Figure 3. The total invertebrate density was significantly greater ($P = .038$) in plots where *D. geminata* was present (black bars) compared to plots where it was removed (white bars). Total invertebrate biomass was not significantly different between treatments. Each bar is the mean of 8 plots and both sampling dates for a treatment. Error bars are 1 standard error of the mean.

Figure 4. There was a significantly higher density ($P = .004$) of Chironomidae in plots where *D. geminata* was present (black bars) compared to plots where it was removed (white bars). Each bar is the mean of 8 plots and both sampling dates for a treatment. Error bars are 1 standard error of the mean.

Figure 5. There was a significantly higher biomass ($P = .018$) of Chironomidae in plots where *D. geminata* was present (black bars) compared to plots where it was removed (white bars). In contrast, Heptageniidae mayflies had significantly lower biomass ($P = .037$) in plots where *D. geminata* was present compared to plots where it was removed. Each bar is the mean of 8 plots and both sampling dates for a treatment. Error bars are 1 standard error of the mean.

Figure 6. Rarefied richness was significantly lower in plots with *D. geminata* compared to plots where it was removed. Bars represent 95 percent confidence intervals.

Figure 7. The growth rate of *Baetis spp.* was not significantly different in treatments with and without *D. geminata*. Each point represents one of 6 chambers for each treatment. Regression lines are shown.

Figure 8. *Epeorus deceptivus* had a slower growth rate in the *D. geminata* treatment, but this difference was not statistically significant. Each point represents one of 7 chambers with *D. geminata* and 8 chambers without *D. geminata*. Regression lines are omitted for clarity.

Fig. 1.

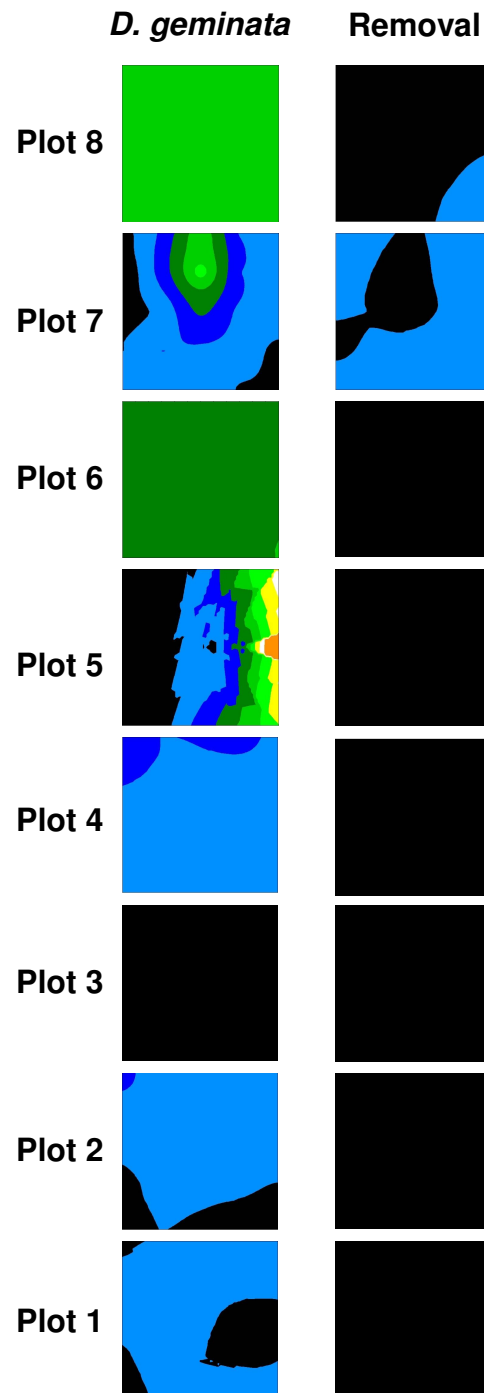
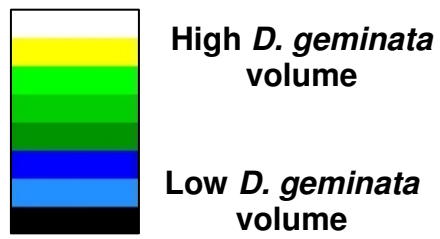


Fig. 2.

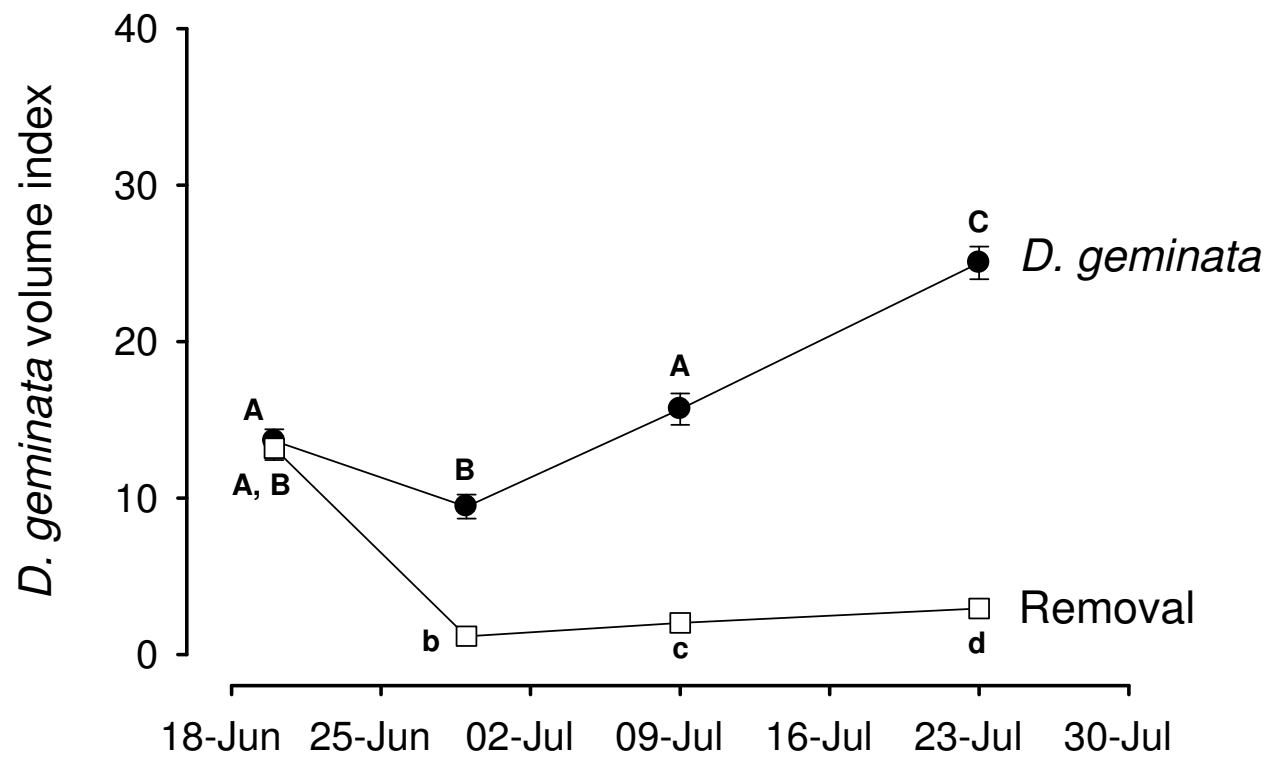


Fig. 3.

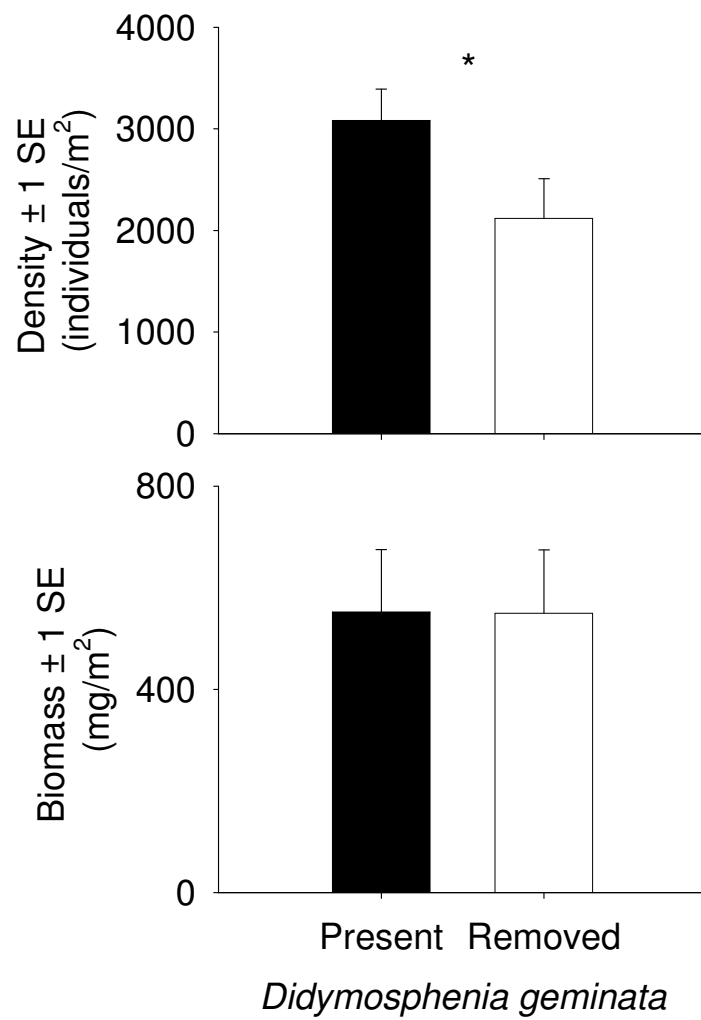


Fig 4.

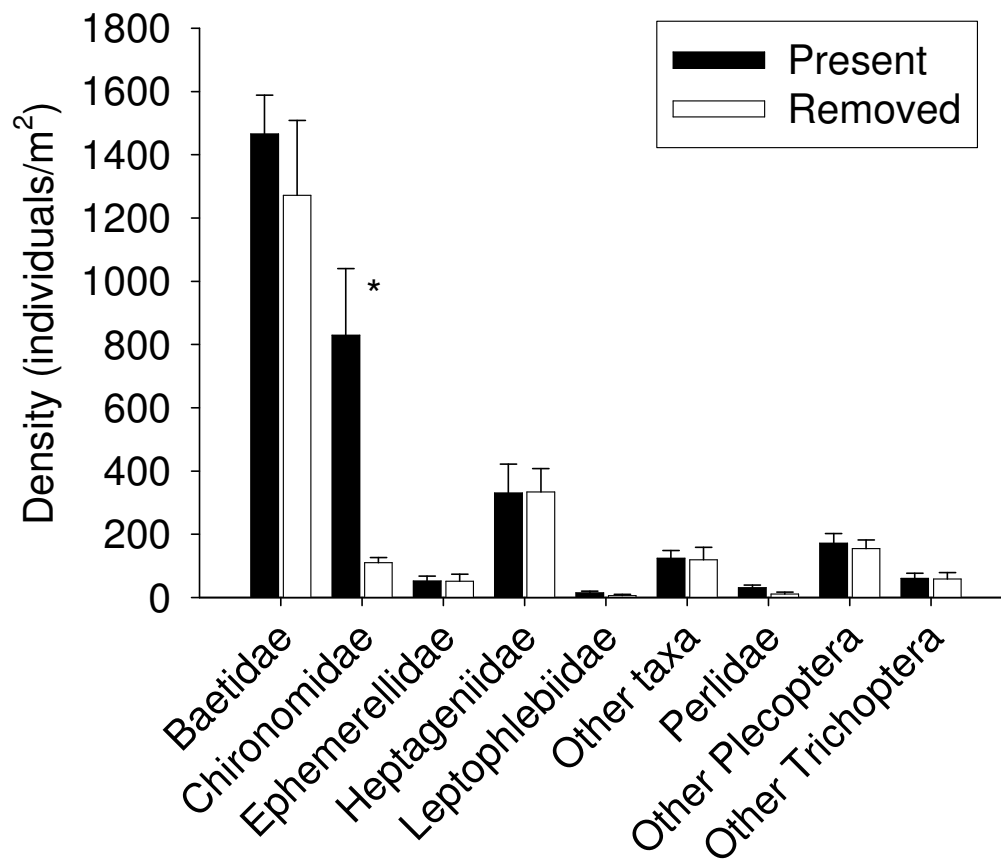


Fig. 5.

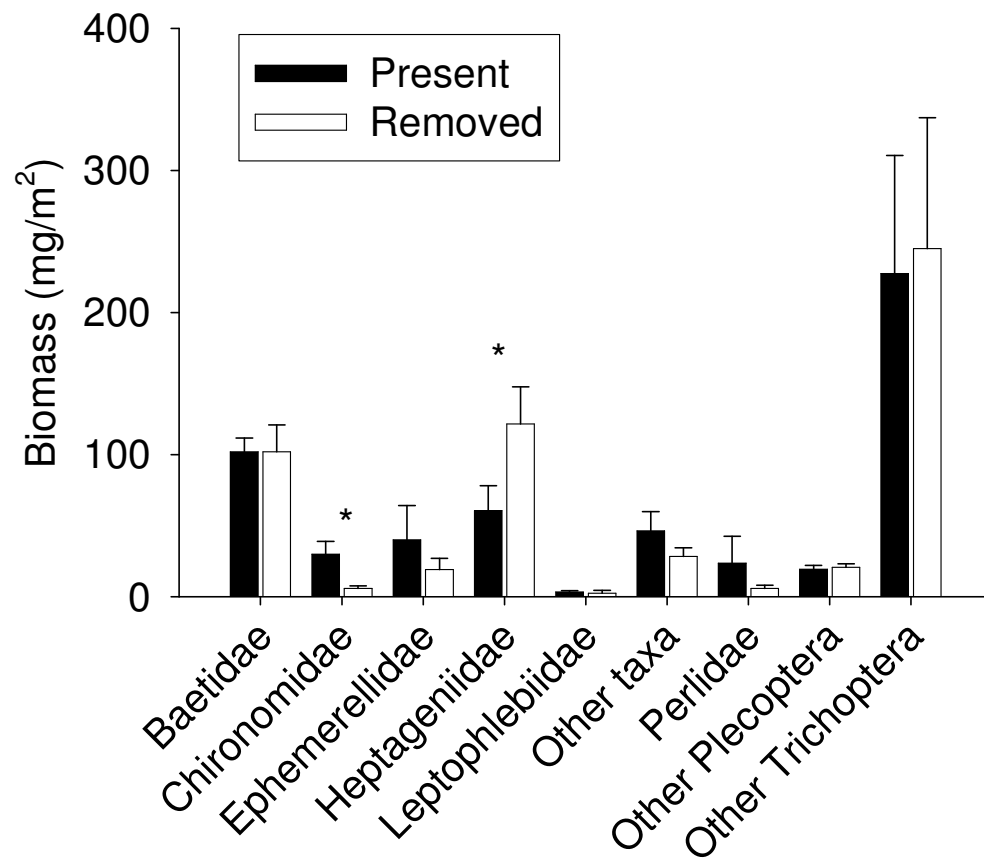


Fig. 6.

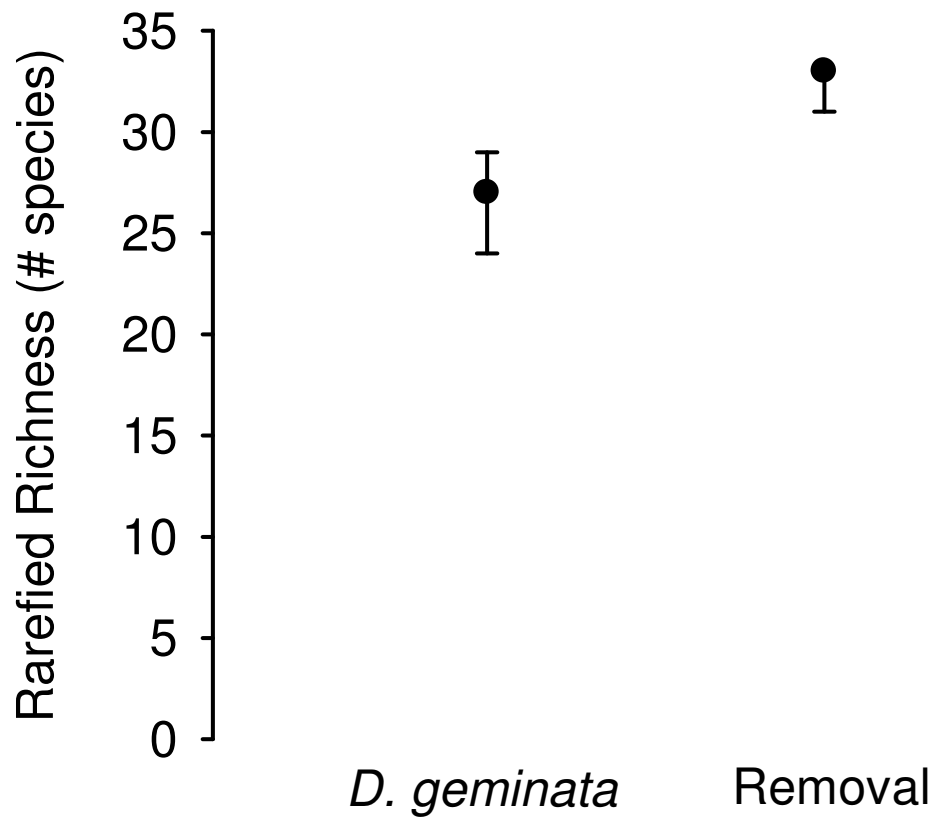


Fig. 7.

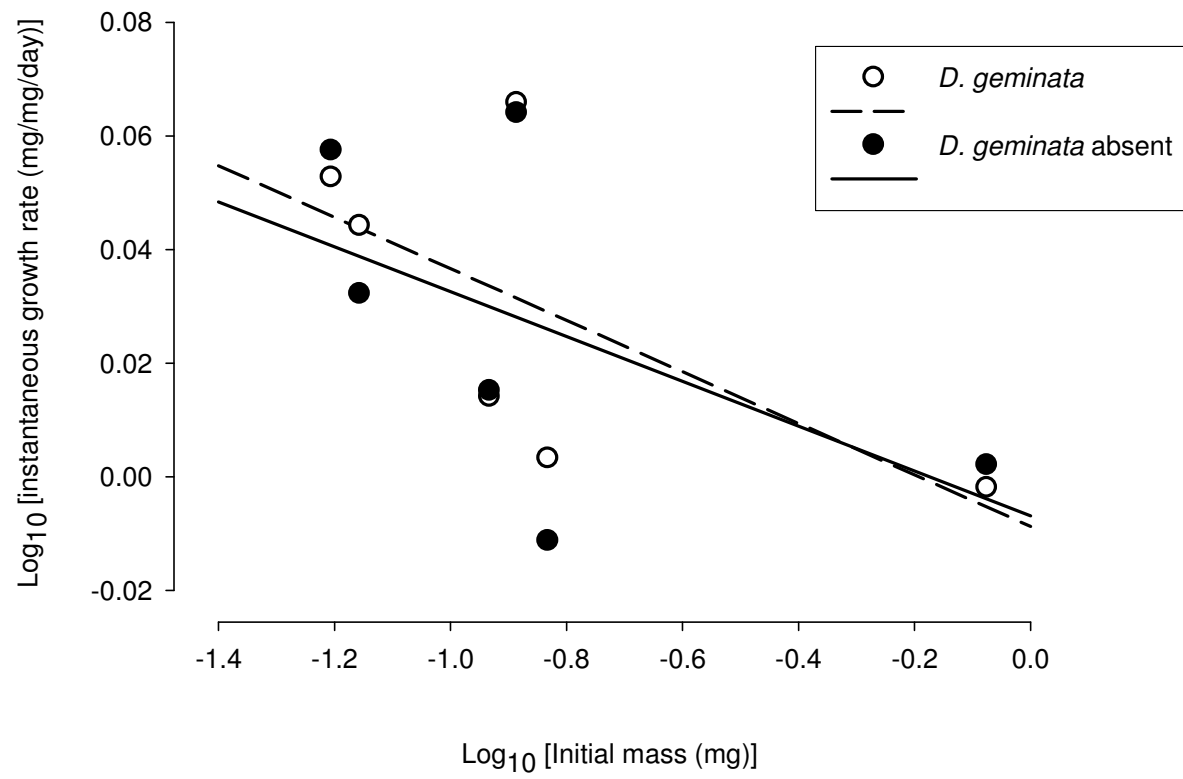


Fig. 8.

