

**Effect of diatom, *Didymosphenia geminata*, on
Invertebrate Abundance and Composition in
Rocky Mountain Streams**

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Abstract

The diatom *Didymosphenia geminata* has become an increasing concern for aquatic habitats. The algae has spread within its native environment and exhibit invasive characteristics, as well as invade ecosystems outside of its native range. *D. geminata* does not appear to affect the species richness of an ecosystem, however it does appear to effect the abundance of specific taxa. Three taxa belonging to the Heptageniidae family, specifically Rhithrogena, Epeorus and Cinygmula, decrease in the presence of *D. geminata*. Chironomidae larva appeared in much more larger densities in places where *D. geminata* was present. The over all trend the experiment exhibited was a decrease in larger taxa in the presence of *D. geminata* and an increase in smaller invertebrates in the presence of *D. geminata*. In order to explain this trend we conducted a predator refuge experiment. In this experiment we tested to find if chironomids find refuge in a *D. geminata* imitation from stonefly predators. The experiment revealed significant evidence that chironomids do benefit from *D. geminata* as a refuge.

Introduction

Photosynthetic plants are the primary source of energy in most food chains. Their ability to convert sunlight into energy indirectly supports every organism in the food web. Algae are at the base of the food chain of most aquatic ecosystems, and often serve as an important source of energy for the consumers that depend on the algae for survival. Diatoms are unicellular algae that are considered to be the most important photosynthetic eukaryotic producers on our planet. Although diatoms only compose a small portion of the Earth's photosynthetic biomass, they produce almost $\frac{1}{4}$ of the oxygen we breathe and can fix the same amount of carbon per day as terrestrial plants. However, while diatoms do contribute greatly to the carbon budget (Spaulding and Elwell 2007) and play a central role in sustaining our ecosystems, aquatic algae, if introduced as an invasive species, has the ability to alter the chemical and physical features of the environment (Dudley 1986; Mulholland et al. 1994;

Sabater et al. 2000).

An invasive species is an organism that invades a non-native ecosystem and has the potential to cause harm to the economy, environment, or human health. Invasive species change native habitats and compete with native wildlife ultimately altering the functional dynamic of the ecosystem. The U.S. fish and Wildlife Service is concerned about the impacts invasive species are having on our fish, wildlife, and plant resources.

The diatom *Didymosphenia geminata* has been of increasing concern over the past several years due to outbreaks of nuisance blooms, masses of cells and stalks that extend for greater than 1 km and persist for several months of the year (Spaulding and Elwell 2007). The presence of diatoms is usually dependant on the availability of silicon in the water which the organism inhabits in order to generate the diatoms silica cell wall. Typically, diatoms thrive only in nutrient rich waters where silicon, nitrogen and phosphorus are rich in concentrations. Silicon is normally the first of these essential nutrients to be expended in waters where nutrient concentrations are low. *D. geminata* has always been considered unique because unlike other primary producers, blooms have occurred in stream habitats considered pristine or low in nutrients (Johsson et al. 2005). However, over the past several years, *D. geminata* has adapted to high nutrient rivers outside of the organisms previously noted ecological range of the cool temperate waters of northern North America and northern Europe. The algae has spread within the limits of its native distribution to other areas of Europe and North America, as well as beyond limits of its native distribution including New Zealand, where in 2004, the first ever account of *D. geminata* in the Southern Hemisphere was documented (Spaulding and Elwell 2007).

D. geminata secretes a mucopolysaccharide stalk through the apical porefield. This trait of *D. geminata* is essentially the cause of the public's increasing concerns (Spaulding and Elwell 2007). The stalk can attach to rocks or other submerged substrates. When the diatom divides, the stalk does as well, eventually creating a large mat of algae which can cover up to 100% of the streams surface with a thickness of 20 cm. This feature gives *D. geminata* the potential to alter both physical and biological conditions of streams (Spaulding and Elwell 2007). The large mats of algae may reduce the number of suitable habitats and decrease the growth of other diatoms, causing a change in the biodiversity of the community (Biosecurity, New Zealand 2005).

Previous studies have shown a difference in the invertebrates that inhabit areas with and without *D. geminata*, particularly, in the abundance of chironomids, which is significantly higher in streams where *D. geminata* present. The reason for this is unknown, but it is hypothesized that the cotton like algae may act as a refuge for the tiny chironimids against predators. We conducted an algae removal experiment to test if *D. geminata* has an effect on the invertebrate species abundance and composition in the East River along the Gothic townsite of Colorado. Furthermore, given the influx of chironomids in streams colonized by the algae, we have conducted an experiment to test the refuge hypothesis, and determine if there is evidence that *D. geminata* acts as a retreat for the small bodied chironomids against predators.

Methods

Removal Experiment

We removed the algae, *Didymosphenia geminata*, from the stream bottom of 8 plots in the East River, in order to manipulate the environment and determine if the presence of *D. geminata* has an effect on the abundance and composition of the invertebrate environment. The plots were placed at two sites in the East River. Plots one through four resided 30 meters above Vera falls, and plots five through eight were placed several hundred meters downstream. In order to prevent bias, the plots were chosen when in mid June when the river's water level was high, (roughly 40 centimeters) and the stream bottom was difficult to see. The plots at each site were placed two meters away from each other, beginning downstream and moving upstream. Each plot measured 0.5 m x 0.5 m and had an adjacent control where no *D. geminata* was removed during the experiment. The control plots were placed 1 meter away from the corresponding experimental plot where the velocity and depth of the water were similar between the two plots. The upper corners of each plot were marked two steel bars placed 0.5 meters away from each other. During removals the plots were defined using a 0.5 m x 0.5 m grid which was placed over the steel bars and submerged to outline removal area. We used glass viewing box to identify where in the quadrat *D. geminata* was present and metal forceps to eradicate the algae from the substrates of the removal plots. To control for disturbance, we placed the quadrat over the adjacent control (non *D. geminata* removal) plot and touched each of the rocks within the designated area with the same forceps without

removing any *D. geminata*. We visited the sites regularly (every 2 to 4 days) to remove any *D. geminata* growth in the experimental plots. The removals took place from July 3rd to July 27th, just over three weeks. We used a volume index to determine the amount of *D. geminata* growth in each of the plot over the three weeks. To do this, we placed the 0.5 x 0.5 meter quadrat over the plot. The quadrat is divided into 25 10 x 10 cm squares. We visually determined the amount of *D. geminata* inside each individual square at each plot along with the depth of the algae (mm) in the particular square. The volume index was calculated by multiplying the percent cover by the depth for each of the 16 plots. The plots were first scored on July 3rd (prior to removal) and continued to be scored every 7 to 10 days until July 27th. On July 28th we sampled the center of each of the 16 plots for benthic invertebrates using a 31 cm by 31 cm Hoffman sampler with 375 micrometer mesh and electrobug sampling methods (Taylor et al. 2001). We separated the invertebrates by plot and taxa in the field and preserved the samples in water. We used a dissecting microscope to identify and count the invertebrate. Most of the taxa were classified to genus or species using Ward et al. (2002) and Peckarsky et al. (1985), except for chironomid larvae which we identified to family. The differences in abundance between plots were tested using a Hotelling's T2 test. Species diversity metrics (e.g., richness, evenness, and dominance) will be rarefied using EcoSim, pooling across all 16 plots and differences assessed using generated 95% confidence intervals from EcoSim software (Gotelli and Enstminger 2004).

Predator Refuge Experiment

We used a polyester material to act as *Didymosphenia geminata* to determine if there is evidence that chironomids find refuge in the algae. We first placed polyester covered rocks in the East River and removed them a day later to confirm whether the polyester would be colonized by chironomids and other invertebrates, and could serve as a suitable imitator of *D. geminata*. Upon confirmation, we conducted an experiment in artificial flow-through channels containing rocks either covered or not covered in the polyester material. The filters of the channels were covered in 45 micrometer mesh to keep the chironomids from escaping. The polyester was fastened with rubber bands to the top of the rocks where *D. geminata* would grow. The stonefly predator used in the experiment was hesperoperla, which were caught via kick netting in the East River. The chironomids were also picked from the East River. We

established four experimental treatments: polyester-covered rocks + chironomids + stonefly; non polyester covered rock + chironomids + stonefly; polyester-covered rock + chironomids, no stonefly, and non polyester- covered + chironomids, no stoneflies. The latter two served as control treatments to estimate the natural mortality rates of chironomids. Each treatment had 12 replicates. Each tank had two rocks, either bare or covered in polyester, depending upon the treatment and 5 chironomids. The chironomids were given 6 hours to settle in the tanks before the predator was added to the appropriate tanks. The stoneflies remained in the tanks for 15 hours to feed. After this time, the chironomids were counted. To do so, each tank was poured into a separate pan and sifted through in order to find the chironomids. We began with the control treatments, and then the experimental treatments. When counting the experimental treatments we emptied the tanks two at a time, one tank with polyester-covered rocks and one without in order to control for the feeding time of the stoneflies throughout the treatments. We placed the polyester from the treatment tanks into cups which we labeled by tank number and returned to later to check if any chironomids had been caught in the polyester during the initial count. We corrected the predator-induced mortality rates for natural mortality using the two corresponding control treatments. We used an ANOVA to determine whether any of the treatments were statistically different. Based on the results of the ANOVA we ran a Tukey-Kramer HSD to determine which treatment is statistically significant.

Results

Predator Refuge

When I removed the imitation *D. geminata* (polyester covered) rocks from the East River they had been colonized by an assortment of aquatic invertebrates including chironomids. The Predator refuge experiment showed that there is evidence that *Didymosphenia geminata* does provide a refuge for chironomids against the predator *Hesperoperla*. An ANOVA performed in JMP showed that there were statistically significant differences within the four treatments ($F < .0001$, $df=3$). The results of a Tukey-Kramer HSD Post Hoc test revealed that the mean amount of chironomids eaten in the no Didymo no Stonefly treatment (.667 +/- SD) was not statistically different from the mean amount of chironomids eaten in the Didymo no stonefly treatment (.167 +/- SD). This provided evidence that there was no significant difference in the

natural mortality of the chironomids in the control treatments (the treatments without predators). However, the mean amount of chironomids eaten in the Didymo Stonefly treatment (1.917 +/- SD) is statistically different than the amount eaten in the no Didymo Stonefly treatment (3.833 +/- SD). The amount of chironomids eaten in treatment tanks with a predator are significantly different between tanks with and without fake *D. geminata* ($\alpha = .05$).

Algae Removal

Manual removal of *Didymosphenia geminata* was an effective in creating differences in algal densities in between control and experimental plots. On July 3rd, before any algae had been experimentally removed from the stream bottom, the substrate of control and removal plots were covered in similar amounts of *D. geminata* ($P = .7401$). After only one week of algae removal, the experimental and control plots were significantly different in their algae content ($P = .0002$). Over the remaining duration of the experiment, removal of *D. geminata* from experimental plots continued to have a statistically significant effect on the amount of *D. geminata* present in the plots ($P < .0001$).

Invertebrate Abundance

The Hotelling's T2 test showed that there was no difference in the density of invertebrates collected from removal and non removal plots (309.73 insects/m² in control plots and 369.44 insects/m² in removal plots). Heptageniidae were found to be significantly more abundance where *D. geminata* was removed with densities of 20.8333 insects/m² in control plots and 42.5 insects/m² in removal plots ($P = .0564$). Chironimidae larva were found in high densities in plots where *D. geminata* had not removed and low densities where *D. geminata* had been removed (48.6111 insects/m² in control plots and 8.3333 insects/m² in removal plots, $P = .0507$). Perlidae hesperoperla, Hydropsychidae, Pteronarcyidae, and Rhyacophilidae were only found in plots where *D. geminata* was present, however the difference in abundances were not significant (5.5556 insects/m², 2.7778 insects/m², 1.3889 insects/m², and 2.0833 insects/m² respectively), while Elimidae was only present in plots where *D. geminata* had been removed (1.3889 insects/m²). Baetidae (68.0556 insects/m² in control plots and 83.333 insects/m² in removal plots), Chloroperlidae (8.333 insects/m² in

control plots and 15.74707 insects/m² in removal plots), Ephemerellidae (1.0417 insects/m² in control plots and 8.333 insects/m² in removal plots) did not demonstrate a significant difference in abundance between treatments, however did appear in higher densities in removal plots, while Nemouridae (9.7222 insects/m² in control plots and 1.3889 insects/m² in removal plots), Perlodidae (1.8519 insects/m² in control plots and 1.7361 insects/m² in removal plots), Simuliidae (4.8611 insects/m² in control plots and 2.7778 insects/m² in removal plots) were more abundant in the presence of *D. geminata*. Siphonuridae showed no difference in its abundance between plots (1.3889 insects/m² in control plots and 1.3889 insects/m² in removal plots).

Discussion

Our study focused on the effect of *Didymosphenia geminata* on stream invertebrate communities. We asked two questions in order to assess what role *D. geminata* plays in altering the dynamic of invertebrate communities, 1) Does *D. geminata* effect in vertebrate abundance and composition, 2) Does *D. geminata* provide a refuge for chironomids from stonefly predators. We asked question two based on previous studies that found a significant abundance of chironomids in habitats where *D. geminata* was present. The objective of was to find evidence for a possible explanation for the abundance of chironomids.

Our *D. geminata* removal experiment found that insects that are not affected by the blooms of *D. geminata* were predicted to be found in greater abundance in plots where *D. geminata* had not been removed. Chironomidae are very small, and probably not affected by *D. geminata*'s decreasing effect on mobility. Like Chironimidae, Baetidae are very small and strong swimmers who likely don't experience the effects of *D. geminata* on their mobility. Baetidae and Chironimidae probably find the stalks beneficial as refuge from predators, as confirmed in the Predator Refuge experiment, and possibly profit from *D. geminata* as a source of food as well. Flat-bodied Heptageniidae don't appear to find habitats where *D. geminata* is present as suitable as habitats where the algae are not present. Heptageniidae mayflies are also known as clinger nymphs. They have strong legs and typically inhabit turbulent waters, where they cling to the rocks on the stream bottom. It is possible that the substrate becomes unsuitable for the clinging nature of Heptageniidae when it is covered in the cotton like *Didymosphenia geminata*. Family Nemouridae demonstrated a trend that

suggested that this stonefly prefers habitats where *D. geminata* is present. Nemouridae are accustomed to habitats with slow- flowing waters and aquatic macrophytes that offer both food and shelter. It is possible that *D. geminata* alters the stream in a way that becomes suitable for the members of the Nemouridae family.

Our experimental data suggests that *D. geminata* does have an effect on the abundance and composition of the invertebrates in the habitat. Some invertebrates may find *D. geminata* to be a suitable and beneficial habitat while others may find *D. geminata* as a nuisance.

Invertebrates are an important food source for fish and birds, and other organisms. Their presence is not only important to the habitat in the stream, but outside of the stream as well. The presence of *D. geminata* seems to increase the abundance of small taxa, such as Chironomidae and Baetidae, and decrease the abundance of larger taxa, such as Chloroperlidae and Heptageniidae. A population composed mostly of small-bodied taxa may affect the abundance of fish and other organisms that consume invertebrates, in a way that small invertebrates are not as energetically efficient as larger invertebrates. Due to the spread of *D. geminata*, it is important to know and understand the impact that it has on an ecosystem in order to predict how it may alter ecosystems in the future.

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Figure Legend

Figure 1. An ANOVA followed by a multiple comparisons tested revealed that before any *D. geminata* was removed from the plots the amount of algae cover was not statistically different ($P=.7401$). When plots were sampled for invertebrates on 7/28/09 the amount of algae cover was statistically different in the removal and non removal plots ($P<.0001$). Points with the same letters were statistically similar in the amount of *D. geminata* cover.

Figure 2. Difference in density of 14 invertebrate families occur in different abundances where *D. geminata* is or is not present. The removal of *D. geminata* from the 8 treatment plots had an effect on the invertebrate composition and density. The data was natural log transformed.

Figure 3. The Predator refuge experiment showed that there is evidence that *Didymosphenia geminata* does provide a refuge for chironomids against the predator *Hesperoperla*. An ANOVA revealed significant differences among treatments ($P<.0001$). Tukey's posthoc showed treatments assigned the same letters are significantly similar ($\alpha=.05$).

Figure 1

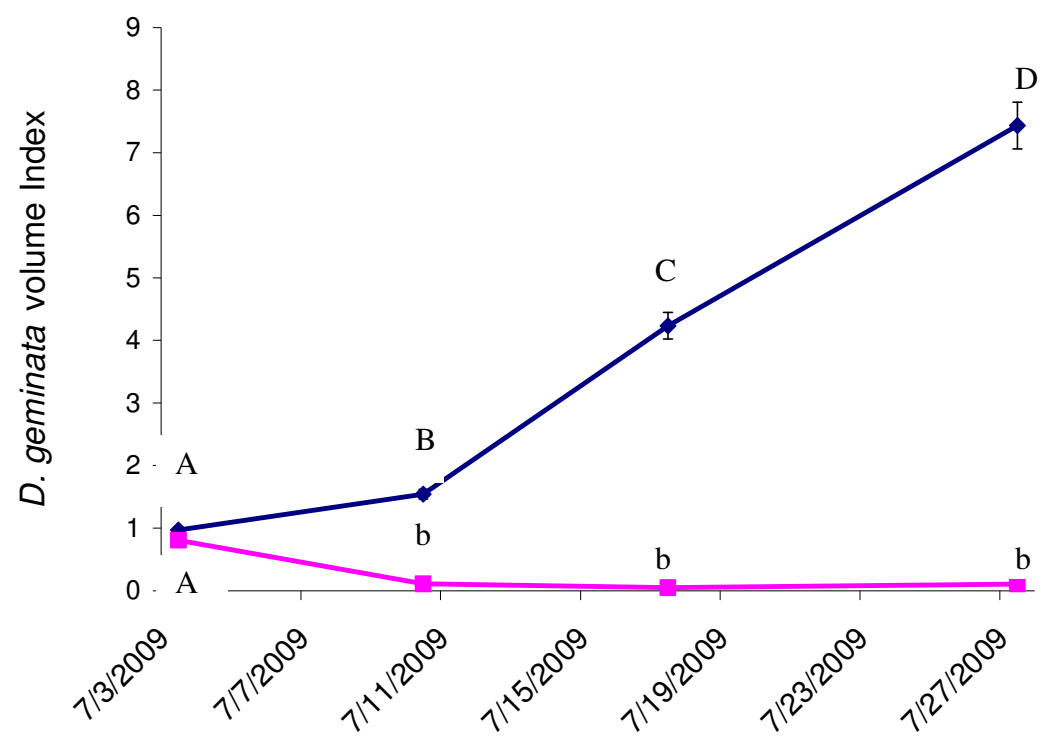


Figure 2

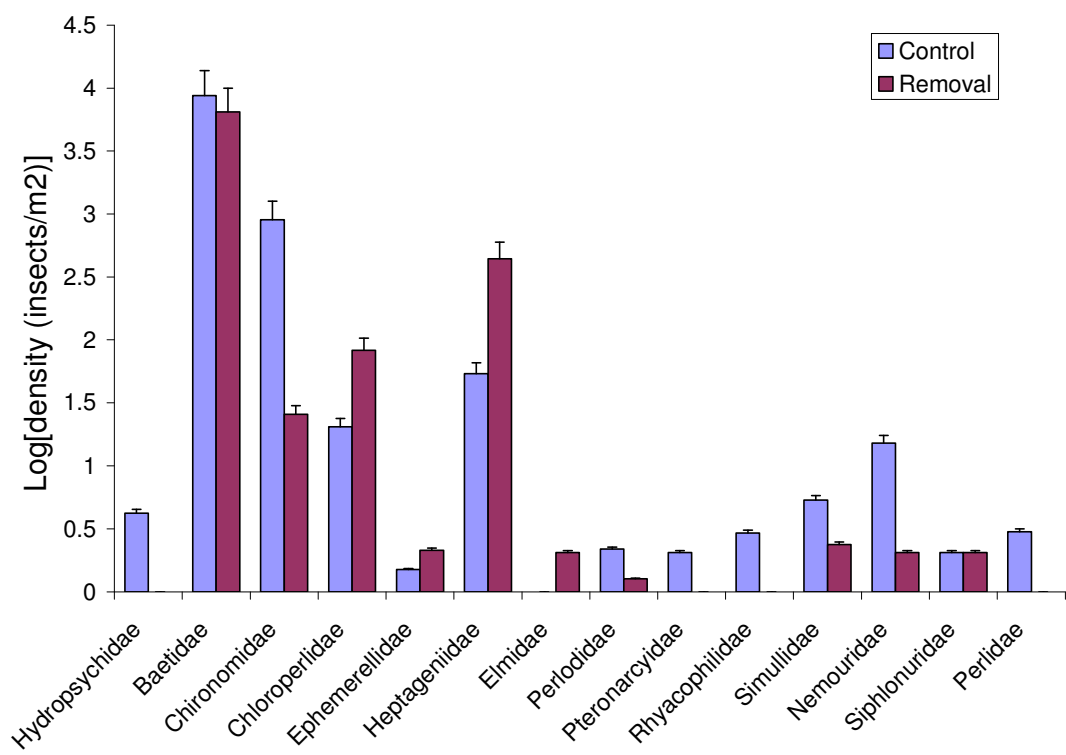
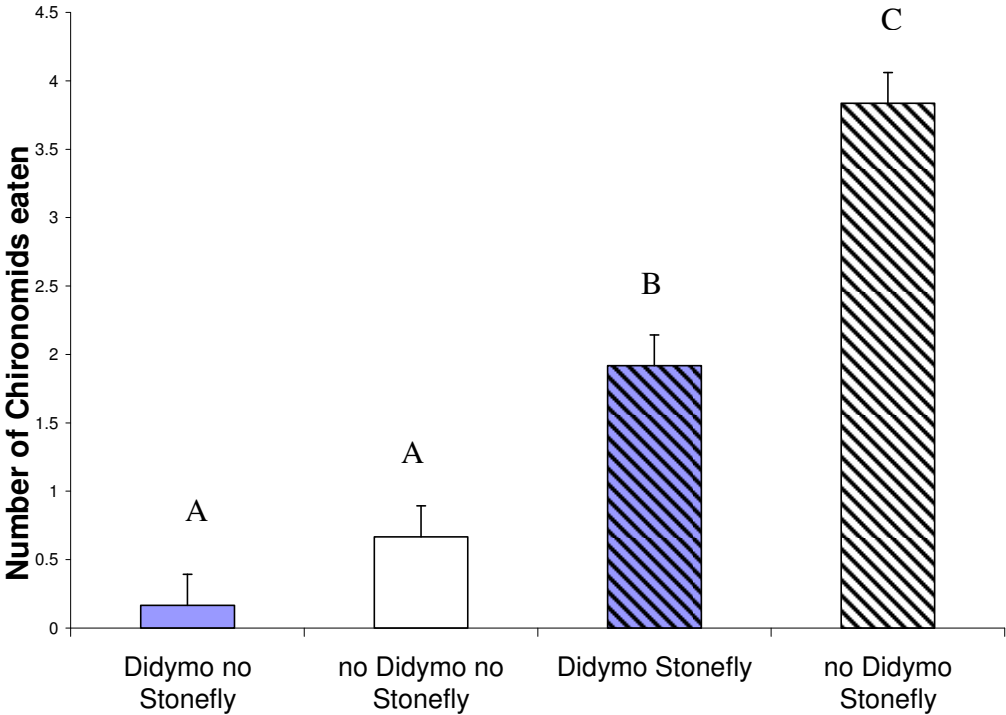


Figure 3



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