

The impact of *Didymosphenia geminata* on the community structures of invertebrates in streams
around the Rocky Mountain Biological Lab

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Abstract

The long-term relationships between animal and plant species and their relationships to their abiotic environment are closely tied to climate change. Stream macroinvertebrates are excellent indicators of freshwater conditions and respond measurably to environmental changes. Previous studies have observed that the Holarctic diatom *Didymosphenia geminata* (*Didymo*) exhibits nuisance growth patterns (putting out polysaccharide stalks, blanketing the bottoms of streams in thick algal mats), and proliferations vary with stream flow and nutrient composition (especially present in low-flow, low-phosphorus streams). As climate change accelerates weather extremes intensify and we see more low-flow years that exacerbate *D. geminata* blooms. *D. geminata* may affect the mobility and food acquisition of freshwater invertebrate indicator species. For 45 years a study of the streams around Rocky Mountain Biological Lab has recorded macroinvertebrate community composition as well as a number of abiotic factors, and more recently, *D. geminata* growth. This study looks at the relationships between macroinvertebrate community composition, specific families and *D. geminata* blooms during the 2020 season, and community composition and *D. geminata* data collected from summers 2015-2020 to explore relevant relationships. Questions addressed in this study include: 1) How does *D. geminata* affect community composition (focusing on community shifts); 2) What is the relationship between *D. geminata* growth and Chironomidae abundance; 3) What is the relationship between *D. geminata* growth and Heptageniidae abundance; and 4) What is the relationship between *D. geminata* growth and Baetidae abundance. Results show that proliferation of *D. geminata* causes significant shifts in the community composition of stream invertebrates; invertebrates in the family Chironomidae (Diptera) benefit from the *Didymo* mats as a refuge habitat within which they can forage on epiphytic diatoms and detritus; while Heptageniidae (Ephemeroptera), which require hard substrates to forage on diatoms in stream beds, are inhibited by it; free-swimming families like Baetidae (Ephemeroptera) are unaffected by the presence of these blooms. The impact of this diatom may reach across its native region and also into zones where it is considered invasive. *D. geminata* may be classified as an ecosystem engineer due to the physical alteration and invertebrate community shifts associated with it. These changes introduce ripple effects on other organisms like trout, which experience lower growth rates in streams dominated by low quality food (Chironomidae). As climate change accelerates and flow variability increases the drastic impacts of this nuisance diatom will in turn accelerate. Consequently, understanding the effects of this organism is key to understanding the impact of climate change on streams across the Holarctic zone.

Introduction

Climate change has complex implications for the community composition of ecosystems. As abiotic factors change ecosystems, flora changes directly affecting fauna (and visa-versa). In addition to overall warming, climate change also increases the severity and variability in weather events as well as flow conditions in streams. As winter precipitation shifts so does snow pack reducing potential snowmelt and decreasing the albedo (or reflective property) of surrounding areas increasing evaporation and lowering river flow (Milly and Dunne, 2020). For example, in Western Colorado we have noticed extreme variability in stream flow among years. Years with low streamflow (related to reduced precipitation from snow in the winter and therefore reduced snowmelt in the spring and summer) are associated with intense blooms of the of the freshwater nuisance diatom *Didymosphenia geminata* known more commonly as *Didymo* or “Rock Snot”. Low phosphorus conditions cause the diatom to produce long polysaccharide stalks in order to maximize nutrient gain (KilRoy and Bothwell 2012); and low water flow enables *Didymo* blooms to avoid the shear stresses of higher flow. These blooms may affect key invertebrate indicator species in affected streams.

Issues of biological diversity must be focused at every trophic level due to the complexity of the world’s ecosystems and the influence of trophic cascades (Polis et al. 2000). Emphasizing food webs over food chains, even a minor change in a subset of a community can trigger a trophic cascade (Polis et al. 2000). For example, invertebrate species affected by changes in growth form of *D. geminata* can affect fish communities feeding on those invertebrates; such ripple effects are indicative of complex and fragile trophic systems. Understanding the complex impacts of this nuisance diatom will enable us to understand not only the specific effects of its proliferation, but also the far-reaching consequences of climate change.

D. geminata, native to regions across the Holarctic zone dating back to the last glaciation, is a stalk-forming diatom that grows on stream bottoms (Bishop and Spaulding 2017; Taylor and Bothwell 2014). This once fairly rare species has popped up as a nuisance one since the 1990s in streams across the Holarctic and as an invasive one in various regions including New Zealand (Kilroy and Unwin 2011). *D. geminata* blooms are unique to other diatom blooms in their occurrence under oligotrophic rather than eutrophic conditions. While not fully understood, researchers theorize that *D. geminata* siphons energy to produce these stalks in order to increase overall nutrient acquisition in low-nutrient—specifically low phosphorous—conditions (Kilroy and Bothwell 2012; Bothwell, et al. 2014).

Another area of fascination with this particular diatom comes in its highly transportable nature. There is a strong correlation between 1990’s *D. geminata* blooms and increased fishing as well as the introduction of felt-soled boots in British Columbia (Bothwell, et al. 2009). Additionally, *D. geminata* can be spread with the introduction of just one cell (Chau and Spaulding 2015). *D. geminata*’s easy spread, and nuisance and invasive blooms may damage invertebrate communities.

For decades scientists have used indicator species to evaluate the health of ecosystems. Specifically, in stream ecosystems, macroinvertebrates play a key indicator role due to their high diversity and variability among species in their vulnerability to disturbance. By studying the community composition of invertebrates and investigating changes we can detect the health of streams. Macroinvertebrate consumers occupy an intermediate position in the food webs of stream ecosystems and are thereby key to ecosystem function, because they are affected by both

top-down and bottom-up forces (Wallace and Webster 1996). Generally, grazers demonstrate a positive relationship with increased algal growth (Wallace and Webster 1996). However, the method of feeding and movement of these groups of invertebrates may explain their more complicated relationship with *D. geminata* algal blooms. Our study of the effects of this diatom focused on its nuisance growth in streams around RMBL and the consequences for community composition of invertebrates.

For the past 45 years researchers collaborating with Dr. Bobbi Peckarsky at the Rocky Mountain Biological Lab (RMBL) have conducted a study of the community composition of invertebrate species in the surrounding streams to monitor the conditions in streams protected by non-consumptive water rights (NCWR) obtained by the RMBL in 1976. Dr. Brad Taylor and his students have more recently compiled data on the presence of *D. geminata* in streams near RMBL. In addition, surveys of *D. geminata* have been added to the NCWR sampling protocol for the past eight years. We have anecdotally noticed an increase in Chironomidae family in the presence of *D. geminata* blooms and a decline in surface crawling species such as the mayfly family Heptageniidae. Interestingly, in contrast, the mayfly family Baetidae seems relatively unaffected by the presence of *D. geminata*. In this study I formally investigated these observed differences and generated the following explanative hypotheses: Increases in *D. geminata* proliferations will be associated with:

- 1) shifts in invertebrate community composition,
- 2) increases in Chironomidae abundance,
- 3) decreases in Heptageniidae abundance,
- 4) no significant effect on Baetidae abundance.

Methods

Study System

We conducted this study in three streams/rivers surrounding the Rocky Mountain Biological Lab; Copper Creek (Second Crossing, above Judd Falls, above the bridge in Gothic), East River (Oregon Mine Road Crossing, near Avery, near Levi Cabin, and above the bridge south of Gothic) and Rustler's Gulch (above the confluence with the East River) — across an elevation gradient of ~2,800m to ~3,200m (Figure 1). These sites have been sampled since 1976 as part of a long-term data set focused on the community composition of invertebrate species. These sites are sampled twice a summer (early and late in the season) to capture species with different seasonal phenologies.

The most abundant mayflies that exist as larvae in streams near RMBL are Heptageniidae and Baetidae. Heptageniidae move laterally across stream substrate and prefer smooth, flat rocks. Specifically, the species *Epeorus* prefer smooth slate rocks for foraging and both *Cinygmula* and *Epeorus* prefer the undersides of rocks (Peckarsky and Cowan 1995). Because of the morphology of these species, understory algae are more vulnerable to mayfly consumption than overstory algae (Chuang et al. 2014). Therefore, they may be inhibited by the dense *D. geminata* stalks. In contrast, Baetidae—a free swimming family of grazing mayflies—have not been observed to be negatively affected by *D. geminata* blooms, because they can climb on the stalks and consume epiphytic diatoms. However, Baetidae exhibit positive relationships with

periphyton (Richards and Minshall 1988). Given this natural history information, we expected a decrease in Heptageniidae and no effect on Baetidae with increased *D. geminata*.

The family Chironomidae represent a group of thousands of species of true flies (Diptera) that inhabit freshwater lakes and streams in their larval stage (Armitage et al., 1995). Chironomidae are poor swimmers and may seek refuge from flow in the *D. geminata* mats; so, we expected an increase in Chironomidae abundance with increased *D. geminata*.

Samples/data collection

We took four samples of invertebrates at every site twice throughout the summer using a Hoffman Sampler—a 0.104 m² area metal box open on two sides, with stainless-steel mesh upstream and a 364 μ m mesh drift-net fastened to the downstream opening. We disturbed the substrate inside the sampler for one minute allowing resident invertebrates to drift into the net. We then counted and identified every invertebrate present in the sample in the field and released them back into the stream.

We estimated the biomass of *D. geminata* at two scales: within each Hoffman sample, and along three transects at each site. For the per sample scale, we placed an aquagoogie™—a glass-bottomed viewing box—inside each Hoffman sample and estimated the biomass of *Didymo* before taking the invertebrate samples. For the whole site estimates we stretched a meter tape across the length of the stream. At every half meter we used the aquagoogie to estimate the percent *D. geminata* coverage at that spot. At both scales we measured the height of the *D. geminata* and used mean of the products of the percent cover x the height (mm) to obtain a standing crop index. This standing crop index is directly correlated to overall biomass and represents a sufficient metric (Brad Taylor, personal communication).

Statistical Approach

To analyze shifts in community composition I used a Permutational Multivariate Analysis of Variance or PERMANOVA to test if there was a significant effect of *D. geminata* on the community composition of invertebrates. I applied this analysis on two scales: the microhabitat (per sample), short-term scale of *D. geminata* data taken in 2020 and the macrohabitat (per site) long-term scale across 5 years of data (2015, 2017, 2018, 2019, and 2020). As compared to other permutational methods PERMANOVA is more powerful for detecting changes in community structure (Anderson and Walsh, 2013). To visualize the effects of *D. geminata* on invertebrate community structure I constructed a Non-metric multidimensional scaling (NMDS) ordination plot.

To better understand the underlying mechanisms of observed shifts in community structure, I used generalized linear mixed-effects models to evaluate the relationships between *D. geminata* and abundance of specific invertebrate taxa across 5 years of invertebrate and *D. geminata* data (at the macrohabitat scale of sites, using mean *D. geminata* standing crop index data from three transects). Responses of taxa to *D. geminata* biomass were tested at the family level, which was the finest level of resolution practical for Chironomidae. As the response variables are count data, and previous years indicate a high proportion of zeros, the distribution of the data did not fit a normal or a Poisson distribution. Using Akaike information criterion (AIC) to compare models with Poisson and negative binomial assumptions, a negative binomial

(glmer.nb) was the best fit for these data. Each final model includes a random effect for site and year to avoid issues created by the similarity of samples taken at the same site within the same year. When conducting this analysis at the macrohabitat scale (data = macrohabitat), we summed the samples taken at the same site on the same day to come up with a density (per 0.416m² area); we did this to avoid issues of pseudoreplication, while capturing the intra-site variability in distribution of invertebrates in these heterogeneous streams.

To evaluate the significance of the effect of *D. geminata* on Heptageniidae abundance I constructed Model 1:

```
Hept.glmer.nb <- glmer.nb(f.heptageniidae ~ scale(Standing_crop_index) +  
(1|Site) + (1|Year))
```

To evaluate the significance of the effect of *D. geminata* on Chironomidae abundance I constructed Model 2:

```
Chiro.glmer.nb <- glmer.nb(f.Chironomidae ~ scale(Standing_crop_index) +  
(1|Site) + (1|Year))
```

To evaluate the significance of the effect of *D. geminata* on Baetidae abundance I constructed Model 3:

```
Baetidae.glmer.nb <- glmer.nb(f.baetidae ~ scale(Standing_crop_index) + (1|Site)  
+ (1|Year))
```

All analyses were conducted using an RMarkdown file in R Studio Pro (version 1.3.1056-1) using R (version 4.0.2).

Results

Community Shifts

Microhabitat/Short-term Scale (2020 samples)

There was a significant effect of *D. geminata* on invertebrate community structure as well as a significant interaction with Site. The site interaction indicates that the impact of *D. geminata* depends on the site being evaluated, because the community structures of different sites shift differently as *D. geminata* increases. The combined R-squared value of *D. geminata* and the *D. geminata*:Site interaction is 0.24952 (*D. geminata*, 0.14344 and Site: *D. geminata*, 0.10608). This value indicates that approximately 25% of the variation in community structure can be explained by *D. geminata* and by the interaction of *D. geminata* at the site level.

The NMDS plot (Figure 2), illustrates that *D. geminata* aligns with axis NMDS 1 such that communities that have low *D. geminata* are on the right and as you move left communities increase in *D. geminata*. This plot indicates that *D. geminata* is a fairly strong variate as you move across multidimensional space.

Macrohabitat/Long-term Scale (multiple year sites)

I also observed a significant effect of *D. geminata* on invertebrate community structure at the macrohabitat/long-term scale with an R-squared value of 0.05418. This result indicates that

approximately 5% in the variation in community structure can be explained by *D. geminata* alone. On the macrohabitat/long-term scale we saw no significant interaction of site.

Similarly, the NMDS plot (Figure 3) illustrates that *D. geminata* aligns with axis NMDS1 such that the affinity of communities for *D. geminata* increases as you move from right to left across the plot. Again, *D. geminata* is a fairly strong variate as you move across multidimensional space.

Mechanisms explaining effects of D. geminata on Invertebrate Community Structure

I evaluated the impact of *D. geminata* on the macrohabitat/long-term scale using 5 years of data. Analysis of the effects of *Didymo* on specific invertebrate families showed a significantly lower Heptageniidae abundance at sites with high *Didymosphenia geminata* (Figure 4). For every one standard deviation increase in scaled standing crop index of *D. geminata* the log-number of Heptageniidae decreased by 0.19705 ± 0.06829 ($P < 0.01$). The line predicted by model 1 is illustrated on the plot.

In contrast, there is significantly higher Chironomidae abundance at sites with high *Didymosphenia geminata* (Figure 5). For every one standard deviation increase in scaled standing crop index of *D. geminata* the log-number of Chironomidae increased by about 0.2269 ± 0.1125 ($P < 0.05$). The line predicted by model 2 is illustrated on the plot.

Finally, there is no significant relationship between scale standing crop index and Baetidae abundance (beta: -0.01790 ± 0.07079 , $p: 0.8$), indicating that no significant correlation between *Didymosphenia geminata* and Baetidae abundance (Figure 6). In this case I did not include a trend line because this trend was not significant.

Discussion

I observed significant shifts in overall community structure as *D. geminata* increased on both a microhabitat, short-term scale and a macrohabitat, long-term scale, such that communities with high *D. geminata* standing crop index were alike and communities with low *D. geminata* were alike. I also observed significant shifts in specific taxa as *D. geminata* increased: Heptageniidae decreased as *D. geminata* proliferated and Chironomidae increased as *D. geminata* biomass increased. Below I elaborate on these major findings and compare them to other studies.

On both a macrohabitat/long-term and microhabitat/short-term scales, significant shifts in community structure as *D. geminata* biomass increased are consistent with our hypothesis and other studies of the effects of *D. geminata* blooms. For example, a study of invasive *D. geminata* in New Zealand concluded that *D. geminata* blooms decreased the overall diversity of invertebrate communities as a consequence of inhibition of their movement (Ladrera et al. 2018). Here, further analyses of specific taxa contributes to our understanding of the mechanisms causing observed shifts in invertebrate community structure.

First, consistent with our hypothesis there was a significant decline in Heptageniidae abundance as *D. geminata* biomass increased. Heptageniidae use a variety of mechanisms to feed on benthic algae, but generally require smooth, hard substrates to forage effectively (McShaffrey and McCafferty 1998; Peckarsky and Cowan 1995). Heptageniidae of the genus *Rhithrogena* (Chuang et al. 2014) move laterally across stream bottoms and may be inhibited by *D. geminata*

blooms potentially explaining the observed negative relationship between *D. geminata* blooms and these mayflies.

In contrast, there was no significant effect on Baetidae as *D. geminata* biomass increased, which is also consistent with our hypothesis. Baetidae is another family of grazing mayflies; however, they are highly mobile, more flexible feeders and are most affected in their foraging and dispersal behavior by the presence of predators (Alvarez and Peckarsky 2014; McIntosh et al. 2002; Peckarsky and Cowan 1995). Specifically, Baetidae are most abundant on rocks with high periphyton abundance (Richards and Minshall 1988). Baetidae are also excellent swimmers overall and their mobility improves exponentially as they grow (Kutash and Craig 1998). These attributes enable Baetidae to feed on epiphytic diatoms that grow on *D. geminata* stalks; so they are not likely to be harmed by its presence, nor to seek refuge from flow in its mats, providing a parsimonious explanation why Baetidae are unaffected by the presence of *D. geminata*.

Finally, we observed a significant increase in Chironomidae abundance as *D. geminata* increased, also consistent with our hypothesis. Chironomidae, though they exhibit a number of different feeding modes, most commonly act as collector-gatherers (Armitage et al., 1995). This family of invertebrates show high diversity in their dietary habits and nutritional needs and variable functional feeding habits may indicate the flexibility of Chironomidae species (Armitage et al. 1995). Additionally, Chironomidae are poor swimmers, incapable of directional movement, and are very much at the whim of the stream flow (Armitage et al. 1995). They, therefore, may benefit from the dense stalks of *D. geminata* blooms which may provide them a refuge from streamflow.

The impact of *D. geminata* on invertebrate communities has notable ripple effects that further affect vertebrate communities in streams with dense proliferations. For example, as *D. geminata* increases in abundance, brook trout growth rate is reduced by 44%, as a consequence of a significant decline in prey caloric value (Taylor, unpublished data). Another indirect effect is a rise in the prevalence of whirling disease in trout in streams with *D. geminata* blooms, caused by an increase in oligochaete density, the intermediate host species of the parasite causing the disease (Taylor, unpublished data).

Ecosystem engineers are organisms that drastically and obviously alter their habitat resulting in consequences to other organisms. Ecosystem engineers traditionally include examples of behavioral and consumptive modification of the environment: like beavers (Naiman et al. 1986), earthworms (Darwin 1882; Ransom 2010) and woodpeckers (Jusino et al. 2016). Recently consumers, like detritivorous fish who significantly alter the substrate of streams have been classified as ecosystem engineers (Flecker 1996). What all of these ecosystem engineers have in common is a physical alteration of their habitat resulting in significant alterations in community structures. *D. geminata* significantly alters stream flow: increasing shear-stress just above the mats and decreasing velocity within mats (Larned et al. 2011), and qualitatively changing the substrate available for grazing invertebrates from smooth rocks with a thin layer of diatoms to thick mats of entangled polysaccharide stalks, which affects ability of macroinvertebrates to forage, both positively and negatively. We contend that the combination of those physical alterations and the resultant significant shifts in invertebrate community structures with consequences to trout growth and disease prevalence observed as *D. geminata* proliferates qualifies it to be considered an ecosystem engineer.

Finally, the impact of this diatom is not limited to the streams in the RMBL basin; we should see similar effects as *D. geminata* blooms across its native Holarctic zone (Whitton et al. 2009, figure 3). *D. geminata* also has been introduced to non-native regions and has exhibited

similar, but invasive, blooms changing communities in streams in New Zealand (Kilroy and Bothwell 2012; Ladrera et al. 2018) and in certain regions of Quebec (Gillis and Chalifour 2009). With climate change increasing the variability of weather events—importantly, the increase in variability of winter precipitation—streamflow will continue to vary drastically across years. And the directional trend toward warming and drying globally should result in extremely low flow occurring more frequently and result in increases of proliferation of *D. geminata*. Therefore, the ripple effects of physical alterations on invertebrates and other organisms like trout could result in streams as we know them being completely altered by the introduction and proliferation of *D. geminata*.

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Figures



Figure 1: Map of the eight sites sampled. Sites indicated with pink polygon. Eight sites distributed across three rivers: Copper Creek (Second Crossing, above Judd Falls, above the bridge in Gothic), East River (Oregon Mine Road Crossing, near Avery, near Levi Cabin, and above the bridge south of Gothic) and Rustler's Gulch (above the confluence with the East River).

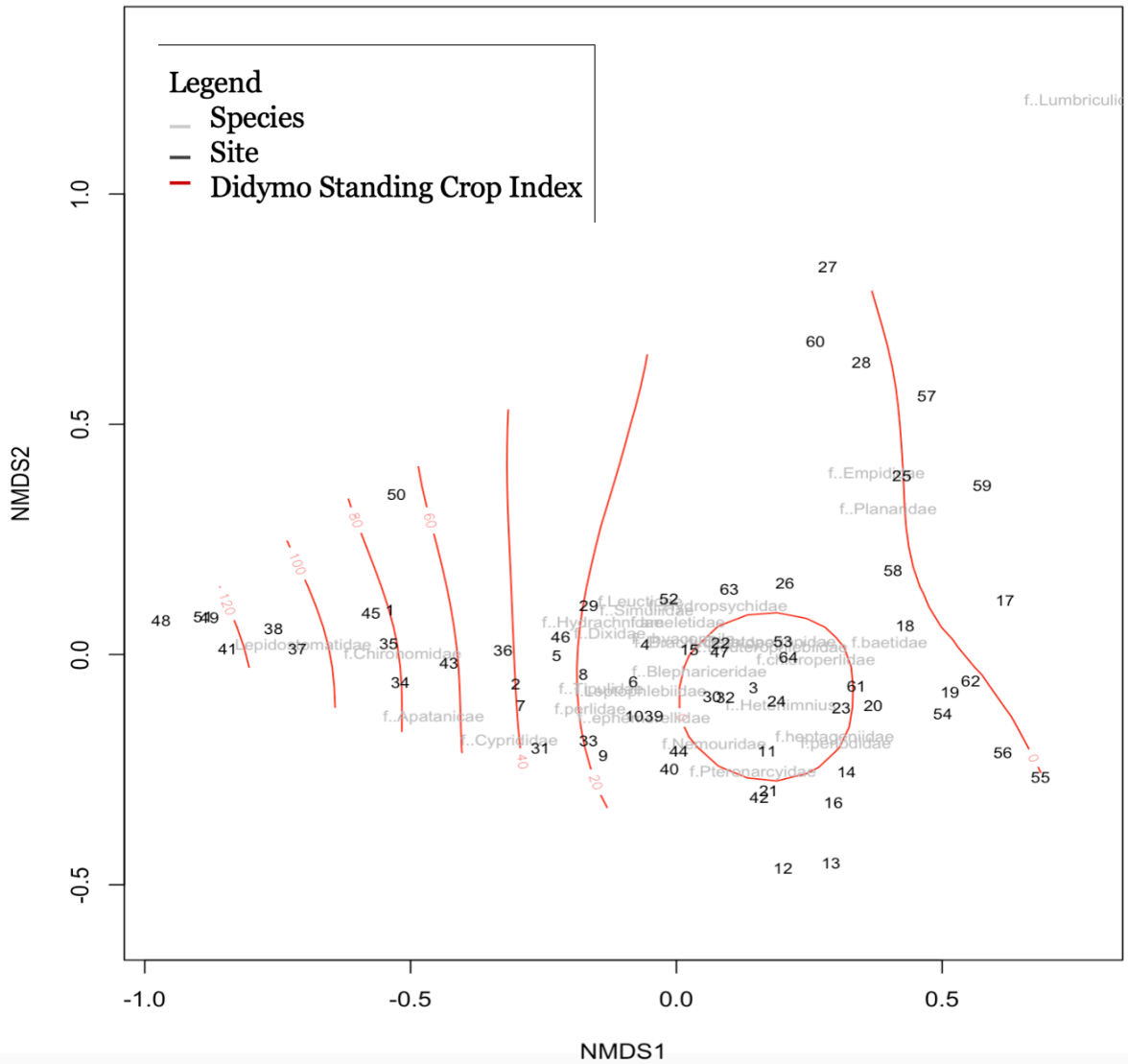


Figure 2: NMDS plot of community structures of stream invertebrates on the microhabitat/short-term scale. Red contour lines indicate increasing *D. geminata* as the graph moves right to left.

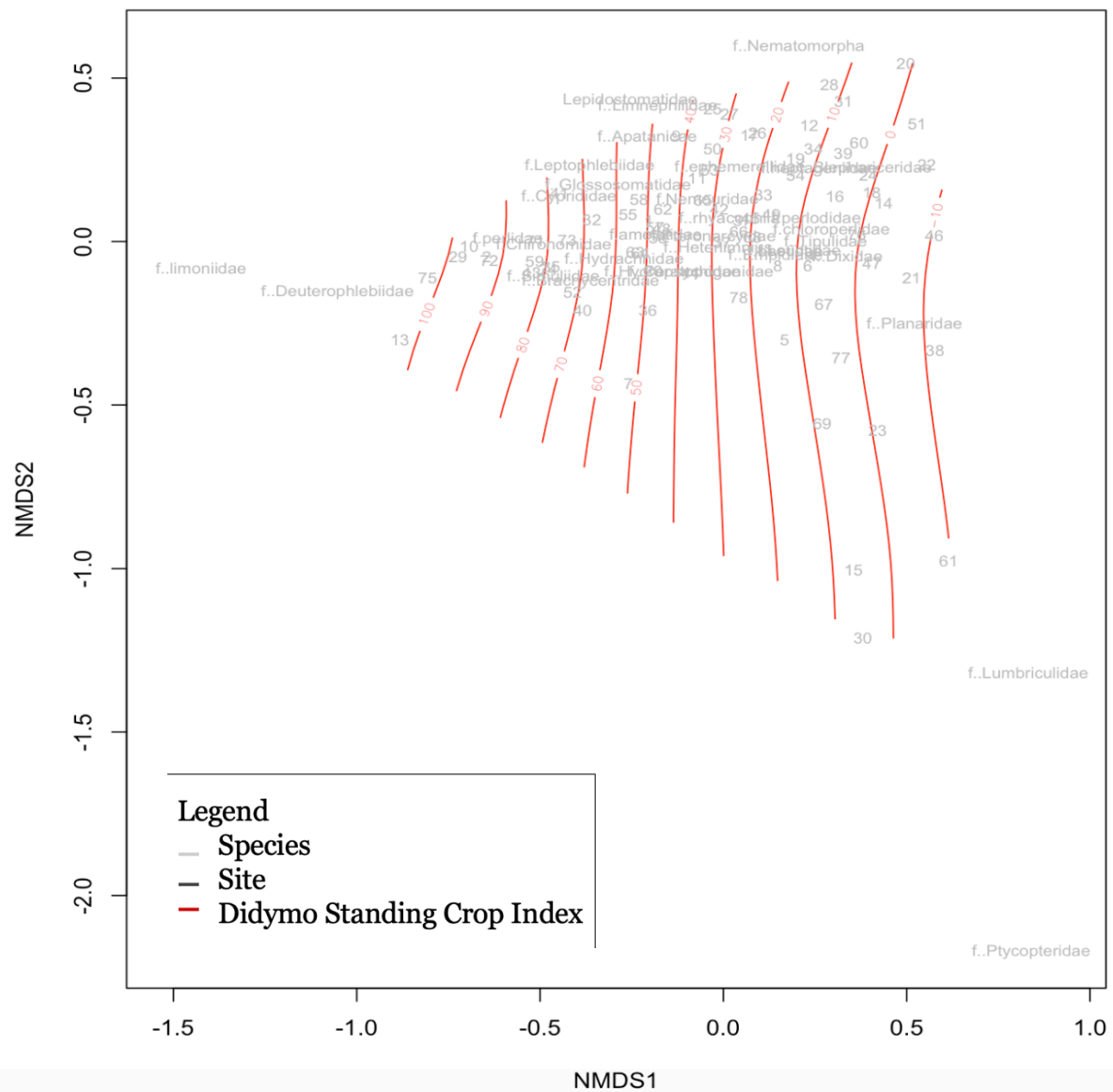


Figure 3: NMDS plot of community structures of stream invertebrates on the macrohabitat/long-term scale. Red contour lines indicate increasing *D. geminata* as the graph moves right to left.

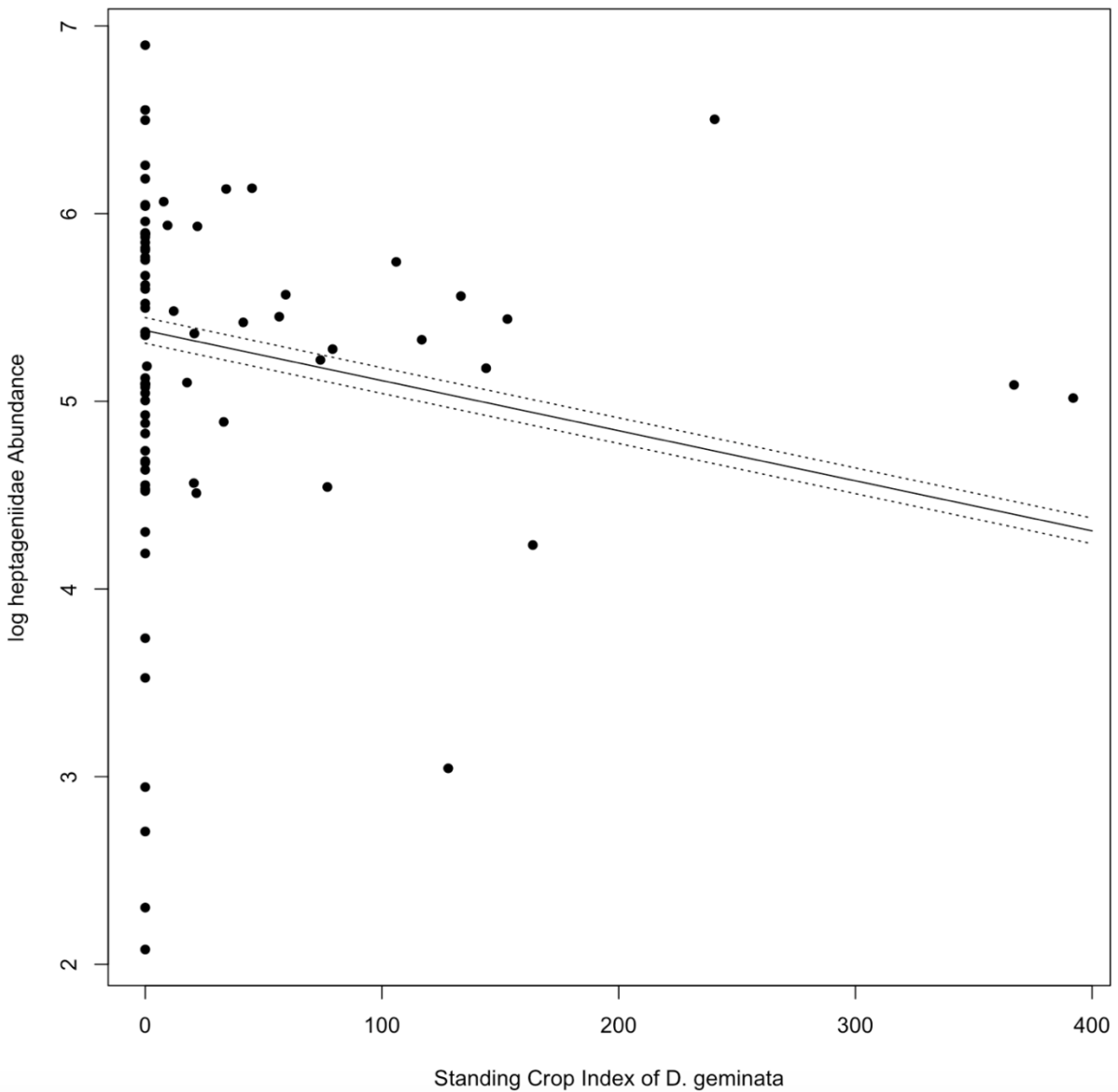


Figure 4: Relationship between standing crop index of *D. geminata* and log Heptageniidae abundance. According to the generalized linear mixed-effects model there is a significant correlation between *D. geminata* and log Heptageniidae abundance such that Heptageniidae abundance decreases in response to *D. geminata* (beta: -0.19705 ± 0.06829 , P: 0.00391) standard error indicated on the graph by dotted lines.

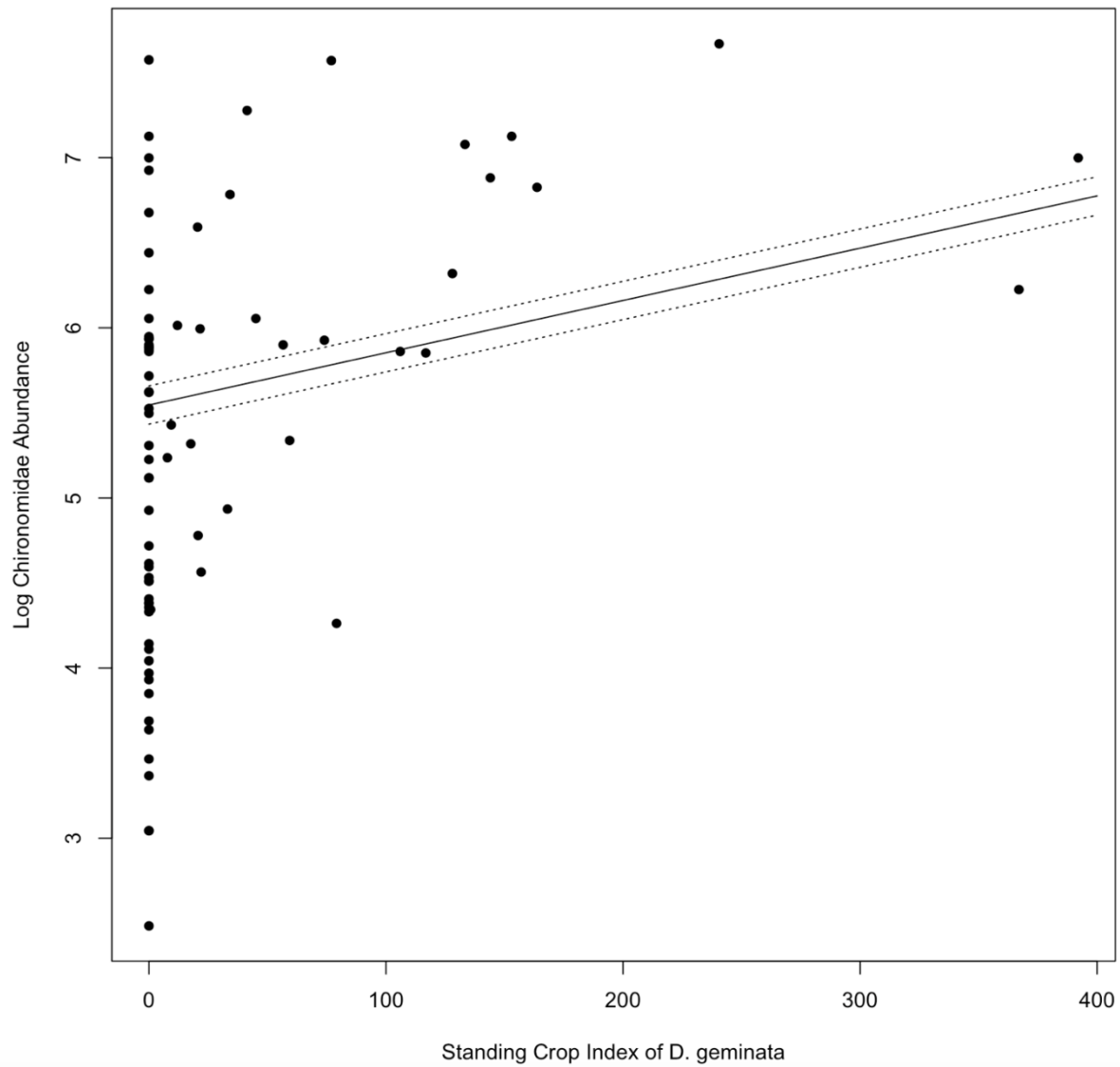


Figure 5: Relationship between standing crop index of *D. geminata* and log Chironomidae abundance. According to the generalized linear mixed-effects model there is a significant correlation between *D. geminata* and log Heptageniidae abundance such that Heptageniidae abundance increases in response to *D. geminata* (beta: 0.2269+/- 0.1125, P: 0.0437) standard error indicated on the graph by dotted lines.

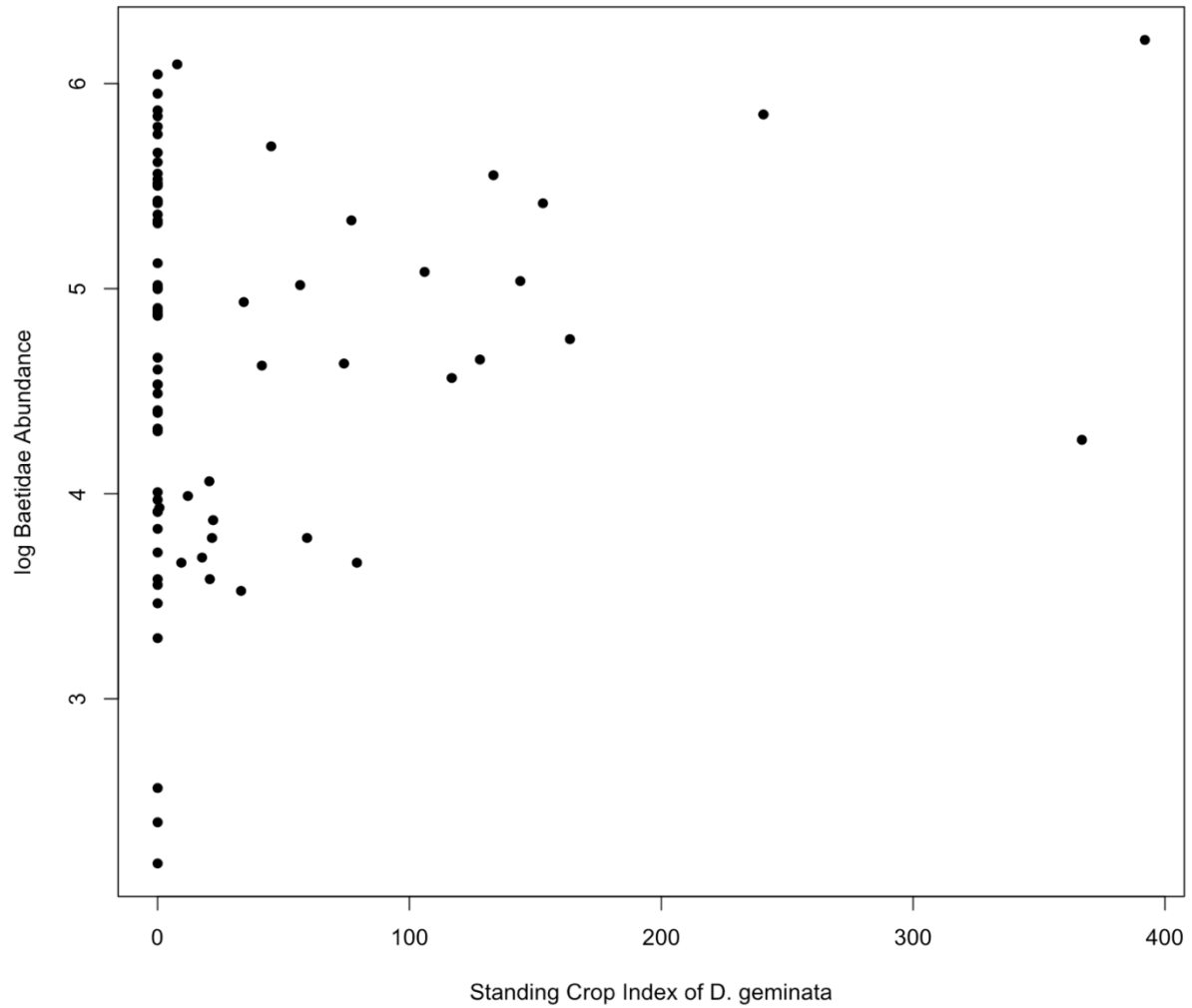


Figure 6: Relationship between standing crop index of *D. geminata* and log Baetidae abundance. According to the generalized linear mixed-effects model the relationship between standing crop index of *D. geminata* and Chironomidae abundance shows a nonsignificant negative trend (beta: -0.01790 ± 0.07079 , P: 0.8).