

**Direct and Indirect Effects of Nuisance Blooms by the Diatom, *Didymosphenia geminata* on
the Whirling Disease Intermediate Host, *Tubifex tubifex***

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Abstract

Didymosphenia geminata, a stalk-producing diatom has increased in frequency worldwide. The nuisance stalks that *D. geminata* forms, may create an environment preferable to oligochaetes such as *Tubifex tubifex*. Previous studies have shown that oligochaetes abundance is higher in areas with higher percent cover of *D. geminata*. This research investigated the mechanisms by which nuisance blooms by the stalk-forming diatom *D. geminata* facilitate a host, *Tubifex tubifex*, of the parasite, *Myxobolus cerebralis*, that causes whirling disease in ecologically and economically important salmonid fishes. To test the hypotheses that *D. geminata* may be providing *T. tubifex* a refuge from predation by the stone fly *Hesperoperla pacifica*, I performed an experiment testing whether a mimic of *D. geminata* mats provides *T. tubifex* a refuge from predation by the stonefly *Hesperoperla pacifica*. The results revealed that predation was 100% lower in *D. geminata* mimic treatments. Implications of this research may help in the management of salmonid populations at risk for whirling disease by avoiding stocking in streams with *D. geminata* mats.

Introduction

Didymosphenia geminata, a stalk-producing diatom has increased in frequency worldwide. Nuisance blooms of this algae have been reported in North America, Europe, Asia, and New Zealand and it is believed to be expanding its geographic range in North America where little research has yet to be conducted (Spaulding 2007). These single-celled algal blooms result from excessive extracellular stalk production by individual cells that form a contiguous mat covering the stream bottom (Spaulding 2007). *D. geminata* is now causing concern because of the possible impacts on rivers where blooms occur and the salmonid fisheries in these rivers.

D. geminata blooms have the potential to alter stream ecosystems by altering the ecosystems metabolism, nutrient cycling, hydraulics and food web (Spaulding 2007). The thick mats (e.g., 2-5 cm) that can cover much of the streambed lead to changes in invertebrate species diversity and population sizes that may propagate up the food web to affect fish populations (Spaulding 2007).

T. tubifex, an aquatic oligochaete tubificid worm is more abundant in streams with *D. geminata* mats (Kilroy et al. 2009), and *T. tubifex* is a key host in the two stage life cycle of *Myxobolus cerebralis*, a myxozoan parasite that causes whirling disease in salmonid populations in the United States (Gilbert 2003). Moreover, brook trout in streams with *D. geminata* blooms have higher *M. cerebralis* prevalence than brook trout in streams without blooms (Taylor unpublished data).

Whirling disease is spread by the death of infected salmonids near susceptible *T. tubifex* and salmonids and it has been found that the death of just one infected salmonid upstream is sufficient for establishment of *M. cerebralis* in a stream (Hallett 2007). The effects from *M.*

cerebralis and *T. tubifex* have the potential to affect aquatic community structure by altering the outcome of interactions among the hosts and other organisms (Minchella 1991). McGinnis, 2013 found that the oligochaetes prefer slow-moving water velocities and areas with deposits of fine particles, which is the type of habitat that *D. geminata* mats create in streams that usually lack these types of habitat.

Whirling disease is on the rise since its introduction in the United States in 1958 and is a health problem both in fisheries and in wild populations of salmonids (Gilbert 2003). While much is already known about *M. cerebralis* and its salmonid host, less is known about *M. cerebralis* and its *T. tubifex* host (Gilbert 2003). For example, many environmental variables including substrate properties can influence the habitat selection by tubificids, and studies on the influence of substrate properties on habitat selection by tubificids have produced conflicting results (McMurtry 1983). This research project tested hypotheses that investigated why oligochaetes are more abundant in streams with *D. geminata* blooms and the mechanisms by which mats of *D. geminata* facilitate a host, *Tubifex tubifex*, of the parasite, *Myxobolus cerebralis*, that causes whirling disease in ecologically and economically important salmonid fishes.

To understand why oligochaetes are more abundant in streams with *D. geminata* blooms, I experimentally tested the hypothesis that the long filaments, or stalks that *D. geminata* produces may provide a refuge for oligochaetes against predatory invertebrates (Taylor unpublished data). With this, I hypothesized that oligochaete mortality from invertebrate predators would be lower when *D. geminata* stalks are more abundant.

In Colorado, recreational fishing generates millions of dollars in economic activity each year, and since whirling disease has been introduced some locations in Colorado (e.g., Gunnison River) have experienced declines in salmonid density and biomass by as much as 90% (Nehring, 2003; Nehring, 2006). Furthermore, knowing more about the interactions between this disease and *D. geminata* will help in the management of threatened populations of salmonids, such as directing stocking efforts away from streams with *D. geminata* mats.

Study Organisms

This study involved interactions among the algae, *D. geminata*, the oligochaete worm *T. tubifex*, and the parasite *M. cerebralis* which is the causative agent of whirling disease in salmonid fishes. *M. cerebralis* has a complex life cycle with two hosts and two intermediary spore stages (Kerans 2002). *T. tubifex* distribution is strongly correlated to the distribution of leaf litter (Lazim 1987) and the deposition of fine particles (McMurtry et al. 1983; Bartholomew et al. 2005). Thus, *D. geminata* has the potential to increase the available habitat for oligochaetes such as *T. Tubifex*, and thereby increase the prevalence of *M. cerebralis*.

Myxobolus cerebralis-

The life cycle of *M. cerebralis* starts when myxospores are released from infected fish (Fig. 1). Next, myxospores must be ingested by *T. tubifex*. The germ cell in the myxospore then migrates to the intercellular space of the intestinal epithelium where it undergoes reproduction and development into a triactinomyxon (TAM) over a (70-120 day period). TAMs are released from feces of *T. tubifex* and then enter the water column. TAMs are short lived and attach to fish and penetrate the skin. Once in the fish, reproduction takes place in the epidermis and the

parasite migrate through central nervous system to the associated cartridge where they mature to plasmodia containing vegetative nuclei and generative cells. Approximately 80 days after TAM exposure, the generative cells initiate sporogenesis to produce myxospores at which stage whirling disease manifested. Importantly, the only way fish can become infected is if *T. tubifex* ingest the myxospores and shed the TAMs (Gilbert 2003).

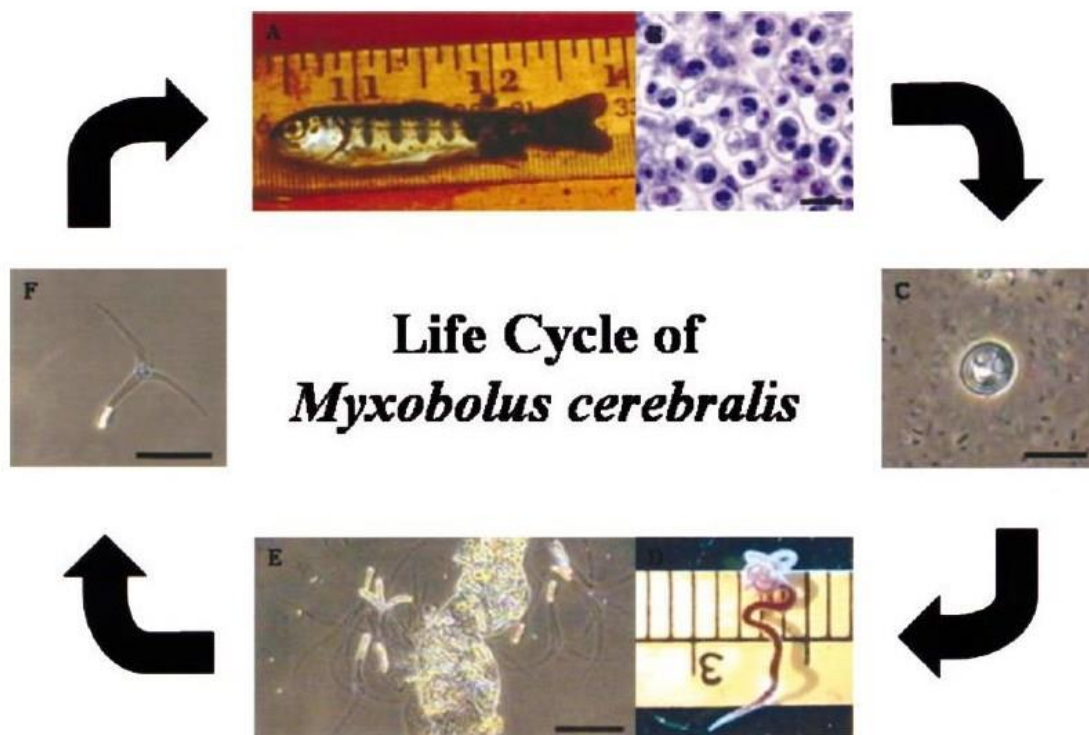


Figure 1. Life cycle of *M. cerebralis* from Gilbert, 2003.

***Tubifex tubifex*-**

T. tubifex is a habitat generalist and is extremely tolerant of a wide range of environmental conditions (Kerans 2002). McMurtry 1983 found a significant correlation between abundance of heterotrophic aerobic bacteria in sediment and tubificid preference and

believes worms were attracted to the leaves because of the microfloras associated and provided bacterial food. *T. tubifex* prefers silt and clay substratum to coarser substratum and have been found to reproduce faster in silt sediment than other substrates (Arndt. Et al, 2002), and *D. geminata* traps these fine sediments. Lemmon and Kerans, 2001 found that finer sediments hold *M. cerebralis* better than courser particles which facilitate their consumption by *T. tubifex*. This illustrates that the environmental effects on *T. tubifex* abundance and the distribution of *M. cerebralis* may also explain the relationship between infected *T. tubifex* and fine sediments (Kreuger 2006).

Didymosphenia geminata-

D. geminata forms thick mats that cover >75% of the stream area and extend for 1 km and may persist for several months of the year (Spaulding 2007). *D. geminata* cells produce copious amounts of extracellular stalk material that form thick benthic mats, or blooms. *D. geminata* thrives in a wide range of conditions such as both low and high nutrient lakes and slow moving shallow water to waters with greater depth and increased flow throughout various areas of the United States has also expanded its geographic range within North America and Europe and has even invaded New Zealand (Figure 2). *D. geminata* mats alter the water velocity along the stream bottom in ways that may be important to invertebrates such as oligochaetes that prefer slow-moving water and areas with stable sediments (Larned 2011). Hiner, 2011 found that water flow rate has an effect on the propagation of *M. cerebralis*, and habitats with lower velocity were found to promote higher prevalence of infection and greater proliferation of its invertebrate host leading to greater severity of infection in fish (Hallett 2007). *D. geminata* may also provide a food source for *T. tubifex* by trapping particulate organic matter that is readily colonized by

bacteria and *D. geminata* mats may also provide refuge for *T. tubifex* from invertebrate predators by seeking protection in the long stalks that *D. geminata* produces. *D. geminata* is a good resource for *T. tubifex* due to their preference for fine silt and clay substratum (i.e., depositional areas) that are areas with abundant microflora, which are a source of bacterial food for oligochaetes (Kreuger 2006).

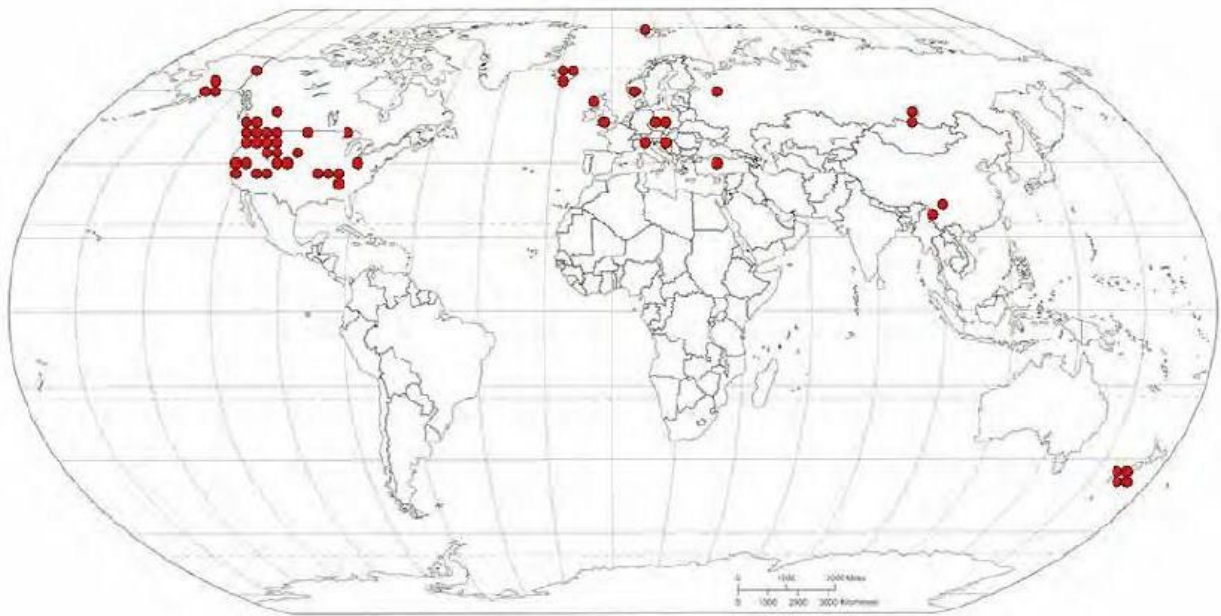


Figure 2. *D. geminata* distribution (image from Spaulding, 2007)

Materials and Methods

D. geminata as a refuge from predation for *T. tubifex*

To test the hypothesis that *D. geminata* mats provides a refuge from invertebrate predators for oligochaetes such as *T. tubifex*, I performed an experiment testing the effects of *D. geminata* and the predatory stonefly *Hesperoperla pacifica* on *T. tubifex* mortality. Located in Gothic, Colorado on the upper East River near the Rocky Mountain Biological Lab, I used 80

streamside flow-through tanks where streamwater was gravity fed into the tanks from a stream located 200 m upslope draining the side of Gothic Mountain into the tanks inside of a portable weatherport. I randomly assigned four treatments to each tank where twenty tanks received substrata covered with polyester pillow stuffing that mimic the structural properties of *D. geminata* mats and one *H. pacifica* stonefly, twenty tanks received substrata without the mimic material and one *H. pacifica* stonefly, twenty tanks received substrata covered with the mimic material and no predators and the remaining twenty tanks received substrata without the mimic material and no predators, which served as controls for losses due to factors other than stonefly predation. Three *T. tubifex* collected from the East River were added to each tank in the morning of 29 July 2013 and *H. pacifica* collected from Avery Creek were added to specific predator assigned treatments three hours later. After ~18 h I removed the stoneflies and counted the number of *T. tubifex* remaining in each tank. Because no *T. tubifex* were dead or missing from the control treatments, and there was no significant block effect associated with the tank arrangement, I used a t-test to test for differences in stonefly-induced mortality between the treatments with and without the *D. geminata* mimic.

Results

Patterns of oligochaete abundance and D. geminata cover

Oligochaete abundance increased along *D. geminata* cover gradient in the East River. (Figure 3). A regression analysis was done to show the variation of invertebrates found in various samples, and the results showed that there is a statistically significant relationship in the percentage of *D. geminata* and the oligochaetes found per square meter.

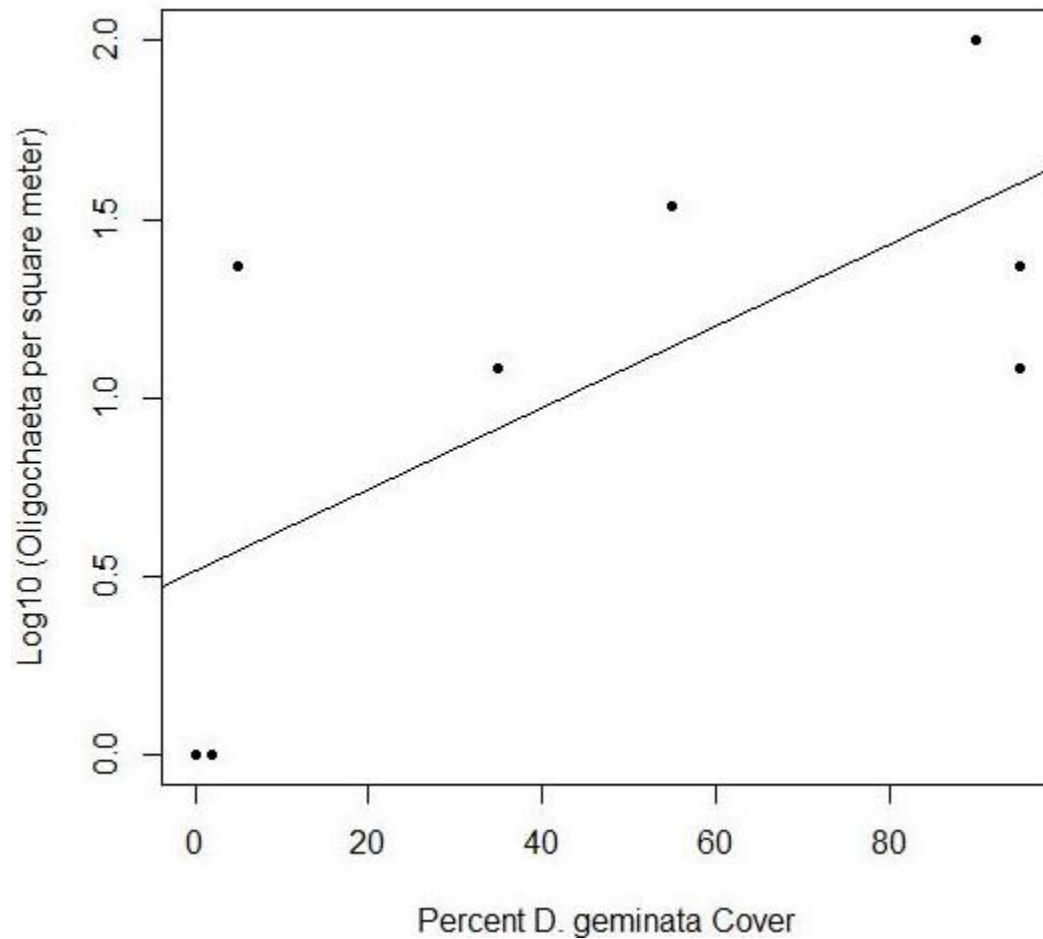


Figure 3. Relationship between the percent of *D. geminata* cover and the number of oligochaetes found along a *D. geminata* cover gradient. ($R^2=0.46$, $P=0.06$) The y- axis was log 10 transformed.

D. geminata* as a refuge from stonefly predation for *T. tubifex

The stonefly *H. pacifica* consumed, killed, or injured nearly twice as few *T. tubifex* in treatments with the *D. geminata* mimic relative to treatments without *D. geminata* mimic ($t_{38} =$

3.19, $P = 0.0014$; Fig. 4). The body size of *H. pacifica* was not different between treatments ($t_{38} = 0.43$, $P = 0.6$).

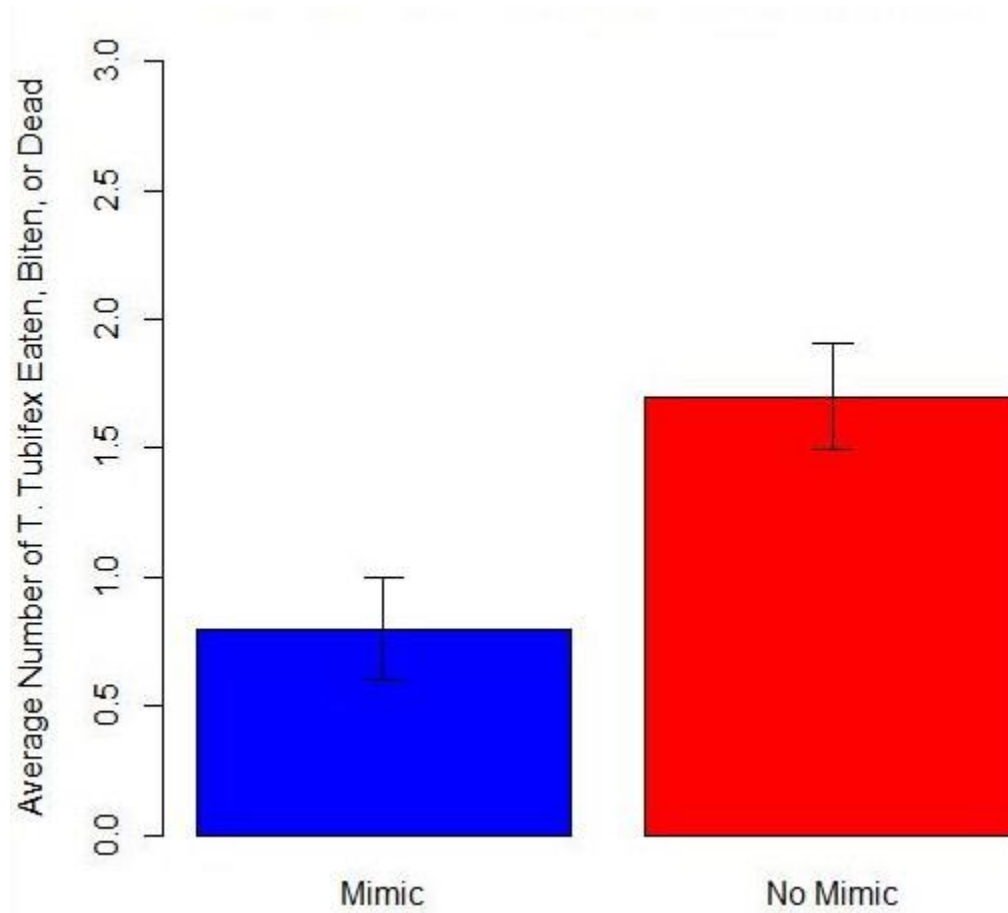


Figure 4: The predatory stonefly *H. pacifica* consumed, killed, and injured more *T. tubifex* on substrata lacking the *D. geminata* mimic. ($t_{38}=3.19$, $P=0.0014$)

Discussion

This study focused on the effects of the diatom *D. geminata* on the intermediate host of whirling disease *T. tubifex*. To find evidence for the abundance of *T. tubifex* in the presence of *D. geminata* I asked the question if *D. geminata* provides refuge from predators.

The oligochaete abundance sampling data showed that *D. geminata* did have an effect on the abundance of oligochaetes found in relation to the percent of *D. geminata* cover present. This pattern of increased abundance of oligochaetes could be explained by the habitat preference of oligochaetes to fine particles that *D. geminata* mats trap.

The predator refuge experiment data illustrates that treatments with the *D. geminata* mimic did provide a refuge for *T. tubifex* from the predatory stonefly *H. pacifica*. The higher mortality rate of *T. tubifex* in treatments without the *D. geminata* mimic shows that *T. tubifex* was found eaten, dead, or bitten more often due to a lack of protective environment to find refuge in. This pattern observed in a controlled experimental setting is to illustrate the habitat refuge that *D. geminata* may be providing for oligochaetes in East River from predatory stoneflies. I speculate that with more *T. tubifex* finding refuge in *D. geminata* mats, and not getting consumed by *H. pacifica*, that there may be more *M. cerebralis* infected *T. tubifex* that could be present which could lead to a higher chance of spreading of whirling disease in salmonids.

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