

Regrowth of *Didymosphenia geminata* after a removal event

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Abstract:

Didymosphenia geminata is a diatom native to the U.S. Rocky Mountains, and is found and considered an invasive species in many parts of the world. It grows in massive mats that carpet large portions of the streams it is found in. I tested the effects of removal on *Didymosphenia* because past studies have suggested that the mats may not recover after a disturbance event, but nothing had been decisively proven. In this study, I removed the mat from 8 different rocks across 3 streams in the East River Valley, and observed how the *Didymosphenia* regrew over a 40 day period. I also took microscope samples from each stream to analyze how it was reacting on a cellular level. At the end of the observation period, the *Didymosphenia* had grown back slightly, but had not recovered to the level of an untreated, *Didymosphenia*-covered rock. Also, the microscope data showed that the cells from the removed had a higher rate of cell division and had much shorter stalk attached to them. This suggests that the trigger for *Didymosphenia* to grow the stalked mats is not present in July-August when the observations took place, and that the growth is most likely triggered by a cue earlier in the year.

Introduction:

Didymosphenia geminata, commonly known as “rock snot,” is a freshwater diatom native to the U.S. Rocky Mountains (Spaulding 2007). However, it is viewed as an invasive species in many areas of the world, and has recently become a nuisance species in its native habitats. Its massive spread through rivers worldwide has caused changes in numerous ecosystem properties, including invertebrate community composition, and may have strong negative effects on fish populations (Brown 2007; Spaulding 2007, Kilroy 2009). *Didymosphenia* has also been shown to have an economic impact in areas where there are blooms. Blooms often occur around dams and canals, and will often clog up the waterways and require frequent cleaning. It affects tourism due to its unsightly appearance marring what would be otherwise thought of as pristine bodies of water, and thus, is projected to cause losses of \$57 to 285 million in New Zealand over the next few years (Spaulding 2007). Also, the cost to control and eradicate a nuisance species such as this in the United States can be up to \$1 billion for a single species (Spaulding 2007). These potential ecological and economic effects have generated much of the recent research on *Didymosphenia*.

Because *Didymosphenia* has become incredibly widespread, but is still poorly understood, many researchers are trying to determine the cause of blooms, and how to potentially control of the blooms in affected streams. One method that has been considered is adding small amounts of inorganic phosphorus to infested areas to stop the growth of the algae, because *Didymosphenia* tends to grow in low phosphorus environments with a high percentage of inorganic phosphorus (Whitton 2009; Bothwell 2011). Biocides have also been considered and used in streams to remove *Didymosphenia*, and although some have proven to be effective at removing *Didymosphenia*, they have also been highly toxic to the fish and invertebrates in the streams as well (Spaulding 2007, Jellyman 2011).

A previous *Didymosphenia* disturbance experiment at the Rocky Mountain Biology Laboratory that focused on the effects of disturbance limited *Didymosphenia* showed that at the end of a 19 day period, *Didymosphenia* on rocks that were scrubbed once at the beginning of the period had regrown the same amount of algae as the *Didymosphenia* on rocks that had been scrubbed every 3-4 days, but both treatments had significantly less algae than the rocks that were left unscrubbed (Brown 2007). This suggested that a low frequency of disturbance may limit the growth of *Didymosphenia*. Controlled bed-scouring floods during spring from a dam on the Front Range of Colorado also suggest disturbances limits *Didymosphenia*; however, *Didymosphenia* blooms returned later that year (Miller 2009). These differing recovery times show that the effects of disturbances on *Didymosphenia* still need to be intensely studied. It is possible that the cues responsible for promoting increased stalk production and hence bloom formation were not present during the first disturbance experiment, given no graded response to the frequency of disturbance.

The main objective of this study was to determine the effects of a late-season disturbance on *Didymosphenia geminata* in multiple streams in the East River valley. To investigate this, I removed *Didymosphenia* from a number of rocks across three streams in the area, and then observed if and how it grew back on both a macro level and a microscopic level. I hypothesized that *Didymosphenia* would not grow back after being removed from rocks across the streams because the cue for blooming was not present.

Methods:

I haphazardly selected 8 sets of 4 rocks in both the East River and Copper Creek on the edges of the Gothic townsite. Each set of rocks was selected to be at a similar depth and water flow velocity. Two rocks were heavily covered with *Didymosphenia* and two rocks were selected that were lightly covered (less than 50%) with *Didymosphenia*. I placed a painted and numbered rock immediately downstream of each treated rock as a marker. I applied four different treatments to each of the eight replicates (i.e., individual rocks). The rocks with thick mats of *Didymosphenia* were either a removal rock (R), which I removed *Didymosphenia* from with a toothbrush, or a rock with *Didymosphenia* (D), that I did not manipulate as a reference. The other treatments were a control (C), which I gently scrubbed with the toothbrush to determine if it was disturbance affecting the regrowth of *Didymosphenia*, and other treatment (N) was an unmanipulated rock with low *Didymosphenia* percent cover, which was used as a reference to quantify new *Didymosphenia* growth. All rocks in the East River and Copper Creek were treated on 3 July, 2012. All the rocks in Washington Gulch were treated 12 July, 2012. On each treatment date, I estimated the percent cover of *Didymosphenia* and measured water depth to the top of the rock.

After treating the rocks, I let them sit for 5-6 days before going back to observe them again. When I observed them, I recorded the visual estimated percent cover again, and starting on 12 July, 2012, I also measured the thickness of the *Didymosphenia* matt on the rock. I then went back out and observed every site once every 3-4 days until 10 August, 2012. For each date I collected data, I multiplied the percent cover of each rock by the thickness to get a biovolume index to roughly compare the total amount of *Didymosphenia* on each rock. Throughout the

period I also took one-time measurements of depth and stream flow. Also, once 10 days after treating the rocks, I took a sample of the algae off of 3 randomly selected D and R rocks from each stream, preserved it in Lugol's solution, and observed it under a microscope in order to determine the makeup of the *Didymosphenia*. I looked at approximately 300 *Didymosphenia* cells for each sample and determining the total amount of stalk in the viewed sample, average length of 10 haphazardly selected stalks, and the percent of cells undergoing cell division and the percent in each stage of cell division.

I ran a number of statistical tests to analyze the data I collected throughout the observational period. For the one-time measurements (depth, stream flow, and biomass), I ran an ANOVA with the block as a random factor. For measurements that spanned the entire period, I ran a MANOVA with time as a repeated measure factor. I ran a one-way ANOVA on the data I collected from the samples analyzed under the microscope, comparing average stalk length and total percent cells dividing amongst the treatments. All of the above tests were run with SAS JMP.

Results:

Observed response to removal

The one-time measurements showed that there was no statistical difference in depth among treatments in Copper Creek ($F_{x,xx}=3/21$, $p=0.36$), East River ($F_{x,xx}=3/21$, $p=0.47$), or Washington Gulch ($F_{x,xx}=3/21$, $p=0.47$), and no difference in the stream velocity among treatments in Copper Creek ($F_{x,xx}=3/21$, $p=0.08$) and Washington Gulch ($F_{x,xx}=3/21$, $p=0.72$). However, there was a significant difference in water velocity among treatments in the East River ($F_{x,xx}=3/21$, $p=0.009$), which a Tukey's posthoc test revealed was due to lower water velocity associated with the D compared with the other three treatments .

Each stream exhibited slightly different results for the change in the *Didymosphenia* biovolume index over time. East River showed a significant difference between the treatments ($F_{x,xx}=3/28$, $p<.0001$) but had no significant difference over the observed period ($F_{x,xx}=8/21$, $p=.1063$) and had no interaction between time and treatment ($F_{x,xx}=24/61.5$, $p=.4979$). Plotting the average index for each treatment over time showed that the D-treated rocks were the significantly different treatment, and the R, C, and N rocks were all similar (Figure 1).

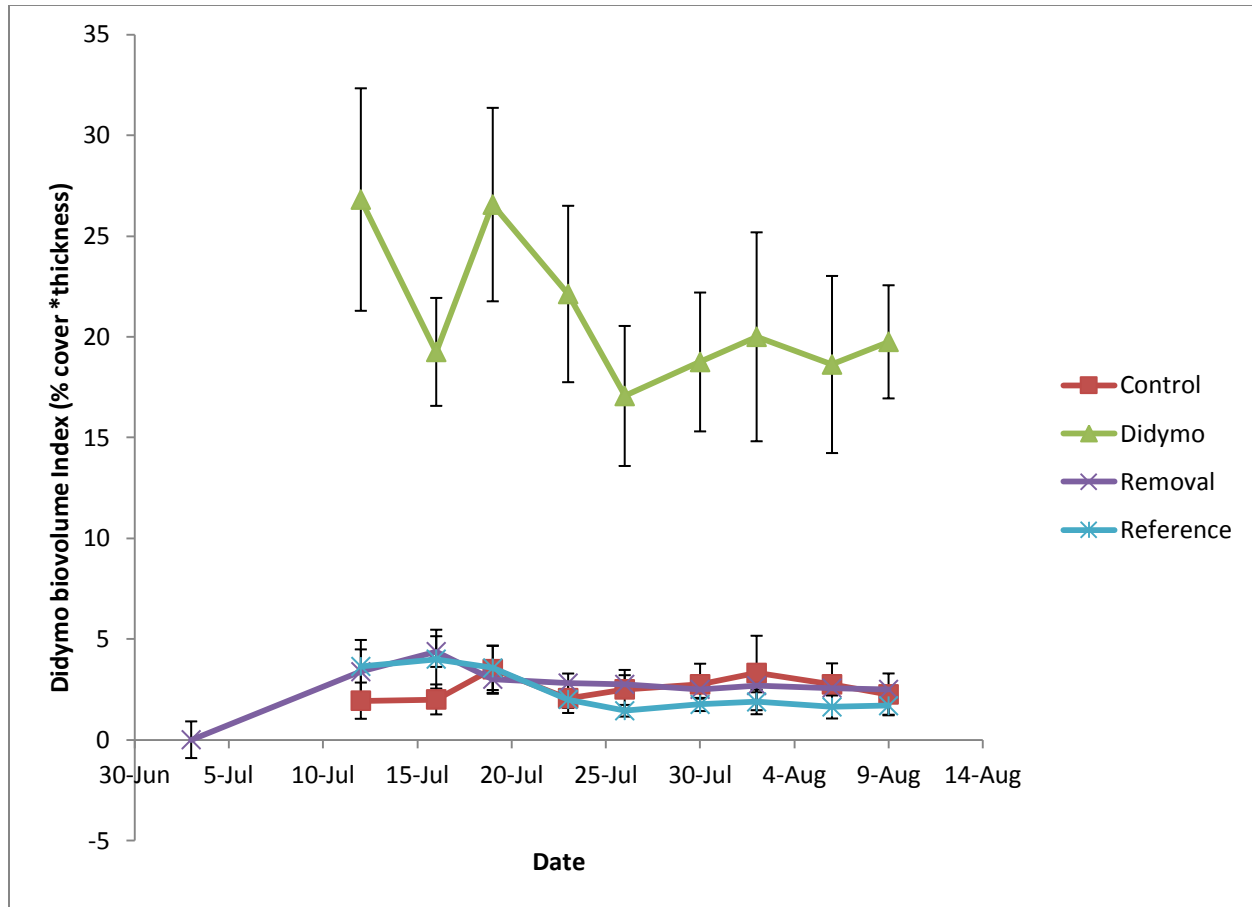


Figure 1: Average *Didymosphenia* biovolume index ($\pm$ SE) of each treatment for East River over the sampling period

Copper Creek showed a significant difference between the treatments ($F_{x,xx}=3/28$, $p<.0001$) and a significant difference over time ($F_{x,xx}=8/21$, $p=.0015$), but no significant interaction between treatment and time ($F_{x,xx}=24/61.5$, $p=.3708$). Plotting the average index for each treatment over time showed that the D-treated rocks were the significantly different treatment, and that the C, R, and N treatments were all similar. It also showed that the change over time was an increase in index over the period (Figure 2).

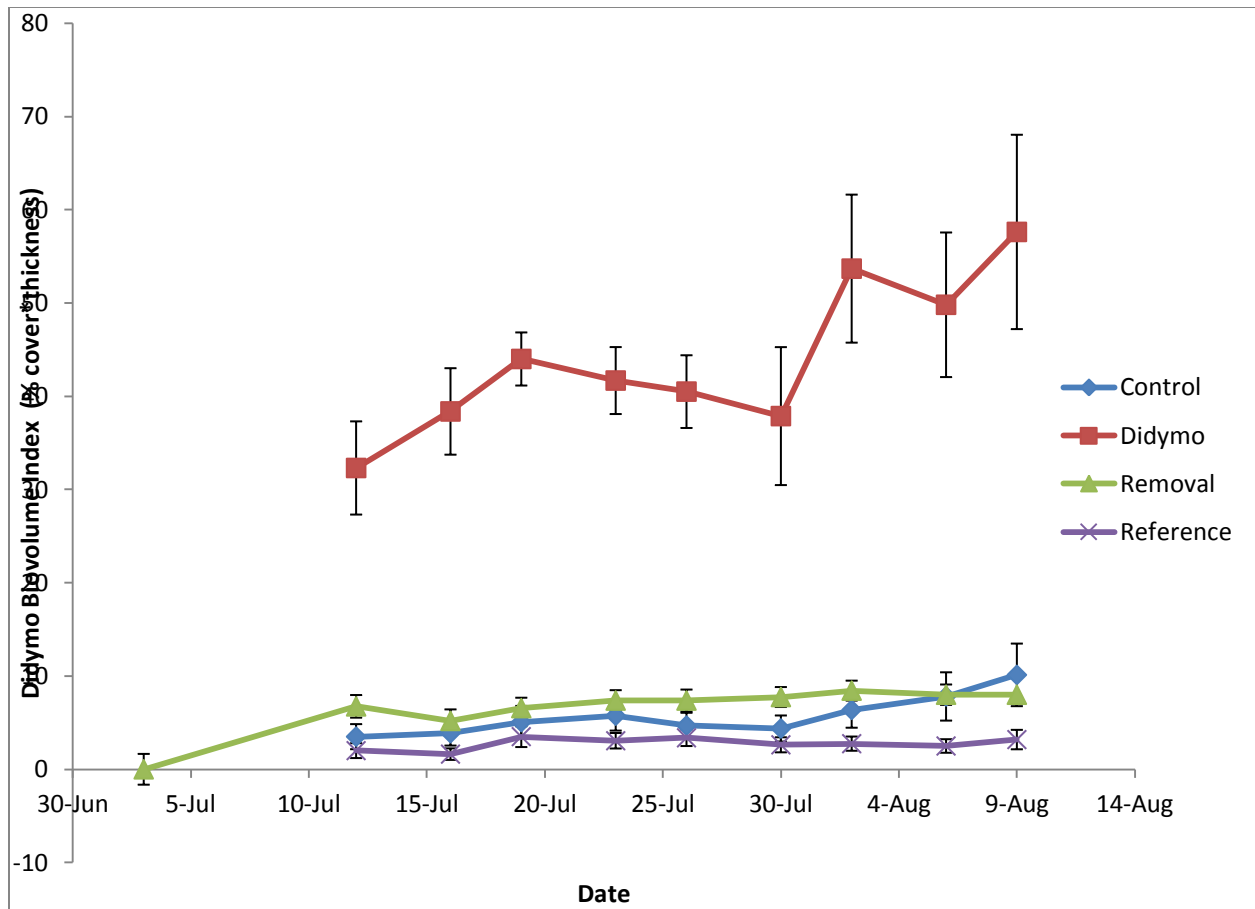


Figure 2: Average *Didymosphenia* biovolume index ($\pm$ SE) of each treatment for Copper Creek over the sampling period

Washington Gulch showed a significant difference between the treatments ($F_{x,xx}=3/28$, $p<.0001$), a significant difference over time ($F_{x,xx}=7/22$, $p=.0003$), and a significant interaction between treatment and time ($F_{x,xx}=21/63.7$, $p=.0267$). Plotting the average index for each treatment over time showed that the D-treated rocks were the significantly different treatment, and that the C, R, and N treatments were all similar. It also showed that the change over time was a decrease in index over the period, particularly in the D-treated rocks (Figure 3).

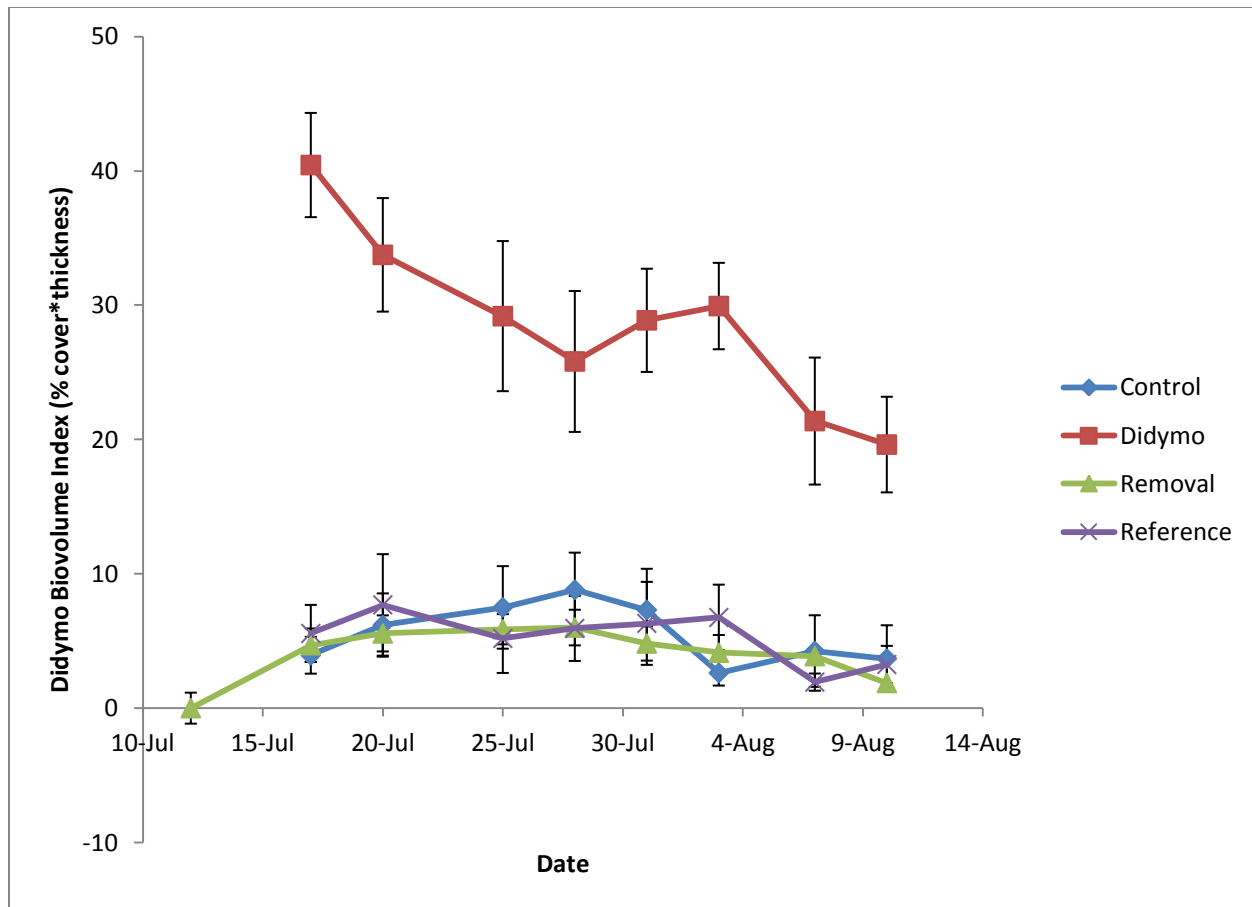


Figure 3: Average *Didymosphenia* biovolume index ($\pm$ SE) of each treatment for Washington Gulch over the sampling period

Microscopic response to removal

The difference in total percent of cells dividing varied among the streams. In the East River, the difference was not significant ($F_{x,xx}=1/4$, $p=.1735$). In Copper Creek, the difference was still not significant, but suggests a possible trend ($F_{x,xx}=1/4$, $p=.0767$). Washington Gulch did have a statistically significant difference between the treatments ($F_{x,xx}=1/4$, $p=.0131$). In all three streams, the average total percent dividing was higher in the R samples than the D samples (figure 4).

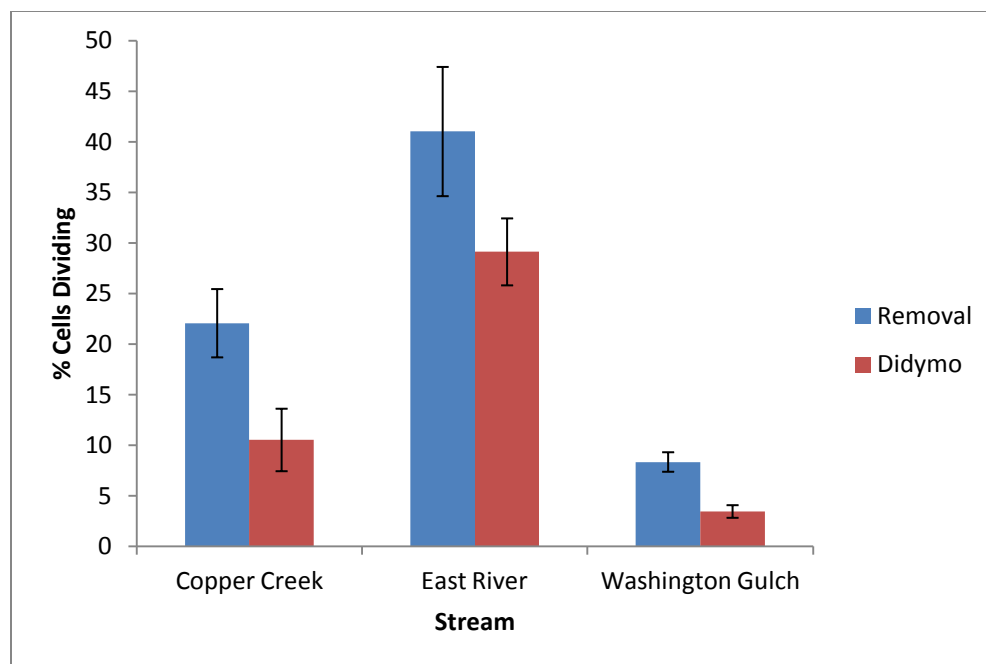


Figure 4: Average total percent cells dividing ($\pm$ SE) for removal and *Didymosphenia* rocks sampled in all three streams.

The difference between average stalk length in the two treatments was significant in East River ($F_{x,xx} = 1/4$, $p = .0444$), Copper Creek ($F_{x,xx} = 1/4$, $p = .0091$), and Washington Gulch ($F_{x,xx} = 1/4$, $p = .0032$). In all three streams, the average stalk length was higher in the D samples than the R samples (figure 5).

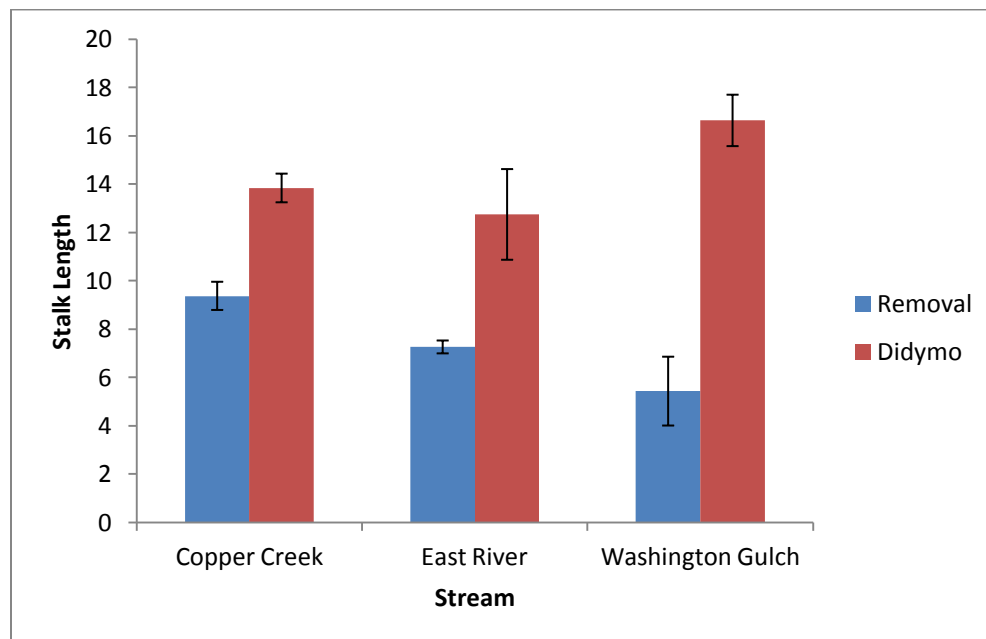


Figure 5: Average stalk length ($\pm$ SE) for removal and *Didymosphenia* rocks sampled in all three streams

Discussion:

The primary conclusion of this experiment is that *Didymosphenia geminata* does not grow back in any appreciable way after being removed mid-season. After 40 days, none of the removal rocks produced the mats that were present on the rocks before treatment. This was not a matter of not having enough time to grow back, because *Didymosphenia* has been shown to grow three to four days after a removal event (Bothwell 2011). This suggests that the cue for the mats to form was not present over the entire period of the experiment, and that the cue for the mats to bloom most likely occurs earlier in the year.

Over the observation period, in all three streams the reference and control do not strongly change, and stay similar to the removal rocks over the whole period. This suggests that as well as not growing on a rock that has had the mat removed, mats possibly will not grow on any rock that was not highly covered by the time the cue to bloom had past. If future studies like this one are done, it would be interesting to analyze these kinds of rocks under a microscope as well to see if the cell activity is more similar to the removal rocks or the untouched *Didymosphenia* rocks.

Another interesting result is the trend toward higher rates of cell division in the removal treatments. It has been shown that *Didymosphenia* divides less when producing stalk (Bothwell 2011). It makes sense that if the cells are not expending their energy on producing stalk, that they would instead produce more cells to increase their rate of reproduction in order to increase their fitness. The difference in stalk length between the two treatments may also be indicative of how *Didymosphenia* grows back after being removed, but it is difficult to tell whether or not the shorter stalk length is simply a result of the stalk being scrubbed off and some smaller pieces remaining, *Didymosphenia* regrowing stalk and simply not having as much time to grow as the untouched samples, or *Didymosphenia* actually growing shorter stalks as a result of being removed. In future studies, it would be interesting to look at multiple samples over time to see if the cellular responses change over time as the season progresses.

If the signal being absent at this point in the year is in fact the case, it has serious implications for future studies done on *Didymosphenia*. If a researcher wanted to do a removal and regrowth experiment to determine the effects of different treatments, such as adding phosphorus to a stream, and they did this experiment after the cue for stalk growth had passed, they would receive no results. In order to deal with this, both the temporal and physical elements of the cue need to be determined so that the cue can either be replicated for experiments, or experiments can be timed around the cue in order to get results for experiments.

Since the *Didymosphenia* mat did not grow back after removal, it could be considered that a simple mid-season removal would be enough to help control massive blooms. However, attempting to use this method to control blooms would be extremely inefficient, because it would require a very large, time-consuming effort to manually remove the mat from an entire stream. Also, *Didymosphenia* has been shown to recover from a scouring and bloom again the

next season, this kind of massive removal effort would have to be undertaken once every year to keep the blooms under constant control (Miller 2009).

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