

Abstract

Climate change predictions show increases in droughts and extreme precipitation events in the future. These types of events disturb aquatic stream ecosystems, and with increased droughts brings greater chances of stagnant pool forming out of drying streams. To further understand the consequences of increased stagnant pool formation and disturbance on stream algal communities, this experiment tested how stream algae responded to stagnant conditions and stagnant conditions with periods of high flow, mimicking extreme precipitation events. Chlorophyll amounts were used to estimate total amount of algae, and random field identification was used to check community composition. Both treatments were shown to decrease overall biomass of algae, regardless of stagnation duration. Both treatments also showed loses in *Fragilaria* algae, and streams experiencing the drought only treatment gained larger populations of loosely attached *Mougeotia* and *Merismopedia*. The overall changes in community suggest that highly-attached species of algae suffer in stagnant conditions.

Introduction

Climate change predictions show a large range of consequences in a variety of categories. One of broadest areas of change will be in the severity and commonality of extreme weather situations. Extreme weather situations include heat waves, droughts, and floods (Walsh *et. al.* 2013). Two of these, droughts and floods, are caused by the predicted changes in precipitation patterns. In the United States, long periods of drought, with little to no rain, are to become much more common, especially in historically dry areas and during the summer (Walsh *et. al.* 2013). Likewise, heavy downpours are also predicted to increase, especially in historically wet areas, such as the Midwest (Walsh *et. al.* 2013).

Stream systems will be heavily affected by changes in precipitation (Paerl 1997). Apart from being important sources of drinking water and recreation for humans, streams are also important ecosystems affected by many factors (Gasith and Resh 1999, Hawkins 1993). Many species of fish use streams for spawning, and many insects spend their juvenile stages in stream waters (Hawkins 1993). Streams also play a role as an ecotone between terrestrial systems and the linked aquatic systems (Triska 1993). By moving nutrients, streams become an important part of nitrogen cycling (Triska 1993).

Droughts are considered disturbances, and they are especially problematic to stream environments. During times of drought, shallow areas of streams may dry out (Lake 2003). The three types of stream drying are downstream drying, headwater drying, and mid-reach drying, dependent on which areas of the stream are the first to dry (Lake 2003). Of these, only headwater drying, the drying of the original source of the stream, normally leads to the creation of small pools (Lake 2003). These pools are the remnants of deeper areas of stream that have yet to dry, and have little to no hydroconnectivity (Lake 2003). With a lack of hydroconnectivity comes stagnation, loss of habitat space for aquatic organisms, and build up of detritus (Lake 2003). The water quality decreases, and the diversity of organisms decreases as their interactions become magnified by lack of resources (Lake 2000).

Floods create high flow rates in streams, which mixes the water layers, moves soils, stream plants, and detritus throughout these layers (Lake 2000). Animals can also be injured and moved to new locations due to flooding (Lake 2000). Due to the many different disturbance effects of floods, streams that experience frequent flooding best support species that are resistant to the effects of high currents (Fisher 1982). In stagnant pools, a sudden flood can create extreme negative effects on organisms, as the replacement of hydroconnectivity can move the large build-ups of detritus through different pools and into downstream areas, like larger ponds, that may still have decent water quality (Lake 2000).

In stream ecosystems, changes in precipitation patterns are likely to have a significant effect on algae (Robson and Matthews 2004). Algal species are primary producers in stream systems, and are important prey species for many organisms (Robson and Matthews 2004). Algae are sensitive in changes

in stream flow, so droughts and floods are likely to have large effects on algal communities (Robson and Matthews 2004, Fisher *et. al.* 1982).

The change from stream habitat to stagnant pool habitat may provide algal communities a chance to form algal blooms. Stagnation can raise water temperature, which has been linked to algal blooms (Paerl 1997). Algal blooms can decrease water quality, which has profound impacts on aquatic ecosystems (Paerl *et. al.* 2001). The populations of other aquatic organisms usually decline, sometimes due directly to the lower water quality, and sometimes due to increased competition with the algae (Peperzak 2005). In many habitats, climate change is predicted to increase the number of algal blooms (Peperzak 2005).

This experiment hopes to look into how stream algal communities will be affected by the changes in precipitation predicted to form due to climate change. Specifically, algae in stagnant pools left by streams during times of drought will be researched. Stagnant pools that experience flashy, heavy downpours will also be researched. Algal biomass and genus diversity will be the measurements for community changes. Predicted results for stagnation conditions include increased biomass and decreased diversity, as a bloom may form, but it would consist of only species that can tolerate a drastic decrease in stream flow (Paerl 1997, Fisher *et. al.* 1982). Predicted results for stagnation with flash floods include decreased biomass and decreased diversity, as the algae will be disturbed on a regular basis, allowing no time for recovery and regrowth (Fisher *et. al.* 1982).

Materials and Methods

Equipment Description

At the University of Michigan Biological Station stream lab, water was pumped out of the nearby Maple River for use in our streams. Two large rain barrels had a constant flow of filtered river water pumped into them, and each of these had eight faucets attached to these that could regulate water into the artificial streams. 10 foot long rain gutters were used as artificial stream beds, and each stream bed had 25 clay tiles lined up along the length of the stream bed. The tiles start about half a meter away from the faucets to avoid turbulence. River water was run over the tiles at a flow rate of approximately 90 mL/sec for two weeks to allow a build-up of stream algae. The section of stream housing the tiles were all covered in a layer of shade cloth to mimic the amount of light available to most forest streams.

Flow Rate Determination Method

Water from each faucet was collected in a 1L graduated cylinder for seven seconds. The water measurement was taken and used to calculate the flow of each stream. The stream flows were checked

and recalibrated at least every two days. Control flows were kept in within a range of approximately 75 mL/s and 100 mL/s.

Treatments

Two treatments were randomly assigned to five streams each, and five streams were used as controls. Treatment 1 streams were dammed up at both ends and left to stagnate in a drought treatment. No stream flow was added to these streams. Treatment 2 streams received a similar drought treatment, but had two day periods of extremely heavy stream flow that began every six days. Control streams received a constant, moderate flow.

Data Collection

For each sample, two tiles in each stream were randomly sampled. The same positions were used for each stream for that sampling session. The three tiles closest to the faucets were not used to avoid the effects of turbulence created by the water entering the streams. The two selected tiles were scrubbed with a toothbrush and stream water until no algae remained on their surfaces. The algae and water mixture was collected in a plastic tray and was mixed until it became homogenous. The mixture was then measured for total volume, and a subset was collected in a 30 mL scintillation vial. The toothbrush, tray, and other materials were rinsed with stream water in between each sampling to avoid cross-contamination. 10 mL was taken from each scintillation vial for measuring chlorophyll. The rest was preserved using formalin (3%) for use in algal diversity sampling.

For measuring chlorophyll, the 10 mL subsample was vacuum filtered onto 0.45 micrometer pore nitrocellulose filter paper to remove the water. The filter was then placed in a test tube and dissolved in 10 mL of 90% buffered acetone solution, leaving an algae and acetone mixture that was sonicated and frozen overnight. The test tubes were thawed the next day, and centrifuged if any cloudiness was present in the sample. A multiple wavelength fluorescence test was then performed on the samples to determine the amount of chlorophyll-a present in each sample in mg/L (Weber *et. al.* 1986). This was then used to determine the total mass of chlorophyll present on the two-tile samples.

Diversity tests were conducted by examining a 0.1 mL sub-sample from the preserved scintillation vial samples using a Palmer counting cell. Random fields of view at 450x magnification were observed, and all algae cells in the field were identified by genera. Algae that did not have easily identifiable cells (i.e. *Shizothrix*) were counted by length, with a quarter of the length of the field of view being the unit of measurement. The average number of cells per field was then calculated, and then extrapolated using the known volumes of sample in each field, Palmer cell, and total sample. This last calculation reflected the approximate amount of cells present in the full two-tile samples.

Each stream also was tested for basic abiotic differences. Temperature was measured using a field thermometer. Total nitrogen, nitrates, and phosphorous were also tested using a SEAL AutoAnalyzer 3. Light was also measured outside and under the shade cloth to check that the shade cloth was removing some level of sunlight from the streams, much as the canopy of a forest would block light from a stream.

Statistical Analysis

Total chlorophyll mass data was log10(x+1) transformed to approximate more normally distributed data. For chlorophyll measurements, cell count measurements, and community diversity measurements, only data from the last sampling day was used, as the longer amount of time in the treatments should have produced the highest level of differences between treatments. ANOVA tests and Tukey tests were used when data was shown to be normal, and independent sample Kruskal-Wallis tests were used when data was non-normal.

Results

Abiotic factors between the treatments tended to vary as well. Temperature showed significant differences in mean temperature dependent on treatment (p < 0.001, ANOVA, Figure 1). Streams receiving full drought treatments had significantly higher temperatures on average than streams receiving either drought/flood or control streams (p = 0.015, p < 0.001, Tukey HSD). Streams also received different variations in temperature dependant upon treatment, with treatment 1 streams having the highest variations, and control streams having the lowest (p < 0.001, Levene's Test of Equality of Error Variances).

Phosphorous availability showed no significant differences between streams (p = 0.827, Independent Samples Kruskal-Wallis Test). However, total nitrogen availability was found to be dependent on treatment, with significantly higher concentrations in drought/flood streams than in control streams (p = .018, Independent Samples Kruskal-Wallis Test, p = 0.027, Tukey HSD, Figure 2). A near significant difference was also found between drought streams and control streams for total nitrogen availability (p = 0.071, Tukey HSD). When ln (x+1) transformed, nitrate availability was very close to a difference between treatments, with a p-value of 0.054 (ANOVA). When tested with Tukey's test, a weaker test, a significant difference was found between control streams and treatment 1 streams (0.050).

Comparing the chlorophyll amounts collected on the last sampling date, a significant difference was found between the different treatments (p = 0.010, ANOVA, Figure 3). Specifically, tiles in control streams had significantly higher amounts of chlorophyll than those in streams of either drought or

drought/flood treatments (p = 0.017, p = 0.019, Tukey HSD). The two treatment streams showed no significant difference in chlorophyll amounts (p = 0.998, Tukey HSD).

The number of cells per field showed a significant difference between streams (p = 0.037, Kruskal-Wallis test, Figure 4). However, when cell densities were tested, a nearly significant difference was found, likely due to the higher number of cells (p = 0.077, Figure 5).

Community differences were observed between all three treatments (Figure 6). Control streams tended to have higher amounts of *Fragilaria*, *Naviculoids*, and *Cymbella* (Figure 7). Drought treatment streams tended to have smaller amounts of *Fragilaria* and larger amounts of *Mougeotia* and *Merismopedia*. Drought/flood treatment streams were more similar to control streams in terms of *Mugeotia*, *Merismopedia*, and *Naviculoids*, but had less *Fragilaria*. Treatment 2 streams also had greater proportions of *Achnanthes*.

Discussion

The significant differences shown in chlorophyll amounts suggest that stagnation, even when interrupted by periods of flash flooding, decrease the amount of algae in a stream. The cell counts showed a nearly significant difference, which would also suggest that greater amounts of algae could be found in control streams than in either of the treatment streams.

The algal communities in the streams showed many differences due to treatments. The amount of difference between drought/flood streams and control streams was much smaller than the difference between drought-only streams and control streams, suggesting that the occasional flooding in treatment 2 streams allowed for for level of stability in the algal community. The higher proportions of *Fragilaria* in control streams is logical, as *Fragilaria* does well in environments with constant flow, as it catches on substrate to avoid being pushed downstream (Keithan & Lowe 1985). In drought-only streams, the extremely low amount of *Fragilaria* and the higher proportions of *Mougeotia* and *Merismopedia*, both species that have very little connection with the surface they grown on, also make sense, as absence of flow in these streams would favor algae that do not have a strong connection to the surface of the tiles (Keithan & Lowe 1985). Green algae had just begun to greatly increase during the last week of this experiment. With more time, greater differences between treatments may have been observed.

Past studies have shown that stream flow can greatly change the community make-up of an area, producing fast-current areas with smaller, highly attached diatoms and slow-current areas with less-hardily attached diatoms (Keithan & Lowe 1985). This is directly shown in the data gathered in these streams. However, there are multiple theories about how current could cause changes in overall

abundance of algae. Faster currents have been shown to greatly decrease the immigration rates of algae when compared to areas of slower current; however, data on how these currents compare to an area of stagnation is not yet available (Peterson & Stevenson 1989). Observations during this experiment suggest that stagnation hinders algal immigration. Other theories include the distribution of larger aquatic animals, which were not present in these streams (Poff and Ward 1992).

The algal community in the non-controlled streams could also have changed due to the changes in temperature. Algae that are used to a steady, low temperature may not be well adapted to life in the warmer, more variable waters of a stagnant pool. Past studies have shown that most types of diatoms, excluding eu-eurytherms, can survive only a small amount of temperature variation, ranging from 20°C in difference to less than 10°C in difference (Patrick 1977, Hustedt 1927-1959). The control streams showed a variation of approximately 5°C, compared to the approximately 15°C variation observed in treatment 1 streams. With this much variation, stagnant streams would not be able to support stenotherms or mesostenotherms, which have low thresholds of temperature variation tolerance (Patrick 1977, Hustedt 1927-1959). Given that the temperature measurements were all taken during the day, the true variations were likely higher than these, especially for the stagnant streams. Thus, even fewer diatoms would be likely to survive in stagnant streams. Algae also have different temperature ranges in which they can grow, so the varying temperatures in the stagnant streams could have inhibited algal cell growth for the individuals that could still survive in these variations (Seaburg and Parker 1983). Unsteady temperatures may lead to inhibited growth by changing how nutrients can flow throughout the periphyton (Shuter 1979).

Nutrient levels in the streams were also likely affected by the senescence of algae that could not survive in stagnant conditions. As phosphorous levels were similar in all treatments, nitrogen was the nutrient most likely to be limiting in the streams (Marks & Lowe 1993). Streams tend to have lower amounts of chlorophyll in periods of low-nitrogen availability, including in times of low-flow; however, the treated streams of this experiment showed a much higher nitrogen availability along with decreasing chlorophyll amounts (Lohman *et. al.* 1991). Considering that no nitrogen could be added by added water to drought-only streams, which still had increasing nitrogen, it is possible that nitrogen was added to the water as algal cells began to senesce and die. Given the added nitrogen, species that were more well-adapted to the stagnant environment could have begun to increase growth if the experiment had continued.

To better understand the implications of this experiment, more research will have to be done as to what specific factors are most affecting stream algal communities during times of drought and stagnation. It is important to note that streams like those recreated in this study are predicted to have rising temperatures with increased global warming, as well as with increased deforestation around the streams, so future information on how temperature alone changes algal compositions of streams in both stagnant

and flowing conditions (Stefan & Sinkrot 1993). Streams that are particularly at risk should be identified, and once the process is better understood, management plans should be created to avoid the destruction of these communities. More genus-specific research would help narrow down "at-risk" algae, which could help with management plans. Research should also be done on whether or not streams experiencing stagnation can be fully revived, and whether or not the algae communities will be able to rebound to their original state.

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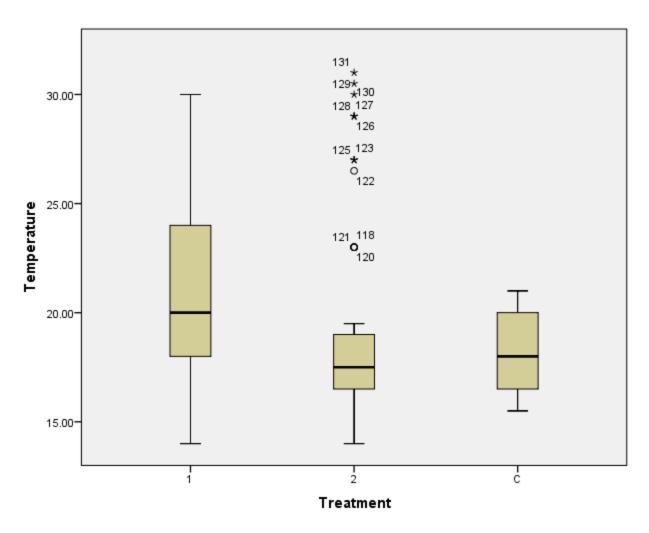


Figure 1. These boxplots show measured temperatures sorted by stream treatments. A significant difference was found between drought (1) streams and control (C) streams. The strong amount of variance in drought/flood (2) streams suggests that, had the experiment gone on longer, a significant difference between these and control streams may have also been found.

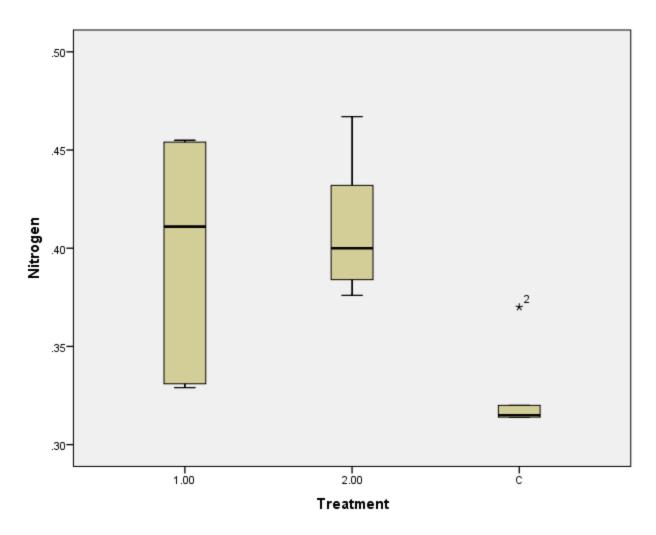


Figure 2. These boxplots show the levels of % nitrogen found in sampled stream water sorted by stream treatment. The drought and drought/flood streams were found to have significantly higher levels of nitrogen than control streams.

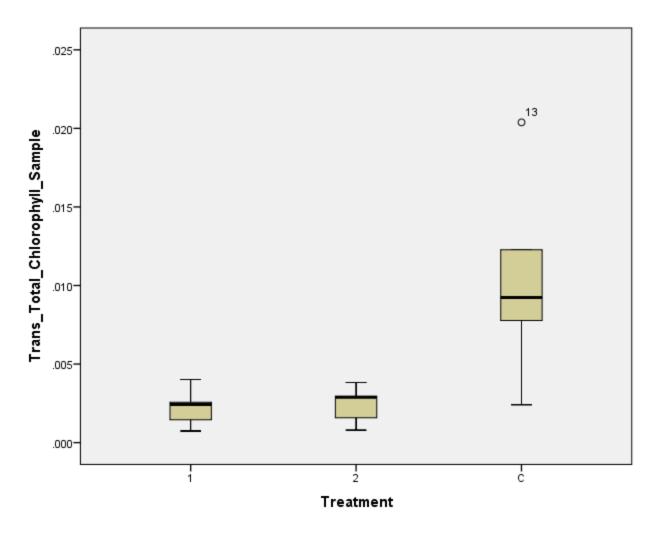


Figure 3. These boxplots show the amounts of total chlorophyll per sample calculated and log10(x+1) transformed separated by stream treatment. Drought (treatment 1) and drought/flood (treatment 2) showed significantly lower amounts of chlorophyll than control streams.

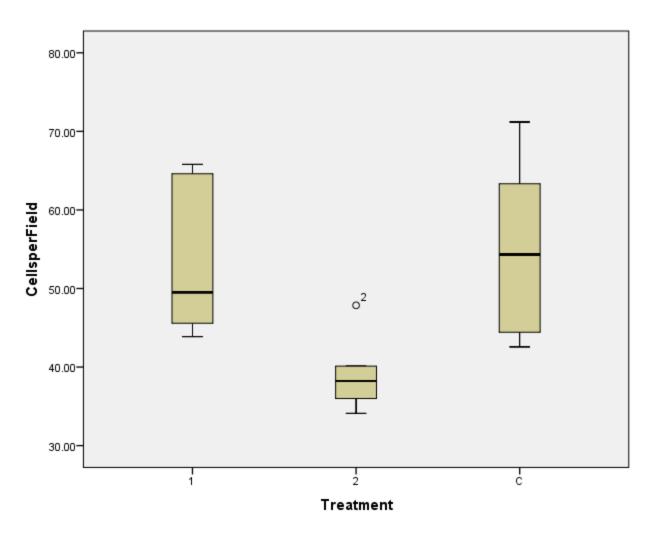


Figure 4. These boxplots show average number of cells per field dependent on stream treatment. Stream treatment was found to significantly affect the average number of cells per field.

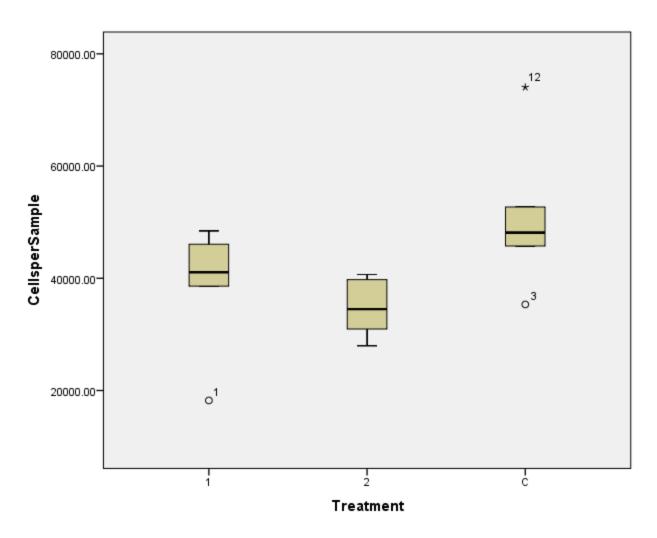


Figure 5. These boxplots show the approximate cell density depending upon stream treatment. A nearly significant difference was found between treatments.

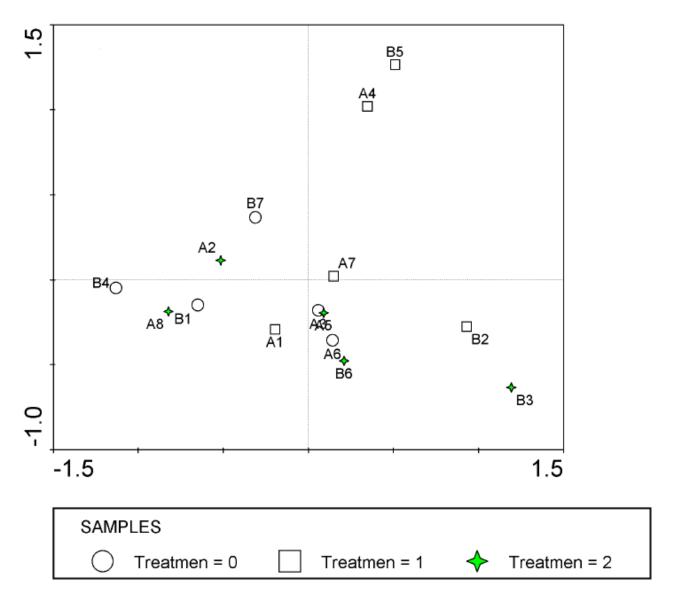


Figure 6. This primary component analysis graph shows the relationships between different streams and the type of treatment given. Most of the control (treatment "0") streams are fairly similar in composition, and thus are close together. Drought/flood (treatment 2) streams have a bit more variability, but are still close to control streams. Drought (treatment 1) streams have the most variability, and were more different from control streams than drought/flood streams.

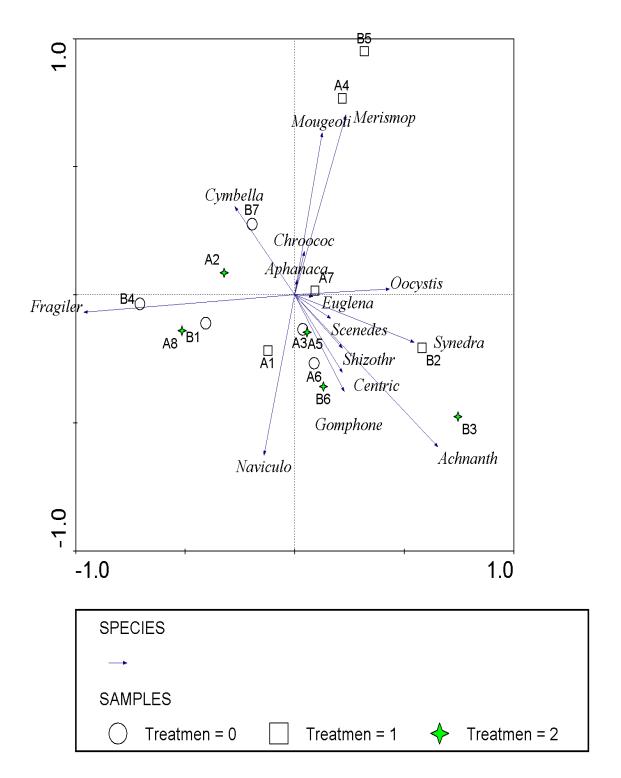


Figure 7. This primary component analysis graph shows which species are most affecting the differences in stream composition. The largest factors are *Fragilaria*, *Merismopedia*, *Mougeotia*, *Achnanthes*, and *Naviculoids*.