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Effects of Microcystis aeruginosa on New Jersey Aquatic Benthic Macroinvertebrates

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Abstract

As increases in anthropogenic eutrophication and climate change contribute to more severe and frequent cyanobacterial harmful algal blooms in freshwater ecosystems worldwide, understanding the effects and consequences cyanobacterial blooms have on aquatic organisms is crucial. *Microcystis aeruginosa* is one of the most common cyanobacteria taxa found in cyanobacterial blooms, producing a number of toxins including Microcystins. This study examined the effects of *Microcystis aeruginosa* on aquatic benthic macroinvertebrates, specifically the pollution intolerant taxa Ephemeroptera, the pollution moderately intolerant taxa Zygoptera, and the pollution tolerant taxa Chironomidae. In a controlled lab environment, macroinvertebrates were exposed to approximately 100,000 cells/ml of *Microcystis aeruginosa*. The survival percentage was lower for macroinvertebrates exposed to *Microcystis aeruginosa* in all three tolerance groups while corresponding with the pollution tolerance levels of the species. These findings support the notion that cyanobacterial blooms have deleterious effects on freshwater ecosystems and can affect aquatic food webs.

MONTCLAIR STATE UNIVERSITY

Effects of *Microcystis aeruginosa* on New Jersey aquatic benthic macroinvertebrates

By

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Introduction

Water Quality and Cyanobacteria

The quality of freshwater around the world has declined in recent decades, decreasing the suitability for human use and for supporting healthy ecosystems. This poses a problem due to fresh water being an invaluable natural resource. Humans rely on freshwater every single day, using it to drink, bathe, and cook as well as for recreational and household purposes. Unhygienic and poor quality of water make up 3.1% of the world's deaths (Pawari and Gawande, 2015). Fresh water also sustains wildlife, including those that are of ecological importance. Aquatic wildlife, particularly plants, insects, and fish, suffer from poor water quality as they can be sensitive to short-term changes. An excess of sediments, road salts, nutrients, and other contaminants can affect the ability for wildlife to persist in freshwater aquatic ecosystems, eventually altering the structure and dynamics of these delicate environments. One major factor related to the decline in water quality is an increase in cyanobacterial abundance in many freshwater systems (Sivonen and Jones, 1999).

Cyanobacteria are organisms with characteristics of both bacteria and algae; they contain blue-green pigments and, unlike other bacteria, perform photosynthesis, giving them the name "blue-green algae" (World Health Organization, 2009). Cyanobacteria have been extant for an estimated 2.5 billion years, making them the oldest oxygenic phototrophic inhabitants on Earth (Akamagwuna & Odume, 2020). Among cyanobacteria taxa, *Microcystis spp.* are among the most pervasive and often produce toxins (Sivonen and Jones, 1999), rendering these species as a common threat to the welfare of freshwater ecosystems throughout the United States, and especially in New Jersey (NJDEP, 2020). *Microcystis spp.* can produce the toxins Microcystins, Anatoxin-a, β-N-methylamino-L-alanine (BMAA), and Lipopolysaccharide (LPS)

(Abeysiriwardena et al., 2018). Microcystin exposure in humans can lead to adverse health effects ranging from a mild skin rash to serious illness; unexplained sickness and death have been observed in dogs and other domestic animals that have come in contact with bodies of water containing high concentrations of microcystins (NJDEP, 2020).

Cyanobacterial Harmful Algal Blooms

When conditions are right, cyanobacteria can experience rapid population growth, called a cyanobacterial harmful algal bloom (CyanoHAB). CyanoHAB are a worldwide phenomenon, commonly occurring in freshwater, estuaries, and coastal marine ecosystems (Bricker et al., 2008). Occurrences of CyanoHAB have become common in watersheds primarily due to increases in anthropogenic sources contributing to heightened inputs of limiting nutrients (Huisman et al., 2005). Increases in limiting nutrients, such as nitrogen and phosphorus, can lead to eutrophication, which is a major stressor in freshwater ecosystems caused by runoff containing agricultural fertilizers, urban pollution, and sewage discharge (Stankovic et al., 2020). This enrichment of nutrients in waterbodies provides cyanobacteria the resources to rapidly multiply and create a bloom (Paerl, 2018). Water temperature is also a major contributing factor to CyanoHAB. Blooms usually occur from spring to fall when water temperatures are warm but can last year round, especially in New Jersey. Warmer temperatures prevent water from mixing in waterbodies that tend to be stagnant or slow moving – including lakes, reservoirs, and ponds – which is known as stratification, allowing cyanobacteria to grow faster and in thicker mats (Hudnell et al., 2010). Climate change has intensified CyanoHAB around the world through warming and increasing hydrologic variability (Paerl, 2018). Changes in rainfall patterns have led to increased precipitation frequency and magnitude, heightening nutrient runoffs into waterbodies (Kunkel et al., 1999).

The World Health Organization (WHO) has determined that human exposure at a density between 20,000 cells/ml and 100,000 cells/ml can result in a moderate probability of acute health effects (WHO, 2009). The New Jersey Department of Environmental Protection (NJDEP) defines a CyanoHAB event as a cyanobacterial cell density of 20,000 cells/ml or higher (NJDEP 2020; Loftin et al, 2008). The NJDEP has developed a 5-tier system, Alert Levels (Watch, Alert, Advisory, Warning, Danger), that is based on a bloom's cyanotoxin level and cyanobacterial cell concentrations (NJDEP, 2020). This system provides guidance and advice for recreational exposure to CyanoHAB and their toxins. The NJDEP considers a CyanoHAB containing 100,000 cells/ml or more in the advisory, warning, and danger tiers, capable of producing very high adverse health effects (NJDEP, 2020). In 2019, New Jersey saw a 45% increase in confirmed CyanoHAB events compared to 2017 and 2018; events included 25 bathing beaches and 4 drinking water sources (Mcgeorge, 2020). 73% of those CyanoHAB events were above 100,000 cells/ml, the highest cell count being 56,300,000 cells/ml at Rosedale Lake (Mcgeorge, 2020). CyanoHAB are problematic for humans, reducing access to clean and safe water for drinking and recreational purposes (Qin et al., 2010), reducing fisheries production potential (Lee and Jones, 1991), and negatively affecting human health through cyanotoxin accumulation in edible fish tissue (Chueng et al., 2013).

Benthic Macroinvertebrates

CyanoHAB have led to the mortality of fish, benthic organisms, and other wildlife that live in and near freshwater ecosystems through direct cyanobacteria toxin exposure and bioaccumulation of toxins in food webs (Akamagwuna and Odume, 2020). One group that is particularly vulnerable to cyanobacteria are aquatic benthic macroinvertebrates, which are a vital trophic link between primary producers, detritus, and consumers (Poirier and Cattaneo, 2010).

Due to their high taxonomic diversity, position in freshwater ecosystem food webs, and variety of feeding habits and habitat tolerances, benthic macroinvertebrates are functional ecosystem health indicators (Akamagwuna and Odume, 2020), commonly used as bioindicators to assess ecosystem health and determine if an ecosystem is experiencing anthropogenic ecological degradation (Jonsson et al., 2018).

Benthic macroinvertebrates regulate water quality, decomposition, and nutrient cycling in freshwater ecosystems (Wallace and Webster, 1996). They comprise different functional feeding groups due to variation in behavioral mechanisms of acquiring food. These groups include scrapers, shredders, gatherers, filterers, and predators, all of which provide a balance to the trophic dynamics of a benthic ecosystem by consuming a variety of foods such as algae, detritus, leaf litter, suspended organic particles, and other small organisms (Barbour et al., 1999). When an imbalance of this community structure occurs, the detrital food web and rate of decomposition can be disrupted, slowing down the recycling of nutrients and organic material.

The United States Environmental Protection Agency (USEPA) has categorized benthic macroinvertebrates into three groups based on their tolerance to pollution: intolerant, moderately intolerant, and tolerant. CyanoHAB can lead to mortality in benthic organisms through clogged respiratory structures, oxygen depletion, and toxicity (Kroger et al., 2006). Cyanobacteria, specifically *Microcystis spp.*, has led to mortality in invertebrates such as *Daphnia spp.* (DeMott et al., 1991), brine shrimp, *Artemia salina* (Metcalf et al., 2002), and copepods (Reinikainen et al., 2002). *Microcystis spp.* has also been observed to kill the aquatic mosquito larvae, *Aedes aegypti* (Kiviranta et al., 1993). However, an understanding of how cyanobacteria can disrupt communities of benthic macroinvertebrates across pollution tolerance groups remains incomplete.

This study aims to investigate the effect of *Microcystis aeruginosa* on the survival of benthic macroinvertebrates, across three pollution tolerance groups, intolerant, moderately intolerant, and tolerant. For this study, I tested immature stadia of the pollution intolerant taxa Ephemeroptera, mayfly nymphs, pollution moderately intolerant taxa Zygoptera, damselfly nymphs, and pollution tolerant taxa Chironomidae, midge larvae (Voshell, 2002), in a lab setting. Mayflies, damselflies, and midges share a natural habitat in New Jersey freshwater ecosystems and are each in a different macroinvertebrate pollution tolerance group. Mayflies are considered to be important in freshwater ecosystems and are commonly used as ecological indicators as they are sensitive to environmental changes (Menetrey et al., 2008). Mayflies are primarily collector-gatherer detritivores but can also be herbivores and carnivores (McShaffrey and McCafferty, 1991). They mainly scrape periphyton off substrate and consume algae and detritus (McShaffrey and McCafferty, 1990). Mayflies are incredibly important in aquatic food webs as they are the main food source for many fish species (Morse et al., 1997). Damselflies are instrumental to aquatic ecosystems. Their life histories change when their habitats switch from aquatic to terrestrial environments; they are predators in aquatic ecosystems and prey in terrestrial ecosystems (Butler and deMaynadier, 2008). Midges are considered to be the most abundant and diverse group of benthic macroinvertebrates (Milosevic et al., 2013). Midges are opportunistic omnivores, consuming decomposed plant and animal matters, diatoms, and algae (Peckarsky et al., 1990). Their different feeding behaviors, usually gathering, filter feeding, and sometimes predatory, contribute to the recycling of organic matter, making them an integral part of aquatic food webs (Stankovic et al., 2020).

CyanoHAB may affect benthic macroinvertebrate community structure by reducing populations of less pollution-tolerant macroinvertebrate species, thus disrupting the composition of benthic macroinvertebrate communities. Such disruptions may affect the structure and function of entire food webs. The objective of this study is to determine if by exposing benthic macroinvertebrates from different pollution tolerance groups to *Microcystis aeruginosa*, as compared to the control group that was not exposed to *Microcystis aeruginosa,* the benthic macroinvertebrates would sustain a significantly higher mortality which would roughly correspond to their respective pollution tolerance levels.

Methods and Materials

Study Organisms

Ephemeroptera nymphs, Zygoptera nymphs, and Chironomidae larvae were collected from a manmade lake, Lake Wapalanne, in Sandyston, New Jersey in November of 2019. Located in a watershed within forest landscape and protected land, Lake Wapalanne is fed by Big Flat Brook, with controlled outflow by a weir.

Experiments were conducted on either of the two *Ephemerellidae* genera, *E. ephemerella* and *E. eurylophella*, as these organisms naturally inhabit the benthos of Lake Wapalanne and its tributaries. *E. ephemerella* and *E. eurylophella* are considered scrapers and collectors, feeding on available periphyton found on substrate (Pennak, 1978). The *Chironomidae* species *Chironomus riparius* larvae were used in the experiment. This species, which are collector-gatherers, feed mainly on detritus (Pery et al., 2009). The experiment examined a combination of the three Zygoptera genera, *Calopterygidae calopteryx*, *Coenagrionidae enallagma*, and *Lestidae lestes*, as these organisms naturally inhabit the benthos of Lake Wapalanne and its tributaries, and have similar habitat preferences, feeding behaviors, and life histories (Pennak, 1978). *C. calopteryx*, *C. enallagma*, and *L. lestes* are predators in their environment, feeding mainly on small invertebrates (Pennak, 1978).

Microcystis aeruginosa was cultured from a stock of *Microcystis aeruginosa*, originally purchased from UTEX Culture Collection of Algae at The University of Texas at Austin, using the media BG-11, manufactured by Sigma Aldrich, in an environmental growth chamber with light and temperature control at Montclair State University.

<u>Experimental Design</u>

Immediately after collection, macroinvertebrates were transported back to Montclair State University's greenhouse. After four weeks of acclimation, benthic macroinvertebrates were maintained in two 95L black plastic bins (one treatment and one control) that contained 28L of dechlorinated tap water in order to mimic the natural environment at the bottom of shallow aquatic ecosystems.

Benthic macroinvertebrates were randomly allocated into 30.5 centimeters tall plastic pipes, each with a diameter of 7.6 centimeters (Figure 1). The three macroinvertebrate pollution-tolerance groups were replicated four times in each bin, making a total of twelve plastic pipes per bin; there were fifteen individuals in each plastic pipe. Two sections, each measuring 5 centimeters wide and 25.4 centimeters long, were cut out of plastic pipes and covered with fiberglass mesh (pore size $1mm^2$), to allow water and *Microcystis aeruginosa* cells to flow freely through plastic pipes but keep macroinvertebrates inside (Figure 1). Gravel and *Potamogeton perfoliatus* (claspingleaf pondweed) were taken from the lake and placed in the plastic pipes to mimic a natural lake environment.

CyanoHAB usually take place in the summer and fall months when water temperature is warm, therefore experiments were conducted in a temperature-controlled greenhouse. Cyanobacteria and its growth rate show a strong response to high water temperatures, usually above 25 degrees Celsius (Paerl, 2018); thermometers and water heaters were used to maintain a water temperature of 20 to 25 degrees Celsius. Aerators were used to keep water oxygenated and circulated. In this study, the treatment group was inoculated with *M. aeruginosa* at a concentration of approximately 100,000 cells/ml. *Microcystis aeruginosa* cell count was monitored every three days and maintained as needed using a microscope. Macroinvertebrate survival was checked every six days for a 36-day period to determine macroinvertebrate survival and mortality. Individuals were confirmed dead by the absence of body or gill movement. Macroinvertebrates that emerged into terrestrial adults during the experiment were considered alive during data analysis.

Statistical Analysis

To test for differences in survival percentages among the treatments (insect type and cyanobacteria presence), I used a generalized linear model with a binomial distribution which approximates a logistic regression with categorical data. A binomial distribution is often used when testing for differences in percentages or ratios among groups. I then conducted a Tukey's posthoc analysis to test for specific differences among the groups, comparing each species and the treatment and control groups for each species. One-way ANOVAs were used to test for differences in means in total surviving macroinvertebrates between control and treatment groups. A p-value of less than 0.05 is considered statistically significant for this study. For this analysis, I used the *glm* and *TukeyHSD* functions, along with the *Anova* function in the *car* package in *R*.

Results

Benthic Macroinvertebrate Survival

Microcystis aeruginosa presence (χ^2=5.8809, *df*=1, *p*= 0.01531) and insect type (χ^2=1.6738, *df*=2, *p*=0.43305) predicted the survival percentage of the macroinvertebrates, but there was no interaction (χ^2=0.3232, *df*=2, *p*=0.85079) between *Microcystis aeruginosa* presence and insect type (Table 1). The Tukey's posthoc test revealed that the presence of *Microcystis aeruginosa* had a significant effect on the number of surviving mayflies (p< 0.001), damselflies (p< 0.001), and midges (p<0.001) (Figure 2). The Tukey's test also revealed a significant difference between midge survival percentages and the survival percentages of both mayflies and damselflies, but the difference between mayfly and damselfly survival rates was not statistically significant. This demonstrates that mayflies (pollution intolerant) and damselflies (moderately intolerant) had similar responses to *Microcystis aeruginosa* in terms of mortality. In the treatment, there was no significant difference between the survival percentages of each macroinvertebrate pollution tolerance group.

The mean survival percentage for treatment groups exposed to *Microcystis aeruginosa* was substantially lower than the mean survival percentage for control groups not exposed to *Microcystis aeruginosa* (p<0.001). Mayflies had a survival percentage of 38% in the *Microcystis aeruginosa* treatment group compared to a 98% survival percentage in the control group (Figure 3). Damselflies had a survival percentage of 43% in the *Microcystis aeruginosa* treatment group compared to a 98% survival percentage in the control group (Figure 3). Midges had a survival percentage of 72% in the *Microcystis aeruginosa* treatment group compared to a 100% survival percentage in the control group (Figure 3).

In the treatment group, the percentage of individuals that died corresponded with their respective pollution tolerance levels: mayflies (pollution intolerant) died earliest on average and in the highest total number of deaths, and midges (pollution tolerant) died the latest on average and in the lowest total number of deaths, while these values for damselflies (moderately intolerant) fell in between (Figure 4). In the control group, there was only one death recorded for mayflies and damselflies, which occurred at the seventh week (Figure 4).

Number of Surviving Insects:

These results can also be demonstrated in terms of mean surviving insects of 15 macroinvertebrates, including insects that emerged (Table 2). A notable dissimilarity in the number of emerged macroinvertebrates occurred between treatment and control groups. Among treatment mayflies, zero emerged, whereas seven emerged in the control group. One damselfly emerged in the treatment group, and nine emerged in the control group. No midges emerged in either group. The several emerged individuals in the control group could be a result of normal macroinvertebrate growth in a healthy environment. *Microcystis aeruginosa* appeared to have a substantial effect on either the growth or ability to emerge for certain macroinvertebrates (Figure 5). Due to the emergence of insects, some individuals did not remain present in the full experiment, which should be taken into consideration.

Discussion

A previous study on the survival of mayflies (Smith et al., 2007) when exposed to microcystin-LR documented similar findings, where 20% of egg hatchlings died in a seven-day period when exposed to lower toxin concentrations of 0.01 μg/ml; 100% of egg hatchlings died within five days when exposed to a higher concentration of microcystin-LR of 10.0 μg/ml. *Microcystis aeruginosa* had a negative effect on the survival of the macroinvertebrates in this experiment, reducing the survival by more than 60% in the most sensitive group, Ephemeroptera.

Mayflies are particularly sensitive to poor water quality and pollution and had the lowest survival percentage, 38%.

In a previous study where the different mayfly larval stages were exposed to microcystins, the eggs required two extra days for 90% to emerge (Smith et al., 2007). In addition, 20% of the egg hatchlings (young nymphs) exposed to typical bloom conditions (0.01 μg/ml) died within a seven-day period at the highest concentration (10 μg/ml), 100% of egg hatchlings died after five days (Smith et al., 2007). Microcystins, therefore, had a negative effect on the emergence time of mayfly eggs and the survival of egg hatchlings thereafter. A similar response occurred in this study where *Microcystis aeruginosa* delayed or prohibited nymphs from emerging into terrestrial insects. Since benthic macroinvertebrates have different larval stages that are a part of aquatic and terrestrial ecosystems, exposure could affect both food webs in a natural setting.

CyanoHAB have been shown to reduce feeding rates for not only filter feeding taxa (Yang et al., 2006), but for visual predators due to reduced water quality and light (Jiang et al., 2014). Damselflies are visual predators and thrive in waters that have limited suspended particles, high visibility and high oxygen levels. CyanoHAB create an unfavorable environment for damselflies, with increasing suspended particles, decreasing visibility, and deoxygenating bottom waters (Akamagwuna and Odume, 2020). Midges and mosquito larvae are both pollution tolerant. In a study, where mosquito larvae were exposed to *Microcystis aeruginosa* and toxic strains of *Anabaena circinalis* and *Oscillatoria agardhii*, the larvae developed lesions in their epithelial cells just after a 24-hour exposure period (Saario et al., 1994). *Microcystis aeruginosa* and cyanotoxins that it produced can affect benthic macroinvertebrates by being ingested or diffused across the cuticle, egg, or gill membranes (Smith et al., 2007). However, the exact nature of the effects of *Microcystis aeruginosa* and other cyanobacteria exposure is difficult to determine due to the limited information available regarding the toxicity to benthic macroinvertebrates. A consideration for future research on the effects of *Microcystis aeruginosa* on benthic macroinvertebrates is that studies should take into account that each macroinvertebrate species likely has a unique tolerance to *Microcystis aeruginosa*. Although *Microcystis aeruginosa* had an effect on these specific species of macroinvertebrates, that may not be the case for other species of the same taxonomic order.

The survival results for each species of macroinvertebrates in this experiment corresponded with the pollution tolerance levels of the species, and the mean number of pollution-tolerant midges was significantly different than the mean number of surviving mayflies and damselflies, indicating that pollution tolerance should be a consideration in future studies of benthic macroinvertebrates and exposure to CyanoHAB, especially when *Microcystis aeruginosa* is present. In the control groups, there is no statistical difference in survival means between any macroinvertebrate species. These results further suggest that measures to mitigate or control CyanoHAB, specifically their monitoring and evaluation components, should consider the relationship between macroinvertebrate survival percentages as it pertains to *Microcystis aeruginosa* exposure and pollution tolerance levels. Further research is needed to determine how broader populations of pollution tolerant macroinvertebrate species such as midges are affected by CyanoHAB in relation to populations of less pollution tolerant macroinvertebrate species. If pollution tolerant macroinvertebrates have higher survival probabilities to CyanoHAB than less tolerant species, this could result in changes to community structure, resulting in changes in ecosystem functions and services. Benthic macroinvertebrates are among the most diverse (Strayer, 2006) and ubiquitous (Voelz and Mcarthur, 2000) organisms in freshwater ecosystems, giving them a vital ecological role. Because benthic macroinvertebrates are an important trophic link between primary producers and consumers (Poirier and Cattaneo, 2010), a shift in abundance and diversity could affect each trophic level to which these macroinvertebrates are connected (Maisto et al., 2017).

As anthropogenic eutrophication and climate change increase the magnitude and frequency of CyanoHAB events around the world, the need to understand how blooms affect aquatic ecosystems is paramount (Briland et al., 2020). Further, *Microcystis aeruginosa* was shown to kill benthic macroinvertebrates at different rates relative to their pollution tolerance group, thus reducing biodiversity and abundance. Because this study suggests a link between CyanoHAB and a reduction in biodiversity among benthic macroinvertebrate communities, climate change should be approached with even greater urgency because of the critical role macroinvertebrate biodiversity plays in an ecosystem. Since macroinvertebrates are environmental indicators, the mortality displayed in this study and previous studies demonstrates that CyanoHAB can have deleterious effects on freshwater ecosystems.

Future studies should be conducted in the lab and field in order to better understand the impacts of CyanoHAB on the structure, function, and dynamics of aquatic as well as terrestrial food webs. In addition, studying the effects that different concentrations of cyanobacteria have on aquatic organisms could help predict its threats to ecosystems, especially in freshwater environments that experience recurring CyanoHAB events within a year. Beyond the direct effects that climate change has on increasing the magnitude and frequency of CyanoHAB, future studies should investigate how other aspects of climate change, such as warmer temperatures, more or less rainfall, species migration, etc., interact with cyanobacteria and its effects on benthic macroinvertebrate communities. Finally, future research on the effects of *Microcystis* *spp*. should take into account that each macroinvertebrate species likely has a unique reaction to cyanobacteria.

Conclusion

The present study demonstrated that *Microcystis aeruginosa*, in high concentrations causes increased mortality in benthic macroinvertebrates in correspondence to their pollution tolerance levels. Additionally, *Microcystis aeruginosa* delayed or prevented macroinvertebrate nymphs from emerging into terrestrial stadia. Previous studies have also shown that *Microcystis aeruginosa* and other cyanobacteria cause mortality in aquatic fauna, including insects, fish, and vegetation. However, many questions about how CyanoHAB affect the structure of both aquatic and terrestrial food webs remain unanswered. Future research on the effects of *Microcystis aeruginosa* should take into account that each benthic macroinvertebrate species likely has a unique reaction to cyanobacteria. Although *Microcystis aeruginosa* had an effect on these specific species of macroinvertebrates, that may not be the case for other species of the same taxonomic order. Moreover, cyanobacteria seem to have the ability to take advantage of an increasingly warming world. Researchers should focus on how this advantage will affect freshwater ecosystems ecologically and economically in the future as bloom events continue to increase.

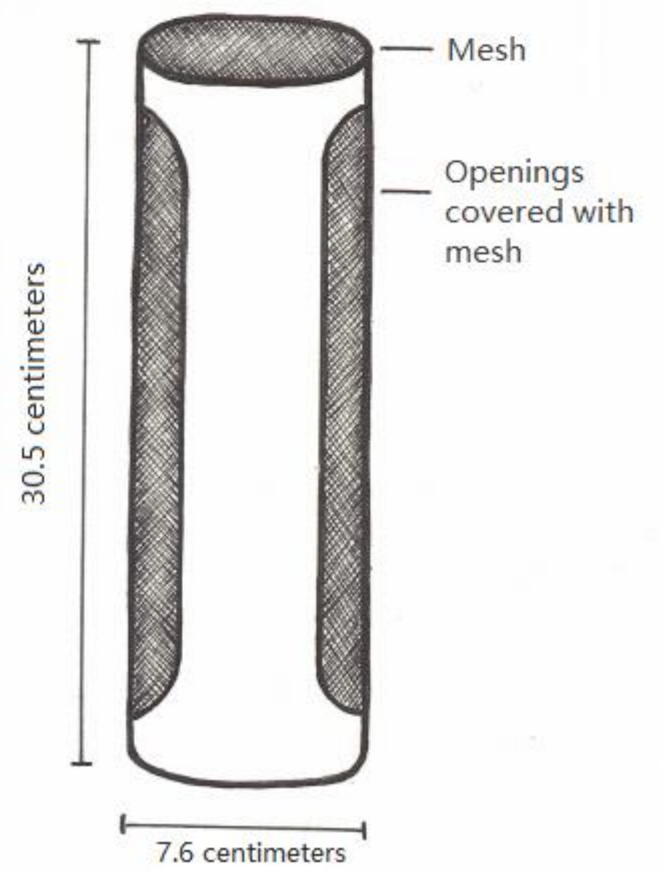

Figure 1- Diagram of experimental design.

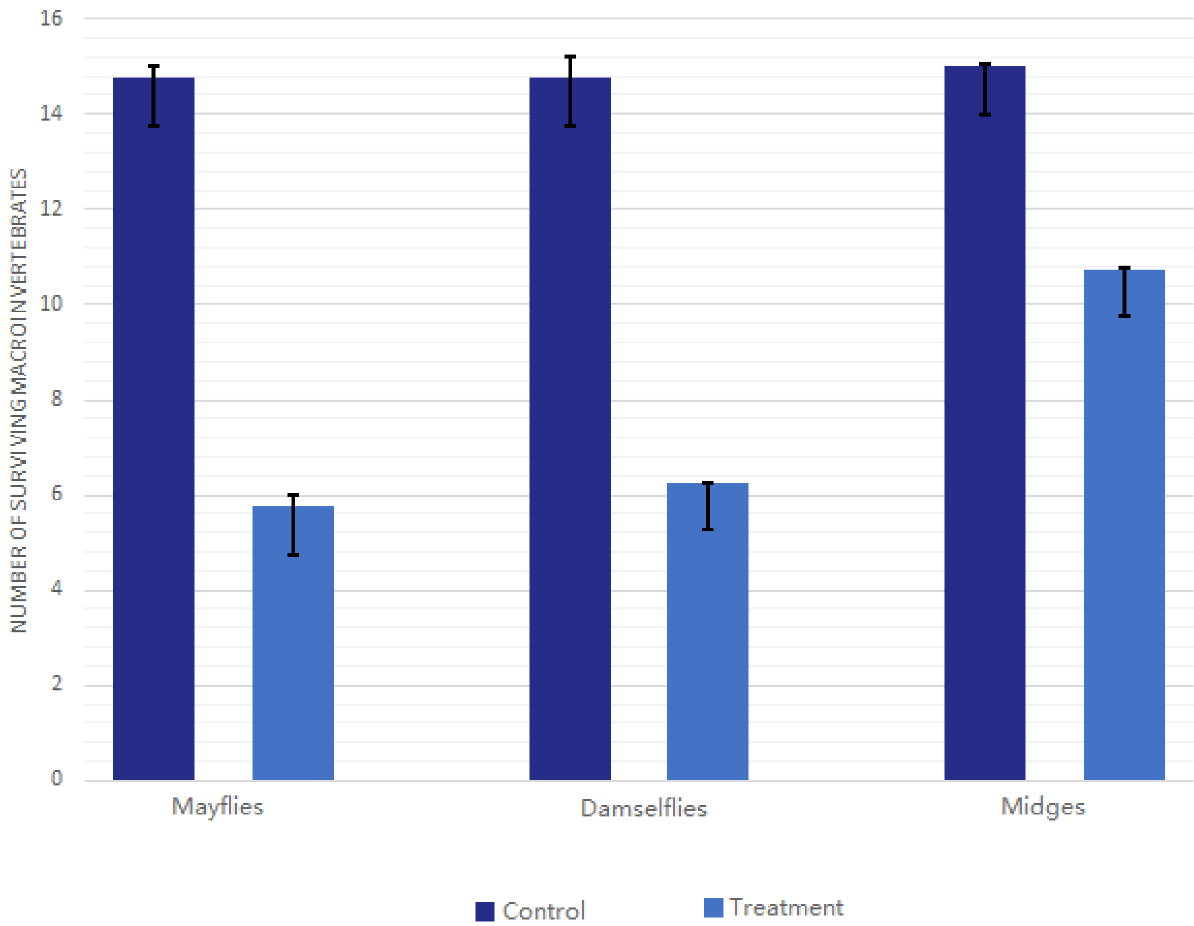

Figure 2- The mean number of surviving mayflies, damselflies, and midges after being exposed to *Microcystis aeruginosa* compared to the mean number of surviving mayflies, damselflies, and midges in the control group.

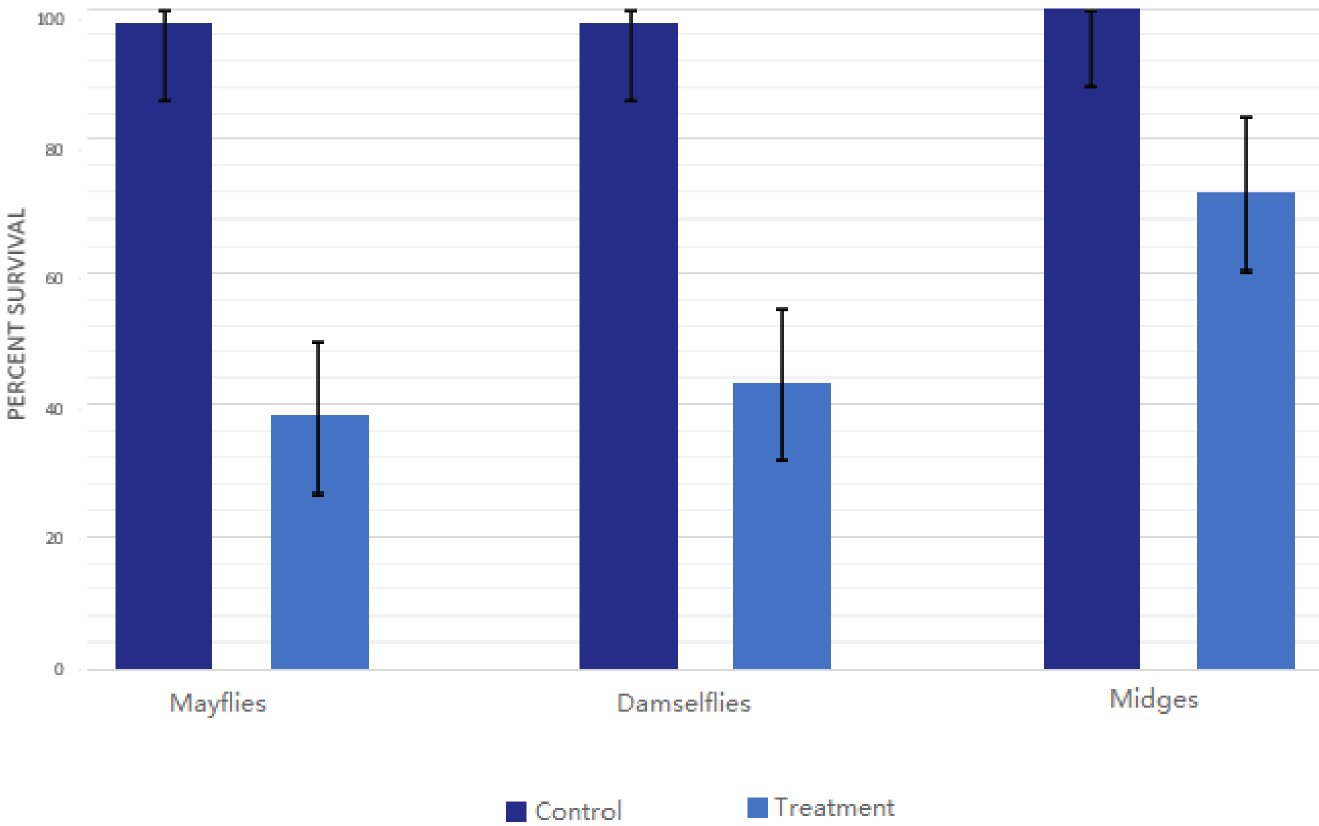

Figure 3- The survival percentage of mayflies, damselflies, and midges after being exposed to *Microcystis aeruginosa* compared to the survival percentage of mayflies, damselflies, and midges in the control group. Figure includes standard error.

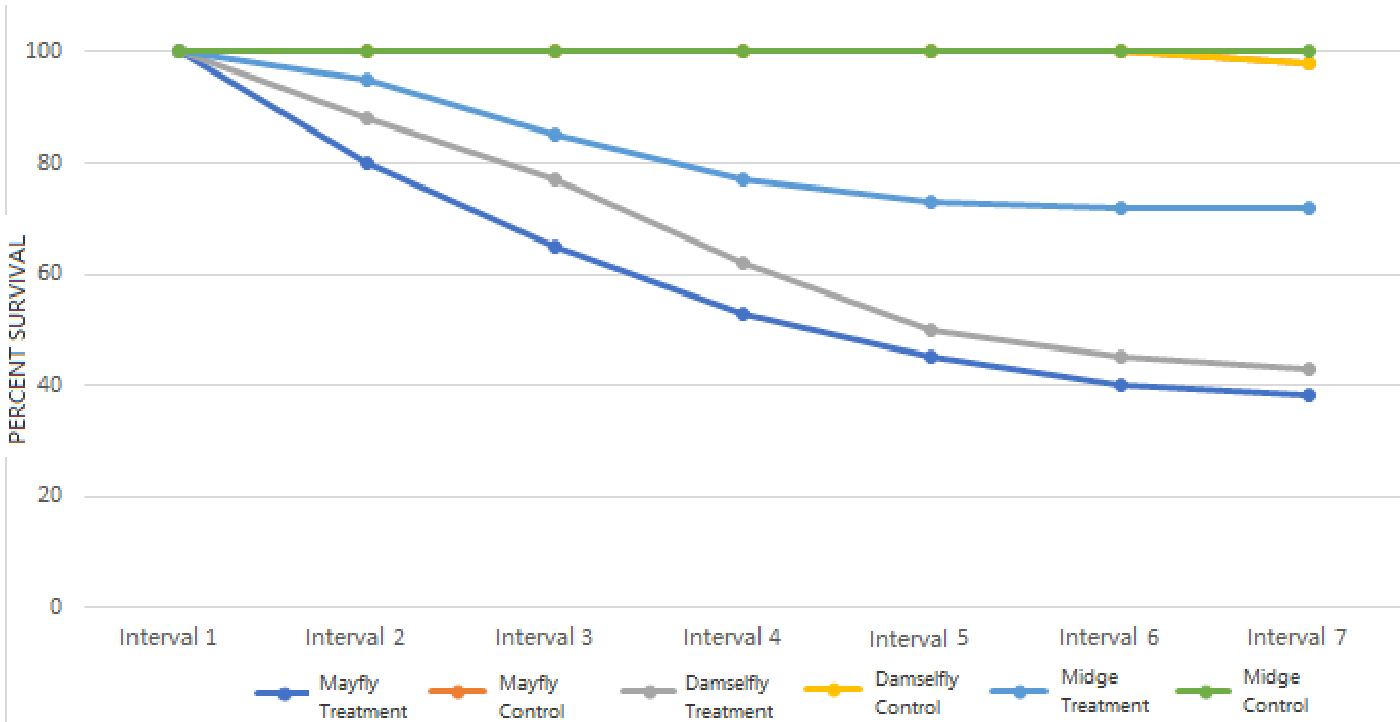

Figure 4- The comparative mean survival percentage of mayflies, damselflies, and midges exposed and not exposed to *Microcystis aeruginosa* over a 36 day period, in intervals of every 6 days.

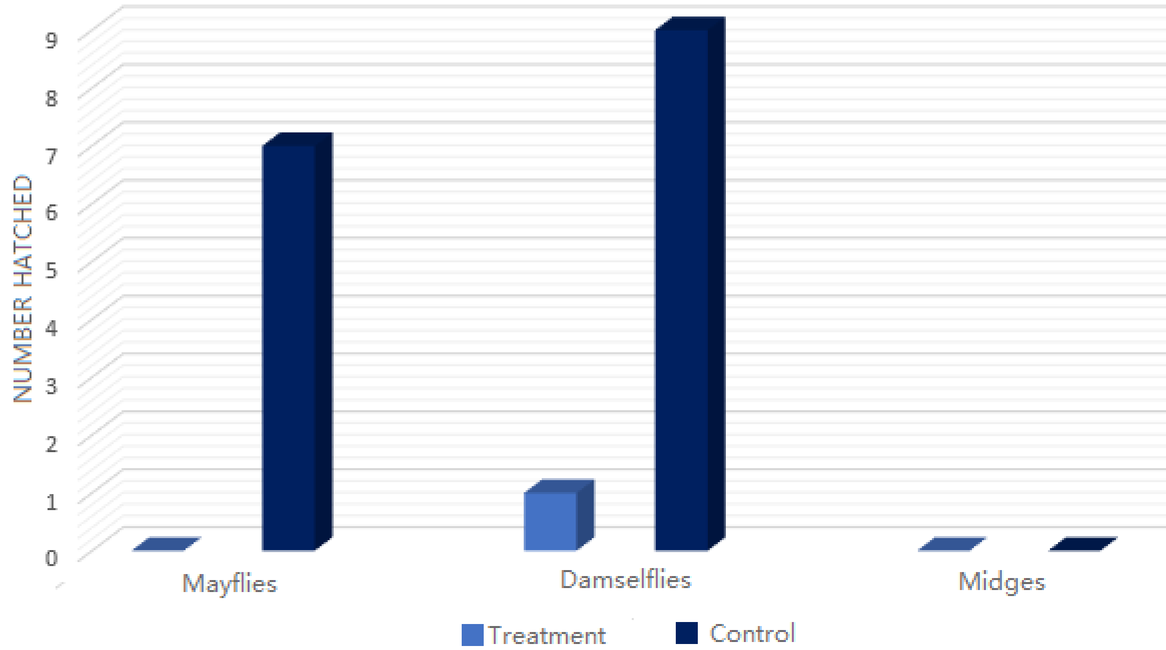

Figure 5- The number of emerged mayflies, damselflies, and midges exposed to *Microcystis aeruginosa* compared to the number of emerged mayflies, damselflies, and midges not exposed to *Microcystis aeruginosa.*

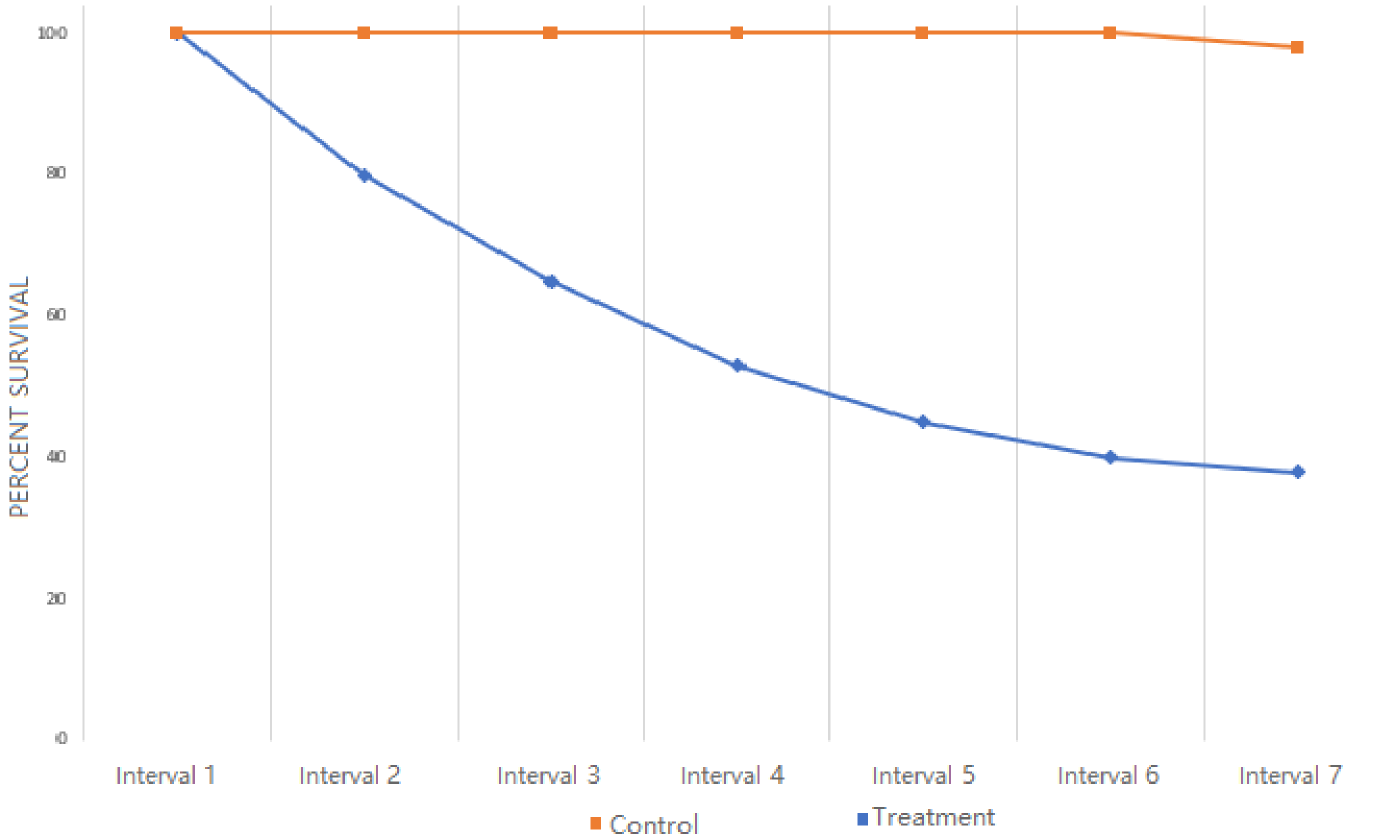

Figure 6- The mean survival percentage of mayflies exposed to *Microcystis aeruginosa* compared to mayflies not exposed to *Microcystis aeruginosa* over a 36 day period, in intervals of every 6 days.

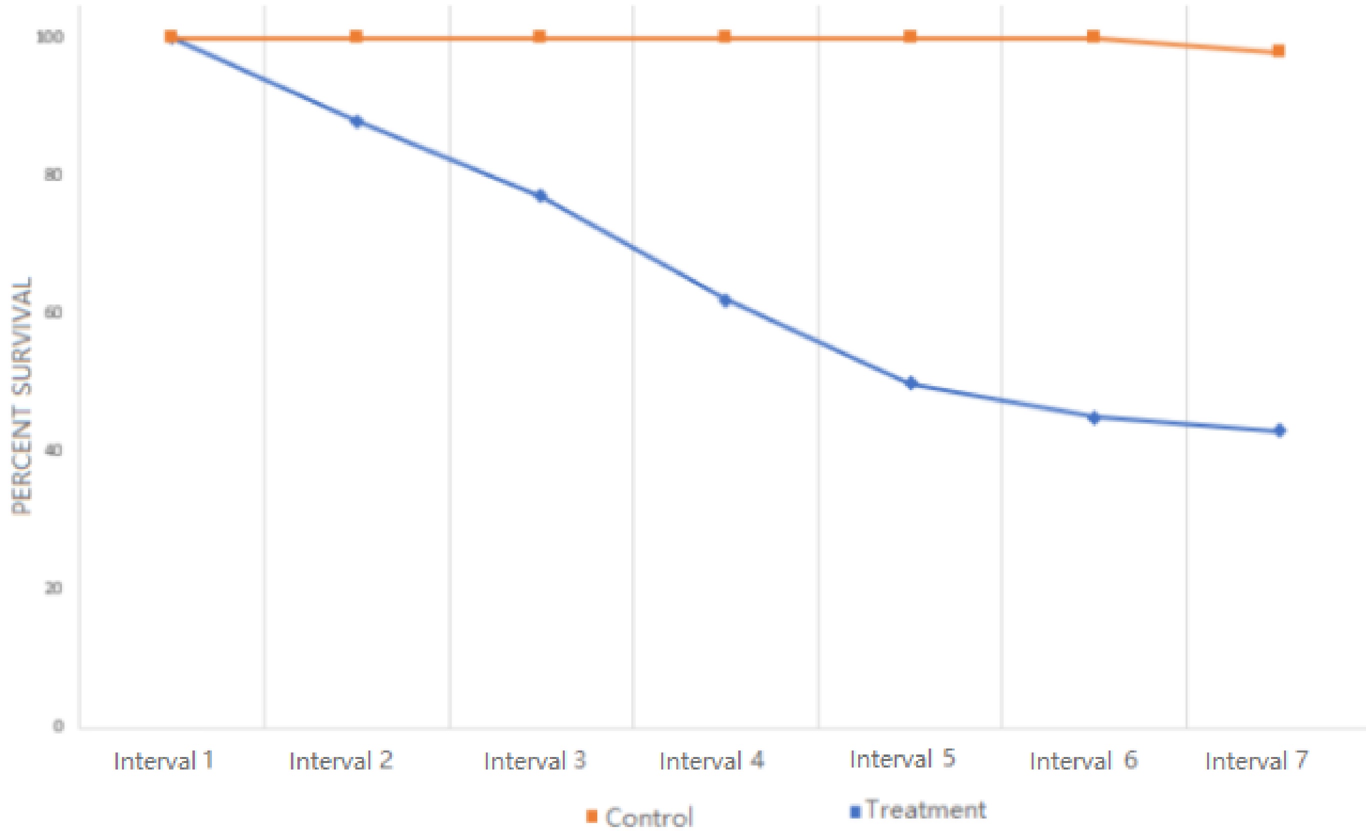

Figure 7- The mean survival percentage of damselflies exposed to *Microcystis aeruginosa* compared to damselflies not exposed to *Microcystis aeruginosa* over a 36 day period, in intervals of every 6 days.

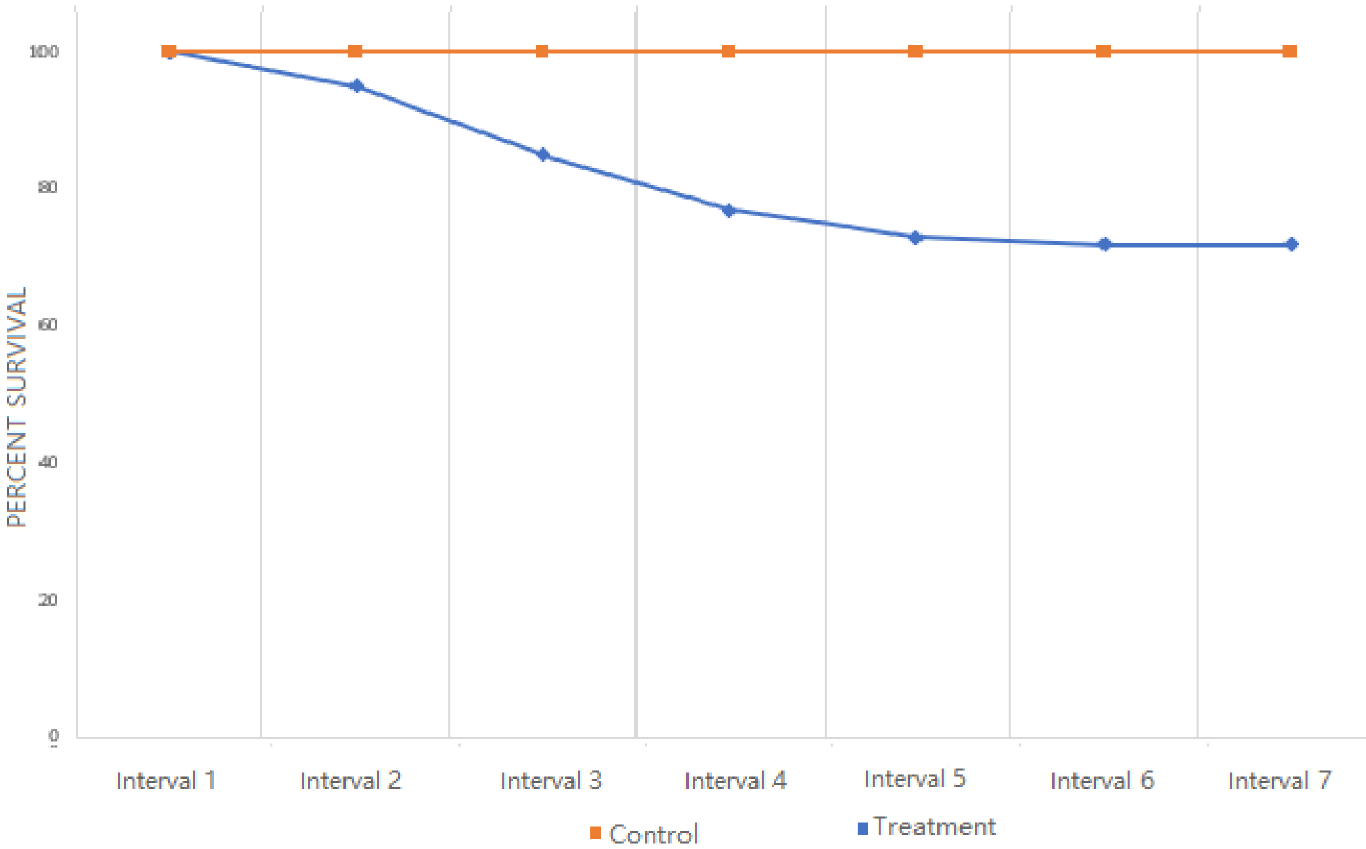

Figure 8 - The mean survival percentage of midges exposed to *Microcystis aeruginosa* compared to midges not exposed to *Microcystis aeruginosa* over a 36 day period, in intervals of every 6 days.

Table 1. Generalized linear model results showing the effect of *Microcystis aeruginosa* on the survival of the different benthic macroinvertebrates. Significant results are in bold.

	χ^2	df	Pr(>Chisq)
Insect	1.673	2	0.433
Treatment	5.880	1	**0.015**
Insect:Treatment	0.323	2	0.850

Table 2. Mean surviving mayflies, damselflies, and midges exposed to *Microcystis aeruginosa* compared to mean surviving mayflies, damselflies, and midges in the treatment group. Table includes number of emerged insects.

	Treatment		Control	
	Avg. Surviving	Number Emerged	Avg. Surviving	Number Emerged
Mayflies	5.75/15	0	14.75/15	7
Damselflies	6.25/15	1	14.75/15	9
Midges	10.75/15	0	15/15	0

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