Determining effects of *Myriophyllum spicatum* on life history characteristics of *Daphnia magna*

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Department of Biology

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A Senior Comprehensive Project in Partial Fulfillment of the Requirements for a Bachelor of Science Degree from Allegheny College

I hereby recognize and pledge to fulfill my responsibilities as defined in the Honor Code and to maintain the integrity of both myself and the College community as a whole.

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Pledge

Approval by the Senior Thesis Committee:

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Abstract

Life history changes are utilized as an anti-predatory behavior of the zooplankton species, *Daphnia magna,* to survive in situations in which horizontal and vertical migration is unfavorable (Dodson, 1989). Macrophytes have been shown to cause zooplankton to alter life history parameters when they are unable to migrate away from macrophyte beds (Burkes *et al.*, 2000). *Myriophyllum spicatum*, an invasive species of macrophyte, was chosen to examine effects on *D. magna* that have never been exposed to it. I hypothesized that (1) clutch size would decrease, (2) life-expectancy would decrease, and (3) age of first reproduction (AFR) and size at first reproduction (SFR) would decrease in *D. magna* when exposed to *M. spicatum* due to a harsh chemical environment created by *M. spicatum*'s chemical secretions. Using four treatments (a control, a plastic plant, a live *M. spictatum* sprig, and senescent *M. spictatum*), 100 total *D. magna* were placed into individual 50 mL aquaria, 25 per treatment, from birth until death and measured for changes in length, egg/neophyte development, and clutch size every other day for the total duration of the experiment. The *D. magna* in the live M*. spicatum* sprig treatment had the largest average size, highest growth rate, largest clutch size, and earliest AFR. Utilizing a multi-generational style of study would have shown all results more accurately than the single generation study I conducted.

Introduction

Macrophytes (vascular aquatic plants) play a key role in the chemical, biological, and physical environments of lakes and influence the distribution and abundance of organisms living in the littoral zone (Orth and Moore, 1983; Carpenter, 1986; Keast, 1984). Zooplankton, small parthenogenic crustaceans that feed on phytoplankton, is a group of organisms that populates lakes, including macrophyte dominated lakes. Zooplankton play a large role in regulating algal growth while also providing a food source to larger animals, including fish, in lakes (Jeppesen *et al.*, 1998). Consequently, factors affecting zooplankton growth and survival are important in assessing functions within the ecosystem. Zooplankton are consumed by both tactile and visually-orienting predators and must use predator avoidance behaviors, specifically horizontal migration, vertical migration, or life history characteristic changes, in order to survive when predator cues, such as kairomones, are present (Burks *et al.*, 2001; Lima and Dill, 1990; Dodson, 1989). Horizontal migration has been documented in plankton swimming close to macrophytes, as well as in zooplankton living in shallow lake systems where they are unable to properly avoid predators via vertical migration (Nihan Tavsanoglu *et al.*, 2012). When lakes are deep enough, zooplankton will vertically migrate so that visually-orienting predators will not be able to see them (Han and StraSkraba, 1998; Dodson, 1988; Slusarczyk and Pinel-Alloul, 2010; Johnsen and Jakobsen, 1987). In other cases, when migration is not possible, zooplankton will change their life history characteristics, maturing at a smaller size and reproducing earlier (Dodson, 1989).

Each predator avoidance mechanism that a zooplankter can utilize comes with a consequence. The consequences of vertical migration include decreased population growth

rate due to eggs being in a sub-optimal temperature for development, and high energy demand of constant migration throughout the water column (Bottrell, 1975; Orcutt and Porter, 1983; Dawidowicz and Loose, 1992; Riessen and Sprules, 1990; Riessen, 1992). Life history characteristic changes in zooplankton are more favorable for survival conditions compared to migration techniques; however, utilizing life history characteristic changes requires time because it only occurs across generations. Horizontal migration often results in utilizing macrophytes as a source of protection from visually orienting predators. However, certain macrophytes secrete chemicals that repel or even increase the mortality rate of zooplankton (Burkes *et al.,* 2000).

In a study completed by Tavsanoglu *et al.* (2012), chemicals from Canadian pondweed (*Elodea canadensis*) were believed to have forced zooplankton to alter life history characteristics rather than migrate into macrophyte beds for protection. These life history characteristic changes often occur when zooplankton are unable to migrate away from an environment. When the environment is toxic, zooplankters experience an increase in mortality rate as well as a decrease in fecundity (Burkes *et al.*, 2000). Chemicals from macrophytes usually make organisms grow slower than normal and delay reproduction, therefore increasing predation risks for these organisms (Burkes *et al.,* 2000). These chemicals force zooplankters to remain near the sediment rather than in the protection of macrophyte beds (Tavsanoglu *et al.*, 2012).

Macrophytes may suppress growth in zooplankton through both chemical and physical mechanisms. A physical mechanism in which zooplankton populations are suppressed by macrophytes occurs when the macrophytes in a lake become so dense that filter feeding

becomes difficult (Orth and Moore, 1983). Zooplankton cannot properly filter feed when macrophytes physically interfere with swimming. An abundance of macrophytes presents a problem when zooplankton species living in shallow systems must seek refuge in macrophyte beds in order to avoid visually orienting predators trying to consume them (Tavsanoglu *et al.*, 2012). One study has found that in the presence of *Myriophyllum spicatum* (Eurasian watermilfoil), *Daphnia pulex*, a species of zooplankton, began reproducing at an earlier age and at a smaller size after a single generation (Cerbin *et al.*, 2007). However, the same study also found that the life history characteristic traits were changed when in the presence of artificial macrophytes (Cerbin *et al.*, 2007). These trait changes were the direct result of improper feeding because the macrophytes were interfering with the *Daphnia*'s ability to filter feed properly.

No research, to my knowledge, is currently available to determine the effects on life history characteristic changes that *Daphnia magna* undergoes in the presence of Eurasian watermilfoil. Furthermore, the effects of Eurasian watermilfoil on *D. magna* un-acclimated to the plant have not been determined. However, very few research studies have verified that it is strictly chemicals impacting the life history characteristics of zooplankton.

The purpose of this study is to determine if life history characteristics in *D. magna* are affected by chemicals secreted by Eurasian watermilfoil. More specifically, I would like to determine what effect Eurasian watermilfoil has on *D. magna* collected in a system free of said species of macrophyte to determine if any changes are due to high macrophyte densities rather than chemical cues. I hypothesize that (1) clutch size will decrease, (2) life-expectancy will decrease, and (3) age of first reproduction (AFR) and size at first reproduction (SFR) will

decrease in *D. magna* when exposed to *M. spicatum* due to a harsh chemical environment created by *M. spicatum*'s chemical secretions.

Methods

Daphnia were purchased from Carolina® and cultured in low light conditions in an aerated aquarium. Select individuals were taken and placed in separate 50mL aquaria and allowed to parthenogenically breed for multiple generations, with each generation being about 10 days, allowing for no genetic variance between each other. After 100 juveniles hatched, they were immediately collected and individually placed into 50mL aquaria to undergo experimental treatment. The diet consisted of 3 drops of homogenized *Spirulina*, ordered from Carolina®, mixed with 10mL lake water collected from Conneaut Lake, located in Crawford County, Pennsylvania, to a concentration of 300 mg/mL dry weight for the duration of the experiment. The *D. magna* were placed in a chamber simulating environmental conditions with a temperature of 20±1°C and a 16:8 hour light/dark schedule. The *D. magna* were measured every other day from head to the base of the spine beginning at birth and ending on the day of death.

M. spicatum was collected from the littoral zone of Conneaut Lake with a rake. The *M. spicatum* was grown in a greenhouse located on the Allegheny College campus to ensure enough specimens for experimentation. *M. spicatum* was grown under grow lights in five, 5-gallon tanks filled with lake water and artificial sand purchased from Carter Lumber, Conneaut Lake, Pa.

The experiment consisted of 4 separate treatments with 25 individual *D. magna* per treatment. The experiment lasted for the lifetime of one full generation in which the

zooplankton were individually extracted via pipette every other day to measure growth (mm), clutch development, and survival. These measurements were used in the calculation of life history characteristics.

The first treatment was the control treatment with only filtered lake water collected from Lake Pleasant, the second treatment had artificial plants, the third treatment had live Eurasian watermilfoil, and the final treatment had senescent Eurasian watermilfoil. The control treatment allowed for a base reading for what life expectancy, clutch size, over-all growth, age of first reproduction (AFR), and size at first reproduction (SFR) in the absence of any chemical or physical effects of *M. spicatum*.

The second treatment acted as a physical control to determine if *M. spicatum* is affected *D. magna* due to the macrophyte making feeding more difficult by taking up more space in the aquarium. Artificial plants were cut between 5 and 6 cm in length and placed in each aquarium in this treatment.

The third treatment was an experimental treatment in which a small sprig of *M. spicatum* between 5 and 6 cm in length was placed in each aquarium. The second treatment was compared to the third treatment to see if there was a correlation between life history changes and full plants in each aquarium.

In the fourth treatment group, a sprig of *M. spicatum* between 5 and 6 cm in length was ground using a mortar and pestle allowing for senescent material to be released. The senescent *M. spicatum* was then placed in filtered lake water and then placed into each of the aquaria allowing the senescent plants to interact with *D. magna*. The third treatment's results were

compared with the results of treatment four to determine how the chemical nature of *M. spicatum* affects life history characteristics of *D. magna*.

The differences in AFR, SFR, clutch size, and life expectancy between treatments were calculated using a 1-way analysis of variation (ANOVA) to determine any difference in life history characteristics of *D. magna* between treatments 1 and 2 due to strictly physical structure; between treatments 2 and 3 due to healthy plant chemistry; and between treatments 3 and 4 due to plant senescence. The average values of each treatment were given with ± Standard Error bars included. An analysis of covariance (ANCOVA) was used to determine the differences between the growth rates of all treatments.

Results

After experimentation, the maximum *D. magna* lifespan was found to be 33 days in the *M. spicatum* treatment. All other treatments yielded a maximum lifespan of 27 days before complete mortality. The survivorship of *D. magna* specimens among treatments was graphed as an average (Figure 1). The overall chance of survival was similar among all treatments with the exception of the plastic treatment which had the greatest survivorship compared to the other three treatments (Figure 1).

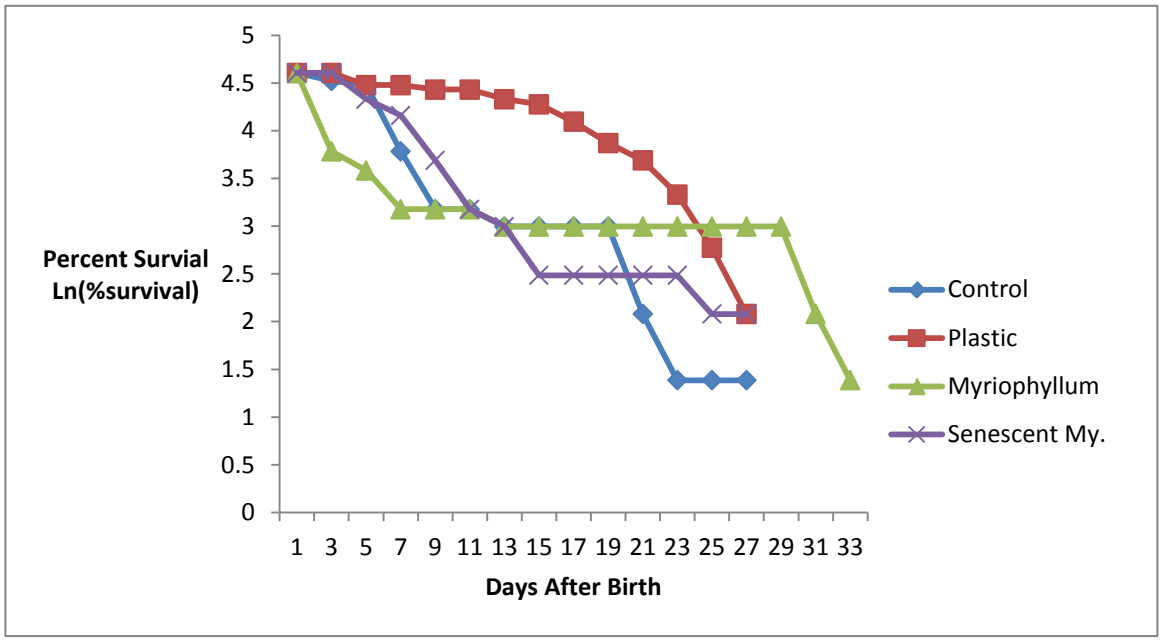

Figure 1. *The chance of survival determined by taking the amount of specimens alive every other day divided by the total number of individuals, multiplied by 100, and then taking the natural log of the resulting number. Values were plotted over duration of every other day until complete mortality occurred among treatments*

The SFR was found to have no significant differences among all treatments. Instead, the average length from birth to death of all specimens was taken for each treatment and compared. The sprig of *M. spicatum* treatment had the largest average length of any other treatment's specimens at 2.715 mm which is 0.616 mm larger than the next largest treatment average (Figure 2). An ANOVA showed that the *M. spicatum* sprig treatment yielded significant differences (when P-value < 0.05) against both senescent (P= 0.0006) and plastic treatments(P= 0.0119) (Figure 2, see Appendix A). Since the average total length only takes into

account one parameter of length, the growth rate was calculated after every other day until death to get a broader view of how each treatment affected a *D. magna*'s length (Figure 3). An ANCOVA revealed significance between the slopes of all treatments except between the control and the *M. spicatum* sprig treatments. The ANCOVA results show that *D. magna* in the senescent *Myriophyllum* treatment grew significantly slower than both the sprig of *M. spicatum* treatment (P= 0.0318) and the plastic treatment (P = 0.0461) (see Appendix D).

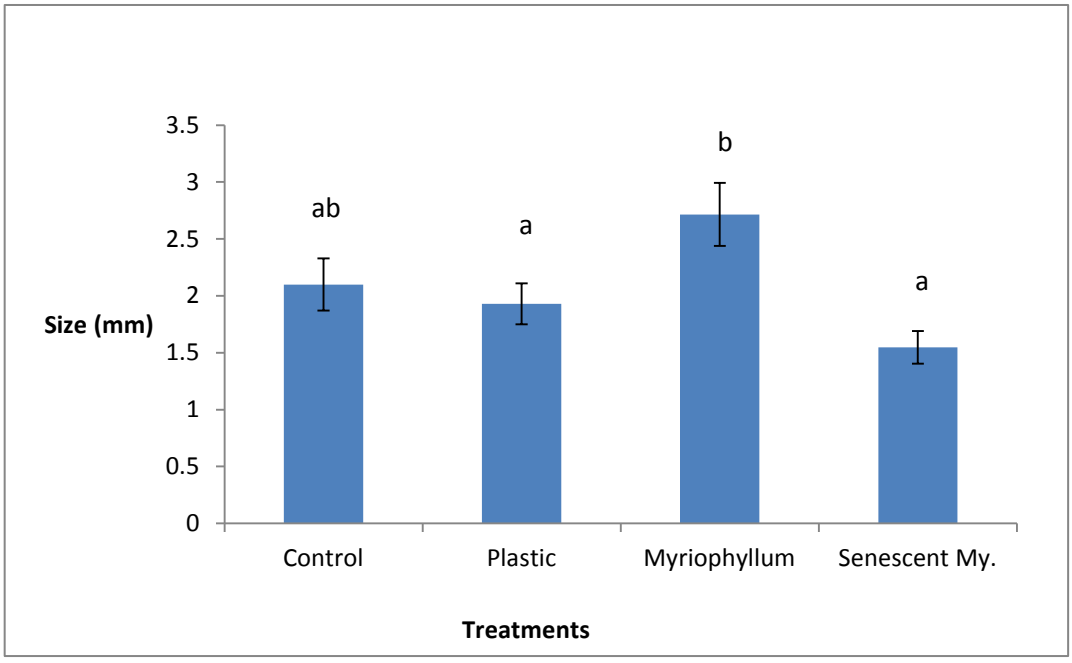

Figure 2. *Average length of all specimens in each treatment at time of death measured from head to base of spine (mm) with corresponding standard error bars. Values above the bar graphs designated with an a, b, or ab represent significance between treatments.*

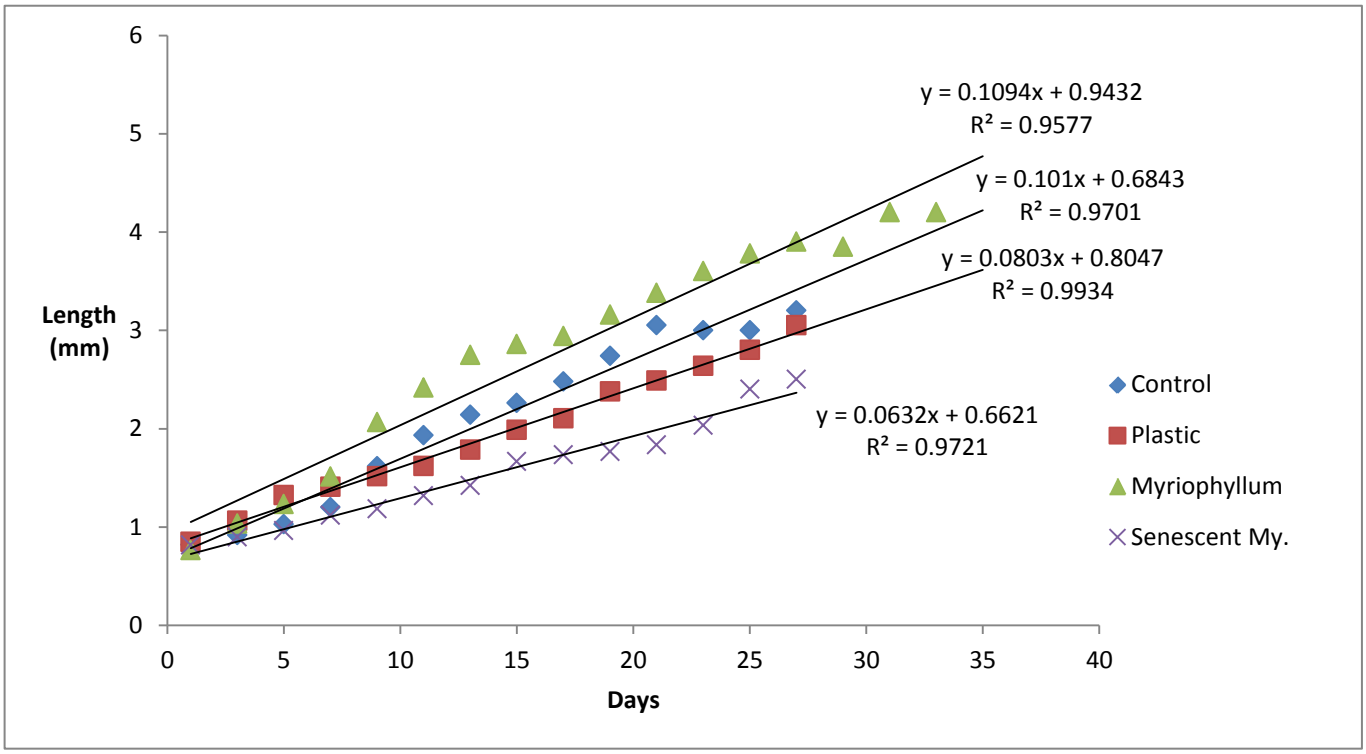

Figure 3. Average lengths of all living *D. magna* specimens calculated every other day until total treatment mortality.

After the growth rates were analyzed, reproductive factors were measured and averaged for each treatment. All treatments had an average brood size relatively close to each other with the exception of the *M. spicatum* sprig treatment. The *M. spicatum* sprig treatment had an approximate brood size of 23 individuals which was 11 individuals more than the next largest average brood size seen in the plastic plant treatment (Figure 4). An ANOVA confirmed that there were significant differences in the *M. spicatum* treatment compared to the control and plastic treatments (P = 0.0005 and 0.0034 respectively) (Figure 4, see Appendix B). Even though the *M. spicatum* sprig treatment had the largest average brood size, it also was the treatment that had the earliest average day of first reproduction at 11 days after birth (Figure 5). The senescent *M. spicatum* treatment had the latest AFR at 19 days after birth. An ANOVA

showed a significant difference in AFR in the plastic treatment compared to the control and senescent *Myriophyllum* treatments (P= 0.0281 and 0.0117 respectively) (Figure 5, see Appendix C).

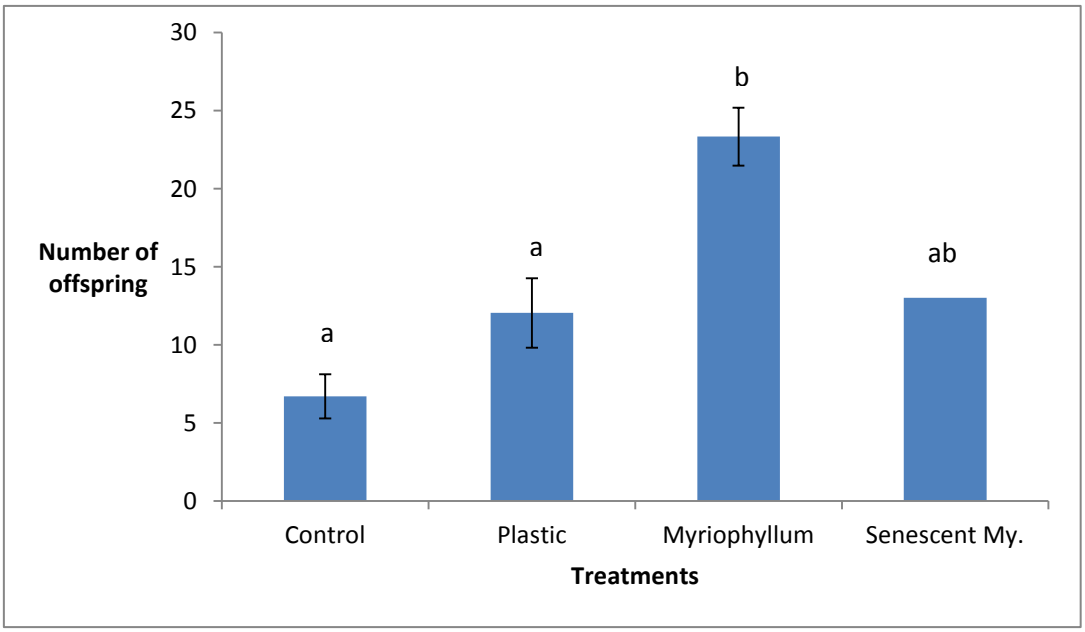

Figure 4. *Average number of offspring each individual creates per treatment over a lifetime with corresponding standard error bars. Values above the bar graphs designated with an a, b, or ab represent significance between treatments.*

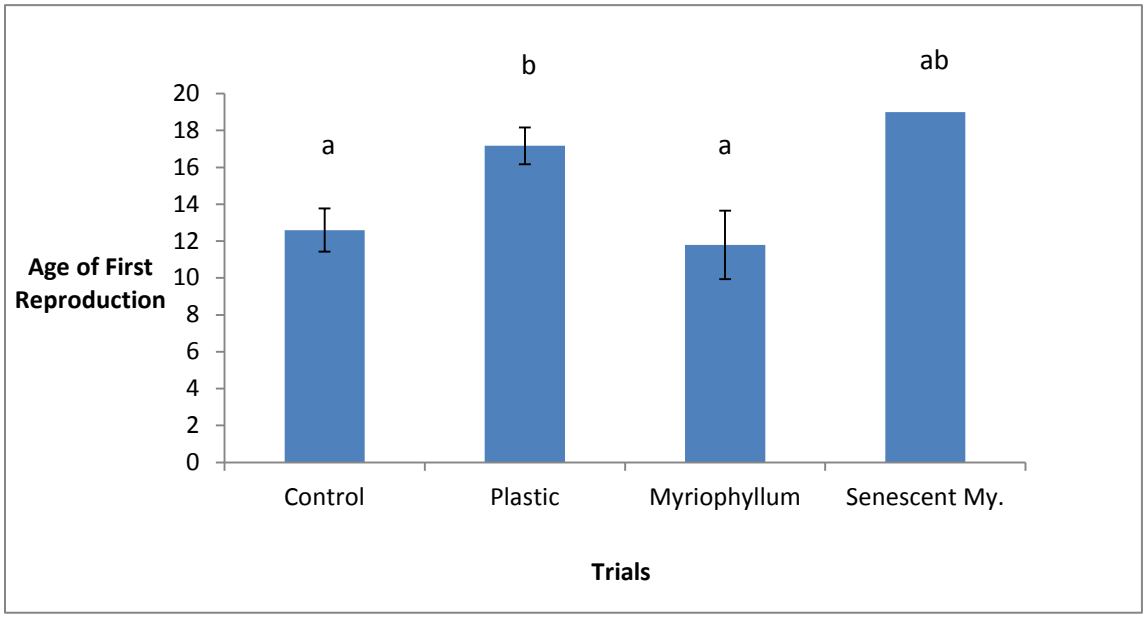

Figure 5. *Average of the first day that egg formation was spotted in each individual separated by treatment and with corresponding standard error bars. Values above the bar graphs designated with an a, b, or ab represent significance between treatments.*

Discussion

The control treatment had the lowest overall survivorship rate based on Figure 1. My second hypothesis, that life expectancy would decrease in the presence of *M. spicatum*, was not supported due to both the *M. spicatum* sprig treatment and the senescent *Myriophyllum* treatment having a higher survivorship ratio in the long term than the control treatment. This result was unexpected due to some chemicals from macrophytes being toxic to many zooplankton species (Burkes *et al.*, 2000; Tavsanoglu *et al.*, 2012). In fact, the *M. spicatum* sprig treatment had the individuals with the longest life, with 3 of the individuals lasting longer than the individuals of any of the other treatments. However, the *M. spicatum* sprig treatment had the lowest initial survivorship statistics for the first 11 days which suggests that certain *D.*

magna individuals within populations may be better suited to live in toxic chemicals. Furthermore, the individuals that live the longest most likely also have the highest number of offspring due to normally high mortality rates within a toxic environment. This theory is plausible based on the statistics showing the *D. magna* in the *M. spicatum* sprig treatment have a significantly larger average brood size than the control and plastic treatments (Figure 4). Since only one Daphnid underwent parthenogenesis in the senescent *Myriophyllum* treatment, the results cannot be used to conclusively link *Myriophyllum* chemical secretions to an earlier AFR.

The *M. spicatum* sprig treatment yielded the largest average sizes by a significant margin, especially over the senescent *Myriophyllum* treatment (P= 0.0006) (Figure 2, Appendix A). The senescent *Myriophyllum* treatment yielded the smallest average sizes which was expected due to more chemicals being present due to homogenized *Myriophyllum*. Although the senescent *Myriophyllum* treatment was expected to show the smallest total average size, the *M. spicatum* sprig treatment was predicted to have the second smallest average size which it did not. Since the plastic treatment shares no significant differences with any treatment besides the *M. spicatum* sprig treatment in average size, it can be determined that having a physical plant present in the treatment does not account for any type of reduced size due to feeding difficulties.

The average growth rate was closely analyzed to see if there were any results that could show less favorability for *D. magna* to live in the *M. spicatum* sprig treatment conditions. However, the *M. spicatum* sprig treatment also showed the largest growth rate while the senescent *Myriophyllum* treatment yielded the smallest growth rate among all treatments (Figure 3). Although my growth results all showed favorability towards the *M. spicatum* sprig

treatments, the results could be skewed due to such a small sample size and only over one generation. It would be interesting to do a similar study over multiple generations to see what types of effects *M. spicatum* has on *D. magna* over time.

The AFR was the last variable that was measured in this experiment. My hypothesis was supported under this variable since the *M. spicatum* sprig treatment had the earliest average day of reproduction compared to all other treatments (Figure 5). Having an early AFR may indicate that the *D. magna* species in that treatment were reproducing more rapidly after sensing a change from a clean water environment to a more toxic and dangerous environment for the specimen. Interestingly, the *D. magna* in the *M. spicatum* treatment not only had the earliest AFR, but they also continued to reproduce for the longest period of time. Reproducing for a long period of time allows for a larger brood size and species survivorship; however, it would also take more energy and resources to continue to do so for such a long period of time. As with the average brood size, the results from the senescent *Myriophyllum* treatment cannot be analyzed with validity since only one individual underwent parthenogenisis.

Although it would have been possible to collect *D. pulex* from Lake Pleasant, Crawford County, Pa, using this species was not preferable due to the translucence of their body in water as well as their significantly smaller body size in comparison to *D. magna*. Without collecting the zooplankton myself, I cannot be 100 percent sure as to what types of conditions the *D. magna* came from, as well as what type of macrophytes they may have been exposed to previously. However, due to *M. spicatum* being a toxic invasive species, there is high probability that the *D. magna* cultured by Carolina® were not exposed to it. A benefit of having ordered the

D. magna was that it ensured that the specimens all derived from a common ancestor giving them little genetic variability.

Research on the impact of invasive species on lakes is important for ensuring the health of not only the organisms living in the lake, but also the people that use the lake. Zooplankton may have the ability to help restore a lake from heavy eutrophication under circumstances that favor their survival (Tuzun and Mason, 1996). This ability can be impacted if a devastating invasive species like *M. spictatum* is introduced into a lake that is in the process of being restored. Future experiments focusing on the impacts of introducing invasive species are important to maintain a healthy lake system. These experiments would benefit from using a larger sample size for each treatment to allow for a larger quantity of results and more definitive proof in the significance of the results. I would also strongly recommend studying the changes over generations to see how these treatments affect zooplankton over more than one generation. Even though results can be measured in the course of one generation within the treatment, generational studies would provide much more definitive proof that all results observed and are continuous over time (Cerbin *et al.*, 2007; Brennan *et al.,* 2006; Civitello *et al.,* 2012).

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Appendices:

Appendix A. Below is my ANOVA table for the overall average length of *D. magna* species over all treatments.

ANOVA Table for Length

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Treatment	3	10.587	3.529	4.708	.0055	14.125	.883
Residual	53	39.726	.750				

Appendix B. *Below is my ANOVA table for the overall average brood size of D. magna species over all treatments.*

ANOVA Table for Residual

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Treatment	3	740.094	246.698	6.136	.0043	18.409	.923
Residual	19	763.862	40.203				

Appendix C. Below is my ANOVA table for the age of first reproduction of *D. magna* species over all treatments.

ANOVA Table for Age of First Reproduction

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Treatment	3	148.364	49.455	3.911	.0259	11.732	.735
Residual	18	227.636	12.646				

Appendix D. Below are my ANCOVA scattergrams between treatments that were found to have significant differences in slopes. They are plotted with corresponding regression lines and R^2 values.

