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## Effects of green algal concentrations on the life history characteristics of *Daphnia magna*

### Abstract

As eutrophication levels continue to rise, it is important to understand how increases in algal biomass will affect other food web components. In this study, we used *Scenedesmus acutus*, a species of green algae, to determine potential implications on the reproductive patterns of *Daphnia magna*. We tested six concentrations of *S. acutus*: 0.1 mg C/L, 0.25 mg C/L, 0.5 mg C/L, 0.75 mg C/L, 1.0 mg C/L, and 2.0 mg C/L. These specific quantities were chosen to represent a gradient of good quality for carbon that is consistent with different levels of eutrophication. Utilizing life-table experiments, we recorded life history characteristics over the course of two weeks. We tested for total reproduction, age at first reproduction, clutch size, and number of clutches. We discovered that higher levels of green algae resulted in greater reproductive characteristics as compared to lower levels. We concluded that *D. magna* grown in the 1.0 mL treatment produced the greatest reproductive output. Additionally, our data showed reproductive patterns were disrupted with more extreme concentrations of green algae, which was exhibited through a decrease in *D. magna* reproduction in the 0.1 mL treatment sample. We did not test for growth characteristics in this study, so future research could account for these characteristics. However, our data clearly shows a relationship between green algal concentrations and the reproductive patterns of *D. magna*, which can be used to better understand the impacts of eutrophication in freshwater ecosystems.

### Introduction

Eutrophication is described as extreme algal growth accompanied by a decreased species presence in bodies of water (Scholten *et al.*, 2005). As such, eutrophication is cause for major environmental concern in aquatic environments. Daphnids, such as *Daphnia magna*, are common in freshwater ecosystems. Daphnids are suspension feeders that filter planktonic algae from water (Ebert, 2005). Zooplankton like *D. magna* are the primary consumers of algae, which form the basis of freshwater food webs. Because these zooplankton feed on algae, previous research has shown that cultivation of Daphnia-rich environments can drastically reduce eutrophication rates in freshwater ecosystems (Scholten *et al.*, 2005). As eutrophic rates

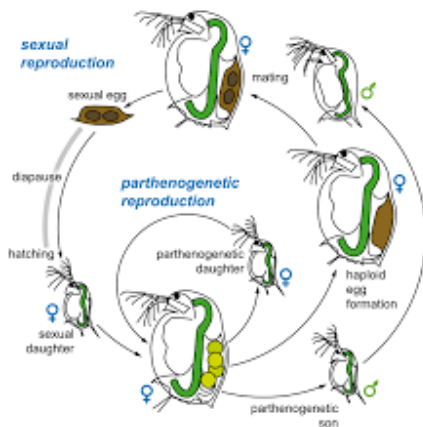
increase, maintaining Daphnid populations is essential to sustaining trophic balance in freshwater ecosystems.



**Figure 1.** Microscopic image of *Daphnia magna* (Peake, 2019).

In order to cultivate higher amounts of Daphnids, peak conditions for reproduction must be attained. *D. magna* can produce both sexually and asexually, but the majority are

usually females that reproduce asexually in peak growth season (Figure 2). Neonates are released from the brood chamber within three days of development, and these new individuals are able to reproduce within ten days. Adult females are also capable of reproducing every four days (Ebert, 2005). Eutrophication can decrease Daphnid life history characteristics, but high-quality algae, like green algae, have been shown to positively influence reproduction (Taipale *et al.*, 2019).



**Figure 2.** Life cycle of *Daphnia magna* (Ebert, 2005).

Additionally, previous studies have suggested that *D. magna* seek out high-quality algal blooms in the midst of eutrophic environments. This allows green algae to be introduced in eutrophic environments to facilitate healthy populations of Daphnids (Taipale *et al.*, 2019). For this study, it was hypothesized that *D. magna* would exhibit higher reproduction and life history characteristics in environments with higher algal concentrations. It was also hypothesized that extremely high algal concentrations would disrupt reproduction, thereby resulting in decreased life history characteristics.

## Methods

### Cultures

The *D. magna* used in this study was ordered from the Carolina Biological Supply Company. This specific species was cultured in the lab for approximately one year prior to this experiment. The lake water that was used in the study was collected from Lake Carl Blackwell in Stillwater, OK. All lake water was filtered and autoclaved to kill all algae and bacteria prior to use. Additionally, *S. acutus* was chosen as the algal species for this particular study because green algae has been shown to be a high quality food source for Daphnids (Ebert, 2005). This algal species was obtained from long term cultures at OSU.

Before completing the life-table experiments, single *D. magna* were placed in plastic cups with 50 mL of lake water. Approximately 1 mL of *S. acutus* was administered to each cup. Cups were changed with fresh water and algae every other day. Natural daylight conditions were maintained throughout the study. Neonates of the original organisms were transferred to other containers upon discovery. Deceased individuals were removed from the cups and replaced with younger individuals. This process was repeated throughout eight weeks to create the neonates for use in the life-table experiments.

### Life-table experiments

Life-table experiments began when neonates from all individuals were born on the same day. Overall, 30 individual neonates were chosen for the life-table experiments. These new individuals were placed in plastic cups with 50 mL of filtered lake water like previously described. Each individual was placed in a single, appropriately labeled cup. Six treatments of various concentrations of *S. acutus* were implemented: 0.1 mg C/L, 0.25 mg C/L, 0.5 mg C/L, 0.75 mg C/L, 1.0 mg C/L, and 2.0 mg C/L. 1 mg C/L treatment corresponds to 1 mL of algae used in the experiment. These concentrations were selected to provide a range of extremely low to high food concentrations. Individuals were selected for each treatment at

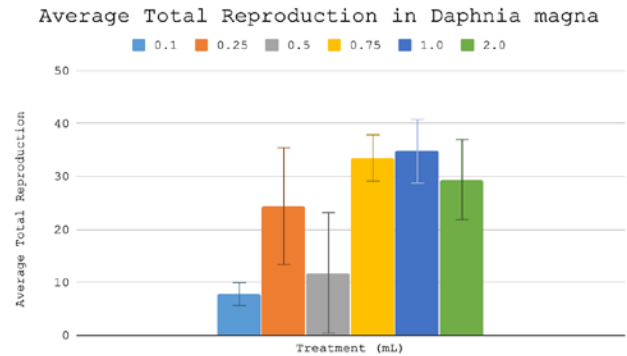
random, and each treatment had five replicates. Cups were changed daily with fresh water and algae. The *Daphnia* were monitored and life history characteristics were recorded for a total of two weeks.

Date of reproduction and number of offspring were recorded when the cups were changed each day. From these data we calculated average total reproduction (total number of neonates produced per female over experiment), age at first reproduction, clutch size, and number of clutches to be calculated. New offspring were immediately removed from the cups upon discovery. In addition, all deceased individuals were removed from the experiment at their time of death.

## Results

### Total Reproduction

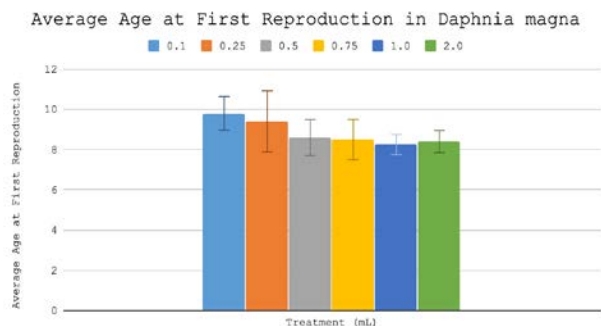
The average total reproduction for each treatment was calculated over the two-week time period. There was a difference in the total number of neonates produced in the lowest and highest algal treatments (Figure 3). The 0.1 mL treatment produced the fewest offspring with an average of 7.80, whereas the 1.0 mL treatment produced the greatest amount of offspring with an average of 34.8. The 0.75 mL treatment had the second highest average total reproduction with 33.5 offspring. The 2.0 mL and 0.25 mL treatments were in the middle with an average of 29.4 and 24.4 offspring, respectively. The 0.5 mL treatment was on the lower end of the spectrum with an average of 11.8 offspring. Therefore, the order of average total reproduction from greatest to least is as follows: 1.0 mL, 0.75 mL, 2.0 mL, 0.25 mL, 0.5 mL, and 0.1 mL (Figure 3).



**Figure 3.** Average total reproduction compared to treatment samples in *Daphnia magna*. Error bars represent the standard deviation.

### Average Age at First Reproduction

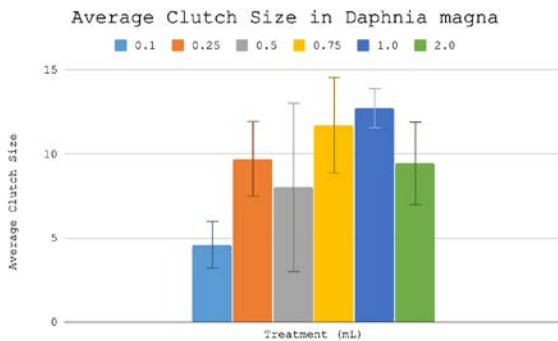
There were no differences in the average age at first reproduction. However, there was a general trend of decreasing average age at first reproduction across the algal gradient (Figure 4). The 0.1 mL treatment exhibited the oldest average age at first reproduction at 9.80 days, while the 1.0 mL treatment exhibited the youngest average age at first reproduction at 8.25 days. All other treatments exhibited similar age at first reproduction values. The 0.25 mL treatment had an average age at first reproduction of 9.40 days, the 0.5 mL treatment was 8.60 days, the 0.75 mL treatment was 8.50 days, and the 2.0 mL treatment was 8.40 days. The youngest average age at first reproduction was the 1.0 mL treatment followed by the 2.0 mL, 0.75 mL, 0.5 mL, 0.25 mL, and 0.1 mL treatments (Figure 4).



**Figure 4.** Average age at first reproduction compared to treatment samples in *Daphnia magna*. Error bars represent the standard deviation.

### Average Clutch Size

All neonates born were accounted for during the two-week time period. This enabled the calculation of the average clutch size for each treatment sample (Figure 5). For this observation, the 1.0 mL treatment exhibited the largest average clutch size of 12.7 offspring. The 0.75 mL treatment followed closely behind with an average clutch size of 11.7 offspring. On the other hand, the 0.1 mL treatment exhibited a drastic decrease in the average clutch size with 4.60 offspring. The 0.25 mL, 2.0 mL, and 0.5 mL treatments exhibited average clutch sizes of 9.70, 9.43, and 8.00 offspring, respectively. In this regard, the order of average clutch size from largest to smallest is as follows: 1.0 mL, 0.75 mL, 0.25 mL, 2.0 mL, 0.5 mL, and 0.1 mL (Figure 5).

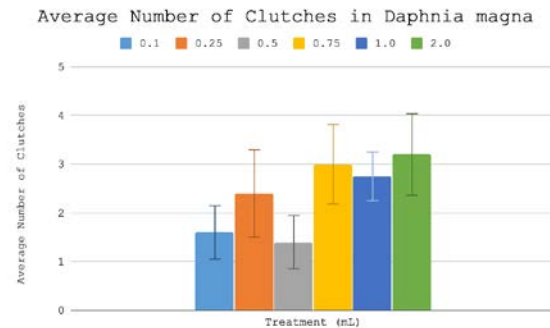


**Figure 5.** Average clutch size in *Daphnia magna* as related to treatment samples. Error bars represent the standard deviation.

### Average Number of Clutches

Because progeny were recorded after each birth, the average number of clutches was calculated over the course of the two weeks (Figure 6). The greatest average number of clutches was recorded at 3.20 for the 2.0 mL treatment sample. The lowest average number of clutches was recorded at 1.40 for the 0.5 mL treatment sample. Once again, the 0.75 mL treatment sample was second for the greatest average number of clutches at 3.00. The 1.0 mL, 0.25 mL, and 0.1 mL treatment samples were

recorded at 2.75, 2.40, and 1.60 clutches, respectively. Consequently, the greatest number of clutches was the 2.0 mL treatment sample followed by the 0.75 mL, 1.0 mL, 0.25 mL, 0.1 mL, and 0.5 mL treatment samples (Figure 6).



**Figure 6.** Average number of clutches in *Daphnia magna* as related to treatment samples. Error bars represent the standard deviation.

### Discussion

Comparison of all data suggests that *Daphnia* exposed to the 1.0 mL treatment had the highest reproductive output. Table 1 shows a summary of the data that supports this conclusion. 1.0 mL is 2.0% of the 50 mL container, so these results suggest introducing this percentage of green algae can increase the reproductive characteristics of *D. magna*. Additionally, the 0.75 mL and 2.0 mL treatment samples showed similar increases in reproductive characteristics. Although the 2.0 mL sample produced more clutches on average, it seemed to result in fewer neonates per clutch. Taipale *et al.* (2019) supports this data as well, concluding that higher levels of algae in eutrophication are more detrimental to the reproductive of Daphnids. Thus, the 2.0 mL treatment sample seemed to be less effective than the 0.75 mL treatment sample, which suggests that extreme algal concentrations are not as effective. This phenomenon is further exemplified in the 0.1 mL treatment sample because this sample resulted in the lowest reproductive characteristics of *D. magna*.

Reproductive Category	Treatment Sample with the Greatest Reproductive Characteristics
Average Total Reproduction	1.0 mL
Average Age at First Reproduction	1.0 mL
Average Clutch Size	1.0 mL
Average Number of Clutches	2.0 mL

**Table 1.** Summary of all reproductive results in this study.

Since the 0.75 mL and 2.0 mL treatment samples are 1.5% and 4.0% of the overall containers, respectively, it can be inferred that the peak for reproductive characteristics of *D. magna* is somewhere in this range. It would be interesting to test a 1.5 mL treatment sample since these results exhibit peak reproductive characteristics at 1.0 mL. In this regard, it could be proven if peak reproduction is actually 1.0 mL or slightly above. Additionally, future studies could test treatment samples between 0.75 mL and 1.0 mL to determine if the finite peak is between these two samples. However, the results of this study indicate that algal percentages of approximately 2.0% serve to create environments for peak reproductive characteristics in *D. magna*.

It should also be noted that two individuals from the 0.5 mL treatment sample and one individual from the 1.0 mL treatment sample died during the study. This could account for the large amount of variation within the 0.5 mL sample. It is unclear if these two individuals would have significantly influenced the data. Yet, various trends in the findings suggest that the 0.5 mL results would have been higher than 0.25 mL but lower than 0.75 mL. In addition, the data for the 1.0 mL sample could be higher, resulting in greater reproductive characteristics for this treatment sample.

Although reproductive patterns of *D. magna* were tested in this study, future research could test for growth patterns in response to changing levels of algal concentrations. The organisms may produce more offspring, but are these offspring ultimately fit for survival? If trophic

levels are essential to freshwater ecosystems, then providing a healthy base through zooplankton is necessary for the survival of other organisms. Larger organisms would serve as a greater source of food. In this regard, length, weight, and size of Daphnids could be measured in response to algal concentrations. Perhaps the organisms in the 1.0 mL treatment sample were larger than others due to the abundance of food, which is why they had the greatest reproductive characteristics.

In conclusion, the results from this study clearly suggest higher concentrations of green algae lead to greater reproductive characteristics as compared to smaller concentrations, which supports one of the hypotheses. The other hypothesis is supported by the data, as well. Here, extreme levels of algal concentrations disrupt reproductive patterns. Together, the results of this study can be implemented to combat rising levels of eutrophication in freshwater ecosystems.

## References

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