Possible Effects of Environmental Change: Survivorship and Biomass Production of *Daphnia* in a Series of Laboratory Simulations

BIOS 35502: Practicum in Field Biology

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Abstract

With the recent environmental changes occurring as a result of human impact on climate and pollution, there are many possible problems that aquatic ecosystems could be facing. These include changes in food availability, dissolved oxygen content, pH, and dissolved organic carbon. Since *Daphnia* are an important link between primary producers and secondary consumers in lentic systems, this study focused on the effect that these four factors have on *Daphnia* biomass production and survivorship. Laboratory experiments were performed in which one of the four factors was changed at a time, and biomass was measured each day along with survivorship. It was found that depleted dissolved oxygen levels have a very negative effect on *Daphnia* survivorship, but that the amount of oxygen depleted was not statistically significant.

Introduction

It has become clear in recent years that the climate of the earth is changing as a result of human actions, and that these changes have both direct and indirect consequences for many parts of the environment (Kratina 2012). In North America, the average temperature has increased by just under 1 degree C, and in far north areas the ice-free season has increased in length (Foley 2012, Schindler 1990). It is predicted that temperatures will continue to rise due to greenhouse warming, which means that the environment will continue to be affected (Schindler 1990). It is relatively easy to observe both biotic and abiotic factors in a lake system, and since lakes tend to be at the lowest point of a landscape, their health is often affected by other things flowing in, such as chemicals and snow runoff (Fischer 2011). An effective way to study climate change and its effects on aquatic ecosystems such as lakes is through research on *Daphnia* (Fischer 2011).

Daphnia are a vital part of lake ecosystems because they provide a link between primary producers and secondary consumers; they consume algae and other phytoplankton and are consumed by higher-order predators such as fish (Wojtal-Frankiewicz 2012, Fischer 2011). In addition, they are very abundant in freshwater systems around the world, including streams, rivers, lakes, and ponds, which makes them a valuable study organism (Wojtal-Frankiewicz 2012, Stanley 2012). *Daphnia* are also useful in research because they are highly sensitive to changes in their environment, and because their simplicity, small size, and short generation time allows them to quickly adapt to new conditions (Fischer 2011, Wojtal-Frankiewicz 2012). Since *Daphnia* are such an integral part of aquatic ecosystems, they are good indicators of the health of these systems, and are a very effective study tool in research concerning the effects of climate change on the environment (Wojtal-Frankiewicz 2012, Fischer 2011). Climate change can affect lentic ecosystems in many different ways, a few of which were examined further in this study, including changes in pH, dissolved oxygen content, food availability, and levels of dissolved organic carbon.

As a result of pollution and climate change, aquatic systems have become more acidic over the past thirty years (Saarinen 2010, Locke 1991). Part of this is a result of runoff from terrestrial systems, which can be very acidic due to coniferous forests and peatlands, as well as human activities such as water and air pollution and land use of very acidic soils (Saarinen 2010). Climate change serves to magnify the effect of these acidic terrestrial areas because there is more runoff from ice melting due to higher temperatures (Saarinen 2010). Past studies (both done in the field and in the laboratory) have found that *Daphnia* are intolerant of acidification in their environment (Locke 1992, Locke 1990, Locke and Sprules 2000).

Another way that climate change can affect lake ecosystems is through oxygen limitation (Foley 2012). In addition to increasing the average air temperature around the world, global warming has increased water temperatures in lakes, both on the surface and deeper (Foley 2012).

During the summer, lakes stratify due to warmer temperatures, causing the oxygen obtained from the air to remain on the surface above the thermocline (Foley 2012, Golosov 2012). In the winter when temperatures decrease the lake mixes, evenly distributing the dissolved oxygen throughout the water column (Foley 2012). As a direct result of climate change, temperatures are increasing around the world, meaning that summers are getting longer (Foley 2012). Longer summers mean a longer period of summer stratification in lakes, and shorter mixing period in the winter (Foley 2012). As a result of this lack of mixing, oxygen is being depleted in the deepest layers of lakes (Golosov 2012, Foley 2012). In addition to simply depleting oxygen alone, a lack of mixing dissolved oxygen can cause the activation of anaerobic processes in lakes, which can greatly decrease the water quality even further (Golosov 2012). Therefore, it is important to determine the ability of *Daphnia* to function with less oxygen, and the implications this may have for the aquatic food web (Foley 2012, Nicolle 2012).

The factors already discussed in this paper, lake acidity and lack of dissolved oxygen as a result of climate change and other human impacts, not only affect *Daphnia*, but also impact their food sources by directly affecting the timing of food availability (Nicolle 2012). Phytoplankton is the main food resource for *Daphnia*, and large population booms of *Daphnia* are dependent on high densities of phytoplankton (Nicolle 2012). If increased temperatures as a result of global warming change the timing of phytoplankton blooms, then there is a possibility that *Daphnia* will have significantly less available food than they had in the past, which could cause a decrease in population, and would have further implications for the rest of the system (Nicolle 2012, Wojtal-Frankiewicz 2012).

Dissolved organic carbon (DOC) is important to aquatic ecosystems because it modifies the effects of other chemicals and processes (Stanley 2012). Also, bodies of water with higher

DOC levels are much darker than those with low DOC, which means that less light is able to penetrate through the water column (Stanley 2012). Dissolved organic carbon can have a strong influence on the acidity as well as nutrient availability of lakes, rivers, and streams (Stanley 2012). In landscapes without a lot of pollution or agriculture the major factors affecting levels of DOC in a lake are topography and climate, whereas areas with agriculturally cominated land use, DOC levels tend to be higher due to runoff, soil disturbance, and high Nitrrogen deposition (Stanley 2012; Sinsabaugh *et al.* 2004). Since humans have a definite impact on DOC levels through climate change and therefore excess snowmelt and agriculture runoff, it is important to gain an understanding of the effects that DOC has on aquatic ecosystems.

Because of the importance of *Daphnia* to aquatic ecosystems, it is important to determine the effect of specific environmental change scenarios to the growth and survival of individual *Daphnia*. To answer this question, *Daphnia* were exposed to different amounts of food, amounts of dissolved oxygen, pHs, and levels of dissolved organic carbon. Since they play an important role in aquatic ecosystems and are good indicators of the health of these ecosystems, the results of these experiments could indicate the affect that human-influenced changes (especially those dealing with environmental change) on lentic systems (Wojtal-Frankiewicz 2012). In the following study, short-term laboratory tests were performed simulating possible changes in aquatic systems, including changes in pH, dissolved oxygen, quantity of food available, and amounts of dissolved organic carbon. The specific hypothesis being tested was that changes in these factors will negatively influence the biomass production and survivorship of *Daphnia*. It was predicted that both biomass production and survivorship would decrease at low amounts of food, low levels of dissolved oxygen, extreme acidic and basic levels of pH, and high levels of organic carbon.

Materials and Methods

To test the effects of amounts of food, oxygen, pH, and dissolved organic carbon on daphnia survivorship and biomass production, *Daphnia* were collected and kept individually in glass containers with 20mL of lake water. Both *Daphnia* and lake water were collected from Bay Lake on the University of Notre Dame Environmental Research Center (UNDERC) property.

In order to test whether food availability affects *Daphnia* survivorship or biomass production, groups of individual daphnia were fed 0, 10, 20, 30, 40, or 50 µL of algae suspended in water. The algae were obtained from Tenderfoot Creek on the UNDERC property. The individual *Daphnia* were photographed every other day with Leica ® imaging software, and measured with imageJ measurement tool. *Daphnia* were also checked for survival.

To determine the effect of water oxygen content on daphnia survivorship and biomass production, oxygen was displaced from Bay Lake water using nitrogen gas for either 0, 15, 30, or 45 seconds. Using a dissolved oxygen meter, the oxygen content of the water was determined for each time period. A list of the dissolved oxygen concentrations equivalent to each bubbling time interval is found in table 1. After being bubbled and sealed, the samples were observed for 30 minutes, then were checked every ten to fifteen minutes until there was 0% survival.

To test the effect of pH on *Daphnia* survivorship and biomass production, the pH of water from Bay Lake was altered. Sulfuric acid and sodium hydroxide were used to obtain lower and higher pHs, respectively. Individual *Daphnia* were measured for length then placed into pHs 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12. In order to avoid the shock of a sudden change in pH, each *Daphnia* that was placed in a pH more acidic than 7 or more basic than 9 was slowly adjusted to the new pH of their environment. D*aphnia* were placed in increasingly acidic or basic water and given one hour in each to acclimate until they reached their assigned pH. They were kept for four days and their length was measured each day.

In order to determine whether *Daphnia* survivorship or biomass is affected by the dissolved organic carbon (DOC) content of their water environment, water was used from three different lakes on the UNDERC property. These lakes were Hummingbird (high DOC), Long (medium DOC), and Bay (low DOC). The specific DOC values for each lake are listed in table 2. Each *Daphnia* was again placed individually in a bottle containing water from one of the three lakes with a known DOC concentration, then measured for length every day.

During each test, each individual *Daphnia* were measured every day (or every other day during the food test) until all had died. The length was then converted to biomass using length-weight regressions given in Downing and Rigler (1984), with the following equation:

$$(1)\ \text{biomass}\ (\mu g) = e^{(1.9445 + (2.72 * \text{length (mm)}))}$$

Statistical methods

For the tests of food limitation, pH, and dissolved organic carbon, an ANOVA was used to determine if there was a difference in the total change in biomass over the experiment. In addition, ANOVAs were run to test for a significant difference between the percent survivorship in each treatment. For the oxygen test, an ANOVA was used to determine if there was a difference in the number of minutes survived in water with different oxygen contents. Tukey’s honestly significant difference test was used as well to determine specifically which oxygen levels were different from one another.

Results

In the food limitation experiment, it was found that there was no significant effect of amount of food on total change in biomass over the experiment ($F_{2,3}$=0.223, p=0.95) (Figure 1).

In addition, there was no significant difference in survivorship between low (0 or 10µL), medium (20 or 30µL), and high (40 or 50 µL) amounts of food, with a p-value of 0.563 ($F_{2,3}$=0.70).

In the oxygen limitation test, there was a significant difference between the amount of time *Daphnia* could live in water with different levels of dissolved oxygen content ($F_{3,20}$=11.284, p=0.0002) (Figure 2). There was a significant difference between water with full oxygen content and water with oxygen displaced for 15 (p=0.001), 30 (p=0.0006), and 45 seconds (p=0.0005).

In the pH test, there was no significant difference in total biomass change between *Daphnia* in different pHs ($F_{7,22}$=0.799, p=0.597)(Figure 3). In addition, there was no significant difference in survivorship between very acidic (pH 3, 4, 5), neutral (pH 6, 7, 8, 9), and very basic (pH 10, 11, 12), with a p-value of 0.329 ($F_{2,7}$=1.31).

In the test of dissolved organic carbon, there was no significant difference between total biomass change in *Daphnia* in different levels of DOC ($F_{2,15}$=0.77, p=0.48) (Figure 4). There was also no significant difference between the percent survivorship of *Daphnia* in the three levels of DOC ($F_{1,1}$=0.535, p=0.598).

Discussion

In the experiment testing the effects of food limitation, the non-significant results suggest that *Daphnia* are rather resilient and able to live without much food. However, due to the very short-term nature of the experiment, the results can be explained by a lack of time and number of replicates. There was a high mortality rate in all the tests at around the fourth day, meaning that the mortality was due to some other factor, not the lack of food. It is possible that there was not enough water in each jar to keep the *Daphnia* alive for more than four days, or the temperature in the laboratory was too high for them to live for more than a few days inside.

In the test in which dissolved oxygen content was manipulated, there was a significant difference in the survival time between *Daphnia* in water with different amounts of dissolved oxygen. By running a Tukey's HSD test, it was found that the only significant differences in survival time were between the replicates in water that had no oxygen removed and the replicates that had some oxygen removed. There was no statistically significant difference in survival times between *Daphnia* in bottles with some amount of oxygen displaced. This evidence suggests that *Daphnia* are sensitive to any reduction in dissolved oxygen in their environment, and it does not really make a difference how much the reduction is. This could have very negative implications for *Daphnia* health as well as the health of entire aquatic systems. Since climate change has the potential to cause a decrease in the dissolved oxygen content of lake systems, it may become a very widespread problem for aquatic systems if the *Daphnia* cannot survive in lakes with lowered oxygen levels (Foley 2012).

The test of the effect of pH on *Daphnia* biomass and survivorship had no significant statistical results. Much like the food limitation experiment, this can be attributed to a small sample size and short time frame. It has been shown in other studies such as one performed by Andrea Locke that lake acidification has a negative impact on *Daphnia* size, reproduction, and abundance (2000). But one thing that was learned from the pH manipulation study was that the *Daphnia* could only survive in pHs between 5 and 11. Each *Daphnia* that was placed in a pH of 3, 4, or 12 died very quickly even though they were given one hour to adjust to each successively stronger pH. These observations provide evidence that *Daphnia* can adjust to a relatively wide range of pH for at least a short period of time, however when the pH levels become too extreme they can no longer survive.

In the experiment that manipulated the dissolved organic carbon levels, there was again no significant statistical result in either change in biomass or survivorship. This can be attributed to the small sample size and short time frame for the experiment. In this experiment especially, almost all of the *Daphnia* that began the experiment did not survive to the end of it. It was only in the lowest DOC level that more than half of the *Daphnia* survived for the duration of the experiment. It would be interesting to do further testing with more replicates over a longer time period to find out if this is still the case. If it is, further experimentation could be done to explain why the decreased survival rates occurred. Since it has been shown that DOC affects lake systems primarily by influencing other factors such as light absorbance and chemical processes, more testing will be needed in order to determine the effects (both direct and indirect) of DOC on *Daphnia* and therefore entire aquatic systems (Stanley 2012).

If I were to perform this experiment again, there are a few things I would change. First of all, I would use many more replicates to ensure that even if there was a high mortality rate among the daphnia I would still have enough replicates for each treatment to perform more powerful statistical tests. In order to include more replicates, I would have needed my own microscope camera to use, and assistance taking pictures and measuring all the *Daphnia* because so many pictures had to be taken every day. In addition, I would like to expand quite a bit on the results that I obtained. I would like to explore the effects of the four factors tested on *Daphnia* reproduction in addition to the production and survivorship. I would also like to test the effects of temperature on *Daphnia* biomass, survivorship, and reproduction. Finally, I would like to test the effects of pH, oxygen, DOC, food availability, and temperature in the field rather than in the lab. It would be more reliable to obtain data from a whole lake that has been altered in some way rather than trying to simulate the environment in glass jars in a lab.

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Appendix

Table 1. Dissolved oxygen values equivalent to the number of seconds oxygen was displaced for.

Number of seconds	Dissolved oxygen content (mg/L)
0	4
15	2.85
30	1.70
45	1.03

Table 2. Dissolved organic carbon values for each lake.

Lake	DOC (mg/L)
Bay	5.72
Long	8.11
Hummingbird	26.04

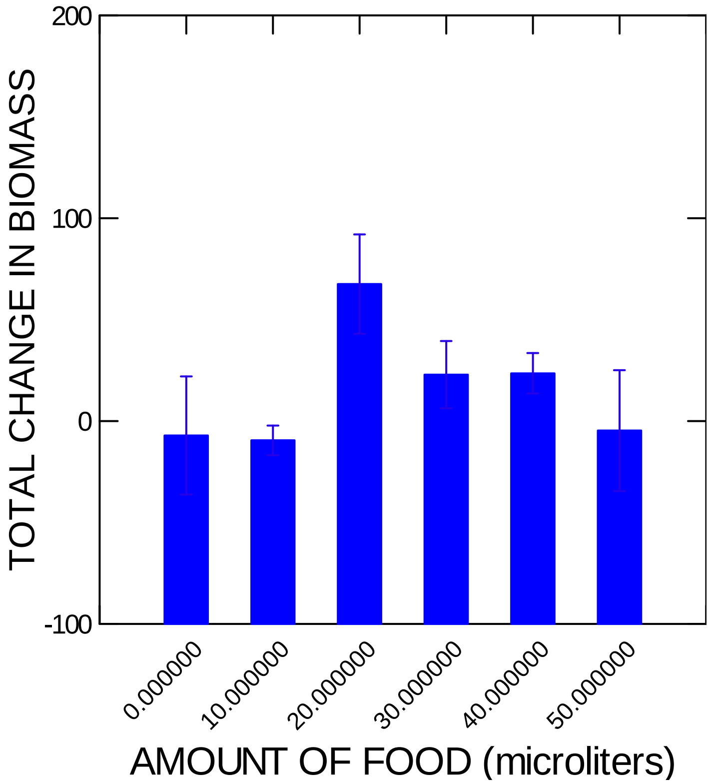

Figure 1. The amount of food had no significant effect on the change in biomass of *Daphnia*.

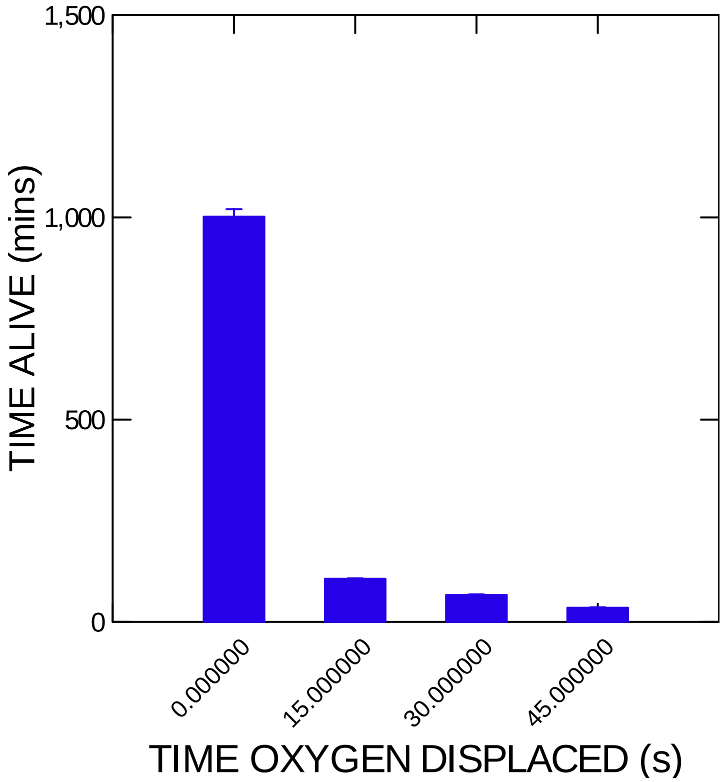

Figure 2. Survival of *Daphnia* placed in water without displaced oxygen was significantly higher than the survival of *Daphnia* in water that had some displaced dissolved oxygen.

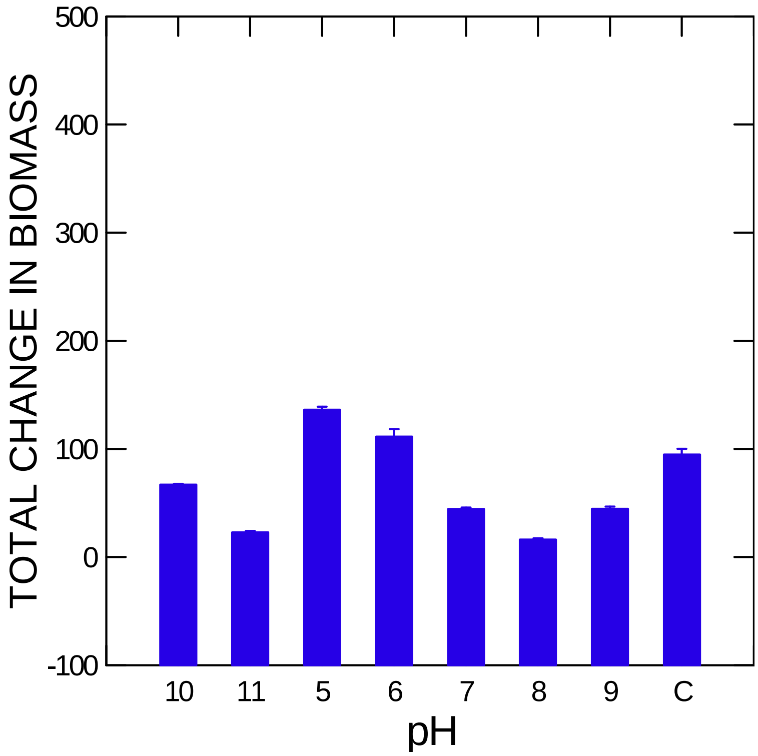

Figure 3. There was no significant difference in *Daphnia* biomass change between different pHs.

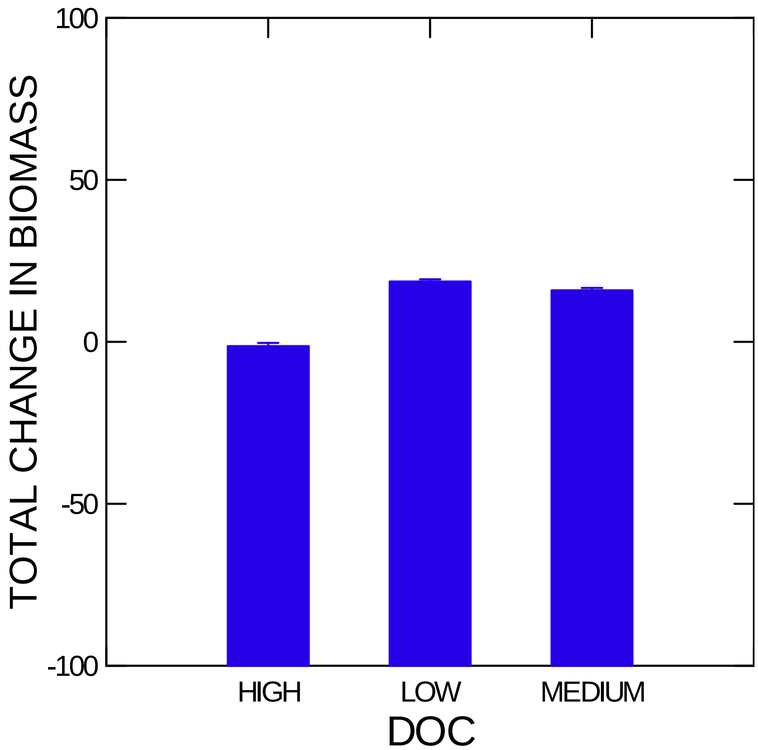

Figure 4. There was no significant difference in *Daphnia* biomass change between different levels of dissolved organic carbon.