Daphnia pulicaria as an indicator of temperature's effect on temperate lake ecosystems

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Kyle Madoni

Advisor: Ben Clifford

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Abstract

The physical factors, light, temperature, and precipitation, are constraints on the types of vegetation that will grow, which then determines the organisms at higher trophic levels. With the climate changing, due to both natural and anthropogenic causes, so too are the environments that depend on certain climatic conditions. For that reason, scientists are working to understand the mechanisms behind climate change and how they will influence environments. One way of studying an environment is through a model organism or a keystone species, a genus of zooplankton, *Daphnia,* is both. *Daphnia* are a keystone herbivore in aquatic ecosystems that participate in vital biogeochemical processes and are sensitive to changes, especially because they are affected by both bottom-up and top-down controls. Even with the increasing interest in climate change, research on temperature's effect on aquatic environments, in which water resources are sensitive to physical changes, is only now emerging. This study was designed to understand the changes in a lake ecosystem through climate warming by use of an important aquatic indicator, *Daphnia pulicaria*. As temperature increased, *Daphnia* activity increased but then began to decrease. Initially, there was a decrease in dissolved oxygen, pH and phytoplankton abundance, yet at the higher end of the spectrum these changes were less apparent. This suggests that there is an intermediate temperature in which Daphnia productivity is at a maximum, and with further increase in temperature there is a decline. The changing climate could shift seasonal aquatic regimes and adversely affect said environments through trophic cascades.

Introduction

A globally changing climate has the potential to alter biological communities, becoming an increasing challenge to ecologists (Moore and Folt 1993). Climate change can affect water resources, one of the most highly sensitive environmental components, directly through changes in temperature, light, suspended particles, precipitation, and dissolved substances, and indirectly through species composition and trophic interactions (Przytulska et al. 2015, Straile and Geller 1998, Modenutti et al. 2013). For example, a warming climate may stimulate algal growth, causing higher productivity and suspended particles. This will cause a lack of light and eutrophication of the waterbody, causing population reductions up the trophic structure. Furthermore, lakes have been suggested to be effective integrators and regulators of climate change, helping to provide valuable information on how the ecosystem is being altered (Williamson et al. 2008, Williamson et al. 2009). However, even with the increasing interest in climate change, the combined effects of such direct and indirect factors on ecosystem processes, specifically zooplankton-phytoplankton interactions, is inadequately understood (Przytulska et al. 2015).

Daphnia have been used as a model organism, over the past couple decades, to study the effects of environmental stressors on zooplankton communities because they are important in the trophic structure of aquatic ecosystems (Lampert 2006, Nevalainen et al. 2014). Cladocerans, including the genus *Daphnia*, often dominate the zooplankton communities and are keystone herbivores in temperate lake ecosystems (Przytulska et al. 2015; Straile et al. 2012, Nevalainen et al. 2014), acting as important drivers of seasonal phytoplankton succession and community composition (Straile et al. 2012). Additionally, species of *Daphnia* participate in crucial biogeochemical processes that are linked to ecosystem functioning, and are sensitive to environmental changes because they are subjected to both bottom-up and top-down controls

(Nevalainen et al. 2014). Fluctuations in the *Daphnia* population are strongly associated with large-scale atmospheric oscillations (Scheffer et al. 2001, Straile 2002), specifically latitude in North America, and even reaching maximums when surface water temperatures reach 18.5°C (Straile et al. 2012). This suggests that *Daphnia* population dynamics and phenology are strongly controlled by water temperatures and are sensitive to climate warming (Straile et al. 2012). The warming climate changes the temperature and stratification of aquatic environments, potentially causing the *Daphnia* to be more active. This increased activity will increase respiration rates that will use up more oxygen and leave more free hydrogen ions in the water, causing a decrease in oxygen and acidification of the waterbody.

This study examines the grazing of *Daphnia* and their impact on an aquatic ecosystems along a temperature gradient. I would like to determine temperature's role in a temperate lake ecosystem through observing the activity of *Daphnia* and how they might change the water chemistry. I hypothesize that as temperature increases *Daphnia* will exhibit higher activity, causing a decrease in oxygen, pH, and phytoplankton abundance.

Materials and Methods

Study Site

In this study, Tenderfoot Lake, located on the University of Notre Dame's Environmental Research Center (UNDERC) which is on the border between Michigan's Upper Peninsula and Wisconsin (46°22′N, 89°53′W), was used for sampling zooplankton and phytoplankton populations (***Figure 1***). Tenderfoot Lake is a mesotrophic, temperate lake surrounded by Eastern Hardwoods forest, and has an area of 453 acres and a maximum depth of 33 feet (*Wisconsin DNR*). The lake acts as a drainage basin, with water flowing in from Palmer Lake in the

southeast and out in the north through Tenderfoot Creek. Additionally, the substrate profile consists of 65% sand, 15% gravel, 10% rock, and 10% muck (*Wisconsin DNR*). The mean water temperature between June and July 2015 was 18.5°C ±5.5°C.

Sampling Procedure

The zooplankton used in this study were *Daphnia pulicaria*. Throughout the end of June and early July, additional zooplankton samples were collected for use in trials. The samples were collected in the northeastern part of Tenderfoot Lake (***Figure 1***) with an integrated tow, generally four to five tows in 1-L of lake water. Then, large, adult *D. pulicaria* were transferred individually from the lake sample into 120-mL glass jars where they were allowed to rest. This helped to minimize the stress, specifically thermal, caused from collection, transportation, and introduction into aquariums.

Microcosms

To test the interaction between zooplankton and phytoplankton at different temperatures, three microcosms of temperatures roughly 14°C, 20°C, and 23°C were designed. The 20°C and 23°C microcosms consisted of a 10-gallon (~37.85-L) aquariums filled with 17-L of lake water, filtered for aquatic invertebrates, and contained three 1-L mason jars. The water surrounding the mason jars helped to maintain the water temperature, acting as a buffer between the water and the air, acting as a water bath. To match the water level in the jars, 17-L of water was added. The 20°C aquarium was maintained by the room temperature and the 23°C by Tetra Submersible Heaters for aquariums. The 14°C microcosms consisted of a 380-gallon cylindrical tank of well water (~12°C) surrounding the aquarium filled with lake water and three mason jars. The well water helped to lower and maintain and colder temperature, the lake water in the aquarium acted

as a buffer between the jars and the large tank. The well water in the tank was changed every 12 hours and brought up to the water level in the aquariums, helping to maintain the lower temperature. After the microcosms were set up they were allowed to stabilize for 24 hours, ensuring that the tanks were maintaining a constant and desired temperature before the *D. pulicaria* were introduced.

Measured Variables

Within each tank there were three replicate jars from which variables (date, time, temperature, dissolved oxygen, presence of *D. pulicaria*, pH, and chlorophyll sample) were measured at the beginning (0 hours) and the end (24 hours) of a trial. Generally, the samples were taken between 8-11pm, the time when the sun was going down and the *Daphnia* were rising to the surface of the lake. The temperature was measured using a Fisher Science thermometer. The dissolved oxygen was measured using an YSI 800 meter and the pH by the EcoTestr pH1 meter, both were kept in the jars until there was a stable reading. Additionally, 50-mL of water was filtered by a syringe and 25-mm Easy Pressure syringe filter holder and ran through G.E. Whatman Grade 1 (2.5-cm) filter paper. The filter papers were placed in 5-mL black microtubes and stored in the freezer, preventing growth through lack of light and the cold temperature. After the trials were completed, 50-mL black canisters were filled with 20-mL of anhydrous methyl alcohol (CH_3OH) ("methanol") and one filtered sample, allowed to extract overnight, and then analyzed for chlorophyll *a* through spectrophotometry. Then, 5-mL of the methanol extraction liquid was transferred from the canister to small test tubes and then measured for raw fluorescence by a fluorometer (Turner Designs, Trilogy Module: Chl NA). In order to infer the amount of chlorophyll *a* in the samples, standards with a known chlorophyll

amount were run through the fluorometer. The chlorophyll and raw fluorescence were plotted (*Figure 2*), fitted with an equation, and then used to convert from RFU's to chlorophyll *a*.

Statistical Analysis

To test the effectiveness of the temperature and sampling time (0 or 24 hours) on the different tank types, a two way ANOVA was run. To analyze the activity of *D. pulicaria*, a two way ANOVA was run to test the effect of *Daphnia* presence/absence and tank type (14°C, 20°C, and 23°C) on dissolved oxygen. To test the effect of *Daphnia* presence/absence and tank type on pH, a two way ANOVA was run. To test the effect of *Daphnia* presence/absence and tank type on fluorescence, a two way ANOVA was run. To test the effect of *Daphnia* presence/absence and tank type on the amount of chlorophyll present, a two way ANOVA was run. All statistical analyzes were performed with SYSTAT statistical software (SYSTAT 2013).

Results

The three tanks maintained average temperatures of 14°C, 20°C, and 23°C, only varying within a degree of two between trials and less than a degree within trials (*Figure 3*). Thus, the temperature within the tanks remained stable over the twenty-four hour periods and did not vary significantly (F-ratio=0.548, p=0.461, df=1) within tanks; however, they did vary significantly between tanks (F-ratio=1,261.256, p=0.000, df=2). Additionally, the interaction between the time and the tank temperature did not vary significantly (F-ratio=0.651, p=0.534, df=2).

The dissolved oxygen differed between trials, ranging from a high of 5.87-mg/L to a low of 1.55-mg/L, with the highest amounts observed in the 14°C tank and the lowest in the 23°C tank. The average changes in dissolved oxygen for the 14°C, 20°C, and 23°C tanks without *D. pulicaria* present were -0.15, -0.88, and -0.88 and with *D. pulicaria* present were -0.04, -0.58,

and +0.46-mg/L, respectively (*Figure 4*). Overall, the greatest change in dissolved oxygen was observed in the 20°C tank, and there was a greater change at higher temperatures; however, there was a greater decrease in dissolved oxygen from the 14°C tank to the 20°C tank, but an increase in dissolved oxygen for the 23°C tank. The dissolved oxygen within tanks and with the presence/absence of *Daphnia* varied significantly (F-ratio=8.363, p=0.000, df=2; F-ratio=8.424, p=0.004, df=1), but did not vary significantly with the combined effect of both variables (F-ratio=0.141, p=0.849, df=2).

The pH values of all the tanks and respective trials made up a tight range of 1 pH unit, with the lowest value of 6.4 and the highest of 7.4. The average pH value of each tank was roughly 6.8. Additionally, the average changes in pH without *D. pulicaria* present were +0.06, +0.04, and +0.04 and with *D. pulicaria* present were -0.14, -0.09, and -0.08 for the 14°C, 20°C, and 23°C tanks, respectively (*Figure 5*). The greatest average change in pH was observed in the 14°C tank, then 20°C, and finally the lowest in the 23°C tank. The pH did vary significantly within tanks for the presence/absence of *Daphnia* (F-ratio=112.060, p=0.000, df=1) but did not vary significantly between tanks (F-ratio=1.273, p=0.284, df=2); however, the pH did vary significantly through the interaction of the tank type and presence/absence of *Daphnia* (F-ratio=4.192, p=0.018, df=2).

The concentration of phytoplankton in the samples greatly varied, with the lowest raw fluorescence of 3.55-RTU, or 0-µg/L of chlorophyll, and the highest of 143.42-RFU, or 6.557-µg/L chlorophyll. On average, the change in RFU for the 14°C, 20°C, and 23°C tanks without *D. pulicaria* present were -8.94-RTU's (or -0.46-µg/L) , +0.44-RTU's (+0.02-µg/L), and -9.65-RTU's (-0.50-µg/L) and with *D. pulicaria* present were -33.88-RTU's (-1.75-µg/L), -25.24-RTU's (-1.31-µg/L), and -11.88-RTU's (-0.61-µg/L), respectively (*Figure 6*) (*Figure 7*). There

was a greater change in fluorescence and chlorophyll with *D. pulicaria* present, and the most observed in the 15°C tank. The fluorescence varied significantly between tanks (F-ratio=4.614, p=0.012, df=2) and for the presence/absence of *Daphnia* (F-ratio=47.815, p= 0.000, df=1). Additionally, the fluorescence varied significantly for the interaction between tank type and the presence/absence of *Daphnia* (F-ratio=3.810, p=0.025, df=2). The chlorophyll varied significantly between tanks (F-ratio=4.614, p=0.012, df=2) and for the presence/absence of *Daphnia* (F-ratio=47.815, p= 0.000, df=1). Additionally, the chlorophyll varied significantly for the interaction between tank type and the presence/absence of *Daphnia* (F-ratio=3.810, p=0.025, df=2).

Discussion

There was a greater decrease in dissolved oxygen between tanks and with presence of *D. pulicaria*, decrease in pH with the presence of *D. pulicaria*, and greater decrease in fluorescence with the presence of *D. pulicaria*. This supports the hypothesis that over a natural daily cycle (period of 24 hours) *Daphnia* will respire and graze phytoplankton. This reduces the concentration of phytoplankton and oxygen, increasing the concentration of carbon dioxide (CO_2), which then causes a decrease in pH. The carbon dioxide, when dissolved in water, forms carbonic acid (H_2CO_3) that will readily give up hydrogen ions ("weak acid"), lowering the pH.

A constant temperature was maintained for all three tank types (14°C, 20°C, and 23°C) throughout the twenty-four hour periods. Thus, the design was well suited to maintaining constant temperatures, allowing for the observation of temperature's effect on the interaction between zooplankton and phytoplankton. It is interesting to note that the range of temperatures (14°C to 23°C) used in this study were observed in Tenderfoot Lake during the months of June

and July. While this natural variation in temperature may occur, warming will cause changes in the zooplankton populations which could then cause trophic cascades in the temperate lake ecosystem.

As temperature increased there were lower dissolved oxygen values observed. There was a greater change, in this case a decrease, in dissolved oxygen for the higher temperatures because of the increase in vaporization. It was to be expected that the warmer the temperature the more energy the water molecules would have, causing a greater amount of water to vaporize. Also, there was a greater change in dissolved oxygen between tanks, due to temperature, and with the presence/absence of *D. pulicaria*. The change in dissolved oxygen increases and levels out without *D. pulicaria* present, and begins to do so with *D. pulicaria* present as the temperature warms until it reaches the higher temperature (23°C). It is interesting to note that there was a positive change in dissolved oxygen for the 23°C tank with *D. pulicaria* present. Temperature is a limiting factor of primary production, where, generally, increasing temperature increases primary productivity. Thus, the increased photosynthesis of the phytoplankton may be overshadowing the opposite effect, a decrease in dissolved oxygen, created by the zooplankton.

While the pH was relatively similar between tanks, it was highly varied between the presence and absence of *Daphnia*. The pH without *Daphnia* present was relatively constant between tanks, all increasing by similar amounts over the twenty-four hour period. On the other hand, the pH was relatively constant between tanks with *Daphnia* present but showed a decrease in pH. So, *Daphnia* lowered the pH in the tank. It is also interesting to note that the average change in pH, whether positive without *Daphnia* present or negative with *Daphnia* present, was less than 0.20 of a pH unit. However, even with small changes in pH, *Daphnia* still had a significant effect on the pH.

The fluorescence, and chlorophyll, was relatively constant between tanks without *Daphnia* present, decreasing by less than 10-RFU's, or 0.50-μg/L. The fluorescence, and chlorophyll, significantly decreased with *Daphnia* present, and the decrease in fluorescence was less severe with increasing temperature. These results suggest that *Daphnia* grazed more phytoplankton the lower the temperature. It has been suggested that because Cladocerans use their thoracic limbs to both feed and obtain oxygen (Moore and Folt 1993). Then, at elevated temperatures the thoracic limb beats more quickly to supply the increased metabolic demand for oxygen, causing the gut to fill sooner. However, because the rates of the gut passage may not be able to increase it limits the rate of food processing and also food intake.

In a similar study, Przytulska et al. (2006) evaluated both temperature and the shift in the phytoplankton community effects on zooplankton communities. While they found that temperature did not significantly affect the growth rate-food concentration curve, the combined effects of warming and increase in food abundance did result in a significant change in the slope of the growth-food curve. This study shows that not only does temperature affect the phytoplankton community but that it also indirectly affects the zooplankton community through phytoplankton. Additionally, in a related study done by Straile et al. 2012 they found that an increase in temperature is an important factor affecting the growth rates of *Daphnia*, whereas food quantity and quality only played a secondary role. While the results of the two studies may conflict slightly, it is obvious that temperature plays a role in the phenology of *Daphnia* and in lake ecosystems.

For future studies, it is recommended that aquarium devices, potentially heaters, be used to help maintain desired temperatures, as they showed the least amount of variation in temperature. Additionally, it would be helpful to measure the temperature for more intervals than

just the beginning and end of the trial, hopefully helping to provide a clearer picture of the temperature in the tank. In general, it may be helpful to monitor physical, chemical, and biological variables throughout the whole twenty-four hour period, as this would help to provide a better picture of how temperature may affect the daily cycle of *Daphnia*. Additionally, while it is helpful to look at the individual effects of one Daphnia, it would also be helpful to increase the population size, thereby providing a clearer picture for the whole lake. Lastly, I would recommend that be done with the water coming in to the Wet Lab from Tenderfoot Lake, as this caused a high mortality of *Daphnia*, when compared to collecting water directly from the lake. Daphnia are sensitive organisms and this study would help further our understanding of how physical and chemical changes will affect aquatic environments.

Finally, the warming climate may change the current phenology of zooplankton, causing trophic cascades up and down aquatic food webs. These changes may even occur more rapidly than the changing climate, as zooplankton communities respond rapidly within hours or days (Moore and Folt 1993). Additionally, the warmer temperatures may stimulate phytoplankton growth and production, causing eutrophication and potential hypoxic zones. There are some studies that suggest that warming will cause increases in low quality food sources, like cyanobacteria, (Przytulska et al. 2015) which will have negative effects on zooplankton. On the other hand, there are other studies that suggest that daphnids benefit from eutrophication more than other zooplankton species (Straile and Geller 1998).

While some studies suggest that temperature will adversely affect aquatic food webs and others that it will increase stratification and lake productivity, it is important to remember that zooplankton are key links in aquatic food webs and that weather conditions coupled with nutrient availability can have synergistic effects. Every lake is different, and these characteristics need to

be taken into account when developing models and forecasts for the future of a waterbody to accurately identify the regime shifts. In the end, the environment will change, one way or another, in the coming years.

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Wisconsin DNR. Tenderfoot Lake Survey Map. July 18, 2015. http://dnr.wi.gov/lakes/LakePages/LakeDetail.aspx?wbic=2962400&page=facts

Tables

Table 1. A stock solution of 626 µg/L of chlorophyll was used to make a series of dilutions (0, 5, 10, 50, and 100-µg/L) with methanol (CH_3OH). Then, 5-mL of the dilution was analyzed in a fluorometer for raw fluorescence. This allowed for the chlorophyll of the samples to be inferred from the raw fluorescence and the standards.

Chlorophyll Standards	
RFU	**Chl Conc. (µg/L)**
2.54	0
113.32	5
243.24	10
955.67	50
1962.43	100

Figures

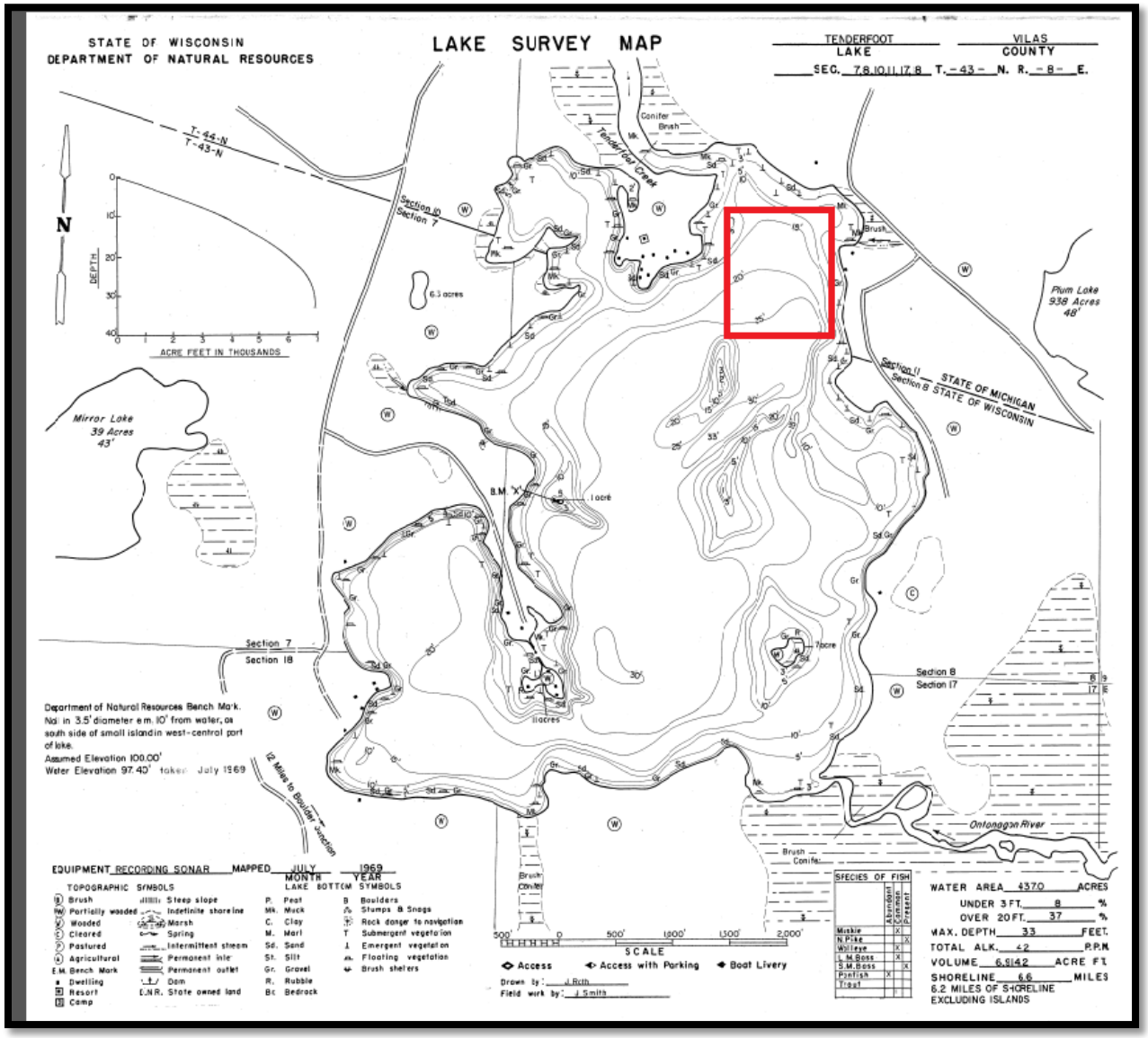

Figure 1. A contour map of Tenderfoot Lake. The red box indicates roughly were the samples were taken.

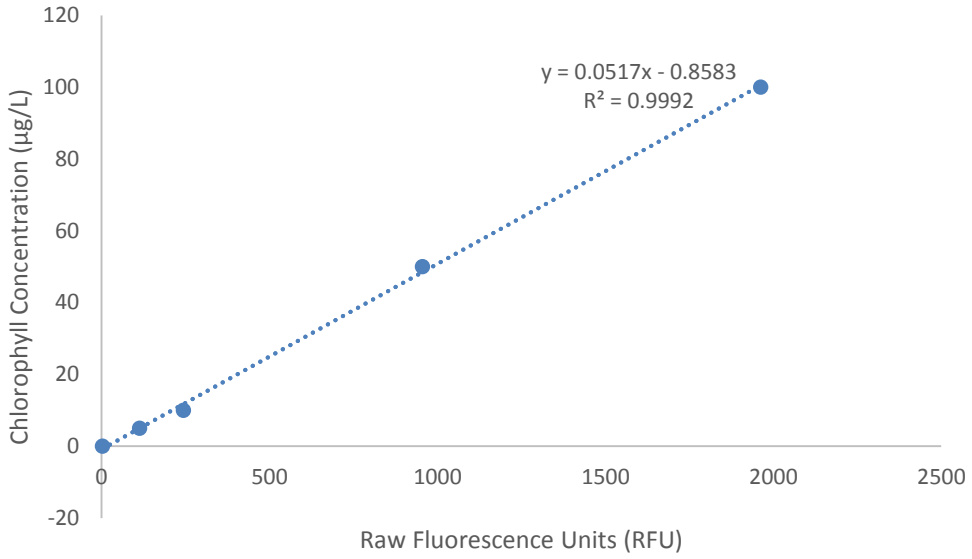

Figure 2. Standards curve for conversion of raw fluorescence units (RFU's) to chlorophyll concentrations. The relationship is given by the equation: y=0.0517(RFU)-0.8583. There is a very strong correlation between the two, as shown by the R-squared value of 0.9992.

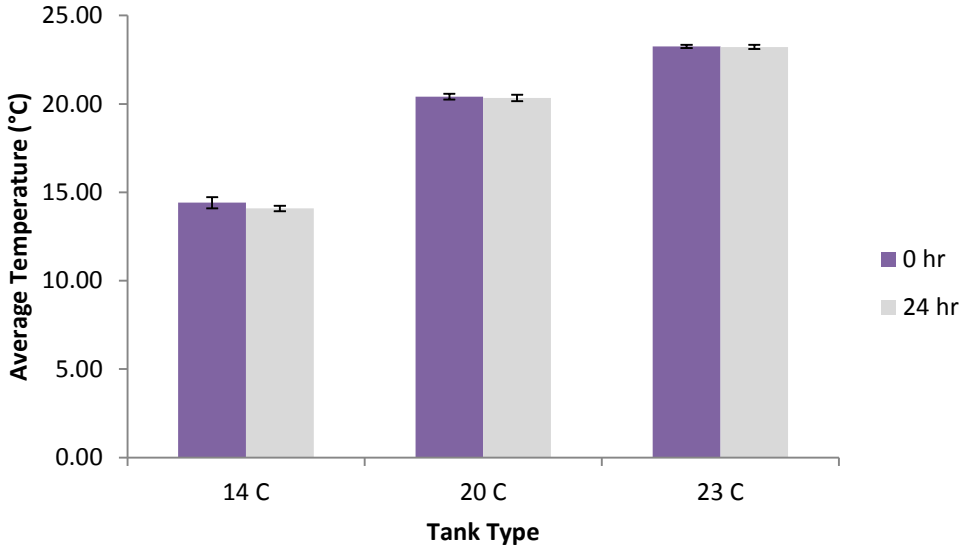

Figure 3. Average temperature at the beginning and end of the trials for each tank type. The 14°C tank averaged 12-14°C, the 20°C tank averaged 19-21°C, and the 23°C tank averaged 23-

24°C. The temperature did not vary significantly over time (F-ratio=0.548, p=0.460, df=1) but did between tanks (F-ratio=1,261.256, p=0.000, df=2), as shown by standard error bars.

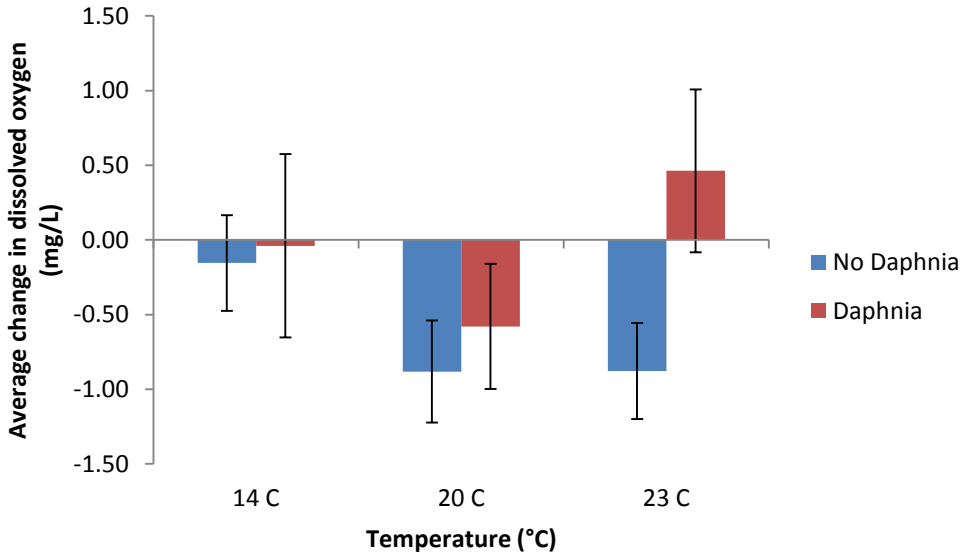

Figure 4. Average change in dissolved oxygen (mg/L) for presence of *Daphnia* at each tank type. The dissolved oxygen (mg/L) varied significantly between tanks (F-ratio=8.363, p=0.000, df=2) and with the presence/absence of *Daphnia* (F-ratio=8.424, p=0.004, df=1), as shown by standard error bars.

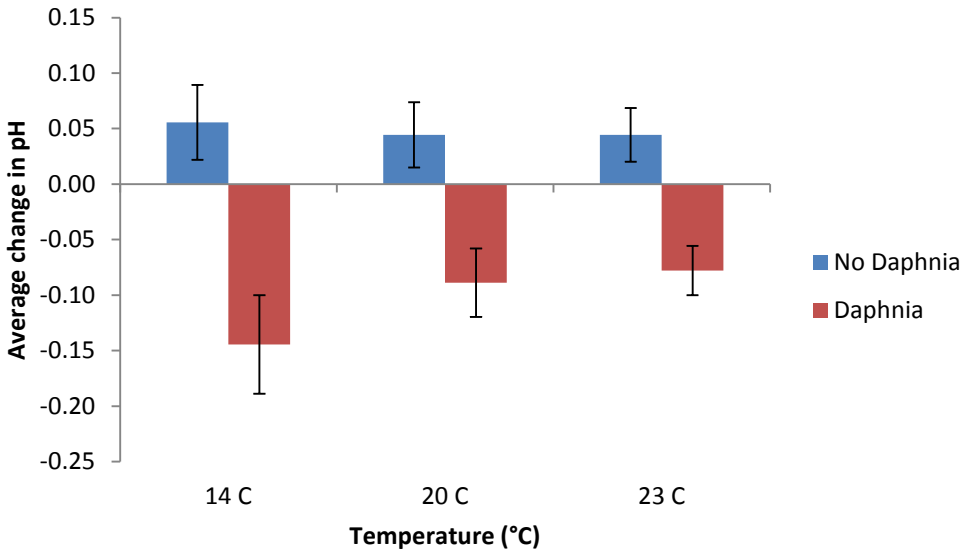

Figure 5. Average change in pH for presence of *Daphnia* at each tank type. There was a greater change in pH observed with the presence of *Daphnia*. The pH varied significantly with the presence/absence of *Daphnia* (F-ratio=112.06, p=0.000, df=1) but did not vary significantly between tanks (F-ratio=1.273, p=0.284, df=2), as shown by standard error bars.

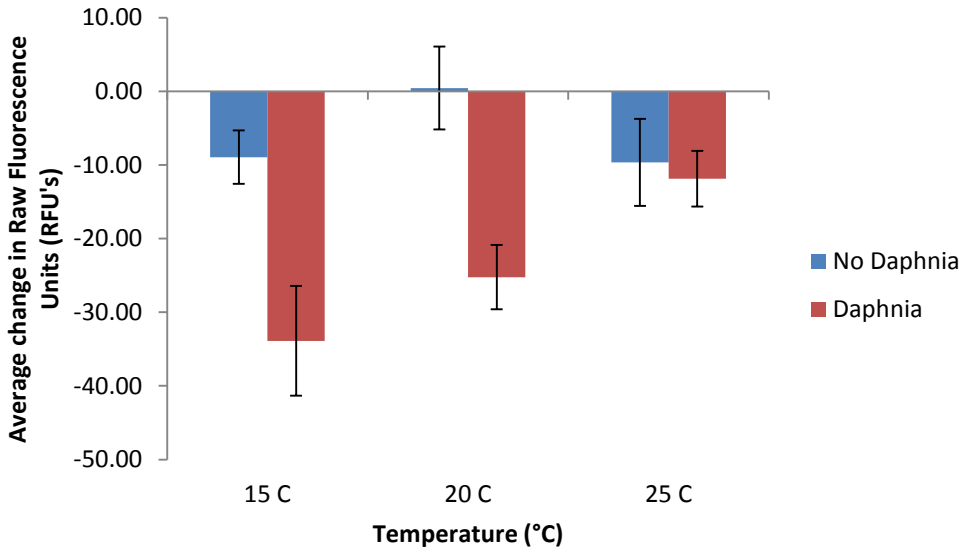

Figure 6. Average change in raw fluorescence units (RFU's) for presence of *Daphnia* at each tank type. There was a greater change in fluorescence with the presence of *Daphnia*, rather than with their absence. The fluorescence varied significantly between tank types (F-ratio=4.614, p=0.012, df=2) and with the presence/absence of *Daphnia* (F-ratio=47.815, p=0.000, df=1), as shown by standard error bars.

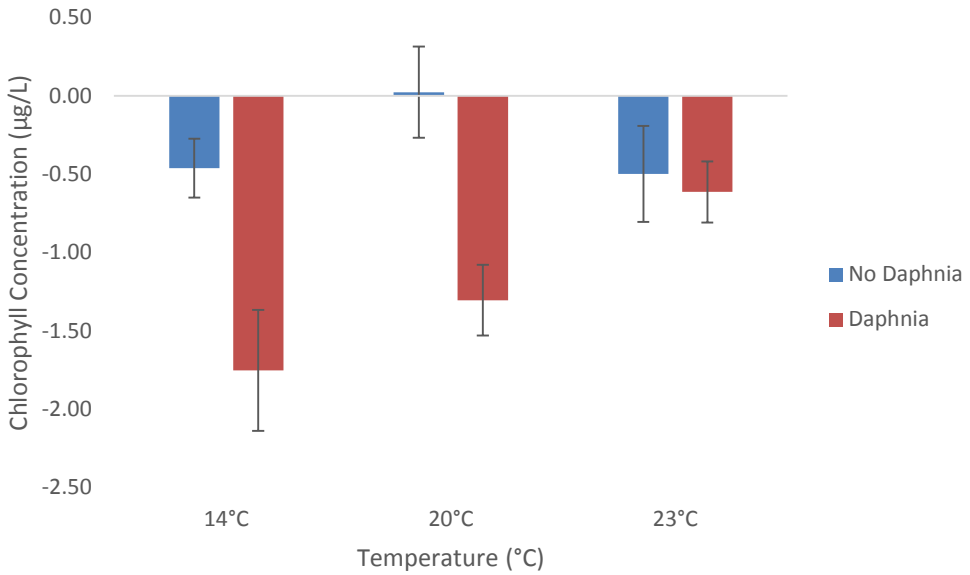

Figure 7. Average change in chlorophyll concentration for presence/absence of *Daphnia* at each tank type. There was a greater change in chlorophyll concentration with the presence of *Daphnia*, rather than with their absence. The chlorophyll concentration varied significantly between tank types (F-ratio=4.614, p=0.012, df=2) and with the presence/absence of *Daphnia* (F-ratio=47.815, p=0.000, df=1), as shown by standard error bars.