The Effect of Water Quality on Daphnia

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Abstract

Water quality is essential of both humans and aquatic organisms. Examining and determining the quality of water that surrounds communities around the country is important considering over 2 million Americans do not have access to safe drinking water free of contaminants. Organisms such as daphnia are used as biomarkers for toxins within water of aquatic ecosystems. Daphnia are transparent organisms that are sensitive to environmental change which makes them great biomarkers for contaminants in waterways. With their heart rate and behavior being shown to be affected by environmental change, the quality of two water sources in Farmville, Virginia were accessed using daphnia. It was expected that the daphnia heart rate and locomotion would increase in response to polluted water. It was found that there was no statistical difference in daphnia heart rate or locomotion. This provides opportunity for additional research in this field to examine the specific effects within the water sources.

Introduction

With the human population growing in number, the use of water around the world is also increasing. Considering water is an essential component of virtually every living organism on earth, the quality of water in which each organism has access to, can affect their overall health. The need for water to produce goods to provide for the growing human population increases the risk of the water quality to be susceptible to contaminants. In addition to the production of goods, water also provides a source of recreation and transportation. When water is exposed to additional human use, the water quality is at risk of decreasing because of the introduction of pollutants and destruction of aquatic ecosystems. The quality of water is important when considering human consumption. In the event of suboptimal water qualities, humans are at risk for disease due to specific bacteria or chemicals the water is exposed to. Not only is water quality essential for human health, but plants and aquatic organisms are also affected (Boyd, 2019).

In the United States, over 2 million Americans do not have access to clean water or sanitation (US Water Alliance, 2019). Determining the quality of the water in local areas is essential for the safety of residents. As a result of the lack of drinkable water, individuals are forced to purchase pre-bottled water to ensure safe drinking. This causes inequalities in poorer areas where residents may not be able to afford to purchase water. Not only is this effecting individuals, but it is also affecting the planet. With over eighty-five million water bottles being consumed every day in the United States, the effect of those bottles being discarded contributed to the world pollution abundance and even polluting local waterways (Hu, 2011). With the main sources of drinking water in Virginia being surface water, exposure to contaminants is likely. The state of Virginia's water quality has been assessed by the Environmental Protection Agency (EPA) and with a population over 7 million, 100% of the population had access to safe drinking water in compliance of the EPA regulations during the first 3 months of 2022. Farmville, Virginia is a rural town with a population of approximately 8,000. The water quality of Farmville was also assessed by the EPA, and it was found that there were no drinking water health-based violations for the town (EPA, 2022).

While the quality of safe drinking water is important for humans, the quality of water is also vital for the production of aquatic organisms. Examining the water quality for the health of aquatic organisms can also indicate the condition of the drinking water. Organisms, such as daphnia, have been used as biomarkers for toxicity testing in environmental conditions, specifically polluted waterways. daphnia are transparent planktonic crustaceans that are found in freshwater environments. They play an important role in the aquatic food chain where they are a link between primary and secondary productivity. Their transparent trait allows scientists to examine the effects of contaminants easily by observing changes in heart rate (Sicilliano, 2015). Since daphnia are sensitive to environmental changes, especially contaminants within waterways, they provide a great model organism for examining water quality. Biomonitoring polluted waterways allows scientists to understand the effects of pollutants in an ecosystem for both aquatic species and safety of exposure for humans (Le, 2016). Daphnia are important marine organisms that play an essential role in aquatic ecosystems. Even though they are considered to be on the bottom of the food chain, daphnia provide an important food source to the organisms above them in the food chain. If something, such as poor water quality, affected the survivorship or quality of the daphnia, the rest of the food chain would be disrupted. This could potentially put other organisms at risk of endangerment in the ecosystem where the daphnia are affected (Brookshire, 2016). With the increase in industrialization, the amount of pollution entering waterways is also increasing. With the use of species of daphnia not only has physiological and behavioral effects been determined, but daphnia allow for genomic sequencing to be performed. Daphnia are able to be genomically screened to investigate the effects of pollutants on the genes of the organism. This can also be applied to humans and how it may affect our DNA (Le, 2016). In response to environmental change, daphnia have been shown to exhibit behavioral and physiological change such as an increase in heart rate and as a result a change in behavior (Lovern, 2008). The normal heart rate for daphnia has been identified to be, on average, 200 bpm. Changes in environmental conditions have been shown to elevate or lower the heart rate in daphnia species (Perez, 2019). Behaviorally, daphnia are very responsive to stimuli, such as predators. When presented with multiple types of visual predators, swimming speed was affected (O'Keefe, 1998).

Considering the importance of water quality, this research intends to examine the water quality in two waterways in Farmville, Virginia – Gross Creek and Wilck's Lake. Each water source has different sources of pollutants, therefore a change in the physiology and behavior of daphnia is expected. Gross Creek has been assessed by the EPA and it has been identified that the waterbody condition is impaired with pollutants such as livestock, domestic waste, and wildlife. Gross Creek has been tested for harmful bacteria and E. Coli has been found to be present within the water (EPA, 2022). Wilck's Lake is a recreational area in Farmville, Virginia with its main pollutants being run-off, litter, and recreational activities resulting in damage of the aquatic ecosystem. Based on the knowledge of daphnia susceptibility to contaminants and the overall structure of Gross Creek and Wilck's Lake in Farmville, Virginia, an increase in heart rate and an increase in response to stimuli (locomotion) is expected in more polluted water, specifically Gross Creek.

Methods

<u>Experimental Groups</u>

Daphnia specimens were ordered from Carolina Biological Supply Company and 3 containers of *Daphnia magna* (Item # 142330) and 3 containers of *Daphnia pulex* were obtained (Item # 142304). Water from Gross Creek in Farmville, Virginia and Wilck's Lake in Farmville, Virginia was obtained. In six 1½ gallon aquatic tanks, three different water types from the different water sources around Farmville, Virginia were added to the tanks until the tanks were halfway filled. Two tanks were filled with natural spring water as a control, two tanks were filled with water obtained from Gross Creek, and the last two tanks were filled with water obtained from Wilck's Lake. After the water sources were added each tank was acclimated until the water within each tank reached room temperature. Once the water reached room temperature, the two species of daphnia were added to the tanks. The two species of daphnia, *Daphnia magna* and *Daphnia pulex* were added to each tank with each species being in separate tanks in each water source.

Feeding and Care

Daphnia were fed food pellets obtained from Carolina Biological Supply Company every 2 days (Item # 142316). Before more food was added, the previous food pellets were removed by pipetting the old food pellets out of the water. Once the old food pellets were removed, two food pellets were added to each tank. Water was changed in each tank once a week replacing the water from the same water source. To obtain all daphnia from the tanks, a net was used to separate them from the previous water and transferred to the new, replaced water.

Behavioral Tests

To perform behavioral responses from human approach, red tape was applied on the bottom of each tank at halfway (FIG). To determine the effects of each water source on daphnia nervous system, the time it took for all daphnia to cross the red line away from the human was recorded. The daphnia were acclimated by having no human approaching, passing by, or being near the tanks to limit the risk of previous reactions before data was recorded; daphnia were acclimated for 5 minutes. When the acclimation period was completed, three individuals lined up 10 feet away from the tanks. On cue, the three individuals approached the tanks. Once the tanks were approached, the data recorders started their individual stopwatches. The observers stood at the tanks and watched daphnia magna until all daphnia magna passed the red line away from the recorder. Once all individual daphnia crossed the red line, the timing was stopped and recorded. Only daphnia magna was observed because of their size, being able to be observed easily by the human eye.

Physiological Tests

Heart rate was recorded from each tank to observe the differences in each water source. To observe the heart rate a daphnia was extracted from each tank individually by pipette. The tip of the pipette was cut off to allow the daphnia to fit within the pipette. To extract the daphnia the modified pipette was used, and one daphnia was randomly selected and transferred to a depression slide with little water so the daphnia was stagnant within the depression slide and enough water so the daphnia could survive while the observation took place. Once in the depression slide a compound microscope was used to observe the heart rate. To record the heart rate of the daphnia a 10 second timer was started, and the observer counted the number of beats by utilizing a piece of paper and marker. After each beat a mark was made on the paper with the marker and after the 10 seconds was completed, the number of marks on the paper was counted and multiplied by 6 to estimate the beats per minute. These methods for recording heart rate were repeated from each tank. An ANOVA statistical test was performed to determine the significant difference between groups.

Results

The effect of each water source, Gross Creek, Wilck's Lake, and Control, on daphnia heart rate was examined to determine if the water sources were contaminated with dangerous pollutants. There was no significant difference between the average heart rate of daphnia in each water source. While there was no significant difference between the groups, general trends showed the highest average heart rate in Wilck's Lake and the lowest average heart rate in Gross Creek (Figure 1).

The progression of daphnia heart rate over time was examined to identify the effects of Gross Creek and Wilck's Lake pollutants compared to the control. This was performed to determine the long-term effects of the contaminants. There was no statistical difference between the three experimental groups in which the ANOVA statistical test revealed a p-value of 0.961. Individually, the heart rate over time in the control, spring water, source increased dramatically after one week and stayed the same until the end of the trials. The heart rate of daphnia in water extracted from Gross Creek increased slightly, however, after the second trial the heart rate continuously decreased resulting in a lower heart rate in the end of the trials compared to the control and Wilck's Lake water. daphnia in Wilck's Lake water exhibited no dramatic change in heart rate throughout the trials; the heart rate did not visually change until trial 4 where it decreased slightly (Figure 2).

Locomotion of the daphnia was observed to determine the stress response in differing polluted waters. There was no statistical difference between locomotion in Wilck's Lake, Gross Creek, and the control spring water. An ANOVA statistical test revealed a p-value of 0.779. While there was no statistical difference between the groups, the daphnia within the control showed a longer latency to cross the half-way mark in the tank when presented with a potential predator while the daphnia Gross Creek and Wilck's Lake exhibited faster latency to cross the half-way mark.

Discussion

In response to three different water sources, two from local Farmville, Virginia and one from natural spring water, daphnia species did not exhibit a statistical difference in physiology or behavior overall, however, did show trends in the data. With the trends such as Wilck's Lake having the highest overall average heart rate and the control having the slowest latency to flee away from predators, this information can provide valuable information to the effects of pollutants on daphnia and the quality of water.

The effect of each water source on daphnia average heart rate showed Wilck's Lake with the highest average heart rate overall and Gross Creek having the lowest. These results indicate that Wilck's Lake implemented the most stress on the daphnia, ultimately increasing their heart rate more than the alternative water sources. It was expected that both Gross Creek and Wilck's Lake would have increased heart rates however it was unexpected that Gross Creek would have a lower average heart rate than the control group. Average heart rate for daphnia has been examined in previous research to be approximately 200 bpm (Perez, 2019). In each of the experimental groups the heart rate of the daphnia was above 300 bpm. This indicates that the daphnia were stressed in all conditions. The increase in heart rate may be a result of stress of transferring the organism to the depression slide to examine the heart rate. This implemented a limitation in the study. An acclimation period after being transferred to the microscope depression slide would have been beneficial and may have resulted in more accurate results. Another limitation when examining their heart rate was the speed at which the heart rates beat. The observer and recorder of the heart rates was kept constant to reduce additional errors, however, to receive a precise representation of each daphnia heart rate a high-quality video camera with the ability to slow down the video would have been required to obtain an accurate heart rate reading. The *Daphnia pulex* species in the control, spring water, experimental group did not survive past the 2nd trial. As a result, the future data points involving the *Daphnia pulex* organisms were not analyzed within the overall data.

Previous research has suggested that daphnia heart rate report lower in pond waters compared to control, spring water. This was the opposite for this study as the lake/ pond water exhibited higher heart rate compared to the control (Mauree, 2002). Previous research also suggests that daphnia heart rate increases with low levels of oxygen within the water (Lovern, 2007). Wilck's Lake is surrounded by agriculture and local neighborhoods in which fertilizers and pesticides are often used to treat the crops and plants. Fertilizers, pesticides, and manure can enter the waterways through run-off resulting in a decrease in oxygen levels within the water because they increase microorganism abundance, ultimately consuming the majority of the oxygen within the water (Berg, 2017). When exposed to DDT, a common pesticide used to prevent the presence of mosquitoes, daphnia experienced an overall decrease in heart rate im *Daphnia magna* (Han, 2020).

Locomotion response to stimuli was observed in daphnia being exposed to different water sources around Farmville, Virginia. The results of the study showed no significant difference in locomotion; however, the control group took had longer latency to cross the half-way mark of the tank, fleeing away from the human predator. Latency was the same for Wilck's Lake and Gross Creek. These results suggested a difference of stress levels in spring water compared to polluted water. The decrease in latency in the polluted water suggests that the daphnia were more stressed resulting in faster fight or flight response. Limitations of this experiment included the ability to see the *Daphnia pulex* organisms. Due to this limitation, we could not perform or analyze behavioral data on these organisms due to their size, compared to the *Daphnia magna* could be seen by the human eye. Previous research has analyzed behavior responses in daphnia in response to pollutants. An increase in behavioral movements in daphnia was observed in polluted water which indicated they were responding to an increased risk of predation (Lovern, 2007). It was also found in a study performed by Hussain, in response to 12 pesticides, all resulted in an increase in locomotor activity and a change in swimming patterns (Hussain, 2020). Without the presence of pollutants, the daphnia exposed to spring water were less stressed, therefore their response to a predator would be less compared to Wilck's Lake and Gross Creek where the daphnia are exposed to pollutants, increasing their stress levels, resulting in their response to a potential predator to be quicker.

Future directions of this research would be to complete a chemical analysis of the three water sources to determine the specific toxins within the water source and measure the pH.

Examining these additional factors would allow researchers to understand the what the factors affecting the water source are allowing them to create a plan of action to reduce the toxins in the waterways. Another extension of this research would be to examine the seasonal changes and how they affect the quality of water. With the increase in temperatures in the spring and summer, excess algal growth occurs in the water resulting in eutrophication and a decrease in oxygen levels in the water. Also, excess rainfall increases the risk of contaminated run-off to expose the waterways of toxic chemicals such as pesticides from agricultural farming (Boron, 2016). These continued studies would allow researchers to identify factors that negatively affect the quality of water sources. It has also been recorded that the effects of pervious environmental stressors could have an effect on daphnia transgenerationally. With stress put on daphnia, such as polluted waterways, the symptoms they experience could be passed down to the future generations, even if the conditions are optimal (Jeong, 2015).

This research has concluded the potential affects water quality has on aquatic organisms, which can also be indicators of the effects of the quality of drinking water in local communities. Identifying potential hazards in water sources will allow citizens and aquatic organisms to thrive in their natural habitat. Daphnia provide early detection for water quality hazards and how they may affect other organisms (Jeong, 2019).

Figures

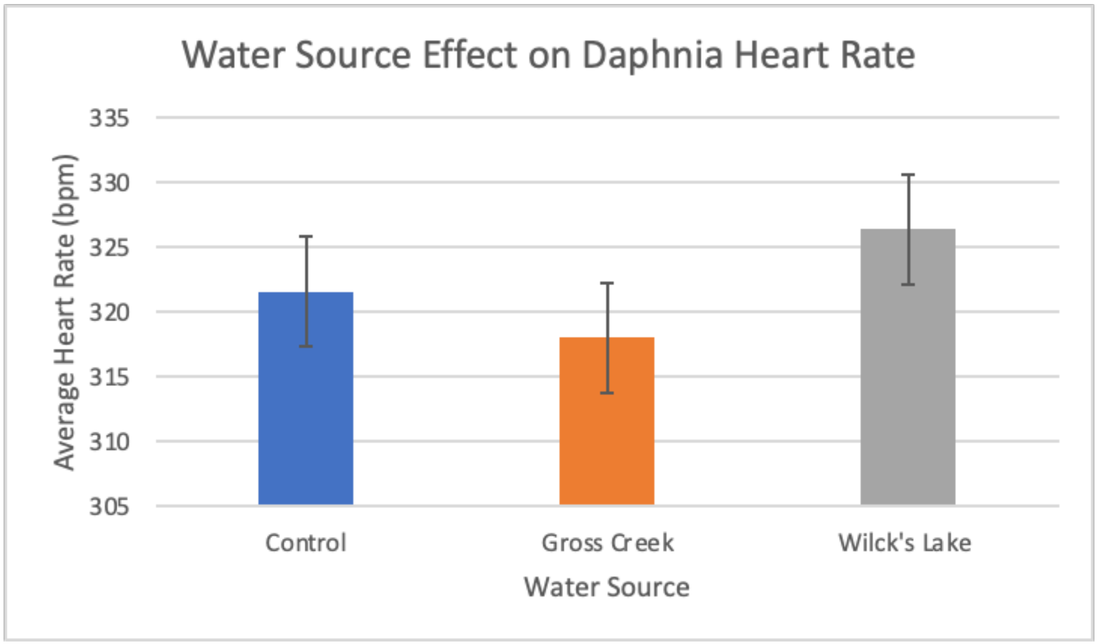

Figure 1. **Effect of Water Source on Average Daphnia Heart Rate.** Comparison of average daphnia heart rate response to different water sources in Farmville, Virginia; Gross Creek and Wilck's Lake. ANOVA statistical test revealed a p-value of 0.943. Error bars represent the standard deviation.

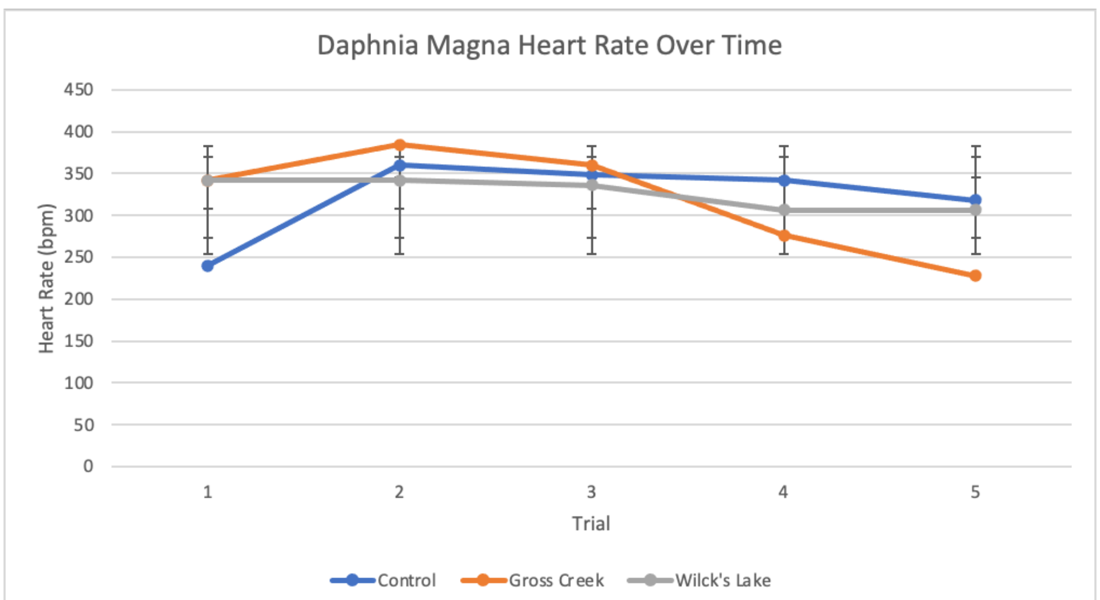

Figure 2. Daphnia Heart Rate Over Time. Change of daphnia heart rate over time in response to different water sources in Farmville, Virginia; Gross Creek and Wilck's Lake. Error bars represent the standard deviation.

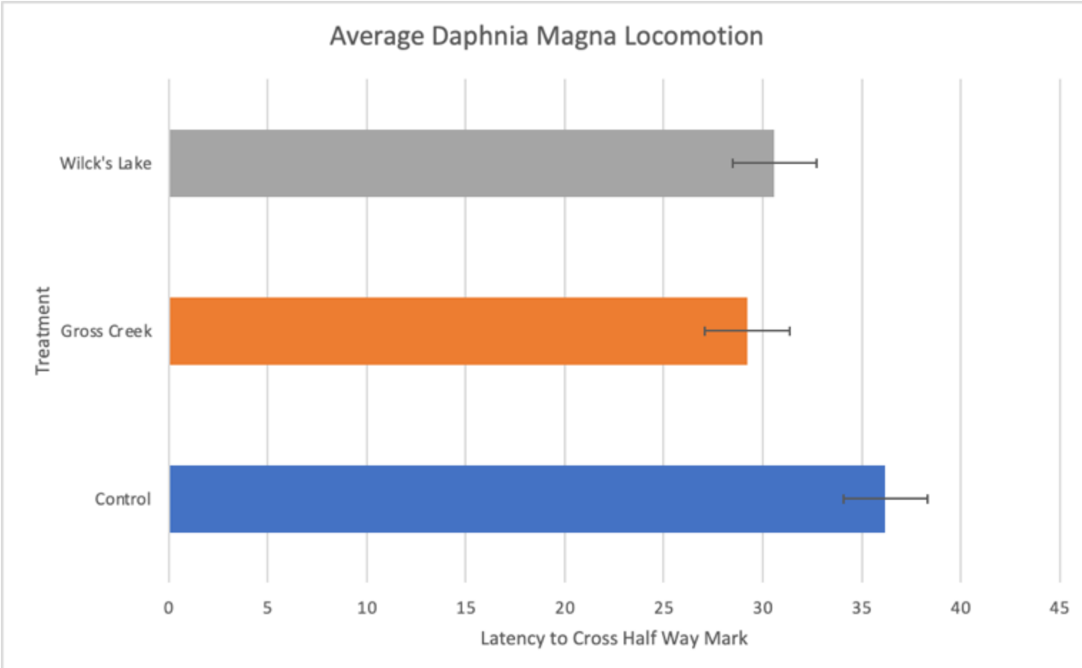

Figure 3. Average Daphnia Magna Locomotion. Comparison of daphnia magna locomotion from control and two treatment waters from Gross Creek and Wilck's Lake in Farmville, Virginia. ANOVA statistical test revealed a p-value of 0.779. Error bars represent standard deviation.

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