Response of *Daphnia mendotae, Daphnia pulicaria*, and *Holopedium gibberum* to presence of

fish kairomones and caffeine

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Abstract

This experiment studied the migration of three types of zooplankton; *Daphnia mendotae, Daphnia pulicaria,* and *Holopedium gibberum* taken from Bay Lake in Michigan. This lab experiment involved placing twenty individuals of each species into a 50 cm tall, 7 cm diameter column and monitoring their vertical migration in four different treatments for one hour. The four treatments included a control, a fish kairomone treatment, a fish kairomone with low caffeine concentration (60.0 ng/L) treatment, and a fish kairomone with high caffeine concentration (357.0 ng/L) treatment. The analysis of variance test using mean depth after an hour showed that treatment significantly impacted the migration of the zooplankton (p value= 0.00000000001630 and $F_{1,50} = 34.523$ with df=3). The high caffeine concentration treatment changed the migration of the zooplankton significantly. The zooplankton migrated deeper in the columns; thus indicating that the presence of caffeine in the water has an impact on *Daphnia* and *Holopedium* migration.

Introduction

Daphnia is a genus comprised of small aquatic crustaceans, sharing the following characteristics: they have a single compound eye, two double branched antennae and a generally translucent carapace (Dodson 1998). They are found in various aquatic environments ranging from freshwater lakes, ponds, streams, and rivers to acidic swamps, *Daphnia* are greatly affected by changes within their environment and can exhibit phenotypic plasticity. One such case is their ability to avoid predation through diel vertical migration (Buerge, 2003; Chetelat 2009; Dodson 1995). When predators such as fish are present in a lake *Daphnia* will suspend at different depths during different times of the day. Generally, the *Daphnia* are deeper during the day and move closer to the surface at night. Vertical migration is also exhibited by other zooplankton, such as

Holopedium. However, *Holopedium* differ from *Daphnia* in that they have a gelatinous casing which scientists hypothesize is to avoid predation by *Chaoborus* (Schulz and Yarusta 1998), which could influence the amplitude of their vertical migration.. *Chaoborus* is a phantom midge which is also capable of diel vertical migration. During the day, *Chaoborus* also remain hidden in the deeper depths of its environment but spread throughout the water columns at night (Knudsen and Larsson 2009).

The driving forces behind *Daphnia* migration have long been disputed. In 1995 an experiment performed by Stanley Dodson showed that chemicals produced by fish, called kairomones, are a major determining factor influencing *Daphnia* migration. According to the results of this experiment, *Daphnia* migrate up or down their aquatic environment, moving in the opposite direction of predators (Dodson 1995). However, Kessler and Lampert (2004) determined it was the temperature gradient rather than the predation level which drove vertical migration. Chetelat and Amyot (2009) concluded in an Arctic freshwater experiment that food supply rather than predation is the primary determinant of *Daphnia* distribution. These different results suggest a trade off: "vertical distributions of zooplankton are the result of a complex interplay of mortality risk, food availability, and abiotic factors" (Kessler and Lampert 2004). Little is known about *Holopedium* migration but it has been hypothesized that they migrate similarly to *Daphnia* (Huss and Nilsson 2011).

The importance of these and similar experiments is to better understand the normal movements of *Daphnia* which may help to detect abnormal movements. A possible disruptor of normal *Daphnia* behavior might be environmental pollutants. One such pollutant, caffeine, has been linked with an increase in estrogen levels (O'Connor 2012). In experiments dealing with increases in estrogen levels due to the breakdown of atrazine it has been proven that it can

change the gonads of certain vertebrates and alter entire ecosystems (Hayes et al 2011). With caffeine increasingly being found in aquatic environments it is hard to predict how it may affect the animals in those environments. For example, in Oregon, the caffeine concentration found in seawater ranged from 8.5 ng/L to 44.7 ng/L, while the concentration in rivers and estuaries went as high as 152.2 ng/L (Del Rey et al 2012). For comparison, the amount of caffeine per cup of coffee usually falls between 29 mg and 176 mg with a median amount of 74 mg per cup (Gilbert et al 1976).

Previous observations of *Daphnia* migration have concluded that they respond to the presence of fish predators and caffeine (Dodson 1995: Corrotto et al 2010). As they respond to both caffeine and fish kairomones it is reasonable to assume that there may be an interaction when both are present. This could mean that the direction of their migration will change or become more pronounced. There may also be some variation in migration patterns between different zooplankton species as they could respond differently to fish kairomones and caffeine. My objective is therefore, to determine if caffeine, as a water pollutant, will have an impact on *Daphnia mendotae, Daphnia pulicaria,* and *Holopedium gibberum* migration to avoid fish predation.

Materials and Methods

The trials were set up and performed in a manner similar to the methods described by Dodson (1995). In my experiment 6 columns made from clear plastic polycarbonate piping, 50 cm high, 7 cm in diameter, and marked off in 5 cm increments, to create 10 strata, were used as the experimental units. The columns had an approximate volume of 1.924 L. Each tube contained twenty of one species from three possible species: *Daphnia mendotae*, *Daphnia*

pulicaria or *Holopedium gibberum*. The zooplankton specimens and fish used for this research were captured from Bay Lake on the morning the trials were to be run. Bay Lake is located in Gogebic County, Michigan. The exact coordinates of the lake are longitude 89.48769°, latitude 46.24369° with the total surface area of the lake being 420 hectares (Elser 1978, unpublished) The zooplankton were captured using a tow net 30 cm in diameter lowered to a depth of 6 m. The *Daphnia* were separated by species microscopically. Individual *Daphnia* and *Holopedium* were used only once.

This experiment had four treatments: a control, fish kairomone, fish kairomone with low caffeine concentration, and fish kairomone with high caffeine concentration. The control received 10 mL of pure water. The fish kairomone column received 10 mL of water from a fish tank containing a fish. The fish kairomone with low caffeine concentration received a caffeine concentration similar to the amount found in the Penha Channel in Brazil of 60.0 ng per liter, in addition to 10 mL of water from the fish tank containing a fish. The fish kairomone with high caffeine group received a caffeine concentration similar to that found in the Brazilian Ramos River of 357.0 ng per liter (Ferreira et al 2005), in addition to 10 mL of water from the fish tank containing a fish.

The fish used was a bluegill, *Lepomis* spp., captured from the same lake as the zooplankton, Bay Lake on the UNDERC (University of Notre Dame Environmental Research Center) property in Michigan's upper peninsula. The fish was housed in a 37.85 L fish tank. The powdered caffeine used for the experiment was ninety nine percent pure and was added to the water in order to acquire the desired concentrations. The water for the trials was well water to eliminate the possibility of fish kairomones being present.

In each trial the specimens were placed into the water twenty minutes before the treatment began. This provided them with an adequate amount of time to acclimatize to the columns and go to where they felt most comfortable. The number of *Daphnia* in each stratum was recorded prior to the treatment. Once the treatment was added, the number of *Daphnia* in each stratum was recorded every 20 minutes for an hour. For the treatments containing caffeine, the caffeine was added to the water prior to the *Daphnia* and *Holopedium* being added. After the caffeine was added, the *Daphnia* and *Holopedium* were once again given 20 minutes to acclimatize before the treatment was added.

There were 8 replicates per treatment for both the *Daphnia mendotae* and Daphnia *pulicaria* and 6 replicates for the *Holopedium gibberum*, totaling 88 trials. The data was analyzed using an ANOVA in the program SYSTAT 13 (Cranes Software). For the ANOVA, the response variable was the mean depth at the end of the experiment. The 2 independent variables were the different species and the four treatments.

Results

Over time *Daphnia mendotae*, *Daphnia pulicaria*, and *Holopedium gibberum* all moved downwards in all treatments except for the control (Figures 1, 2, 3). This movement mainly occurred in the first twenty minutes shown by Figures 1, 2, and 3 and remained relatively constant until the end of the trial. Therefore the ANOVA was calculated using the average depth at time 60 only. The ANOVA indicated that the type of treatment: control, fish kairomone, fish kairomone with low caffeine concentration, or fish kairomone with high caffeine concentration significantly changed the strata in which the species were residing (p value= 0. 00000000001630 and $F_{1,50} = 34.523$ with df=3). Species was not significant in determining the amplitude of the vertical migration (p value= 0.492 and $F_{1,50} = 0.715$ with df=2) and the interaction term for species

and treatment was also not significant (p value= 0.262 and $F_{1.50} = 0.731$ with df=6). Figure 4 shows the average depth of the zooplankton after one hour in the different treatments, this shows that zooplankton in the fish kairomone and high caffeine treatment are significantly lower when compared to the other treatments.

The Post Hoc Test run for the ANOVA was Tukey's Honestly-Significant-Difference Test which used the least squares mean and model MSE of 1.502 with 76 degrees of freedom (Table 1). It showed that there was a significant difference between fish and low caffeine levels (p-value= 0.0000041), fish and high caffeine levels (p-values=0.0000044), and fish when compared with the control trials (p-values=0.0000044). The fish and high caffeine treatments also had significant differences from the fish and low caffeine treatments (p-value=0.00068) as well as from the fish treatments (p-value=0.00070).

Discussion

Migration is important to the survival of many species. When resources become limited, predation too strong, or weather patterns change, species can choose to migrate in order to increase their chances of survival and fitness. Zooplankton also exhibit this form of behavior in order to survive. However they merely migrate up and down in the water column in which they reside rather than moving location entirely.

As predicted for this experiment the treatments caused a downward migration of the species being examined. In the fish treatment, in order to increase their chance of survival the zooplankton moved deeper in the columns away from the fish kairomones. *Daphnia* migration for predator avoidance has long been studied, proving that when in danger from fish predation *Daphnia* will migrate vertically in opposition to their predator throughout the day. *Daphnia* are usually higher in the water column at night and lower in the day when fish predation is at its

strongest (Dodson 1995). However, there is often a tradeoff between this, food, and optimum temperature (Beklioglu et al 2008). As these trials were only conducted over an hour and in room temperature water (approximately 20 °C) these were not factors. There was a significant migration of the *Daphnia* downwards away from the fish kairomone when compared to the mean depth of those in the control. Therefore the hypothesis that *Daphnia* migration is affected by fish presence was validated.

The zooplankton also moved deeper in the columns in the presence of high caffeine concentrations, validating the hypothesis that caffeine would also have an effect on the migratory patterns of the *Daphnia mendotae*, *Daphnia pulicaria*, and *Holopedium gibberum*. This implies that there is a likely interaction between zooplankton migration and caffeine pollution. The increasing amount of contamination from untreated wastewater leakage and the correlating bacterial contamination poses a great threat to drinking water quality worldwide (Hillebrand et al 2012). In studies involving high caffeine concentrations, it was noted that some aquatic species, such as the tadpoles of *Lithobates pipiens*, were smaller in size and exhibited behavioral changes as well (Fraker and Smith 2004). Long term behavioral changes and decreases in size could have unknown impacts on the entire ecosystem. Finally caffeine has been shown to alter estrogen levels (O'Connor 2012). Altered estrogen levels have been shown to alter mating behavior and courtship rituals in some organisms (Guarraci and Benson 2005).

For our experiment, the higher levels of caffeine posed more of a threat to the *Daphnia* and *Holopedium* than the lower caffeine levels. The lower caffeine levels were therefore too low to be an issue and it is only in higher doses that it becomes a problem. Another possible explanation for the exaggerated downward migration in the high concentration of caffeine is that in an experiment studying heart rate and caffeine in *Daphnia* higher concentrations of 0.5% and

2% caffeine heart rate decreased, while lower concentrations of only 0.1% caused no significant changes in mean heart rate (Corrotto et al 2010). Daphnia move upwards by beating their 2nd antenna but sink when this antennae is kept still. If the heart rate decreases, the movement of the antennae would slow down making them sink, explaining their downward migration in the water column (Ebert 2005). Due to the decrease in heart rate it can be theorized that higher concentrations are more damaging overall to the health of these organisms than the lower caffeine levels.

Overall, *Daphnia* and *Holopedium* migration are greatly influenced by the presence of fish. Also, unsurprisingly this is also affected by a pollutant on the rise, caffeine. Zooplankton are often dominant herbivores in the lakes and ponds in which they reside. They are also a large part of fish diets (Dodson 1995). Another possible effect that the presence of caffeine in zooplankton could have on its ecosystem is that of biomagnifiction. Biomagnification is the increase in concentration of a substance that occurs in a food chain resulting from food chain energetic, a low rate of internal degradation of a substance, or persistence (Ikemoto 2008). Through this principal, if zooplankton with caffeine in their systems are eaten in great quantities it could have an impact on the fish predators. Zooplankton, such as *Daphnia* and *Holopedium*, are key members of these aquatic ecosystems and if pollutants, like caffeine, are changing their migratory behavior it could have a large impact on these communities.

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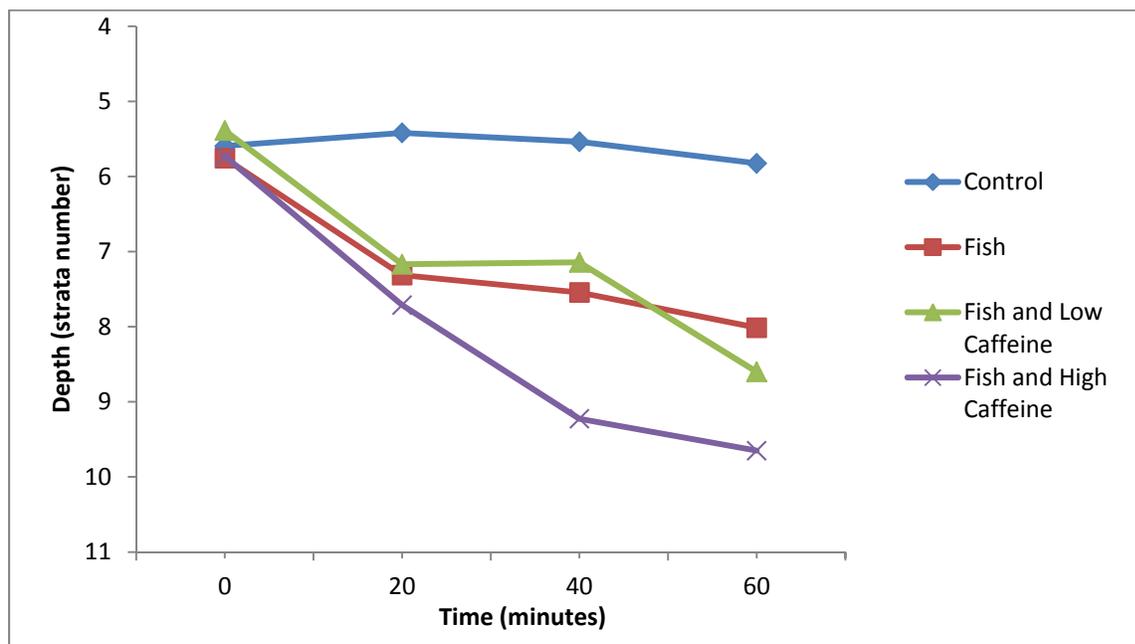
Tables and Figures

Figure 1: Movement of *Daphnia pulicaria* over an hour in the presence of fish, fish and low caffeine (60.0 ng/L), fish and high caffeine (357.0 ng/L), and control. Strata were 5 cm apart in a column 50 cm in height.
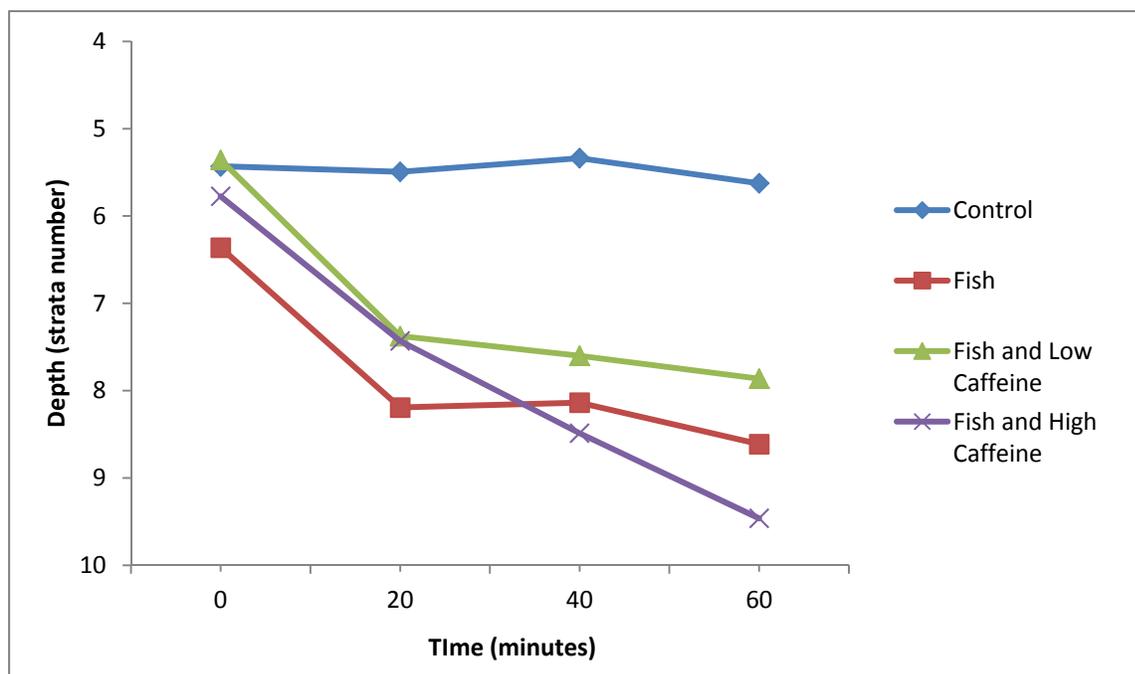

Figure 2: Movement of *Daphnia mendotae* over an hour in the presence of fish, fish and low caffeine (60.0 ng/L), fish and high caffeine (357.0 ng/L), and control. Ezzzzzzzzzach strata was 5 cm in a column 50cm in height.
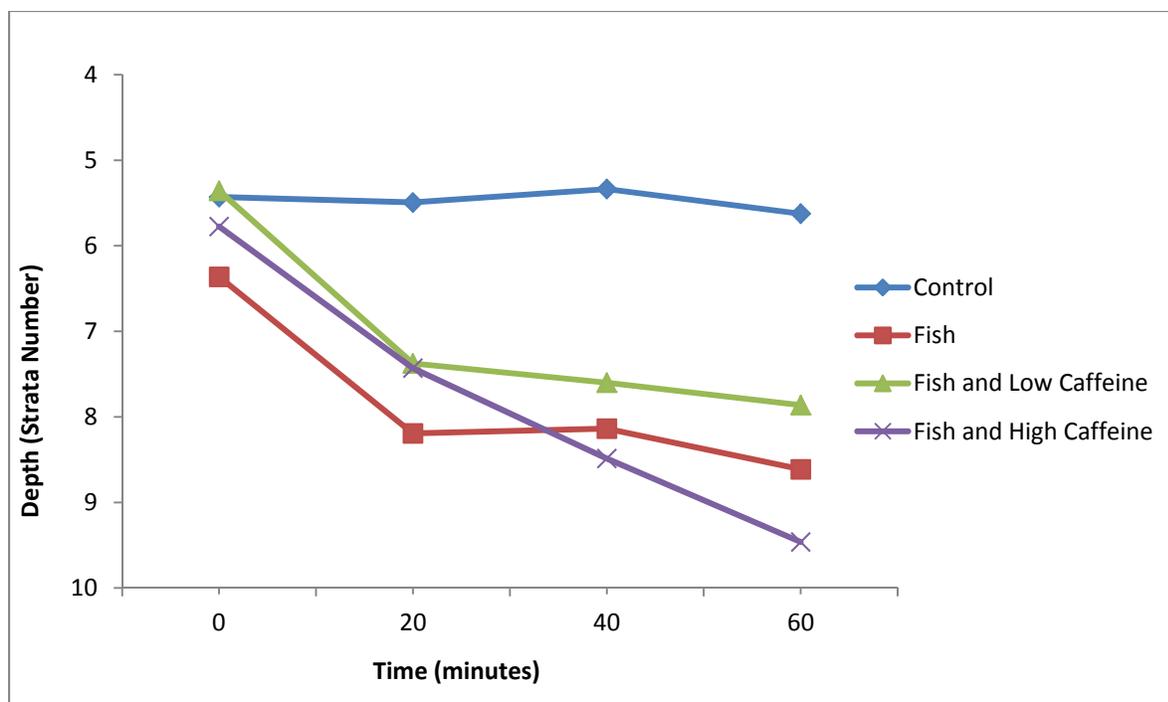
Figure 3: Movement of *Holopedium gibberum* over an hour in the presence of fish, fish and low caffeine (60 ng/L), fish and high caffeine (357.0 ng/L), and control. Each strata was 5 cm in a column 50cm in height.
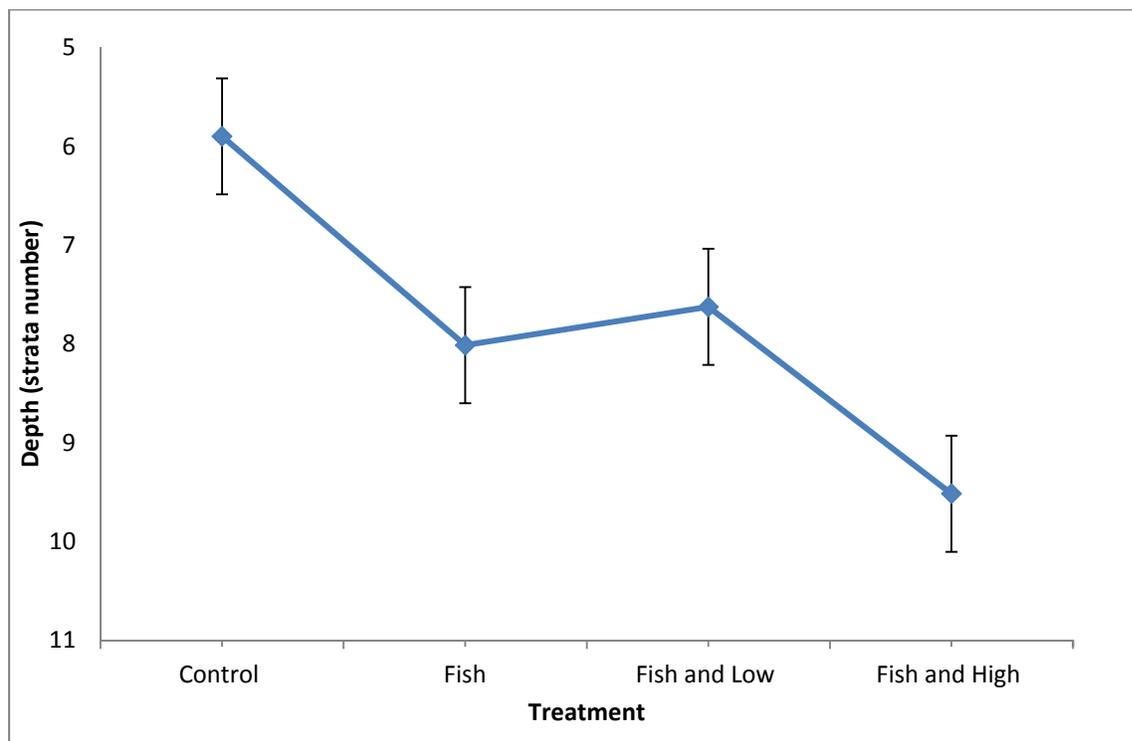

Figure 4: Average strata depths after an hour of *Daphnia mendotae, Daphnia pulicaria,* and *Holopedium gibberum* for control and in the presence of fish, fish and low caffeine (60 ng/L), and fish and high caffeine(357.0 ng/L). Each strata was 5 cm in a column 50cm in height.

Table 1: Comparison from Post Hoc, Tukey's Honestly-Significant-Difference Test. using the least squares means from an ANOVA, crossing species and treatments after an hour. An MSE value of 1.502 and 76 degrees of freedom.

Treatment	Treatment	Difference	p-value	Lower 95% Confidence Interval	Upper 95% Confidence Interval
Control	Fish and High Caffeine	-3.760	0.00000407	-4.730	-2.789
Control	Fish and High Caffeine	-2.246	0.00000437	-3.217	-1.276
Control	Fish	-2.250	0.00000436	-3.220	-1.279
Fish and High Caffeine	Fish and Low Caffeine	1.514	0.00067654	0.543	2.485
Fish and High Caffeine	Fish	1.510	0.00069904	0.540	2.481
Fish and Low Caffeine	Fish	-0.004	0.99999997	-0.974	0.967