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# The Induced Heart Rate Response to Fish Kairomones in *Daphnia pulex*

MICHAEL CULSHAW-MAURER

*Biology Honors Thesis*

## Introduction

*Daphnia pulex* is a cladoceran branchiopod commonly found in the zooplankton of pelagic and littoral zones of many lakes, including those in central Minnesota. While quite small, in the range of 0.5-2.5mm in length, they are still visible to the naked eye, and can be quite abundant in lake water samples. *Daphnia* are one of the main filter feeders of algae and other plankton in lake ecosystems, as well as one of the main food sources for fish.

Planktivores, such as pumpkinseeds, bluegills and other sunfish, feed largely on *Daphnia*, placing them at a critical intersection between fish and the primary producers in lake ecosystems. *Daphnia* are also frequently used as model systems for genetic and evolutionary studies, as they have quick reproductive cycles, broad environmental colonization abilities, and some unique behavioral, morphological and physiological responses to environmental and biological stimuli.

*Daphnia* typically have a characteristic reproductive cycle that includes cyclic parthenogenesis, meaning they can switch back and forth between sexual reproduction and asexual, clonal reproduction (parthenogenesis). Under favorable conditions, they reproduce parthenogenically, producing many clones in a short amount of time, rapidly increasing population at the expense of genetic diversity. Production of clonal populations is another reason *Daphnia* are a popular study species, as experiments can be conducted without genetic variation between subjects. During adverse conditions, *Daphnia* produce resting eggs, forming a tough ephippium, via sexual reproduction. The conditions that push *Daphnia* into one mode of their reproductive cycle or the other are quite varied and well-studied. It has been shown that a complex set of stimuli can trigger sexuality, including low food availability, higher levels of competition, shorter days, and lower temperatures (Ebert 2005; Slusarczyk and Rybicka 2011).

Several studies have shown that kairomones (signaling chemicals that are “eavesdropped” on by other

organisms, and are released unintentionally by the emitting organism) released by fish can trigger the production of sexual resting eggs in *Daphnia* (Slusarczyk et al. 2013) and push them to produce more offspring and reproduce earlier (Castro and Consciencia 2007), suggesting predation by fish is able to induce physiological changes in *Daphnia*. It seems that these changes increase survival of the *Daphnia* population by protecting offspring from fish and potentially offsetting the mortality of young by producing more neonates more quickly.

Another induced response is a morphological modification of the carapace in response to *Chaoborus* kairomones (Krueger and Dodson 1981), making the *Daphnia* harder to handle and eat (Havel and Dodson 1984). *Chaoborus* nymphs, also known as glassworms, are one of the main predators of *Daphnia*, feeding on them by grasping them with modified antennae. The gape of the antennae limits the ability of a *Chaoborus* to grasp and eat *Daphnia*, meaning an enlargement of some dimension of a *Daphnia*'s carapace can make it harder to grasp. Since enlargement of the carapace require significant energy investments (Boeing et al. 2004), they are only beneficial if the threat of *Chaoborus* predation is high, making the kairomone signaling an effective way of inducing defenses only in beneficial situations.

Additionally, studies have shown that the capture efficiency of *Daphnia* by *Chaoborus* decreases as *Daphnia* size increases (Swift and Federenko 1975; Swift 1992). This suggests that predation affects *Daphnia* differently based on their size, as *Chaoborus* will likely target smaller *Daphnia* more frequently in order to reduce the number of failed capture attempts.

Projects done by previous CSB/SJU students in the Biology Department have shown that *Daphnia* have another response to *Chaoborus* kairomones: elevated heart rate. This has been widely studied by students in aquatic ecology, though the methodology for measuring heart rate was rather crude. In 2014, Cody Groen, a St. John's biology student, used video microscopy to measure

*Daphnia* heart rate, yielding much more accurate results than the previous procedures. *Daphnia*'s hearts can be seen through their carapace, meaning their heart rate can be determined visually. By using videography, the video can be saved, slowed down, and heartbeats can be counted much more accurately. He was able to analyze the differential responses between size classes of *Daphnia*, concluding that smaller individuals had a more elevated heart rate in response to *Chaoborus* kairomones than larger *Daphnia* did (Groen, unpublished data). As mentioned previously, this is likely due to larger *Daphnia* being more difficult for *Chaoborus* to physically handle and manipulate, making them less of a target of predation.

Despite the phenomena of elevated heart rates in response to *Chaoborus* and the ability of *Daphnia* to respond to fish kairomones, to my knowledge, no studies have been conducted researching the effects of fish kairomones on heart rate. Additionally, few studies have been done regarding the effects of predation on *Daphnia* across a size gradient. It has been well demonstrated that planktivorous fish select *Daphnia* based on size, preferring larger individuals (Vinyard and O'Brien 1976; O'Brien et al. 1976), so it would follow that induced responses to predation would vary across size classes. As discussed previously, physiological responses to predation are energetically expensive, so a uniform response across size classes despite varying levels of risk could be maladaptive.

Since it has been shown that fish kairomones have significant physiological effects on the reproductive strategies of *Daphnia* and also that *Chaoborus* kairomones can affect *Daphnia* heart rate, my study will focus on the effects of fish kairomones on *Daphnia* heart rate. Since fish and *Chaoborus* differ significantly in their predation styles, if there is an effect on heart rate by fish kairomones, it may follow a different pattern from the one shown in the *Chaoborus* response. Larger *Daphnia* are more visible to fish and provide more energy per individual captured, so, following optimal foraging theory, bluegills should preferentially feed on larger *Daphnia* (Brooks and Dodson 1965; Werner and Hall 1974). This leads me to hypothesize that larger *Daphnia* will have a greater response to fish kairomones than smaller *Daphnia*, as fish present a greater risk to large *Daphnia* than small. I predict the elevation in heart rate from control levels will increase as body length of the *Daphnia* increases.

It is as of yet unknown whether fish kairomones have an effect on the heart rate in *Daphnia*, meaning my

research would help to develop a fuller understanding of the inducible defenses of *Daphnia* in response to various predatory kairomones. My research, combined with that of Cody Groen, could help to demonstrate another physiological response to external stimuli by *Daphnia*, a very well researched and important model organism. A deeper understanding of the relationship between fish and *Daphnia* is especially relevant, considering the role *Daphnia* play as a link between fish in the upper trophic levels and the algae that serve as primary producers in lake ecosystems. *Daphnia* act as a critical point in the exchange of energy up the trophic ladders, meaning any research done on them will ultimately lead to a better understanding of lake ecosystems as a whole.

## Methods

### *Raising Daphnia and fish*

I raised clonal populations of *Daphnia pulex* in jars containing Finken Water Solutions spring water. Individuals were isolated from mixed populations from Trans-Mississippi, placed into separate 130mL jars, and were fed *Nannochloropsis*, a non-motile eustigmatophyte, which was ordered from Carolina Biological Supply. Once the *Daphnia* began to reproduce parthenogenically and the populations grew, I moved them into 425mL jars and eventually to 850mL jars in order to allow the populations to grow further. Populations were fed (*Nannochloropsis* that had been centrifuged to remove the nutrient medium) approximately every 3 days, and water was changed approximately every 2 weeks, depending on accumulation of dead algae, dead *Daphnia*, and exoskeletons. Three 10cm juvenile bluegills (*Lepomis macrochirus*), were kept in an aquarium under standard conditions and fed fish food daily. In order to obtain "fish water", a bluegill would be removed from the large tank and placed in a 1000mL beaker with approximately 750mL of water, along with an aerator, overnight. This would allow the water to be more concentrated with fish kairomones but potentially with fewer waste products built up.

### *Collecting Data*

I removed a single *Daphnia* from the clonal population using a disposable plastic pipette and deposited it on a slide. I used a second disposable pipette with an elongated, narrowed tip to remove the water from the slide until only a thin layer held the *Daphnia* gently in place. I then used a microscope and an ocular

micrometer to measure the *Daphnia's* body length, from the top of the carapace to the tail spine attachment point. The slide would then be removed and several drops of water would be added, either the control spring water or the “fish water”. I then waited 1 minute, removed the water using the narrow pipette, and transferred the slide to the microscope set up for video microscopy. I started a 30-minute timer and took a 12-second recording of the *Daphnia*. After the recording was completed, I removed the slide and added several drops of water, either control spring water or the “fish water”. After 4 minutes and 40 seconds, I removed the water again, transferred the slide to the microscope, and began a 12-second recording at 5 minutes. This process of water replacement, water removal, and recording was repeated every 5 minutes for 30 minutes in total. Three trials were conducted simultaneously, with each timer staggered so that the 5-minute wait time for one trial could be used to conduct the recordings for 2 other trials. During each recording, I spoke the name of the trial and the recording time (5 minutes, 10 minutes, etc.) so that the trial could be identified via audio later on in the video processing. After the 30 minute trial was up, the *Daphnia* was rinsed into a separate collection jar for “used” individuals, since individuals were only used for a single 30-minute trial.

### Analyzing Data

I uploaded the video files to an iMac computer and, using iMovie software, identified each video recording via the audio portion mentioned previously. I clipped each recording to 5 seconds, slowed it down to 25% speed, and played it back, counting the number of heartbeats. The number was entered in an Excel spreadsheet, and the beats per 5 second value was multiplied by 12 in order to extrapolate to beats per minute. The video recordings were 12 seconds long to assure that 5 seconds of usable footage could be clipped out, in case the *Daphnia* moved significantly in the middle of the recording. The final data collected were body length for each individual, and heart beats per minute at 5-minute increments for 0-30 minutes for each individual. Small *Daphnia* control n=9, fish water n=9; medium *Daphnia* control n=10, fish water n=10; large *Daphnia* control n=8, fish water n=8.

## Results

I was successful in rearing stable, healthy *Daphnia* populations throughout the duration of the experiment, made of a wide range of size classes at any given time, especially when the adults gave birth to a new generation. The *Daphnia* handled the experimental procedure well, though I experienced a few deaths of individuals while on the slide, from somehow escaping the water drop while I was working with another slide.

The results show a statistically significant difference between control and fish water treatments only in the smallest *Daphnia* size class. Based on trendlines in Figure 2, larger *Daphnia* tend to have higher baseline heart rates, though the amount of scatter is quite significant, with low R2 values. Even with no genetic variation and consistent experimental treatment, individual *Daphnia* exhibited varying heart rates.

The results demonstrate a significant difference in heart rate between control and fish water trials only in small *Daphnia* (Figure 1). From 10 minutes to 30 minutes, small *Daphnia* treated with fish water exhibited significantly higher heart rates than small *Daphnia* treated with spring water. Over the entire 30 minute period, medium and large *Daphnia* did not exhibit any significant differences in heart rate between control and fish water trials.

Figure 2 shows that there is a large amount of scatter when looking at heart rate vs. a continuous axis of body length. However, the general trends demonstrate a convergence of heart rate of control and fish water treatments as size increases, with a significant difference in heart rate between control and fish trials for the smallest end of the size range.

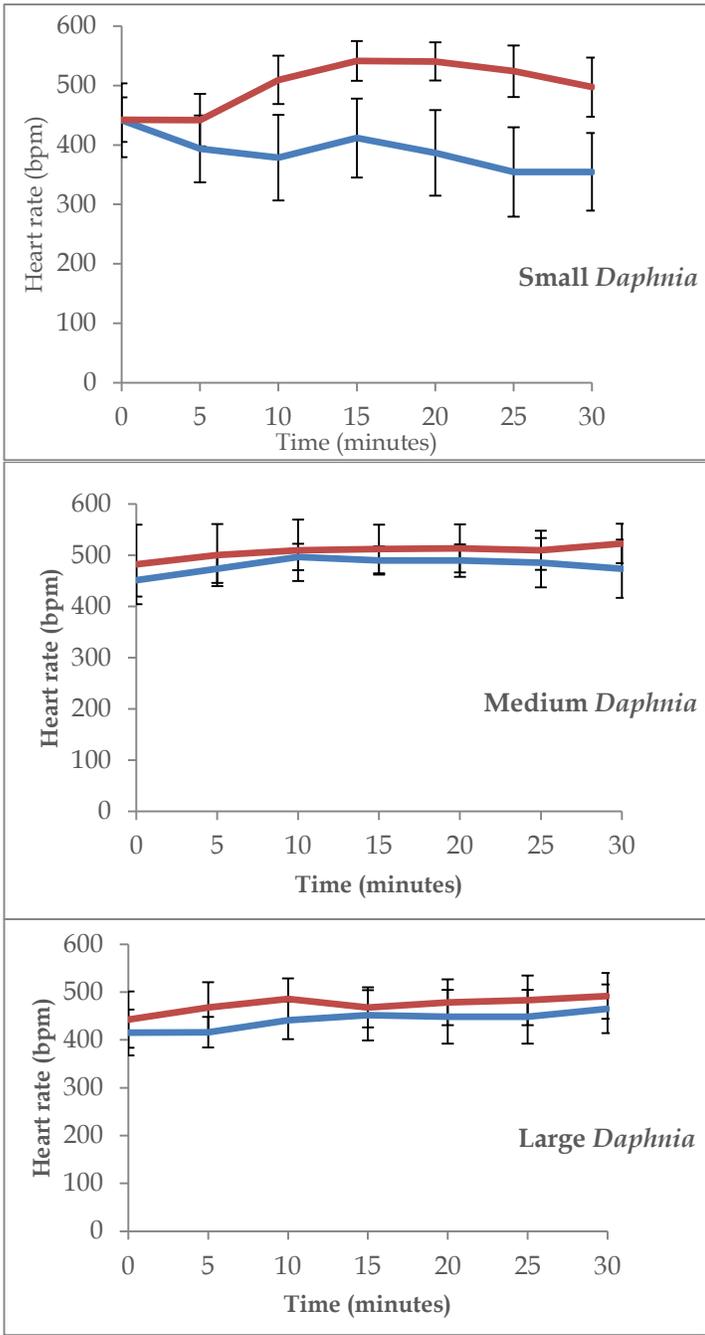


Figure 1. Graphs of mean heart rate over time for each size class, control (blue line) vs. fish water (red line) treatments. Error bars are 95% confidence intervals. Small: 0-1mm; medium: 1.025-1.5mm; large: 1.5mm+.

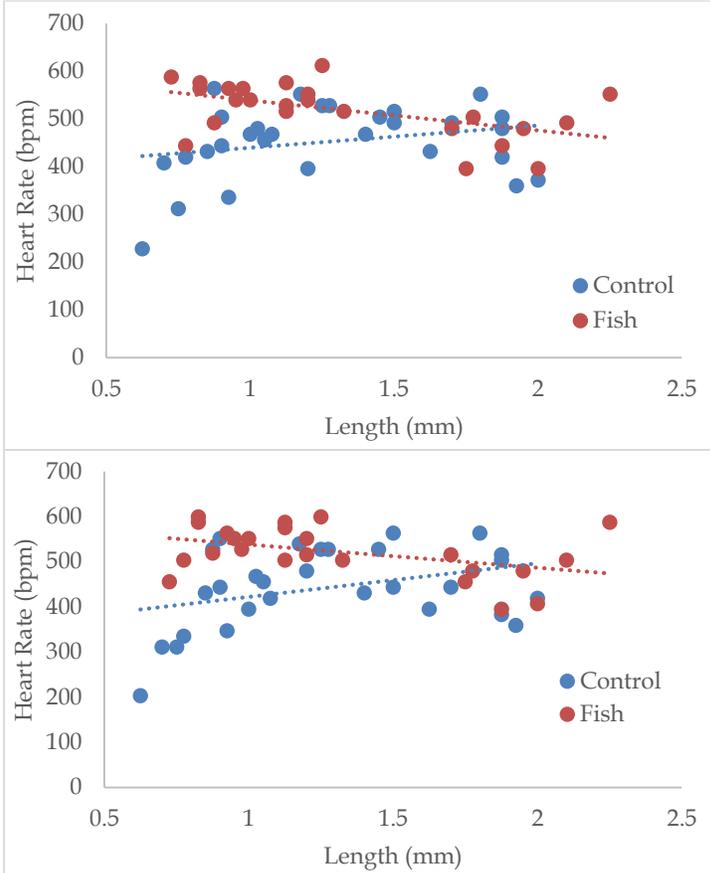


Figure 2. Scatterplots of heart rate vs. *Daphnia* length for discrete time points (15 minutes-top, 20 minutes-bottom). 15 minute control  $R^2 = 0.0644$ ; 15 minute fish  $R^2 = 0.2715$ ; 20 minute control  $R^2 = 0.1261$ ; 20 minute fish  $R^2 = 0.1809$ .

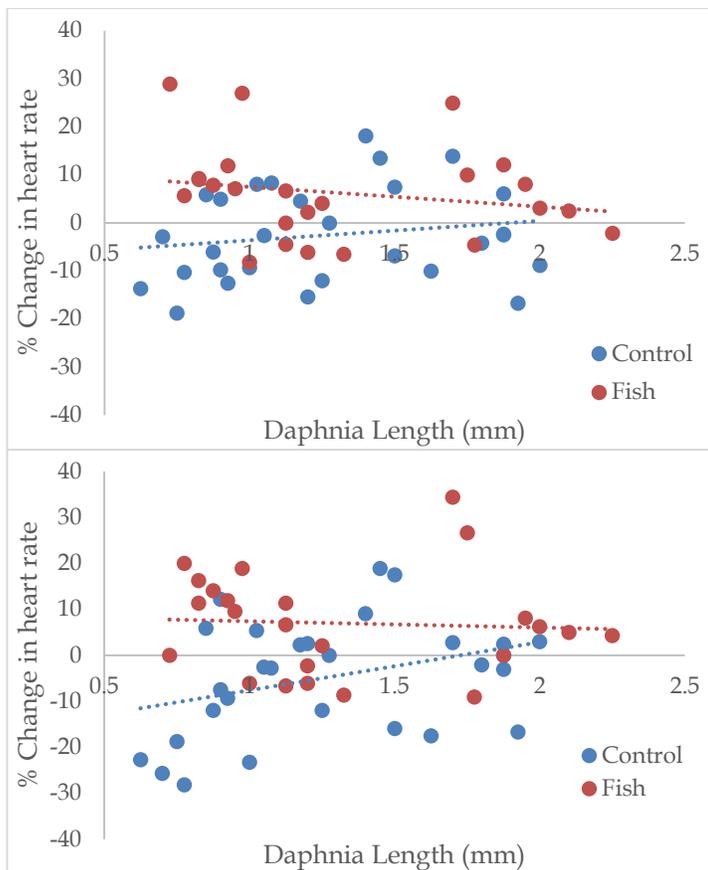


Figure 3. Percent change in heart rate from time 0 to time  $t$  vs. *Daphnia* length for discrete time points ( $t=15$  minutes-top,  $t=20$  minutes-bottom). 15 minute control  $R^2 = 0.0379$ ; 15 minute fish  $R^2 = 0.0379$ ; 20 minute control  $R^2 = 0.1225$ ; 20 minute fish  $R^2 = 0.0033$ .

## Discussion

The results, as a whole, do not match my initial hypothesis, as the large *Daphnia* had no significant heart rate response to the presence of fish kairomones. In fact, only the smallest *Daphnia* size class had a significant response to the fish kairomones, indicating that perhaps there is a different explanation for heart rate elevation. The assumption underlying my hypothesis that larger *Daphnia* would have a greater response is that fish preferentially feed on larger *Daphnia*, so it would be expected that there would be a selective benefit for the largest size classes.

There is a relatively high amount of variability in the data; the average heart rate values for each size class are fairly clear, but the change in heart rate of each individual *Daphnia* varied quite significantly. Obviously, a greater number of replicates would be preferable in order to provide clearer results, though it is likely that the

scatter would remain, as it seems that *Daphnia* simply respond very differently from individual to individual. It is also quite likely that variation in the stress induced by the experimental procedure explains some of the variability in data. However, beyond an automated system, it would be exceptionally difficult to cut down on human error in handling and subsequent stress induction. I spent many hours repeating the procedure in order to achieve a high level of consistency, and I highly doubt a significantly higher degree of precision could be achieved in a reasonable amount of time.

It is entirely possible that there is no ecological reason for differing heart rate response across a size gradient. Smaller *Daphnia* may simply have a greater physiological response to heart stimulants than large *Daphnia*, perhaps due to differences in surface area to volume ratio; it has been demonstrated that numerous chemicals can affect heart rate in *Daphnia* (Baylor 1942), so it is possible that fish release a chemical that increases *Daphnia* heart rate in certain doses, yet only small *Daphnia* have a surface area to volume ratio that allows for absorption of the necessary dose. It is also possible that small *Daphnia* simply have more excitable hearts than their larger counterparts and respond more vigorously to kairomones. If the kairomone itself could be isolated, tests with known dosage could be done across a size gradient to determine the magnitude of response. If small *Daphnia* hearts are more excitable in general, a non-kairomone chemical such as caffeine could be used to determine the magnitude of response across a size gradient.

Predation can be thought of in a series of distinct phases: detection, pursuit, attack, and capture (Holling 1959; Gerritsen and Strickler 1977). When proposing my initial hypothesis, I had assumed that an elevated heart rate would aid in escaping the capture phase of predation, which seems to be what occurs in predation by *Chaoborus*. However, I now believe that an elevated heart rate may be involved in escaping the detection or pursuit phases of predation.

Most planktivores, such as bluegills, feed visually, attacking prey that come within their visual range. Larger *Daphnia* are visible to bluegills from a much greater distance than small *Daphnia* are, making them a much more likely target to encounter (Werner and Hall 1974; Confer and Blades 1975; Vinyard and O'Brien 1976). Since bluegills will preferentially feed on large *Daphnia*, this means that large *Daphnia* represent a more optimal and more easily-detected prey item than small *Daphnia*. Lastly, *Daphnia* in general have a very low escape rate

during the capture phase of predation, meaning a fish that attacks a *Daphnia* will almost always ingest it. Due to these facts, it is highly likely that any large *Daphnia* detected by a planktivorous fish will be eaten. However, smaller *Daphnia* can only be detected by fish at a much smaller distance, and are also a less ideal food choice if larger *Daphnia* are present. I believe it is likely that large *Daphnia* stand no chance of escaping detection; if a fish is present, even if the *Daphnia* can detect it via kairomones, it is almost useless to attempt an escape.

In contrast to large individuals, small *Daphnia* are detectable at a very small distance and might be able to escape detection by fish altogether once they detect kairomones, making an elevated heart rate advantageous in their escape. If a small *Daphnia* is also surrounded by larger *Daphnia*, it becomes a less likely target, making escape advantageous, at least as long as the large *Daphnia* haven't all been eaten. If the smaller *Daphnia* can escape the zone of detection while the fish is focused on larger, more ideal prey choices, then a heart rate response may be advantageous in aiding escape. My initial hypothesis assumed the heart rate response was helping the *Daphnia* to escape the capture stage of predation, whereas this new hypothesis proposes the heart rate response aids in preventing the capture stage from happening at all.

One fundamental assumption of my hypothesis is that an elevated heart rate leads to greater chances of escape, likely through increased ability to do work and flee. However, it is not clear that elevated heart rate actually leads to faster movement. There are several documented methods of increasing *Daphnia* heart rate, including the use of caffeine, so experiments could be done to elevate heart rate and measure swimming speed at varying heart rate levels. In order to determine swimming speed, a long tube of water with a visual length scale could be used with slow-motion video in order to test *Daphnia* swimming speed. Using Drenner's method of simulating the suction created by feeding planktivores (Drenner 1977), an experiment could be performed using different *Daphnia* size classes and treatments of caffeine in order to determine escape success based on heart rate level, which would provide additional insight into the contribution of heart rate elevation to escape ability.

In conclusion, there are several possible explanations for the elevated heart rate of small *Daphnia pulex* in response to fish kairomones, some physiological and some ecological. While the phenomena of heart rate response is well known, little research has been done regarding its connection to fish predation. *Daphnia* are among the most well studied organisms in aquatic

systems, yet there is much that has yet to be understood regarding their induced defensive responses, especially across a gradient of size. A fuller understanding of the effects of predation and induced responses on different size classes of *Daphnia pulex* could give rise to a better understanding of *Daphnia* population dynamics, which contribute greatly to the function of freshwater lake ecosystems.

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