Table of Contents

Section	Page(s)
Table of Contents	1
Abstract	2
Introduction	2-8
Methods	8-13
Capturing the Dragonfly Larvae	8
Obtaining the *Daphnia*	8
Reproduction and Growth Assays	8-11
Behavioral Assay	11
Methods Figures	12-13
Results	13-15
Discussion	15-20
Acknowledgements	20
Literature Cited	21-22
Appendix	23-24

Abstract

This experiment tested *Daphnia magna's* reaction to the presence of different species of dragonfly larvae in terms of growth, reproduction, and behavioral changes by placing the *Daphnia* in dragonfly larvae kairomone containing water (experimental *Daphnia*) and comparing them to *Daphnia* contained in dechlorinated water (control *Daphnia*). The *Daphnia magna* contained in kairomone containing water first focused on growth over reproduction, reproducing slower and in smaller numbers while growing quickly. After reaching a size of 3 mm that allowed them to be relatively safe from consumption by dragonfly larvae, the experimental *Daphnia* reproduced quickly and in larger numbers compared to what they previously did and what the control *Daphnia* did. Both most of the experimental and the control *Daphnia magna* in this experiment did not grow any appendages to protect themselves from predation. Also, the experimental *Daphnia* migrated to deeper depths much quicker than the control *Daphnia* in this experiment. This can all be attributed to the *Daphnia* trying to increase their fitness by maximizing their reproduction capabilities and by conserving energy that could be used for what is needed.

Introduction

Daphnia are herbivorous cladocerans that serve as an ideal organism for scientific studies due to their parthenogenetic mode of reproduction, short generation time, and relatively ease of maintenance with in a laboratory culture. *Daphnia* occupy a central position in the aquatic food chain since they are both important consumers of algal biomass and a crucial food source for both invertebrate and vertebrate predators. The presence of either type of predator induces a wide range of antipredator responses in *Daphnia* including different morphological and

behavioral changes, as well as life history changes. Chemicals, known as kairomones, released by these types of predators are believed to cause these changes (Pijanowska and Kloc 2004).

Daphnia can sense kairomones released by different organisms, thus allowing them to detect the presence of a predator, and to detect what predator is present. The kairomones are sensed in a dose dependant fashion by *Daphnia* (Weber and Declerck 1997). *Daphnia* then react in accordance to what kairomones are present. Different responses that these kairomones may elicit include morphological changes (such as the growth of neck teeth), behavioral changes (such as migration patterns), and life history changes (such as strategic reproduction).

Morphological changes that involve growth and maintenance of tissue can be quite costly (Tollrian 1995). These morphological changes might also cause the organism to be more visible to predators. However, behavioral changes such as migration can also be costly depending on water current flow where a higher current velocity leads to more energy being needed by the *Daphnia* to move in the water. Behavioral changes also do little for the organism if the organism does indeed become exposed to a predator. Life history changes, such as maturing later in life, also have costs or risks. Since the ability to reproduce is directly related to maturity, late maturation leads to late reproductive capability. Thus, death without reproducing is the "cost" of this defense if this defense is unsuccessful. There are conflicting views on the possible costs of *Daphnia's* defenses. No apparent trade-off between growth and reproduction related traits were found in a study by Weber and Declerck with *D. galeata* (Weber and Declerck 1997). However, in a study done by Tollrian using *D. pulex*, faster growth per instar, larger size at maturity, higher fecundity, and longer instar duration occurred in the organisms without neckteeth formation when compared to those *Daphnia* who grew neckteeth, showing a tradeoff between necktooth growth and other traits in the *Daphnia*(Tollrian 1995).

The *Daphnia* response to predation involves changes in the actin and tubulin cytoskeleton and heat-shock proteins. The cytoskeleton plays an important role in regulating cell shape, controlling cell movement, and organization within a cell. In a study by Pijanowska and Kloc (2004), the experimental exposure to predator kairomones reduced the level of all the studied HSPs (Hsp40, Hsp60, Hsp70, ph-H3) in *Daphnia*, the most affected being HSP60. Additionally, a dramatic decrease in actin and tubulin concentration was observed. Their results showed a relationship between the behavioral changes and the level of HSPs and cytoskeletal proteins in *Daphnia* exposed to predation. In general, the presence of kairomones from a predator produces a typical stress response that becomes manifested in the reduction of HSPs that deal with the stabilization of the cytoskeleton. This can then lead to the reorganization of the cytoskeleton to better cope with the threat, leading to a more effective, quicker defensive response (Pijanowska and Kloc 2004).

Morphological changes are made by *Daphnia* in order to avoid predation and to escape if captured. *Daphnia* do this in a number of different ways. In an environment containing fish, mean *D. melanica* body size at maturity decreased. This was also experienced in experiments involving *D. galeata*. However, in the same experiment, *D. galeata* grew relatively quickly when the predator *Chaoborus*, an invertebrate, was also introduced (Weber and Declerck 1997). Similar results were noted in a study conducted by Parejko (1991) where the two invertebrates *Chaoborus* and *Mochlonyx* were introduced to *D. pulex*. Parejko's study also showed that second instar *Daphnia* are most susceptible to predation. This is the instar where neckteeth are commonly grown. Predation on those organisms possessing neckteeth was lower than on those without neckteeth. It is also interesting to note that *Daphnia* with sizes at 0.62-0.67 mm were less susceptible to predation compared to *Daphnia* that were 0.80mm in size. Those above 2 mm

seemed immune to predation by either predator (Parejko 1991). These results can be explained by the differences in predators. Fish, vertebrate predators, are large in comparison to *Daphnia* and are visual predators. Therefore, larger *Daphnia* will be at a disadvantage when fish are around since they will be more easily noticed. Thus it is to the *Daphnia's* advantage to "shrink" or only grow their transparent parts. Invertebrate predators such as *Chaoborus* are not that large in comparison to *Daphnia*, and are limited by mouth gape size, and can sense prey by movements in the water (Weber and Declerck 1997). Thus, it is to *Daphnia's* advantage to grow larger when invertebrate predators are around. Those *Daphnia* 0.62-0.67 mm in length were relatively safe in the study most likely because they were too small for their activity to be sensed. The second instar *Daphnia* were big enough to be sensed, yet small enough to be easily handled by *Chaoborus*. Those that grew neckteeth cause their handling to become harder (Parejko 1991). It is important to note that environments with changing predator types favor morphological changes (Tollrian 1995).

Chemicals released by predators are known to cause a daytime descent to deeper waters in some Northern Hemisphere *Daphnia*. These *Daphnia* then usually return to the surface at sunset to feed. In a study performed by Hendry and Burns (2001), *D. carinata* did not change their descent pattern in response to the predator *Anisops*, an aquatic insect. *D. carinata* did however descend when exposed to two predatory fish, the common bully (*Gobiomorphus cotidianus*) and inanga (*Galaxias maculatus*). It is now generally accepted that this downward migration is a defensive strategy of *Daphnia* used to escape visually hunting predators such as fish during daylight hours. This migration is believed to be triggered by changes in light intensity where more intense light causes the organism to accelerate and thus affects how deep

the *Daphnia* will go. Predator kairomones serve to increase *Daphnia* sensitivity to the light intensity (Gool and Ringleberg 1998).

In the presence of kairomones, planktonic animals may change their life history patterns such as the time it takes them to reach maturity, size at maturity, and released neonate size and neonate population size. In a study performed by Mikulski, Lipowska, and Pijanowska (2004), *Daphnia* exposed to fish kairomones during their third instar released their first offspring earlier and carried them for a shorter period of time as compared to those that were not exposed to these same chemicals. The *Daphnia* were also smaller in size when they released their eggs than the control *Daphnia*. Those exposed to the kairomones during their first or second instar showed a lower reproductive effort than that of the control females. This quickened maturation coupled with remaining small in size until after the first reproduction (also achieved by carrying less eggs for a shorter period of time) is a way *Daphnia* attempt to ensure that they reproduce at least one time. By staying small and maturing quickly, the *Daphnia* were able to avoid detection by the visual predators during their first reproduction in this study. In contrast, *D. pulex* exposed to *Chaoborus*, an invertebrate predator, resulted in the *Daphnia* growing larger and taking longer to reach maturity, as well as having a higher fecundity. This shows that the *Daphnia* invest a high level of energy for rapid growth rather than for reproduction, thus allowing them to reach a size exceeding a typical *Anisop's* mouth gape before the *Daphnia* reproduced. Once they reproduced, they had a higher fecundity to possibly make up for the lost time by having more neonates per clutch (Tollrian 1995).

In *D. galeata*, broad-sense heritabilites, which deal with all the possible genetic contributions to an individual's phenotypic variance, were found to be relatively high for the life history traits size at maturity, neonate size, and number of neonates (Weber and Declerck 1997).

Phenotypic plasticity (morphological changes) also showed this trend. Additionally, Pijanowska and Kloc found tubulin levels to be transgenerational. They also found that offspring of the mothers exposed to predators showed a better escape and alertness performance (Pijanowska and Kloc 2004). This heritability hints at the possibility of evolution potential for some defenses in *Daphnia*.

In general, when a visual predator is present, *Daphnia* tend to decrease their visibility. They achieve this either by decreasing size (morphological), avoiding light areas (behavioral), and decreasing the number and carrying time of eggs (life history). *Daphnia* also tend to mature sooner as to try to reproduce at least once before they grow to a large size and are therefore more easily spotted by visual predators (life history). In contrast, when a nonvisual predator is present, *Daphnia* tend to grow larger and grow neckteeth (morphological) in an effort to exceed the predator's mouth gape (large size), or in an attempt to be too hard to handle (neckteeth). Finally, *Daphnia* tend to mature later and thus reproduce later in order to allow most of their energy to be invested in growth (life history). Once a satisfactory size is reached, they focus more on maturity and reproduction and also are inclined to lay more eggs per clutch to possibly make up for this late reproduction.

Almost all of the studies pertaining to predator avoidance by *Daphnia* have been done using pelagic predators such as fish or planktonic invertebrates. However, *Daphnia* species also inhabit wetlands. Wetlands are typically devoid of fish since they freeze to the bottom in the winter. The dominant predators and threats to *Daphnia* in wetlands include benthic–odonatan larvae (dragonfly larvae) and other similar species. It is reasonable to believe that *Daphnia* are equipped with certain defenses against wetland predators that are similar to their defenses against pelagic predators. That is, I expect *Daphnia* to change their life-history, morphology, behavior,

or a combination of these three in response to benthic-odonatan larvae's presence in almost the same way they would for a typical invertebrate predator. More specifically, I expect *Daphnia* to grow larger in a shorter period of time and to reproduce later when wetland predators are present. I then expect them to increase their reproductive effort after growing to a larger, safer size. However, I expect *Daphnia* to dive in response to the larvae's presence since they are visual predators. I also expect the *Daphnia* to grow appendages to make them harder to handle if they are captured.

Methods

Capturing the Dragonfly Larvae: 32 dragonfly larvae of various species (in order of most obtained to least obtained: *Epitheca cynosura*, *Leucorrhinia intacta*, *Anax junius*, *Erythemis simplicicollis*, and *Libellula pulchella*) and sizes were captured in late October from a lake located in Bousson Research Forest in North West Penssylvania. These larvae were stored in a 40 degree Fahrenheit room in their own separate 7 inch diameter by 3 inch deep glass containers. They were each fed 3 live *Daphnia* of various species (that were also captured from the lake) 3-4 times a week

Obtaining the *Daphnia*: The *Daphnia* used in this experiment were of the species *Daphnia magna* and were ordered from The Carolina Biological Supply Company. They were housed in a 1 Liter glass aquarium filled with pond water and were fed *Daphnia* food which was also provided from The Carolina Biological Supply Company.

Reproduction and Growth Assay Experimental Set-up: The water from the captured dragonfly larvae containers was mixed together and poured into nine 6 inch diameter by 3 inch deep plastic containers. A smaller container made of the bottom 4-5 inches of a 20 fluid ounce

water bottle (similar to a 20 fl. Oz. Aquafina bottle) was placed inside each of these containers. 2 holes about 1/4 inch in diameter were poked in opposite ends of these bottles and mesh netting was used to cover these holes. A dragonfly larva of varying species was ordered from The Carolina Biological Supply Company and was placed on the inside and fed 3-4 *Daphnia magna* 3-4 times a week. 5 *Daphnia magna,* straight from the bottle they were shipped in, were placed on the outside of the dragonfly larvae containers and fed *Daphnia* Food about every 10-12 days (Figure 1). 2 control set-ups were run in 2 of the plastic containers. These set-ups used 5 *Daphnia magna,* also straight from the bottle they were shipped in, and placed each in dechlorinated water in plastic containers like the ones used for the *Daphnia* kept in the kairomone containing water in this assay (Figure 1). Measurements were not taken until the *Daphnia* in either set-up reached a size around 3 mm and after they first reproduced after reaching 3 mm in size. How the *Daphnia* were measured will be discussed in the next paragraph. This size was chosen because this was the size that *Daphnia* could not be preyed on in Parejko's study (1991) and thus should be putting their energy to reproduction and not growth. This simulates post-growth stages in the *Daphnia* and thus these *Daphnia* will be known as either experimental (if they were the *Daphnia* kept in the kairomone containing water) or control if they were the *Daphnia* kept in the dechlorinated water) post growth stage *Daphnia*. This assay was run in a 70 degree Fahrenheit room and was observed every 3-4 days until the *Daphnia* reached 3 mm in size and reproduced once. It was then observed every day until one reproduction cycle was observed. All *Daphnia* reached 3 mm around the same time. These *Daphnia* were checked for helmets, tails, and any other appendage growths under a microscope. All *Daphnia magna* observed in this assay started with a small tail. A tail was then said to exist if a longer, more obvious tail was observed. The *Daphnia* were also measured for reproduction

time and size. Reproduction time was measured in days until reproduction and size was measured in the number of offspring produced. Both of these were determined by viewing the *Daphnia* under a microscope. Reproduction size was determined by counting the number of eggs in the *Daphnia's* bodies. Reproduction time was determined by comparing the number of offspring carrying *Daphnia* to what was seen during the previous day of observation. An attempt was made to track individual *Daphnia* and thus individually observe them by categorizing them by the number of offspring they carried. For example, a *Daphnia* may have carried 8 offspring. If a *Daphnia* was observed the next day with 8 offspring it was assumed to be that *Daphnia*. When no *Daphnia* carrying 8 offspring existed it was assumed to have reproduced. However, some *Daphnia* carried the same number of offspring, so it is impossible to be completely sure that a set of statistics taken represents an individual *Daphnia* or if statistics for *Daphnia* that carried the same number of offspring were swapped between *Daphnia*.

A second part of this assay hoped to simulate the growth-stage of the *Daphnia*. 10 newborn offspring were taken from an experimental set-up and put in their own set-up which was exactly similar to an experimental set-up from the other part of this assay. These *Daphnia* will be known as experimental growth-stage *Daphnia*. The *Daphnia* were observed everyday for 10 days which is by when all the *Daphnia* had reached 3 mm in size and thus left their growth-stage. Growth was measured in days it took them to grow to 3 mm in size. This was measured by putting them in a clear Petri dish, under a microscope, one at a time, next to a dead *Daphnia* which was 3 mm in size. No water was present in the Petri dish to help keep the live organism still. The dead *Daphnia* was preserved in dechlorinated water when it was not being used. The 3 mm size looked for was from the top of the eye to the lowest point on the *Daphnia's* body not counting its tail (Figure 3). Reproduction was measured in days until first reproduction and in

brood size in the same exact way it was measured in the other part of this assay. The *Daphnia* were also observed for neckteeth. It was known when a new *Daphnia* reached 3 mm in size by comparing how many had reached 3 mm compared to the previous count. However, for the same reasons as the other part of this assay, it is impossible to be sure a set of statistics represents a single *Daphnia* if two *Daphnia* carried the same number of offspring. The control *Daphnia* in this part of the assay all died, so the parameters observed from the experimental *Daphnia* in this part of the assay was compared to what the post growth-stage *Daphnia* in the other part of this assay and to what other experiments found.

Behavior Assay: A 110 cm glass tube was filled with kairomone containing water from the reproduction, growth, and maturity assay. Another 110 cm glass tube was filled with dechlorinated water. The downward migration of the *Daphnia* was timed and was measured in cm from the water surface down (Figure 3). 5 *Daphnia magna* taken straight from the bottle they were shipped in were placed in each tube. The water in both tubes topped out at 107 cm. This assay was run in a 70 degree Fahrenheit room where a constant medium amount of artificial light was present. This assay was run twice and was observed for 1 hour after it was begun. It was then checked again 20 hours after it was started. After the first run, the tubes were emptied of their water and refilled with new water. The previously kairomone water filled tube was once again filled with kairomone containing water and the previously dechlorinated water filled tube was once again filled with dechlorinated water, both to 107 cm. All times taken were rounded to the nearest quarter minute.

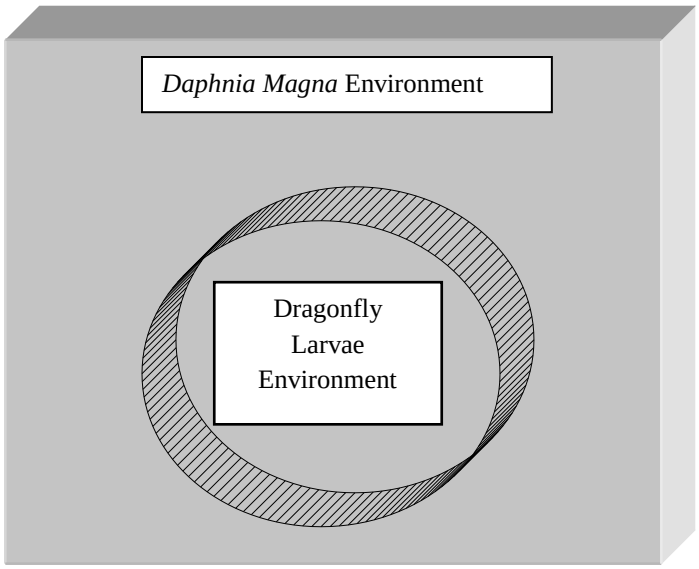


Figure 1: Growth and Reproduction Assay Set-up. In this assay, a dragonfly larva was separated from the experimental *Daphnia* in the bottom of a 20 oz. Aquafina bottle w/ mesh netting lining the inside of the bottle and holes cut in the bottle (but not the netting). The *Daphnia* were located in the environment outside this bottle.

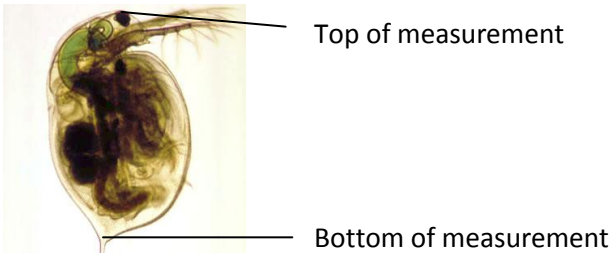


Figure 2: Measuring the Daphnia's size. The sizes of the *Daphnia* were measured under a microscope compared to a previously measured 3 mm *Daphnia*. The 3 mm measurement was taken from the top of the *Daphnia's* eye to the lowest part of its body not counting the tail.

Picture taken from:
www.mblaquaculture.com/assets/images/content/photo_Daphnia_magna.jpg

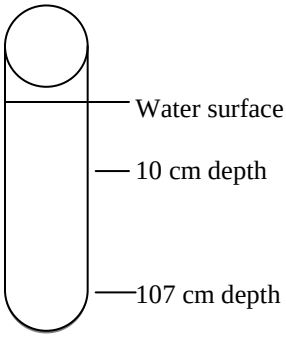


Figure 3: Behavior Assay Set-up. A 120 cm glass tube was filled with 107 cm high of either kairomone containing water for the experimental set-up or dechlorinated water for the control set-up. *Daphnia* migration was measured from the water surface down in centimeters.

Results

Reproduction and Growth Assays: The experimental growth-stage *Daphnia* grew to 3 mm in size on an average of 6.4 days after birth. They reproduced within 5.9 days of birth and released an average of 2.7 offspring per birth (Table 1). No neckteeth were observed on these *Daphnia*. The experimental post growth-stage *Daphnia* in this assay reproduced in an average of 3.76 days after this part of the assay was started. They also released an average of 9.12 offspring per birth. The control *Daphnia* reproduced within an average of 8.1 days after this part of the assay was started. They also released 3.8 offspring per birth (Table 2). The experimental *Daphnia* rarely ever grew tails, never grew helmets, did grow hunchbacks but in less than half the cases. The control *Daphnia* never grew helmets or hunchbacks, but one did grow a tail (Table 2). No other forms of growths such as increased antennae length were observed in either type of set-up. Exact data can be found in Tables 4 and 5 of the Appendix.

	Experimental *Daphnia* (10 total)
Average days until 1st reproduction	5.9 (+/- 0.30 SEM)
Average size of brood	2.7 (+/- 0.29 SEM)
Average number of days to reach 3 mm in size	6.4 (+/- 0.32 SEM)

Table 1: Average Data for experimental Growth-stage *Daphnia*. These growth-stage *Daphnia* exposed to dragonfly larvae kairomones reached 3 mm in size on an average of 6.4 days after they were born, first reproduced on an average of 5.9 days after birth, and had an average first brood size of 2.7 offspring

	Experimental *Daphnia* (25 total)	Control *Daphnia* (10 total)
Avg. Days until 1st Reproduction	3.76 (+/- 0.03 SEM)	8.1 (+/- 0.20 SEM)
Avg. 1st Brood number	9.12 (+/- 0.13 SEM)	3.8 (+/- 0.06 SEM)
Total *Daphnia* with a Tail	3	1
Total *Daphnia* with a Hunchback	9	0
Total *Daphnia* with a Helmet	0	0

Table 2: Average Data for the Reproduction and Growth Assay. The *Daphnia* exposed to dragonfly larvae kairomone had their first reproduction in an average of 3.76 days after reaching 3 mm in size and reproducing once at that size. They released an average of 9.12 offspring and never grew helmets, rarely grew tails, and grew hunchbacks in less than half the cases. The control *Daphnia* reproduced within an average of 8.1 days after their first reproduction after reaching 3 mm in size. They released an average of 3.8 offspring and never grew helmets or hunchbacks, and only grew a tail in one case.

Behavior Assay: The *Daphnia* in the water with kairomone present in it migrated their full distance in an average of 3.92 minutes. All the experimental *Daphnia* migrated almost directly to the bottom without any stops. However, one *Daphnia* did hover at 70 cm below the surface for about 6 minutes before finishing its dive. The *Daphnia* in the dechlorinated water migrated the full distance in an average of 26.00 minutes (Table 3). Only one of these control *Daphnia* migrated directly down with the others hovering at many different various depths. When the tubes were checked 20 hrs after the assay was started, all the *Daphnia* in both set-ups were still at the bottom of the tubes. Exact data can be found in Table 6 in the Appendix.

	Avg. Time to Migrate 107 cm.	Avg. time for experimental vs control set-up *Daphnia*
Experimental Set-up 1 (5 total)	4.20 minutes	
Experimental Set-up 2 (5 total)	3.92 minutes	3.92 minutes (+/- 0.44 SEM)
Control Set-up 1 (5 total)	25.90 minutes	
Control Set-up 2 (5 total)	26.10minutes	26.00 minutes (+/- 1.71 SEM)

Table 3: Average Time for *Daphnia* Migration. The *Daphnia* with dragonfly larvae kairomone present migrated the full 107 cm in an average of 3.92 minutes and the *Daphnia* with no kairomone present did it in an average of 26.00 minutes.

Discussion

In a study by Chopelet, Blier, and Dufresne (2008), growth - stage *Daphnia magna* kept in a 68 degree Fahrenheit room grew to 3 mm in an average of just over 10 days, which is longer than the 6.4 days it took the experimental growth-stage *Daphnia* in this experiment to reach 3 mm (Chopelet, Blier, and Dufresne 2008) (Table 1). After reaching a size of 3 mm that allowed them to be relatively safe from consumption by dragonfly larvae, the experimental post growth-stage *Daphnia* reproduced quickly (3.76 days until reproducing, Table 2) and in larger numbers

(9.12 offspring per brood, Table 2) than the control post growth-stage *Daphnia* (8.1 days, 3.8 offspring, Table 2) and the experimental growth-stage *Daphnia* (5.9 days, 2.7 offspring, Table 1). This all supports my hypothesis of the *Daphnia* first growing quickly and not reproducing much and then increasing their reproductive effort after they had grew to a larger, safer size. Similar results were seen in a study by Parejko (1991) with *Daphnia pulex's* response to *Chaoborus* and *Mochlonyx* and by Weber and Declerck (1997) with *D. galeata's* response to *Chaoborus*. These patterns may occur because they best increase the *Daphnia magna's* fitness.

Fitness of an organism is measured by the organism's ability to pass on its genes. This can be done by either enhancing the organism's ability to reproduce in the short run (being more sexually appealing to the opposite sex for example) or by enhancing the organism's ability to reproduce more often. Since *Daphnia* reproduce parthenogenetically, the only way for them to increase their fitness is to reproduce as much as they can. The *Daphnia* in this study and in the studies mentioned in the previous paragraph all attempted to maximize their reproductions in a similar way. They all allocate most of their energy to growing large to ensure not being eaten as easily by the smaller mouthed predators present. As a trade-off, they are not able to allocate as much energy to reproduction and thus reproduce slower (more days in between reproductions) and in smaller numbers (smaller broods). This seems to go against increasing their fitness, but it does not. If the *Daphnia* would try to reproduce normally or at higher numbers instead of growing to a safe size, the chances that they will be eaten increases. Also, the offspring are very vulnerable because of their size and may be eaten as well. Thus, none of the original *Daphnia's* genes may be passed on to future generations. However, after reaching a safe size, the *Daphnia* allocate more energy to reproduction than growth and reproduce more often and in bigger numbers. This makes up for the possible reproductions they lost while focusing on growth and

thus maximizes their fitness since the original *Daphnia* will most likely not be eaten and continue to be able to reproduce. The fact that the experimental growth - stage *Daphnia* reproduced quicker than the post growth - stage control *Daphnia* can possibly be explained by the fact that the experimental growth – stage *Daphnia* released less offspring than the control post growth – stage *Daphnia* did and thus did not need as much time to develop those offspring as the control post growth-stage *Daphnia* did.

Both the experimental and the control *Daphnia magna* in this experiment did not really grow any appendages to protect themselves from predation (Table 2). This does not support my hypothesis of the *Daphnia* growing appendages to make them harder to handle if captured. In a study by Dodson (1984), adult *Daphnia middendorphianna* (large *Daphnia*) were rarely eaten by the invertebrate predator *Heteroscope* because their large size was too big for the predators' mouths. These *Daphnia* rarely grew appendages such as tails or helmets. Also, examination of the *Daphnia* that survived revealed wounds on consistent certain areas of each *Daphnia* species used in this study. This demonstrates how predators such as *Heteroscopes* can attack smaller, vulnerable parts of *Daphnia* that are too large to normally be eaten in an attempt to weaken or kill the *Daphnia* so as to later turn the weakened or dead *Daphnia* in a manner that will allow them to be eaten (Dodson 1984). This shows that the experimental *Daphnia* in this assay tended to grow hunchbacks in an attempt to increase their width instead of appendages that increased their length such as tails (Table 3). The *Daphnia* were already long enough to not be eaten by the dragonfly larvae, however, they were still relatively thin. The growth of hunchbacks widened the *Daphnia* and thus would cause the handling of the *Daphnia* in any direction to be harder and possibly impossible for the larvae. The *Daphnia magna* also did not grow neckteeth in this assay (data not shown). This, and possibly the fact that they also did not tend to grow tails

or helmets, can be explained by the *Daphnia* allocating this energy to overall body size increase. In a study done by Tollrian using *D. pulex*, faster growth per instar and larger size at maturity occurred in the organisms without neckteeth formation when compared to those *Daphnia* who grew neckteeth, showing a tradeoff between necktooth growth and other traits in the *Daphnia* (Tollrian 1995). Since *Daphnia magna's* best defense against invertebrate predators is its large size, the *Daphnia* would want to allocate most of its energy to growing larger and not to necktooth, tail, or helmet growth. Since *Daphnia* lose their necktooth upon reaching the 3rd instar stage, spending energy on a necktooth seems pointless. Once the *Daphnia* reach a larger size such as 3 mm, they no longer need tails or helmets, so growing these and maintaining them seem pointless too.

The experimental *Daphnia* migrated much quicker (3.92 minutes) than the control *Daphnia* (26.00 minutes) in this experiment (Table 3). This supports my hypothesis of the *Daphnia* migrating to avoid predation by a visual predator such as dragonfly larvae are. It has been observed in other experiments that *Daphnia* migrate to lower depths when a visual predator is present and this quicker migration seems to demonstrate that being the case in this experiment. The light was always on in the room while the assay was being observed so the *Daphnia* maintained this lower depth. The control *Daphnia* also dove, and both the control and experimental *Daphnia* remained at the bottom of the tube after reaching it. This can be explained by the *Daphnia's* desire to conserve energy. The energy used to dive is generally accepted to be the trade-off of escaping predation by diving. However, it has been found that the energy loss is almost negligible and that the main trade-off is the temperature and food presence decrease at the lower depths compared to the higher depths. Planktonic animals that spend more time at the surface are in a better physiological state (higher fecundity, higher protein content) than those

lower in the water (Dawidowicz and Loose 1992). Since no food was present in the tubes and since the water temperature was most likely the same because the entire tube was located in a 70 degree Fahrenheit room, the control *Daphnia* had no reason to swim and spend even a little energy to remain near the water surface. Thus, they eventually sunk to the bottom and remained there after hovering at different depths.

There were a few problems with how this experiment was run, and there are some follow-up experiments that could be run to address these problems or build upon the findings of this experiment. For all parts of the experiment, offspring from 1 parent *Daphnia* should have been used to assure different inherited traits did not account for any difference in changes between the experimental and control *Daphnia* as Weber and Declerck (1997) saw and Pijanowska and Kloc (2004) saw. Also, there should have been a control part to the growth-stage part of the reproduction and growth assay so as to have those *Daphnia* to compare the experimental *Daphnia's* statistics to instead of just comparing them to other experiment's findings and to the post-growth stage *Daphnia*. Growth should also have been measured in the post-growth stage *Daphnia*. No part of the behavior assay tested the *Daphnia's* migration in the dark to see if they would migrate back up to the surface. Also, different migration studies should have been run with a non-lit depth existed to see if migration ceased once that depth was reached. Also, no food or water temperature difference was present in the different depths of water. If all of these existed, it would be possible to better measure the control versus the experimental *Daphnia's* behavior. It would also be better to follow the *Daphnia* throughout their life instead of using different *Daphnia* for different stages and to measure the original size of the offspring to determine if there is a difference in this parameter between the control and experimental *Daphnia*. Follow up experiments could all involve these changes and thus strengthen the

findings from this experiment. Also follow up experiments could use smaller *Daphnia* that might not grow big enough to not be eaten by the larvae along with *Daphnia magna* to see if changes made to try to avoid predation by the larvae exist between two different sizes of *Daphnia*. In the same way, a larger predator could be used with *Daphnia magna* to test the *magna's* changes to a larger predator it cannot avoid predation by growing large.

In this experiment, the experimental growth-stage *Daphnia magna* focused on growth over reproduction, reproducing slower and in smaller numbers while growing quickly during their growth stage. After reaching a size of 3 mm that allowed them to be relatively safe from consumption by dragonfly larvae, the experimental post growth-stage *Daphnia* reproduced quickly and in larger numbers than the growth-stage *Daphnia* and the post growth - stage control *Daphnia*. Both the experimental and the control *Daphnia magna* in this experiment did not really grow any appendages to protect themselves from predation. Also, the experimental *Daphnia* migrated much quicker than the control *Daphnia* in this experiment. This can all be attributed to the *Daphnia* trying to increase their fitness by maximizing their reproduction capabilities and by conserving energy that could be used for what is needed.

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Appendix

	D. magna 1	*D. magna* 2	*D. magna* 3	*D. magna* 4	*D. magna* 5	*D. magna* 6	*D. magna* 7	*D. magna* 8	*D. magna* 9	*D. magna* 10
Average days until 1st reproduction	5	7	6	5	6	8	6	5	5	6
Average size of brood	1	3	3	4	2	3	3	2	2	4
Average number of days to reach 3 mm in size	6	8	6	5	7	8	6	6	5	7

Table 4: Average Data for experimental Growth-stage *Daphnia*. These growth-stage *Daphnia* exposed to dragonfly larvae kairomones reached 3 mm in size on an average of 6.4 days after they were born, first reproduced on an average of 5.9 days after birth, and had an average first brood size of 2.7 offspring. It is important to note that the statistics shown in individual columns may not represent an individual *Daphnia*.

	Experimental Set-up 1 (5 total)	Experimental Set-up 2 (5 total)	Experimental Set-up 3 (5 total)	Experimental Set-up 4 (5 total)	Experimental Set-up 5 (5 total)	Control Set-up 1 (5 total)	Control Set- up 2 (5 total)
Avg. Days until 1st Reproduction	3.6	3.8	3.2	4.2	4.0	7.2	9.0
Avg. 1st Brood number	9.4	12.0	6.2	11.0	7.0	3.4	4.2
Tail	0	2	0	1	0	0	1
Hunchback	1	3	1	2	2	0	0
Helmet	0	0	0	0	0	0	0

Table 5: Average Data for the Reproduction and Growth Assay. The *Daphnia* exposed to dragonfly larvae kairomone had their first reproduction in an average of 3.76 days after their first reproduction after reaching 3 mm in size. They released an average of 9.12 offspring and never grew helmets, rarely grew tails, and grew hunchbacks in less than half the cases. The control *Daphnia* reproduced within an average of 8.1 days after their first reproduction after reaching 3 mm in size. They released an average of 3.8 offspring and never grew helmets or hunchbacks, and only grew a tail in one case.

	Time to Migrate the Full 107 cm				
Experimental *Daphnia* 1	3.3	3.7	3.8	2.4	7.8
Experimental *Daphnia* 2	4.0	3.1	3.5	4.1	4.9
Control *Daphnia* 1	30.75	13.25	24.5	32.25	28.75
Control *Daphnia* 2	28.2	24.8	22.9	31.7	22.9

Table 6: Average Time for *Daphnia* Migration. The *Daphnia* with dragonfly larvae kairomone present migrated the full 107 cm in an average of 3.92 minutes and the *Daphnia* with no kairomone present did it in an average of 26.00 minutes.

Investigating *Daphnia magna's* defensive strategies against benthic-odonatan larvae

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