An Examination of Anti-Predator Defense Mechanisms of Bryospilus *repens* in Shallow Fishless Wetlands



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I hereby recognize and pledge to fulfill my responsibilities, as defined in the Honor Code, and to maintain the integrity for myself and the college community as a whole.

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Approved by:

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Dr. Milt Ostrofsky

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Abstract

Little is currently known about the predator avoidance tactics of *B. repens* in northwestern Pennsylvania wetlands. The present study examines the anti-predator defense mechanisms, and the energy tradeoffs these defense mechanisms require, of *B. repens* in northwestern Pennsylvania wetlands. To compare these defense behaviors, a group of 10 *B. repens* were exposed to *A. junius* (a large visual predator), a second group of 10 *B. repens* were exposed to *C*. *americanus* (a small tactile predator), a third group of 10 *B. repens* were exposed to both *A. junius* and *C*. *americanus*, and a final group 10 *B. repens* were not exposed to any predators and served as a control. The predator exposure was indirect and occurred through a water transfer of the predators' karimones. As predicted, exposure to the karimones from *A. junius* resulted in *B. repens* remaining small compared to the other three study settings. Contrary to expectations, *B. repens* that were exposed to *A. junius* developed no distinguishing structural defenses (i.e., increase in helmet size). Also contrary to expectations, the presence or absence of predators had no effect on *B. repens*' reproduction numbers or quickness to reproduce. These findings indicate that *B. repens* remain small and choose to conserve energy, rather than develop specialized defense responses, when the large predator is nearby. In contrast, when a small predator exists, the *B. repens*' best survival strategy is to grow at the normal rate and develop the normal body structure as mandible size is preventing predation. Together, these results help shape understanding of the population dynamics of wetland species.

Introduction

In pelagic water the anti-predator defenses of Cladocera against both large visual predators and small tactile predators is highly understood (Pastorok, 1981, Brancelj et al., 1996; Krueger et al., 1981). For example, it is commonly accepted that Cladocera can use their vertical position in the water column (deep in the daytime, shallower at night) to avoid visual predators. In addition, Cladocera can enhance their survivability by modifying their life-history strategies such as size at first reproduction, number and size of offspring, body shape/proportions to avoid tactile predators (Barry, 2000; Brancelj et al., 1996). While the adjustment of life history characteristics can occur, it only tends to occur when the preferred defense mechanism, vertical migration, is not possible (Slusarczyk & Pinel-Alloul, 2010).

The study of Cladoceran behavior in wetlands, however, has received much less investigation. Wetland environments pose a different set of survival problems for Cladocera than lake environments (Chow-Fraser, 2005). Unlike lake environments, Cladocera survival cannot be enhanced by migration to greater water depths due to the shallow water column. Without the ability to vertically migrate to darker waters during daylight hours, Cladocera remain in constant threat from their predators and must develop other survival mechanisms. With pelagic species, morphological changes are expressed when the ability to migrate is removed. It is unknown whether the same set of morphological changes will be displayed in wetland environments (Slusarczyk & Pinel-Alloul, 2010). For morphological responses to be displayed an energy tradeoff must be made between growth and reproduction (Enserink et al., 1995).

In lakes, Cladocera detect invertebrate and vertebrate predators by the kairomone chemicals they release (Vuorinen et al., 1989). When this detection occurs the Cladocera can respond and produce offspring with one or more of the following strategies: vertical migration in the water column, developing morphological changes, or reproducing at different ages (Brancelj et al., 1996). These changes are dependent on the type of kairomone chemicals that predators are currently releasing (Covich, 1999). Thus Cladocera defense responses can vary in order to increase their chance of survival.

In wetlands, *Anax junius* (dragonfly larvae) are common predators that sit and wait to capture Cladocera through their ambush tactic. *Chaoborus americanus* (phantom midge) are more abundant predators and actively hunt Cladocera by sensing vibrations of the water. *A. junius* use an extendable jaw to snatch their prey and move them towards the mandible to eat them (Bernardi et al., 1987), while *C. americanus* use tactile sensory cues and strike tactics to capture prey (Vijverberg et al., 2005). Since the invertebrate predators can affect the body size that Cladocera grow to, opportunistic predation is occurring as the Cladocera of a certain size are being targeted (García & Guido, 2000; Pastorok, 1981). When Cladocera grow they pass through instar stages. Predation rates vary based upon the size / stage of the instar, with the most intense predation occurring during the instar stage associated with a size between 0.5-2mm for *Daphnia* (Parejko, 1991). This evidence for gape limited size selection carries across throughout the Cladocera species. To escape this vulnerable state, Cladocera channel their energy into developing a helmet (Figure 1) or growing larger than 2mm (Parejko, 1991). The tradeoff between

growth and defense strategies for northwestern Pennsylvanian Bryospilus *repens* is poorly understood.

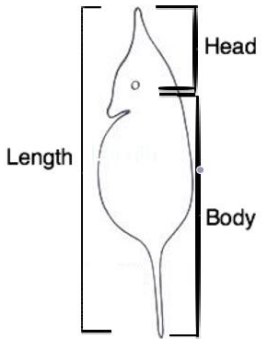


Figure 1. Shows the distance from the top to eye, represents the helmet development and the distance from the eye to the bottom representing the body size.

Bryospilus *repens* is the dominant species of Cladocera that is found in the wetland ponds at Bousson. Although these wetlands typically freeze in the winter, *B. repens* and its non-fish invertebrate predators (e.g. *A. junius* and *C*. *americanus*) can still survive while fish are removed by the winter freeze. *A. junius* and *C*. *americanus* go into a state of diapause to survive in the winter mud (Laurich et al., 2003), making these species persistent wetlands predators. The way *B. repens* respond to these different predators is currently unknown. *A. junius* and *C*. *americanus* are benthic predators that generally exist throughout the shallow water column of wetlands, thus leaving *B. repens* no predator-free habitat to migrate to. To understand the anti-predator defenses *B. repens* use in wetland environments, research that examines potential change in life history characteristics is needed.

Wetland research of *B. repens*' defense mechanisms is important, as *B. repens* populations are a driving force behind the flow of energy in wetland ecosystems (Cedervall et al., 2012; Laurich et al., 2003). While Cladocera defense mechanism

are highly understood in lakes the species make up and environmental factors present in wetlands causes these two bodies of water differ vastly (Brancelj et al., 1996; Laurich et al., 2003).

Research question

Based on these different environmental settings, the following research questions were formed to better understand the anti-predator defense mechanisms of *B. repens*:

RQ 1: What defense mechanisms do *B. repens* display around large, visually-oriented predators such as *A. junius* in the shallow water wetland environment?

RQ 2: What defense mechanisms do *B. repens* display around small, tactilely-oriented predators such as *C*. *americanus* in the shallow water wetland environment?

RQ 3: What defense mechanisms do *B.* repens display around both *A. junius* and *C*. *americanus* in the shallow water wetland environment?

RQ 4: What reproductive strategies do *B. repens display* when faced with no predators in the shallow water wetland environment?

Leading on from these research questions, the following hypothesis are presented:

H1: Greater *B. repens* body lengths occur when the primary predator threat is small tactile predators, than when a large visual predator is the threat.

H2: Greater *B. repens* helmet size occurs when the primary predator threat is large visual predators, than when a small tactile predator is the threat.

H3: Greater numbers of *B. repens* offspring occur when no predatory threat exists, than when it does.

H4: Quicker first reproduction by *B. repens* will occur when there is either no predator or when the primary predator threat is large visual predators, than when a small tactile predator is the threat.

Methods

Study Site

The wetland pond is located in the 283 acre Bousson Environmental Research Forest (N 41° 31’ 1.068’’ W 80° 2’ 53.172’’) of Allegheny College in East Meadville Township, Pennsylvania. The sampled species were collected from the wetland pond (see Figure 2) in October 2015. The pond’s 9,300 liters of water freezes over in the winter, making the pond a fishless wetland. Since the wetland pond has a maximum depth of 1.1 meters, benthic predators exist through the whole water column. In the wetland ponds, *B. repens* are in the Chydoridae family and Cladocera suborder. *B. repens* have an eye spot but no compound eye, hold small clutch sizes, have a large post abdominal claw, and persist as the dominant biomass in the Bousson wetland areas.

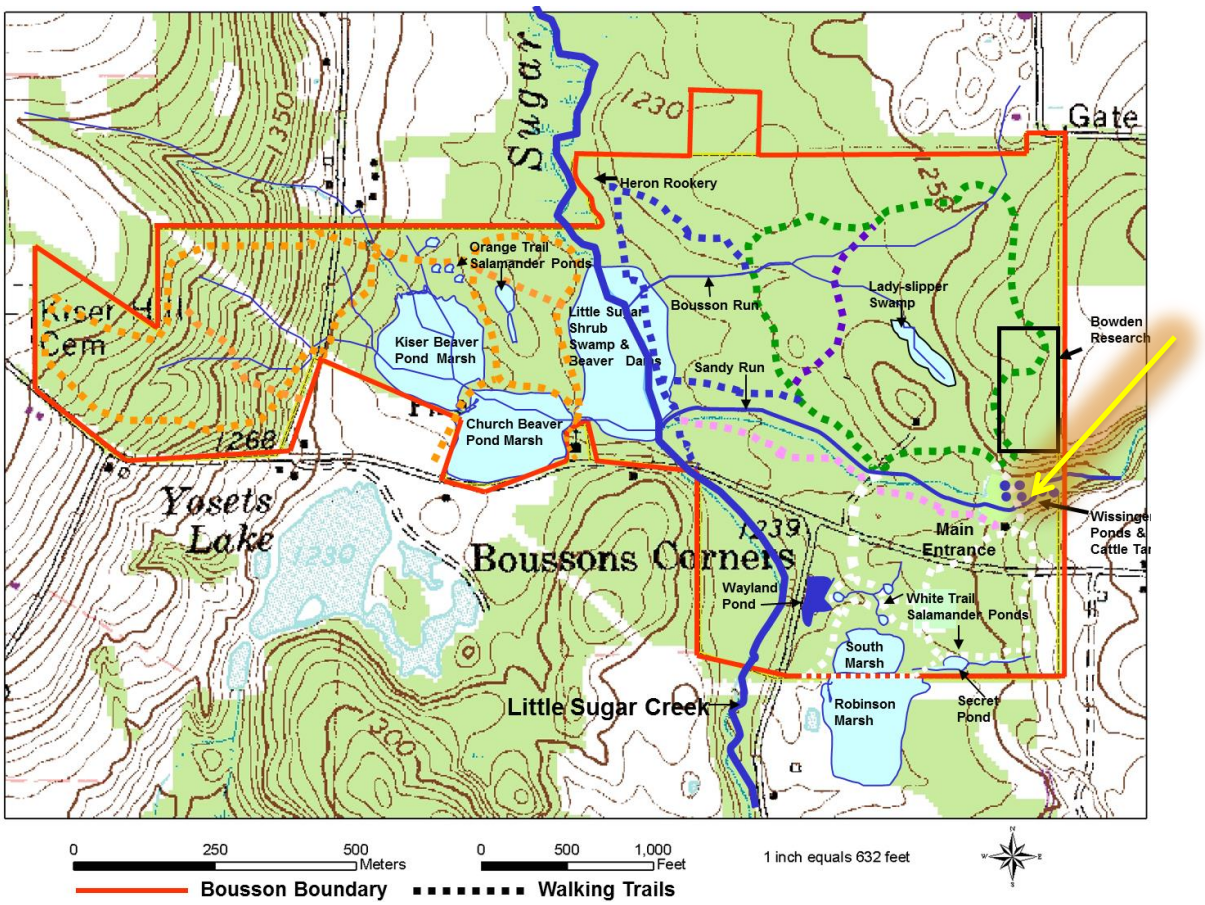


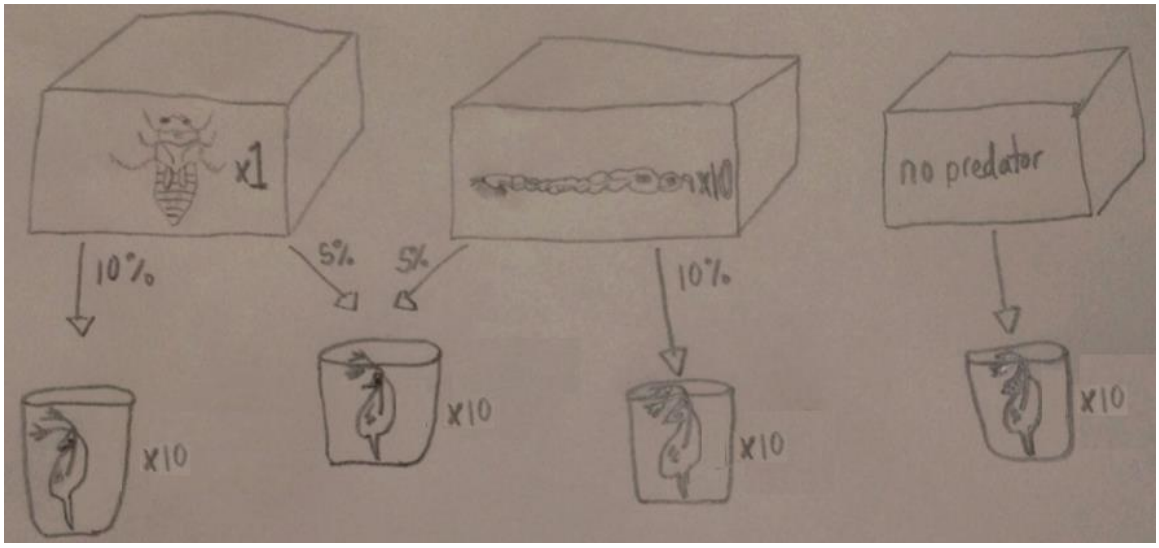
Figure 2. Bousson Environmental Research Reserve near Meadville, Pennsylvania. The yellow arrow indicates the wetland pond that the species were taken from (Goeller, 2010).

Organism Conditions

To examine the anti-predator defense strategies of *B. repens* in Allegheny's Bousson Environmental Research wetland ponds, I replicated the predator prey conditions in the laboratory. The predators (*A. junius* and *C. americanus*) and prey (*B. repens*) came from the wetland pond and were placed in isolated 1L containers. One container contained 1 *A. junius* (large visual predator) and the other container contained 10 *C. americanus* (small tactile predator). The *B. repens* were placed in a larger container to create a colony. Once the *B. repens* began breeding, the second-generation offspring were placed into four separate 1L containers (large containers in Figure 3). In the first container the water from the *A. junius* tank was mixed into

the tank with *B. repens*, in the second container the water from the *C*. *americanus* tank was mixed into the tank with *B. repens*, in the third container the water from both predator tanks was mixed into the tank with *B. repens*, and the final container was used as a control and had no water from either predator tank. The exposure occurred every other day by removing ten percent of the water from each of the three experimental groups' containers and replacing it with water from the container with the assigned predator. This allowed the karimone and exoskeletons of consumed *B. repens* to be present without allowing the predators to feed on the *B. repens*. Once the second-generation *B.* repens, who were chemically exposed to their assigned predators, began reproducing, the third-generation offspring were collected and individually placed into solo cups with 200ml of water so the experiment for that cup could begin (solo cups in Figure 3).

The setup period ended when 40 solo cups of newborn *B.* repens (10 exposed to both predators, 10 exposed to *A. junius*, 10 exposed to *C*. *americanus*, and 10 as a control) had been established. The experiment start date varied by a few days based on the day the third-generation *B. repens* were born. Each group of 10 cups still interacted with its assigned predator by transferring ten percent (20ml) of the water every other day so prey were continuously exposed to predator karimones. No predators, prey, or any food source were involved in this transfer to allow induced defenses to continue to develop for observation.



A. junius Both Predators *C. americanus* Control

Figure 3. A schematic showing the source of B. repens for the four study groups and the source of partial water exchange that occurred ever second day.

To mimic the conditions of wetland ponds, the containers were maintained at 22°C (the average summer temperature of the wetland ponds). The initial water was obtained from the fishless ponds at Bousson, was kept predator free in the lab for a week (so all kairomone chemicals from the wetland faded away), was filtered to remove any organisms, and boiled before the water was used in the 1L containers and 200ml solo cups.

Feeding

The *B. repens* were fed every other day and the predators once a week. The *B. repens's* food consisted of two drops of *Scenedesmus* per each *B. repens*. The food was grown in flasks under the conditions of Bristol's Medium and was pippeted into the solo cups and containers (Urabe & Watanabe, 1990). The predator's food consisted of adding 50 *B. repens* to the two predator tanks once every four days.

Sampling Method

Every day the *B. repens* were observed to identify, count, and remove any offspring that were produced. The age and size of first reproduction, offspring number, and the survival rate was monitored and recorded. The experiment ended when all the third-generation *B. repens* in the solo cups died. The *B. repens* were then measured to examine length, body structure and head structure (Figure 4) under a microscope to identify any presence or absence of induced morphological defenses (Brancelj et al., 1996). To identify body shape change the ratio distance from the top to the eye (helmet) was compared to the distance from the eye to the base (spine) (Figure 1).

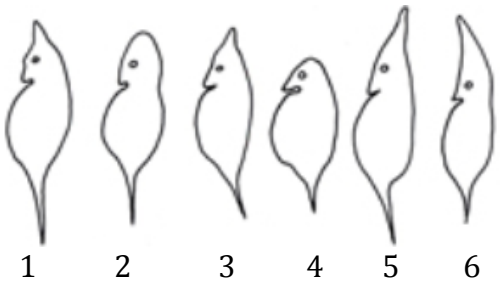


Figure 4. Diagrams of the different body structures that Cladocera can develop. Cladocera #5 has developed the largest helmet (distance from the eye to top of the body), Cladocera #1 has the greatest body size (eye to bottom for the body), and Cladocera #4 is the smallest in length (distance including both helmet and body size).

Statistical Analysis

A one-way analysis of variance (ANOVA), using the IBM SPSS Statistical Data Editor, was performed to test for differences among the four groups of *B. repens*. In the instances where *B. repens* did not reproduce, missing values were entered so that incorrect calculations were not created for the data collected at age of first reproduction. A frequency was run to highlight odd values in the data set so they could be checked and verified. A filter was created to remove the outliers that had a

lifespan of less than 15 days, probably due to handling. A Tukey HSD test was performed to locate the differences among treatments that were significantly different as indicate by the ANOVA. A copy of the full data sheet is shown in Appendix D.

Results

H1: Energy focused on body/length growth

Hypothesis 1 posited that greater *B. repens* body sizes and lengths occur when the primary predator threat is from small tactile predators. The results show that when exposed to both predators, the *B. repens* developed the largest mean body size. The body sizes ranged from 2.01mm to 3.03mm, had a mean of 2.31mm, and a ±0.32mm SD (Figure 5). When exposed to *C. americanus* (the small tactile predator), *B. repens* developed the second largest mean body size. These body sizes ranged from 1.96mm to 2.41mm, had a mean of 2.24mm, and a ±0.16mm SD (see Figure 5). The *B. repens* that grew in the presence of no predators had the third largest mean body size, which ranged from 2.16mm to 2.38mm, had with a mean of 2.29mm, and a ±0.08mm SD (Figure 5). The *B. repens* that were exposed to *A. junius* (the large visual predator), developed the smallest means body size. Their body sizes ranged from 1.65mm to 2.29mm, had a mean of 2.00mm, and a ±0.21mm SD (see Figure 5). These patterns are consistent with H1.

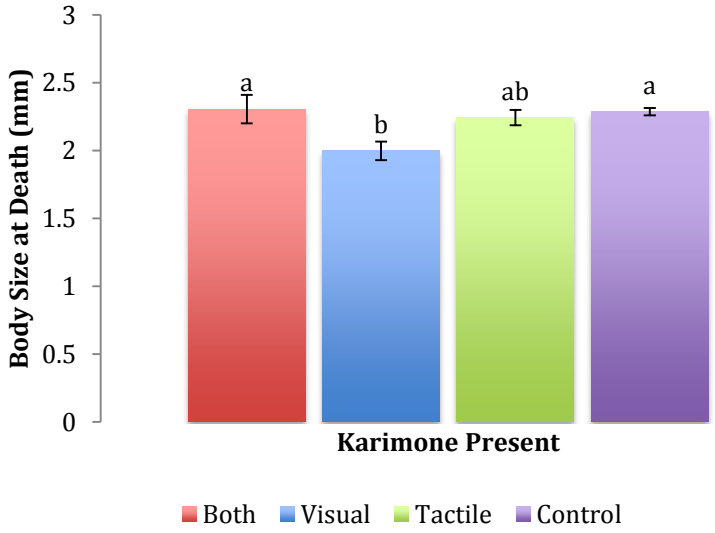


Figure 5. Body sizes at death for the four groups of *B. repens*.

Figure 6 presents the ANOVA results for body size at death. This test looked for intergroup differences. As this table shows, a statistically significant intergroup difference exists ($F=3.94$, $p<.02$). Figure 7 shows the results of the Tukey test that was run to determine which groups had statistically significant differences for body size at death. As Figure 7 shows, there are statistically different body sizes between B. *repens* exposed to both predators and *B. repens* exposed to visual-only predators, with the former group having larger body sizes that the latter group. There was also a statistically significant difference in body sizes between *B. repens* in the control group and *B. repens* exposed to visual-only predators. The control group *B. repens* had larger body sizes than the *B. repens* exposed to visual-only predators. These results provide support for H1.

Body size at Death

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.541	3	.180	3.974	.017
Within Groups	1.361	30	.045		
Total	1.901	33			

Figure 6. ANOVA summary table: body size at death when exposed to different predator karimones

Body Size at Death
Tukey HSD

(I) group exposed to	(J) group exposed to	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1 Both Predators	2	.30778*	.10039	.022	.0348	.5808
	3	.06431	.10348	.924	-.2171	.3457
	4	.01931	.10348	.998	-.2621	.3007
2 Visual Predator	1	-.30778*	.10039	.022	-.5808	-.0348
	3	-.24347	.10348	.109	-.5249	.0379
	4	-.28847*	.10348	.043	-.5699	-.0071
3 Tactile Predators	1	-.06431	.10348	.924	-.3457	.2171
	2	.24347	.10348	.109	-.0379	.5249
	4	-.04500	.10648	.974	-.3345	.2445
4 Control	1	-.01931	.10348	.998	-.3007	.2621
	2	.28847*	.10348	.043	.0071	.5699
	3	.04500	.10648	.974	-.2445	.3345

Figure 7. Post Hoc Test Summary: body size at death when exposed to different predator groups

Differences were also observed between the four groups' body lengths at death. *B. repens* that were exposed to both predators grew to a larger length than *B. repens* that were just exposed to *A. junius*, the larger visual predator. In the perceived presence of both predators, the *B. repens'* body length ranged from 2.18mm to 3.22mm, with a mean of 2.49mm, and a ±0.33SD (see Figure 8). When only exposed to *A. junius*, the *B. repens'* body length ranged from 1.74mm to 2.50mm, with a mean of 2.15mm, and a ±0.24SD (see Figure 8). Exposure to *C. americanus* led to a *B. repens*' body length ranging from 2.10mm to 2.63mm, with a mean of 2.42mm, and a ±0.18SD (Figure 8). In the control setting, the *B. repens*' body length ranged from 2.27mm to 2.56mm, with a mean of 2.42mm, and a ±0.09SD (see Figure 8). These patterns are consistent with H1.

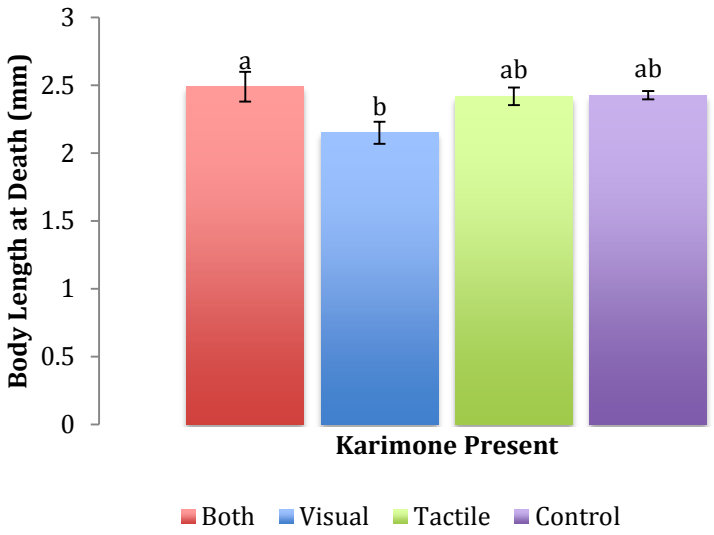


Figure 8. Body lengths at death for the four groups of *B. repens*.

Figure 9 presents the ANOVA results for body length at death. This test looked for intergroup differences. As this table shows, a statistically significant intergroup difference exists ($F=3.78$, $p<.03$). Figure 10 shows the results of the Tukey test that was run to determine which groups had statistically significant differences for body length at death. As Figure 10 shows, there are statistically different body lengths between B. repens exposed to both predators and *B. repens* exposed to visual-only predators, with the former group having larger body sizes that the latter group. These results provide support for H1.

length

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.610	3	.203	3.780	.021
Within Groups	1.615	30	.054		
Total	2.225	33			

Figure 9. ANOVA summary table: body length at death when exposed to different predator groups

length
Tukey HSD

(I) group exposed to	(J) group exposed to	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1 Both Predators	2	.34000*	.10937	.020	.0426	.6374
	3	.07139	.11273	.920	-.2351	.3779
	4	.06264	.11273	.944	-.2439	.3692
2 Visual Predator	1	-.34000*	.10937	.020	-.6374	-.0426
	3	-.26861	.11273	.102	-.5751	.0379
	4	-.27736	.11273	.087	-.5839	.0292
3 Tactile Predators	1	-.07139	.11273	.920	-.3779	.2351
	2	.26861	.11273	.102	-.0379	.5751
	4	-.00875	.11600	1.000	-.3242	.3067
4 Control	1	-.06264	.11273	.944	-.3692	.2439
	2	.27736	.11273	.087	-.0292	.5839
	3	.00875	.11600	1.000	-.3067	.3242

Figure 10. Post Hoc Test Summary: body length at death when exposed to different predator groups

H2: Energy focused on the development of a helmet

The growth of a helmet did not differ among the different *B. repens* exposed to different predators. The development of a helmet (Figure 1) was minutely seen in all groups with a mean of 0.18mm for *B. repens* exposed to both predators, 0.15mm for *B. repens* exposed to *A.* junius, 0.18mm for *B. repens* exposed to *C. americanus*, and 0.14mm for *B. repens* in the control group (see Figure 11). As Figure 12 shows, the ANOVA that was run showed no significant difference in helmet size between any of the four groups. This suggests that no extra energy was being focused into this possible induced defense mechanism. These findings do not support H2.

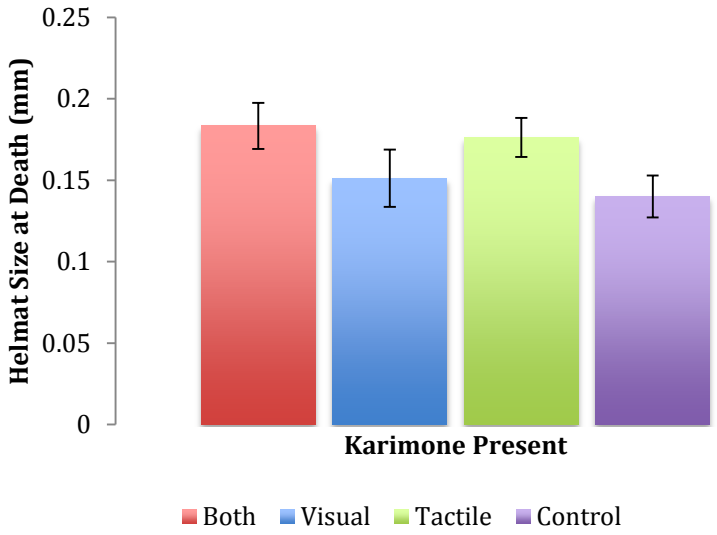


Figure 11. Helmet size at death for the four groups of *B. repens.*

eye-top

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.007	3	.002	1.490	.244
Within Groups	.035	23	.002		
Total	.042	26			

Figure 12. ANOVA summary table: helmet size at death when exposed to different predator groups

As an alternative approach to testing if the different groups of *B. repens* developed a larger sized helmet in response to their predator environment, measurements of the change in helmet size were made. Figure 13 presents a bar chart of the mean change in helmet sizes for each of the four groups. The means are all quite similar. As Figure 14 shows, the ANOVA that was run showed no significant difference in the change in helmet size between any of the four groups. This finding again suggests that no extra energy was being focused into this possible induced defense mechanism. It is again the case that these findings do not support H2.

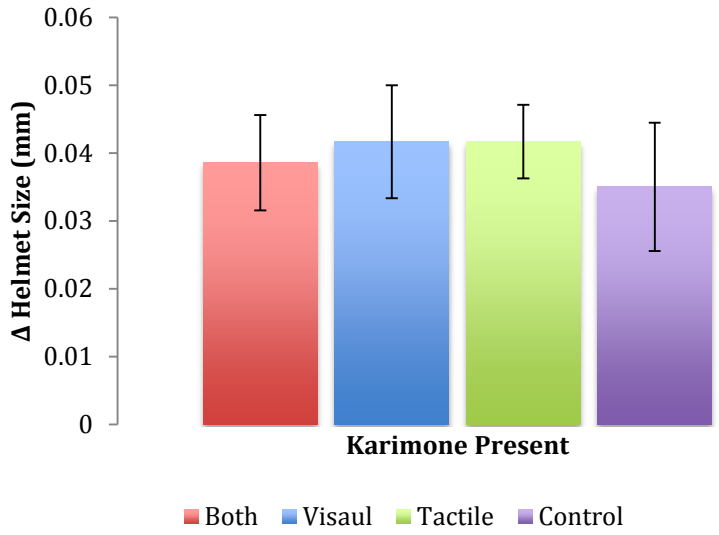


Figure 13. **Δ in** helmet size at death for the four groups of B. *repens*.

Δ helmet size

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	3	.000	.163	.920
Within Groups	.010	23	.000		
Total	.010	26			

Figure 14. ANOVA summary table: **Δ** helmet size when exposed to different predator groups

H3: Energy focused on producing more offspring

The number of offspring produced by the *B. repens* of the four different study groups showed some differences. The *B. repens* exposed to both predators had a mean of 1.67 offspring. *B. repens exposed to A. junius* had a mean of 1.67 offspring. *B. repens* exposed to *C. americanus* had a mean of 1.5 offspring. *B. repens* in the control group had a mean of 2.62 offspring. High variability was observed in the different groups (±2.83 SD to ±3.00 SD). Figure 15 presents a bar chart of the mean number of offspring for each of the four groups.

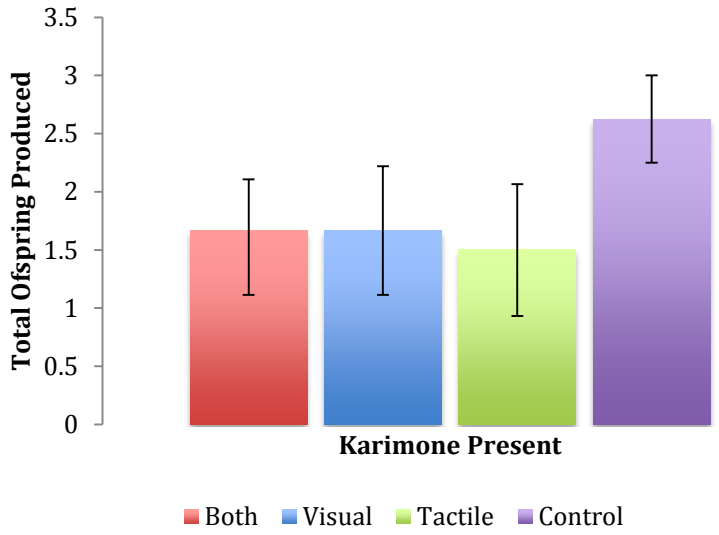


Figure 15. Total offspring produced by the four groups of B. *repens.*

An ANOVA was run to test for differences between the total number of offspring produced by the four different groups. As Figure 16 shows, there are no statistically significant differences between any of the groups. These findings do not support H3.

Total Number of Offspring Produced

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6.390	3	2.130	1.033	.392
Within Groups	61.875	30	2.063		
Total	68.265	33			

Figure 16. ANOVA summary table: offspring produced when exposed to different predator groups

H4: Energy focused on reproducing at a younger age

The age of first reproduction by the *B. repens* of the four different study groups showed some differences. The *B. repens* exposed to both predators had a mean reproductive age of 17.88 days. *B. repens exposed to A. junius* had a mean reproductive age of 17.43 days. *B. repens* exposed to *C. americanus* had a mean

reproductive age of 17.29 days. *B. repens* in the control group had a mean reproductive age of 19.11 days. Low variability was observed in the different groups (±0.06 SD to ±0.23 SD). Figure 17 presents a bar chart of the first age of reproduction for each of the four groups.

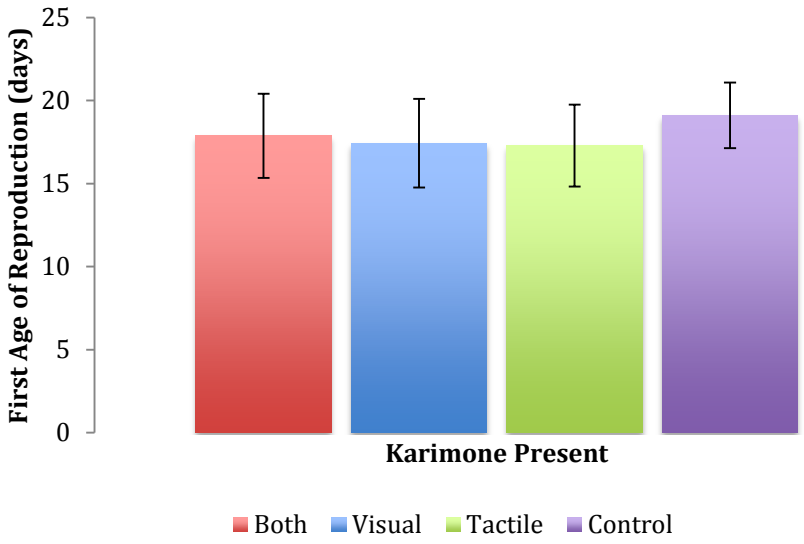


Figure 17. Age of first reproduction for the four groups of B. *repens*.

An ANOVA was run to test for differences between the ages that fist reproducing occurred at by the four different groups. As Figure 18 shows, there are no statistically significant differences between any of the groups. These findings do not support H4.

1st reproductive age

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6.868	3	2.289	.556	.650
Within Groups	94.762	23	4.120		
Total	101.630	26			

Figure 18. ANOVA summary table: first age of reproduction when exposed to different predator groups

Discussion

Change in the body size

This study sought to shed light on behavior of B. *repens* in northwestern Pensalvenia wetlands, a topic that has received little attention in the current literature. The first finding of the present study is that *B. repens* body size and length is influenced by the threat different predators display. *B. repens* that were exposed to both large visual predators and small tactile predators and *B. repens* that faced no predators had significantly larger body sizes and lengths than *B. repens* that lived among large visual predators (Figure 5). It appears that the *B. repens* that were threatened by large visual predators found it advantageous to remain small and avoid reaching the instar size where they were the most vulnerable to predation. By staying small, the larger visual predators' ability to identify and capture the *B. repens* decreased. These findings support H1 and are consistent with the literature on pelagic Cladocera species (Brancelj et al., 1996).

Around both predators *B. repens* reached a larger size (Figure 8). The fact that this response was chosen strengthens the argument that *B. repens* endeavors to avoid predation from *C*. *americanus*, the dominant predator (Helfman, 1989). *B. repens* will outgrow the 0.5mm to 2.00mm size to avoid *C*. *americanus* even though it makes them more vulnerable to *A. junius* (Parejko, 1991). This decision shows that in the presence of both predators *B. repens* will react to grow to a size that prevents *C*. *americanus*' mandible consuming *B. repens*, hence increase the chance of survival.

Change in the helmet size

A second finding of the present study is that there is no difference in *B.*

repens' attempt to develop different helmet sizes in response to the types of predators in their environment (Figure 11). Literature suggested that a larger helmet will hinder the ability of *A. junius* to consume B. *repens* due to the gape limitation of the mandible (Bernardi et al., 1987). When no predator is present it is expected that energy will be focused onto growth and reproduction, as the presence of a helmet is unnecessary. Instead of varying between groups the helmet measurements were similar sizes between all four groups, rejecting H2.

In the presence of *A. junius* energy was focused into functions other than a helmet to increase the ability to survive. While these functions were not morphological, an adjustment was observed in the time spent foraging for food. Consuming less food meant *B. repens* could not expend the same amount of energy on defense mechanisms.

Change in the number of offspring produced

A third finding is that there is no difference in *B. repens*' reproduction habits in response to the types of predators in their environment. All groups of *B. repens* produced a similar number of offspring (Figure 15). This data rejected H3 as *B. repens* did not reproduce earlier when no predators were present. Altering the age of first reproduction was not beneficial because *C. americanus* have the continuous ability of to locate *B. repens* and *B. repens* cannot alter their behavior effectively to avoid predation. Hence there is no advantage for *B. repens* to alter their offspring produced as growing to avoid *C. americanus* is shown to be more important. Instead of altering their reproductive behavior they chose to imitate the reproductive behavior of the other groups since it was the most efficient pattern for survivorship.

Change in the age of first reproduction

A fourth finding is that there is no difference in *B. repens*' age of first reproduction in response to the types of predators in their environment. During the control experiment *B. repens* did not reproduce at a significantly earlier age compared to the three other groups (Figure 17). *B. repens* did not stay smaller nor did they expend energy reproducing. Instead they mimicked the responses showed in other experiments (Appendix C). Even though predation was not an issue the same development of a helmet occurred and the same size was reached rather than reproducing at a younger age.

The first age of reproduction remained constant around visual predator as well (Figure 17). Instead or reproducing before reaching a size they could be visually identified and consumed *B. repens* appeared to conserve energy rather than expending it into early reproduction. It was observed that the *B. repens* were having to conserve energy as they were spending less time feeding in the water column and more time staying stationary on the substrate. By spending less time actively exposed to predators the energy required for defense mechanisms was decreased (Helfman, 1989).

It should be noted that the present study consisted of a simulation of a wetland environment. While every attempt was made to ensure the experimental conditions were the same as the actual wetland being studied, it is accepted that the simulated environment may not have fully reproduced the actual environment. This lack of full reproducibility is acknowledged as a potential study limitation.

Another study limitation is the small sample size. Having only 10 *B. repens*

for each of the four study groups meant that statistical power (the ability to detect effect sizes) was low. Certainly a larger sample size would have been helpful.

In addition to small size, *B. repens* produce significant smaller clutch sizes and produced these clutch sizes less frequently over their lifespan than other Cladocera species (Lemke & Benke, 2003). The average number of offspring produced was 1.85 with the average *B. repens* producing 0.86 times (Figure 15). Since only 29 *B. repens* reproduced this further reduced the results I had for sizes at first reproduction. This made is difficult to measure the tradeoff that was occurring due to the small output of offspring that other defense mechanisms were being compared to (Appendix B & Appendix C).

To better understand the defenses of *B. repens* use in wetland environments further studies are needed on predation rates at different sizes. While defenses can be seen, it is important to know how much they affect survivorship. The cause of these defenses is also still unknown, as the influencing factor could be the karimone of the predator or the karimone of the excreted exoskeleton of the consumed *B. repens*. By comparing the defense responses seen when only the karimone of the predator was present and when only the excreted exoskeleton was present the influencing factor could be understood. The foraging behavior *B. repens* differs from most other Cladocera (Peckarsky & McIntosh, 1998; Peckarsky et al., 2002). The time spent foraging in the presence of different predators needs to be compared as it affects *B. repens* exposure to predators. Foraging increases time exposed to predators due to increased movement, causing *B. repens* to be more vulnerable to ambush and attracting tactile predators. The fact that *B. repens* were observed to be

spending more time on the substrate instead of in the water column decreases exposure to predators so less energy is required for defense mechanisms. By better understanding the defense mechanisms' effectiveness and causes, the ability of *B. repens* to support northwestern Pennsylvania wetlands can be better understood.

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Appendix A

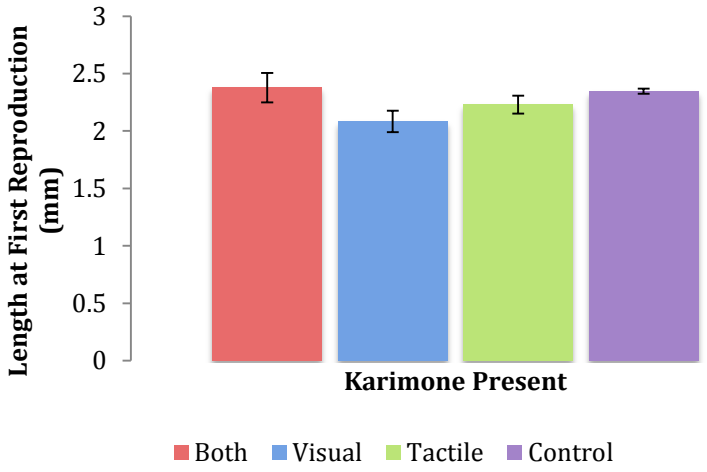

Figure 19. Length at first reproduction for the four groups of *B. repens*

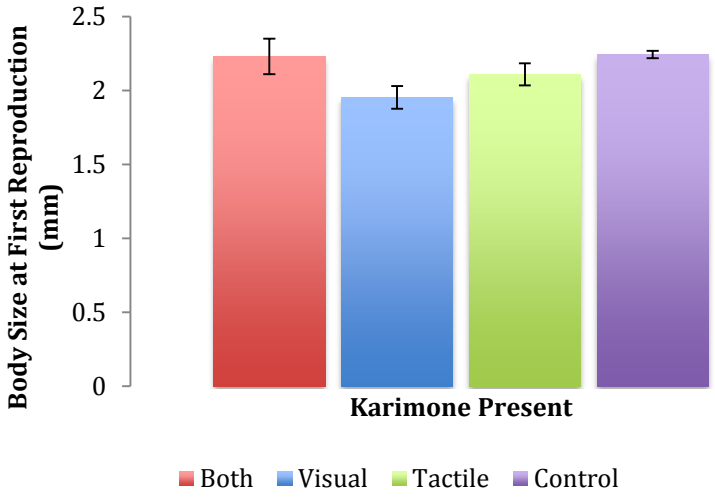

Figure 20. Body size at first reproduction for the four groups of *B. repens*

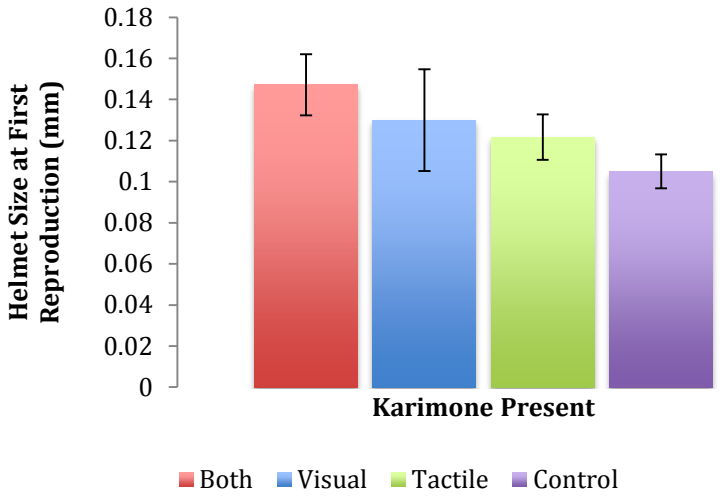

Figure 21. Helmet size at first reproduction for the four groups of *B. repens*

Appendix B

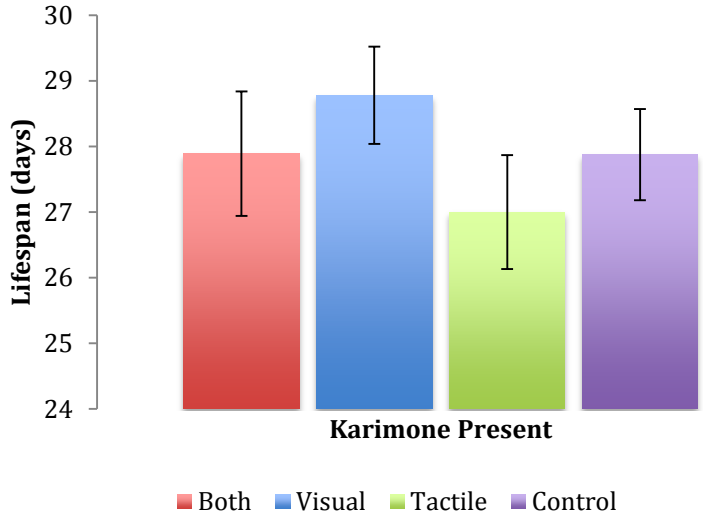

Figure 22. Lifespan for the four groups of *B. repens*

Appendix C

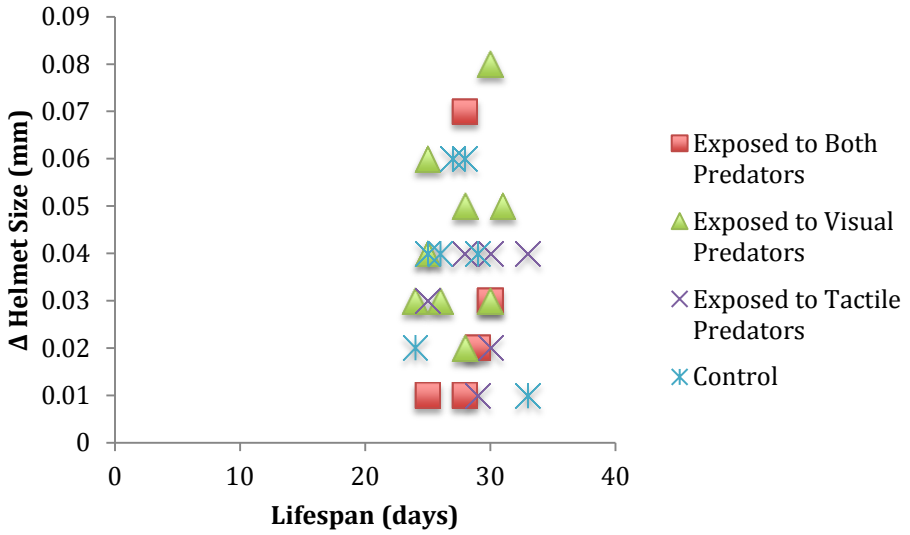

Figure 23. Energy tradeoff between a change in helmet size and lifespan for the four groups of *B. repens*

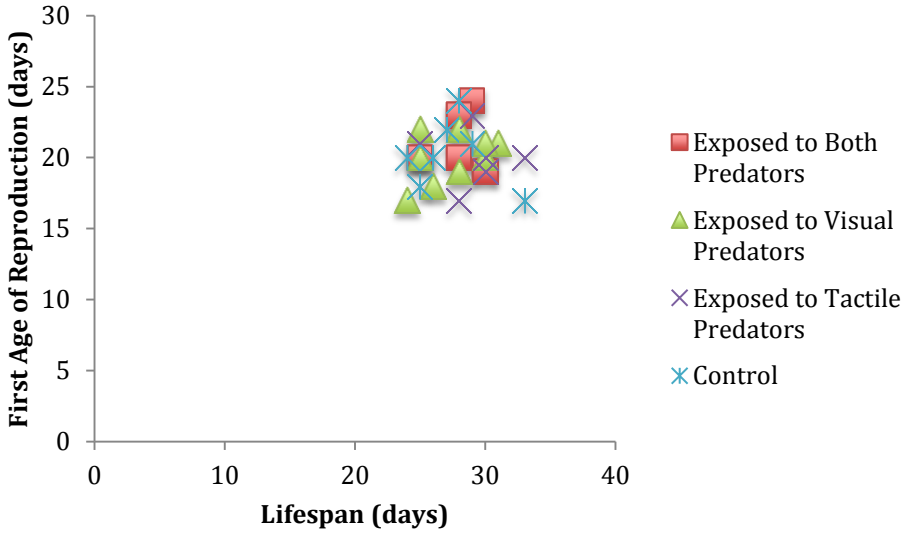

Figure 24. Enregy tradeoff between the first age of reproduction and lifespan for the four groups of *B. repens*

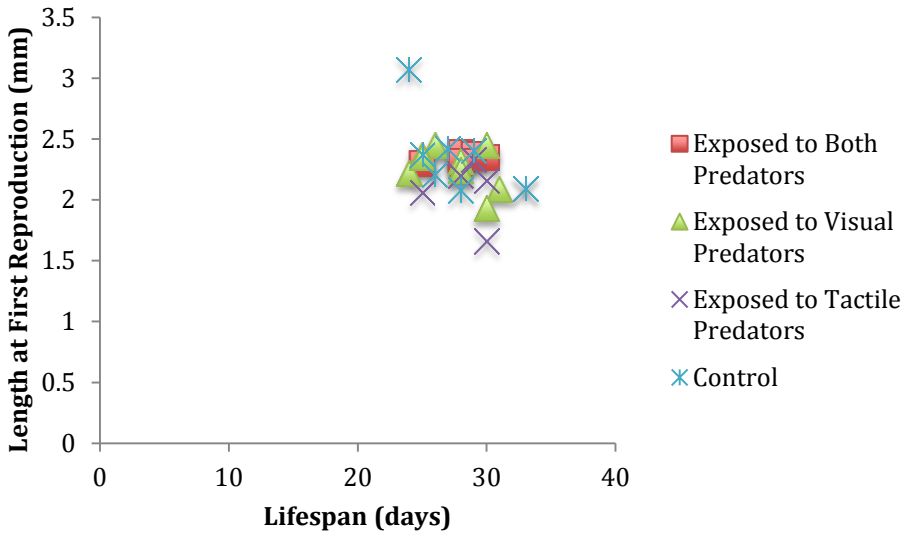

Figure 25. Energy tradeoff between the length at reproduction and lifespan for the four groups of *B. repens*

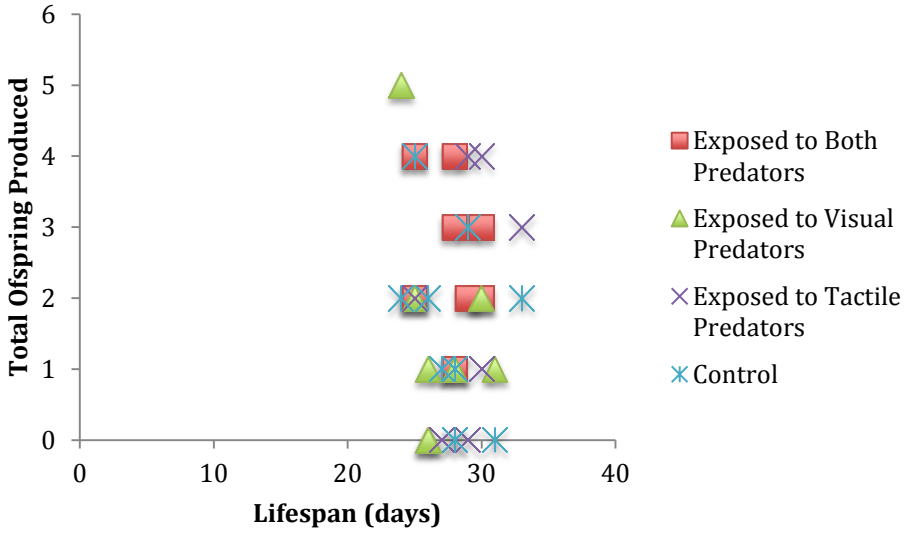

Figure 26. Energy tradeoff between the total offspring produced and lifespan for the four groups of *B. repens*

Appendix D

ID#	group	First Age of Reproduction	Length at First Reproduction	Body Size at First Reproduction	Helmate Size at First Reproduction	Lifespan	Body Size at Death	Helmat Size at Death	Length at Death	Total Ofspring Produced	Δ Helmat Size
1	1	18	2.37	2.27	0.1	25	2.41	0.14	2.55	4	0.04
2	1	24	2.08	1.96	0.12	28	2.19	0.18	2.37	1	0.06
3	1	99	99	99	99	31	2.01	0.17	2.18	0	99
4	1	20	3.07	2.9	0.17	24	3.03	0.19	3.22	2	0.02
5	1	99	99	99	99	13	0.81	0.09	0.9	0	99
6	1	21	2.4	2.23	0.17	29	2.51	0.21	2.72	3	0.04
7	1	99	99	99	99	28	2.13	0.18	2.31	0	99
8	1	20	2.21	2.06	0.15	26	2.11	0.19	2.3	2	0.04
9	1	22	2.42	2.21	0.21	27	2.28	0.27	2.55	1	0.06
10	1	17	2.09	1.98	0.11	33	2.08	0.12	2.2	2	0.01
11	2	20	1.66	1.61	0.05	30	1.69	0.09	1.78	4	0.04
12	2	17	2.2	2.02	0.18	28	2.08	0.22	2.3	1	0.04
13	2	99	99	99	99	27	2.1	0.13	2.23	0	99
14	2	23	2.33	2.13	0.2	29	2.29	0.21	2.5	4	0.01
15	2	99	99	99	99	8	0.7	0.06	0.76	0	99
16	2	99	99	99	99	28	2.03	0.18	2.21	0	99
17	2	21	2.06	1.9	0.16	25	1.97	0.19	2.16	2	0.03
18	2	19	2.16	2.09	0.07	30	2.13	0.09	2.22	1	0.02
19	2	20	2.09	1.97	0.12	33	2.04	0.16	2.2	3	0.04
20	2	99	99	99	99	29	1.65	0.09	1.74	0	99
21	3	18	2.45	2.29	0.16	26	2.34	0.19	2.53	1	0.03
22	3	99	99	99	99	26	2.41	0.22	2.63	0	99
23	3	21	2.09	1.97	0.12	31	2.08	0.17	2.25	1	0.05
24	3	17	2.22	2.1	0.12	24	2.15	0.15	2.3	5	0.03
25	3	99	99	99	99	26	2.38	0.21	2.59	0	99
26	3	20	2.37	2.23	0.14	25	2.31	0.2	2.51	2	0.06
27	3	22	2.32	2.24	0.08	28	2.3	0.13	2.43	1	0.05
28	3	99	99	99	99	6	0.54	0.03	0.57	0	99
29	3	99	99	99	99	12	0.92	0.06	0.98	0	99
30	3	20	1.93	1.82	0.11	30	1.96	0.14	2.1	2	0.03
71	4	22	2.35	2.26	0.09	25	2.3	0.13	2.43	2	0.04
72	4	19	2.24	2.15	0.09	28	2.16	0.11	2.27	1	0.02
73	4	99	99	99	99	14	1.07	0.05	1.12	0	99
74	4	21	2.45	2.35	0.1	30	2.38	0.18	2.56	3	0.08
75	4	19	2.35	2.21	0.14	30	2.24	0.17	2.41	2	0.03
1A	4	99	99	99	99	9	1.02	0.05	1.07	0	99
2A	4	20	2.3	2.17	0.13	25	2.22	0.14	2.36	4	0.01
3A	4	20	2.39	2.29	0.1	28	2.38	0.11	2.49	3	0.01
4A	4	24	2.38	2.31	0.07	29	2.34	0.09	2.43	2	0.02
5A	4	23	2.32	2.2	0.12	28	2.27	0.19	2.46	4	0.07

Figure 27. A table of all measures taken of the *B. repens* trials during their lifespan