Variations in *Daphnia* Phenotype Based On Wetland Phosphorus Levels and Predation Pressures

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Abstract

Daphnia is a model organism for phenotypic plasticity and a key part of many freshwater trophic webs. It is a phosphorus rich food source for many predators. This survey tested the hypothesis that *Daphnia* would display a larger body size, smaller eye spot, and neck teeth in habitats with high aquatic phosphorus due to increased predation levels. Significant phenotypic variations were found between aquatic ecosystems. *Daphnia* in high phosphorus displayed greater body length and larger eye spots. *Daphnia* in ecosystems with high predation densities displayed smaller eye spots. No neck teeth were observed. Experimental manipulation would be required to verify if these observations are directly caused by phosphorus and predation.

Introduction

The zooplankton *Daphnia* is a well known and often utilized model for ecological studies of evolutionary biology (Miner *et al*. 2010). It plays a critical role in most freshwater food webs as a phosphorus-rich food source for secondary consumers and displays extraordinary phenotypic plasticity. *Daphnia* has become a model organism for studying phenotypic responses to changes in ecosystems and, more specifically, trophic interactions within those ecosystems (Miner *et al*. 2010).

In freshwater zooplankton assemblages dominated by *Daphnia*, phosphorus is generally found to be the limiting factor (Sterner and Elser 2002). This is due to *Daphnia*'s importance as a primary consumer of phosphorus dependent algae (Miner *et al*. 2010). It stands to reason that due to *Daphnia*'s significance both as a grazer and as prey, the presence of *Daphnia* plays an important role in the nutrient composition of freshwater ecosystems, and, conversely, nutrient composition plays an important role in the presence and physical structure of *Daphnia*.

Daphnia are known to be a phosphorus rich food source for higher predators, but their phosphorus content is directly influenced by the phosphorus content of their aquatic habitat (Demott*et al.* 2004). Interestingly, aquatic phosphorus not only plays a role in the phosphorus content of these organisms, but in other various phenotypes as well (Weider*et al* 2005). Phosphorus deficiency within lakes, for example, often leads to greater developmental plasticity (Demott*et al*. 2004). That is, *Daphnia* observed in lower phosphorus levels have been shown, in some cases, to display a wider range of phenotypes within a population than those in phosphorus rich areas. Furthermore, there is amulti-generational effect of phosphorus levels on *Daphnia*. Phosphorus deficient parents produce phosphorus deficient offspring (Frost *et al*. 2009).

However, phosphorus alone may not be the key factor in determining *Daphnia* phenotype (aside from internal phosphorus levels). In a lab experiment, *Daphnia* exposed to varying levels of phosphorus, with no other pressures, did not display any significant levels of phenotypic plasticity (Stige*et al*. 2004). This suggests that variations in phenotype are not an inherent response to phosphorus, but rather a response to other factors that may be influenced by phosphorus, like trophic interactions.

Daphnia have been shown to increase in size and grow neck teeth (a spiny row on the back of their neck to protect against predators. See Figure 8.)in the presence of *Chaoborus* (a common zooplankton predator) (Luning 1992). The larger size is an adaptation shown in many organisms from plankton to fish to mammals to deal with gape-limited predators (Craig *et al.* 2008). Jeyasingh and Weider (2005) tested a hypothesis that a combination of both phosphorus levels and trophic interactions are responsible for phenotypic responses. *Daphnia* were exposed to treatments containing both variations in phosphorus levels and the presence or absence of *Chaoborus*kairomones. The *Daphnia* populations that had both high phosphorus levels and high kairomone grew larger than all other treatments. This suggests that there is an interaction between food availability and predation pressures that determines if *Daphnia* will display phenotypes that are designed to protect against predation.

It is important to note that these studies were carried out in laboratory settings. *Daphnia* in a natural ecosystem may exhibit different characteristics. One example of the effect of phosphorus on *Daphnia* and the trophic web occurred at Onondaga Lake in New York. Generally, the lake undergoes a Clear Water Phase (CWP) in the spring, which is a common occurrence in *Daphnia* rich waters, where an abundance of *Daphnia* consumes the phytoplankton in their habitat, causing clearer waters (Droscher*et al*. 2008). In 2009, a severe reduction of phosphorus in this lake led to a crash in the *Daphnia* population and the elimination of the CWP. Carnivorous zooplankton populations crashed as well when their food source, *Daphnia*, disappeared (Wang *et al*. 2010). Clearly, aquatic phosphorus plays a key role in the trophic cascades of *Daphnia*-rich wetlands.

In 1998, this assumption was tested experimentally (Sterner &Elser 2002). Researchers added a higher level carnivore, northern pike (*Esoxlucius*), to a lake in order to decrease the number of *Daphnia*-consuming fish. At the beginning of the experiment, nitrogen was the limiting factor of the lake because cyanobacteria were more dominant than *Daphnia*. As predation decreased, the *Daphnia* population exploded, and phosphorus became the lake's limiting factor. Even more interestingly, more large bodied *Daphnia* were seen over time, due to the decrease in predation pressure by non-gape limited planktivorous fish (Sterner and Elser 2002). This experiment works on two levels: First, it shows *Daphnia*'s important place in the trophic web and its influence on nutrient composition. Second, it shows *Daphnia*'s phenotypic response to changes in the trophic web.

This top-down approach showed that adding a higher level predator to a freshwater ecosystem can change the food web of that ecosystem all the way down to the nutrient level and that these changes are reflected in *Daphnia* phenotype. I took a bottom-up approach by examining the effect of phosphorus levels on *Daphnia* phenotype (although, unlike in the Sterner and Elser study, I did not do any experimental manipulation). Specifically, my research addresses whether phosphorus levels in different wetlands affect *Daphnia* phenotypes that are most likely to vary with predation (body size, eye spot size, and the presence or absence of neck teeth) (Miner *et al.* 2010, Sandlund*et al*. 1987). I tested the hypothesis that freshwater ecosystems with high phosphorus levels can support larger *Daphnia* populations, which will in turn support larger predator populations. Therefore, I expect that phosphorus-rich wetlands will display higher predation pressures, and the *Daphnia* that live in these wetlands will be larger, have smaller eye spots,and have neck teeth.

Materials and Methods

For a fair representation of UNDERC aquatic habitats, I chose to sample for *Daphnia*from three vernal ponds (K, P, and Red Bike), three marshes (Wood Duck, North Gate, and Froschsee) and three lakes (Tenderfoot, Tuesday, and Roach) (Table 10). I sampled using Nitex 153 μm conical plankton tow nets in the lakes and marshes, sampling from all accessible areas. In Red Bike pond, simply dipping the collection bottle into the water was found to be effective in one corner of the pond where *Daphnia* were in especially high concentrations, but I used tow nets throughout the pond as well to minimize bias. In the lakes, I used a larger tow net at various depths. I sampled the entire water column at each depth. Day towing was ineffective, so I repeated the process at night. Of all the ecosystems, only K, P, Red Bike, Wood Duck, and Tenderfoot yielded any *Daphnia*.

Mason Murphy's water samples, collected at multiple points in each body of water, were used for phosphorus. To test for phosphorous, an SRP analysis was used.

In the laboratory, I separated the zooplankton predators in each sample from the *Daphnia*, in order to both prevent predation of the *Daphnia* samples and approximate predation levels in each wetland. I qualified predator density categorically (high or low). I randomly took individual *Daphnia*from each collection and placed them in PCR wells with formaldehyde for preservation. Sample sizes varied depending on *Daphnia* density in each body of water.

I examined and photographed each individual *Daphnia* under a zooplankton microscope. I noted the presence or absence of neck teeth. I measured the length and eye spot area of each individual using Image J computer software.

Statistical Analysis

I used ANOVAs to check whether there were differences in length and eye spot area between each body of water. I followed those ANOVAs with Tukey post-hoc tests to check for grouping. I ran a regression between length and eye spot area to be sure there was not a correlation that I would have to account for. I ran t-tests to compare the phenotypic data to phosphorus levels. Phosphorus was a categorical variable with two categories: high and low. I also used t-tests to compare the phenotypic data to the predation categories (again, high and low).

Results

The ANOVA for length across the different bodies of water showed significant differences between the habitats ($F=5.723$, $p=0.000467$) (Table 2, Figure 2). The Tukey post-hoc test showed grouping for length between Red Bike Pond and Tenderfoot Lake (Table 3).

Eye spot area was also shown to be significantly different across the habitats (F=1.00186, p=0.00000167) (Table 4, Figure 3), with no grouping found in the Tukey test (Table 5). The t-test for phosphorus and length showed a significant difference (t=2.064, p=0.0424) with high phosphorus (Mean length=2.0145+/-0.0818mm) displaying a longer mean length than low phosphorus (Mean length=1.797+/-0.0621mm) (Table 6, Figure 4). The t-test for phosphorus and eye spot area showed a significant difference (t=4.916, p=0.00000507) with high phosphorus (Mean area=0.0274+/-0.00415mm) displaying a larger mean eye spot area than low phosphorus (Mean area=0.0115+/-0.000944mm) (Table 7, Figure 5). The t-test for predation and length showed no significant difference (t=1.038, p=0.302) in mean length between high predation (Mean length=1.935+/-0.0153mm) and low predation (Mean length=1.828+/-0.0918mm) (Table 8, Figure 6). The t-test for predation and eye spot area showed a significant difference (t=3.136, p=0.00244) with high predation (Mean area=0.015+/-0.00138mm^2) displaying a smaller mean eye spot area than low predation (Mean area=0.0211+/-.00260mm^2) (Table 9, Figure 7). No significant correlation was found between body length and eye size (r^2=0.0285, p=0.142) (Table 1, Figure 1). No neck teeth were found on any of the collected specimens.

Discussion

The significant phenotypic variations in body length and eye spot size between each habitat, and even within each habitat, strongly suggest the phenotypic plasticity that *Daphnia* are renowned for (Miner *et al*. 2010). Due to the small sample size and lack of experimental manipulation, I am wary of over-generalizing or inferring causation from the results of this survey, but there are implications that could be addressed in further studies.

Body length was found to be significantly larger in habitats with high aquatic phosphorus than low aquatic phosphorus, which supports my initial hypothesis. However, there was no significant difference in body length between high and low predation, which confounds the reasoning for my hypothesis. It is possible that, rather than large amounts of phosphorus increasing the population size and causing a trophic cascade (as I predicted), phosphorus could be a limitation on body size. With higher amounts of aquatic phosphorus, *Daphnia* have a nutritional base that could allow them to grow larger via their presumably more nutritious food. This would contradict past studies that claim that nutrients do not affect phenotypes inherently (Stige*et al*. 2004) and should be examined further.

Eye spot area was found to be larger in areas with high phosphorus, which contradicts my hypothesis.This again could be due to the increased nutrition provided by a high phosphorus base. However, eye spot area was found to be significantly smaller in ecosystems with high predator density. Eye spot size has been shown to be responsive to fish predation (Sandlund*et al*. 1987) – a factor that was not quantified in this survey - but nothing conclusive has been found about eye spot size relative to zooplankton predation. The results of this survey suggest that this response occurs when zooplankton predators are a factor. Asthis relationship does not appear to be heavily researched, further experimentation may be called for.

Interestingly, with one exception, aquatic ecosystems withhigh phosphorus levels had low predation levels and vice versa. This could be due to phosphorus being taken up in the biomass rather than remaining aquatic. If this is the case, my trophic cascade hypothesis could still be valid, and I was just testing the wrong source of phosphorus. It could also be due to the possibility that I brought up earlier in the discussion that a large amount of phosphorus allows for larger *Daphnia*.For invertebrate predators, larger *Daphnia* would be more difficult to eat than smaller *Daphnia*, which could eliminate all but the largest predators. The one exception to this pattern was Red Bike pond, which displayed low phosphorus and low predation. The fact that there is an exception, and the fact that this exception makes up one fifth of the sample sites in this survey, makes it difficult and presumptuous to draw any solid conclusions. The hypotheses above would have to be addressed in a much more extensive survey.

The lack of neck teeth in this survey is puzzling, given that the presence of *Chaoborus* has been shown to cause neck teeth (Luning 1992) and *Chaoborus* were found in three of the five sites. It is possible that the genetic pathway responsible for the production of neck teeth is absent in the *Daphnia* in this area. A genetic survey of UNDERC *Daphnia* could shed some light on this observation, as well as some of the other observations in this study.

It is important to note that no experimental manipulation was done in this study. In any ecosystem, many different factors are at play, and I was only examining two. The role of inter and intraspecific competition, pH, water temperature, other nutrients, and many other factors are all unaccounted for. Ultimately, this survey presents some interesting hypotheses as to the effects of phosphorus on *Daphnia* and the trophic web as a whole, but it raises more questions than it answers. A controlled experiment that examines the effect of phosphorus levels, predation, and other factors on *Daphnia* phenotypes would be the best way to examine the hypotheses that may, or may not, explain my observations.

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Tables

Table 1: Regression between *Daphnia* length and eye spot area. No correlation was found.

Dependent Variable	LENGTH(1)
N	77
Multiple R	0.168962965667
Squared Multiple R	0.028548483767
Adjusted Squared Multiple R	0.015595796884
Standard Error of Estimate	0.441180236629

Analysis of Variance

Source	SS	df	Mean Squares	F-Ratio	p-Value
Regression	0.428998011347	1	0.428998011347	2.204058819969	0.141838807396
Residual	1.459800008943E+001	75	0.194640001192		

Table 2: ANOVA testing for differences in average *Daphnia* length across the five aquatic ecosystems. Significant differences were found.

Dependent Variable	LENGTH
N	77
Multiple R	0.491167747015
Squared Multiple R	0.241245755708

Analysis of Variance

Source	Type III SS	df	Mean Squares	F-Ratio	p-Value
WATER$	3.625199512842	4	0.906299878211	5.723096292913	0.000467448292
Error	1.140179858794E+001	72	0.158358313721		

Least Squares Means

Factor	Level	LS Mean	Standard Error	N
WATER$	LAKE TF	1.692692307692	0.110369419441	1.300000000000E+001
WATER$	MARSH WD	2.110111111111	0.093795970572	1.800000000000E+001
WATER$	POND K	2.002857142857	0.086838194834	2.100000000000E+001
WATER$	POND P	2.063400000000	0.177965341413	5.000000000000
WATER$	POND RED	1.585295000000	0.088982670707	2.000000000000E+001

Table 3: Tukey post-hoc test to check for grouping in the *Daphnia* length ANOVA. Red Bike Pond and Tenderfoot Lake display similar average lengths.

Tukey's Honestly-Significant-Difference Test				
Sub-Group	**WATER$**	**Group Mean**	**Group Size**	**p-Value**
1	POND RED	1.585295000000	2.000000000000E+001	
	LAKE TF	1.692692307692	1.300000000000E+001	0.000000000000
2	POND K	2.002857142857	2.100000000000E+001	
	POND P	2.063400000000	5.000000000000	
	MARSH WD	2.110111111111	1.800000000000E+001	-0.941887544204

Table 4: ANOVA testing for differences in average *Daphnia*eye spot area across the five aquatic ecosystems. Significant differences were found.

Dependent Variable	EYESPOT
N	77
Multiple R	0.597970545086
Squared Multiple R	0.357568772790

Analysis of Variance					
Source	**Type III SS**	**df**	**Mean Squares**	**F-Ratio**	**p-Value**
WATER$	0.006372884942	4	0.001593221236	1.001856329149E+001	0.000001674608
Error	0.011449938043	72	0.000159026917		

Least Squares Means				
Factor	**Level**	**LS Mean**	**Standard Error**	**N**
WATER$	LAKE TF	0.012120307692	0.003497547682	1.300000000000E+001
WATER$	MARSH WD	0.009392055556	0.002972343980	1.800000000000E+001
WATER$	POND K	0.023189523810	0.002751855800	2.100000000000E+001
WATER$	POND P	0.044896000000	0.005639626180	5.000000000000
WATER$	POND RED	0.012951100000	0.002819813090	2.000000000000E+001

Table 5: Tukey post-hoc test to check for grouping in the *Daphnia* eye spot ANOVA. No significant similiarities were found.

Tukey's Honestly-Significant-Difference Test				
Sub-Group	WATER$	Group Mean	Group Size	p-Value
1	MARSH WD	0.009392055556	1.800000000000E+001	
	LAKE TF	0.012120307692	1.300000000000E+001	
	POND RED	0.012951100000	2.000000000000E+001	-1.168199901097
2	POND K	0.023189523810	2.100000000000E+001	1.000000000000
3	POND P	0.044896000000	5.000000000000	1.000000000000

Table 6: T-test for differences in average *Daphnia*length between aquatic ecosystems with high and low phosphorus levels.*Daphnia* were found to be significantly longer in ecosystems with high phosphorus than low phosphorus.

Variable	P$	N	Mean	Standard Deviation
LENGTH	HIGH	2.600000000000E+001	2.014500000000	0.417420100139
	LOW	5.100000000000E+001	1.797900000000	0.444141578779

Variable	P$	Mean Difference	95.00% Confidence Interval		t	df	p-Value
			Lower Limit	Upper Limit			
LENGTH	HIGH LOW	0.216600000000	0.007578832761	0.425621167239	2.064333221645	7.500000000000E+001	0.042444492530

Table 7: T-test for differences in average *Daphnia* eye spot area between aquatic ecosystems with high and low phosphorus levels. Eye spots were found to be significantly larger in ecosystems with high phosphorus levels.

Variable	P$	N	Mean	Standard Deviation
EYESPOT	HIGH	2.600000000000E+001	0.027363846154	0.021174240213
	LOW	5.100000000000E+001	0.011483196078	0.006739609110

Variable	P$	Mean Difference	95.00% Confidence Interval		t	df	p-Value
			Lower Limit	Upper Limit			
EYESPOT	HIGH	0.015880650075	0.009444935741	0.022316364409	4.915674707417	7.500000000000E+001	0.000005070461
	LOW						

Table 8: T-test for differences in average *Daphnia* length between aquatic ecosystems with high zooplankton predator density and low zooplankton predator density. No significant difference was found.

Variable	PREDATION$	N	Mean	Standard Deviation
LENGTH	High	3.100000000000E+001	1.935064516129	0.475623305813
	Low	4.600000000000E+001	1.827889130435	0.422367587260

Variable	PREDATION$	Mean Difference	95.00% Confidence Interval		t	df	p-Value
			Lower Limit	Upper Limit			
LENGTH	High	0.107175385694	-0.098558939699	0.312909711088	1.037767143242	7.500000000000E+001	0.302712582227
	Low						

Table 9: T-test for differences in average *Daphnia* eye spot area between aquatic ecosystems with high zooplankton predator density and low zooplankton predator density. Eye spots were found to be significantly larger in ecosystems with low predation than high predation.

Variable	PREDATION$	N	Mean	Standard Deviation
EYESPOT	High	3.100000000000E+001	0.010536161290	0.007668293305
	Low	4.600000000000E+001	0.021097434783	0.017633975567

Variable	PREDATION$	Mean Difference	95.00% Confidence Interval		t	df	p-Value
			Lower Limit	Upper Limit			
EYESPOT	High	-0.010561273492	-0.017271009502	-0.003851537483	-3.135613126452	7.500000000000E+001	0.002449199594
	Low						

Table 10: The geographical coordinates for the sites sampled in this survey.

	N deg	N min	N sec	W deg	W min	W sec
Red Bike	46	13	36.97	89	31	24.48
P	46	13	40.99	89	36	36.59
K	46	14	28.47	89	28	40.49
Woodduck	46	14	29.37	89	29	7.68
Frosch-See	46	14	27.00	89	30	46.74
North Gate	46	15	31.21	89	31	51.26
Tenderfoot	46	13	19.26	89	31	20.66
Roach	46	13	35.02	89	31	44.68
Tuesday	46	15	5.66	89	29	47.65

Figures

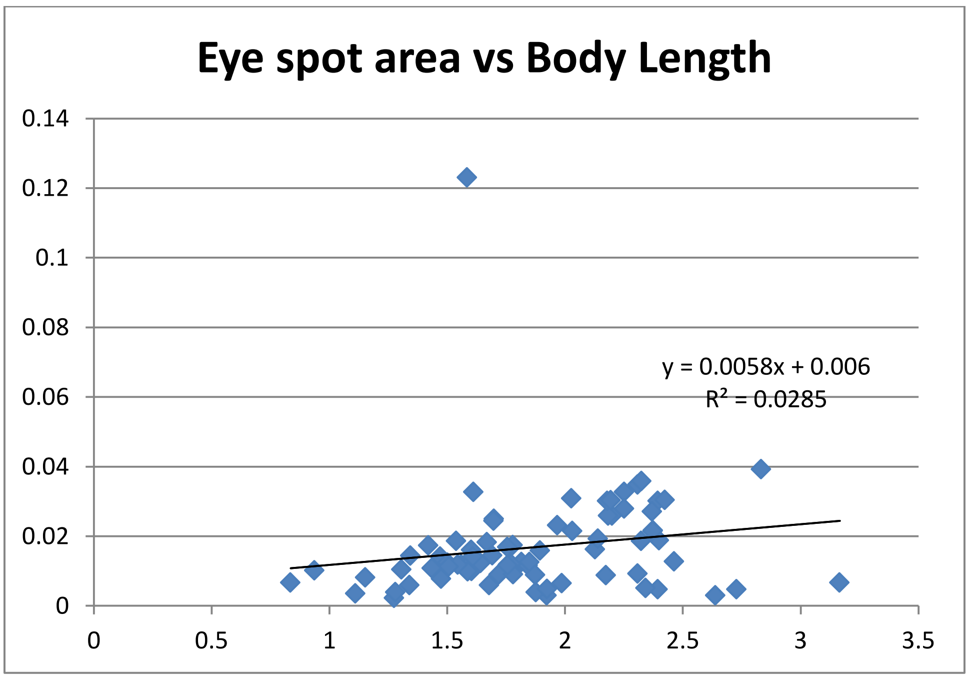


Figure 1: Regression between *Daphnia* length and eye spot area. No correlation was found. ($p=0.142$)

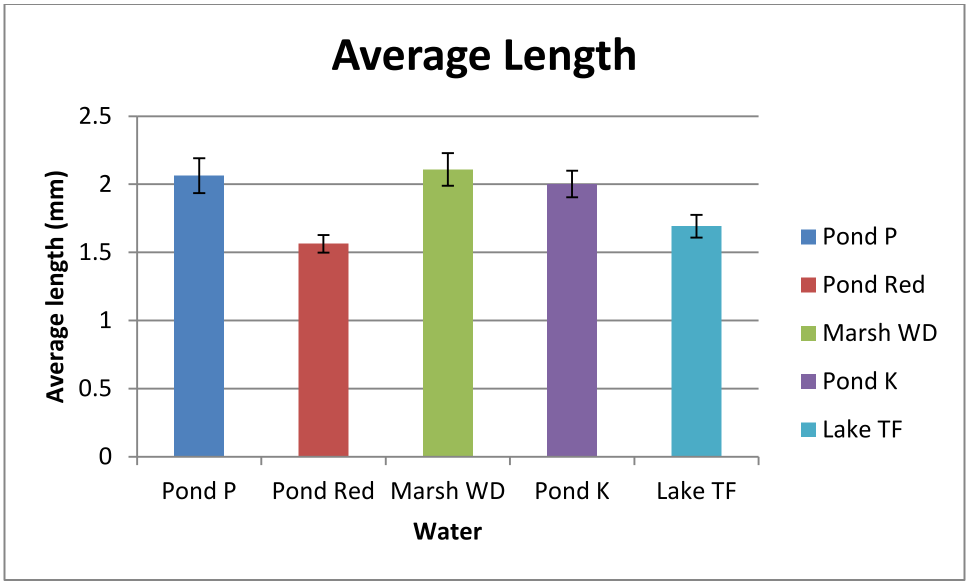


Figure 2: ANOVA testing for differences in average *Daphnia* length across the five aquatic ecosystems. Significant differences were found (F=5.723, p=0.000467). Significant grouping was found between Red Bike Pond and Tenderfoot Lake.

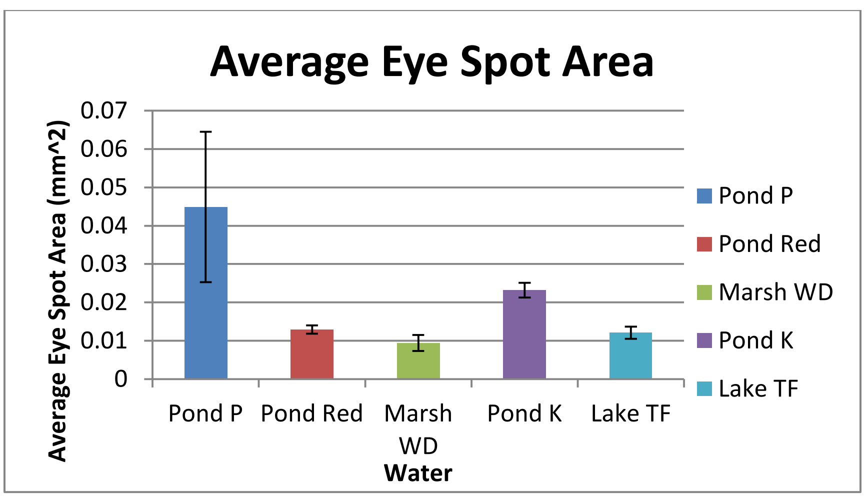
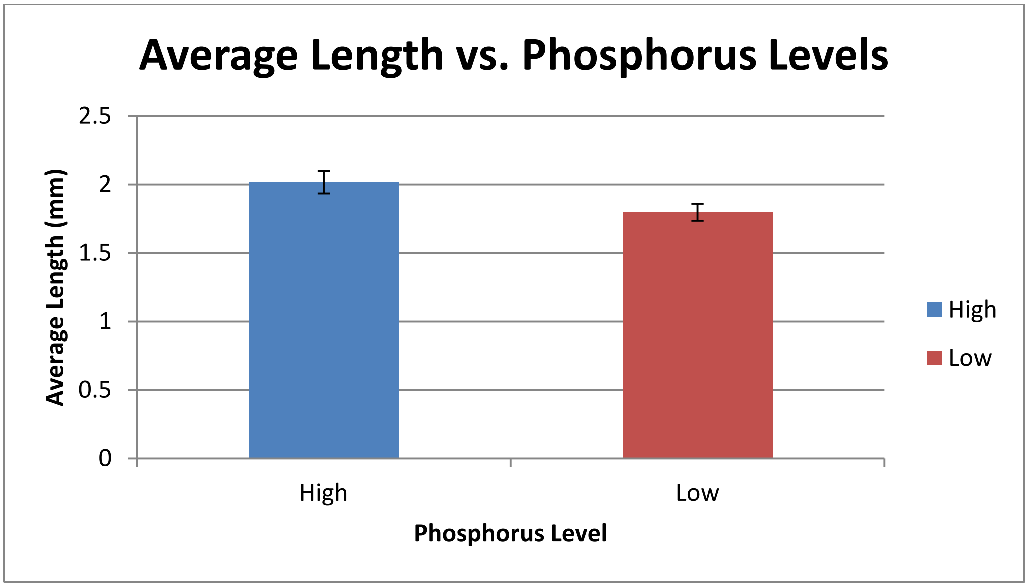


Figure 3: ANOVA testing for differences in average *Daphnia*eye spot area across the five aquatic ecosystems. Significant differences were found (F=1.00186, p=0.00000167).



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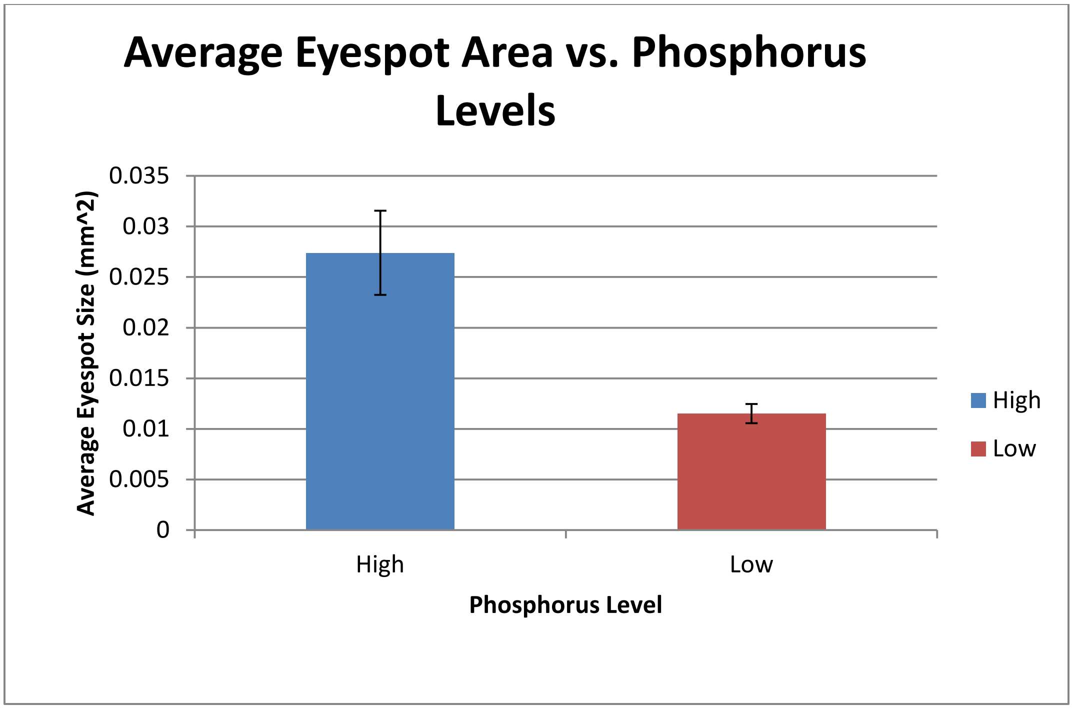
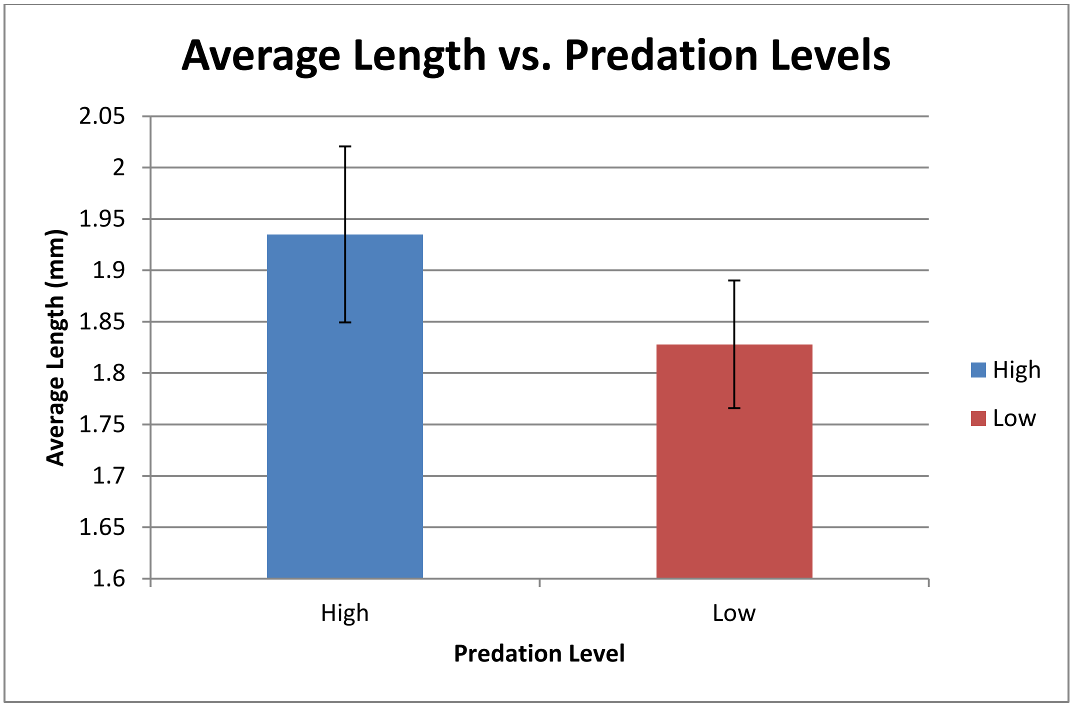


Figure 5: T-test for differences in average *Daphnia* eye spot area between aquatic ecosystems with high and low phosphorus levels. Eye spots were found to be significantly larger in ecosystems with high phosphorus levels (t=4.916, p=0.00000507, High mean area=0.0274+/-0.00415mm, Low mean area=0.0115+/-0.000944mm).



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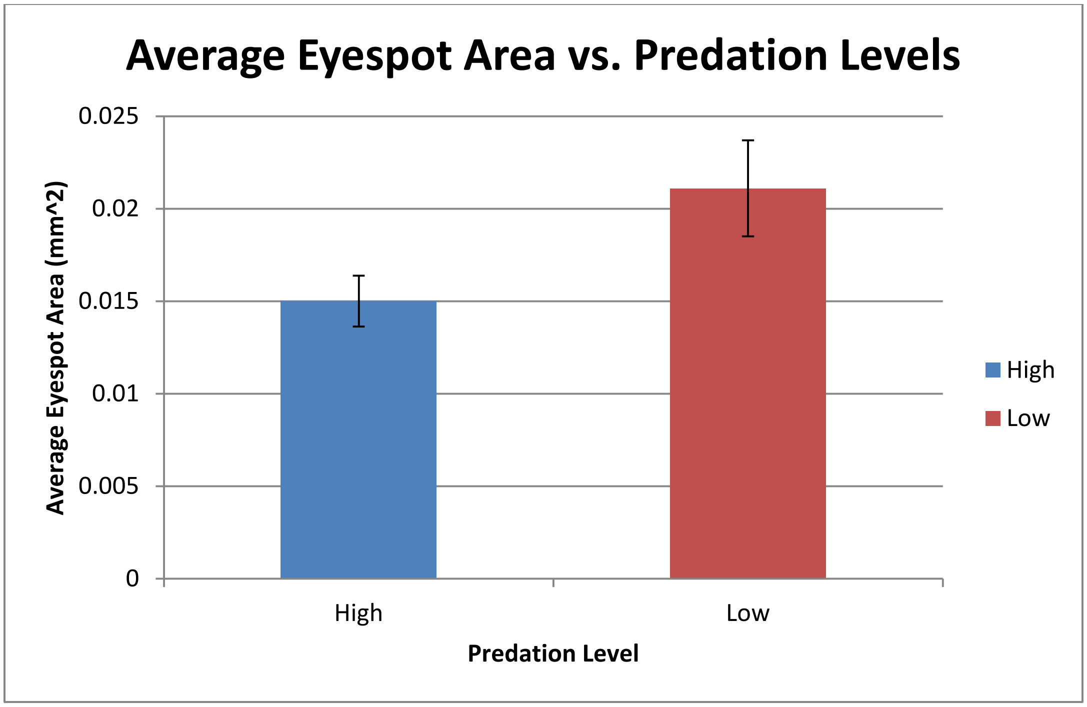

Figure 7: T-test for differences in average *Daphnia* eye spot area between aquatic ecosystems with high zooplankton predator density and low zooplankton predator density. Eye spots were found to be significantly larger in ecosystems with low predation than high predation(t=3.136, p=0.00244, High mean area=0.015+/-0.00138mm^2,Low mean area=0.0211+/-.00260mm^2).

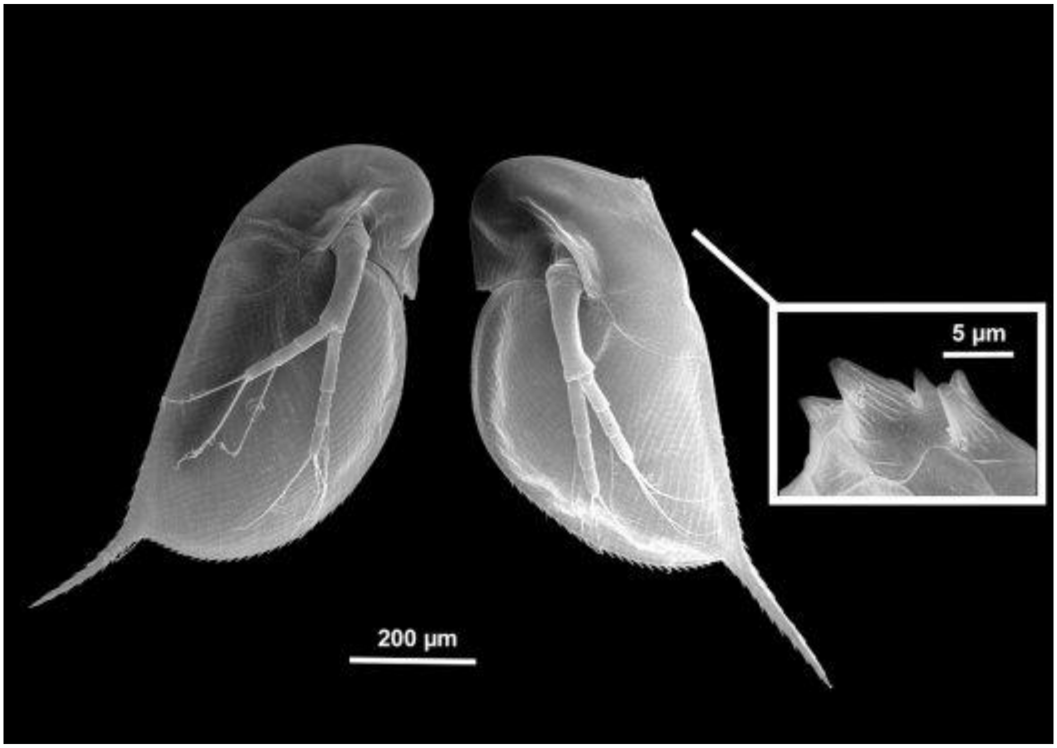

Figure 8: *Daphnia* may grow "neck teeth" in response to predation pressures. Picture taken from http://www.frontsidebus.net/2011/02/04/water-flea-genome-is-the-most-complex-yet-and-may-help-scientists-study-organisms-response-to-stress/