Variations of Growth Rate, Body Length, Reproduction, and Population Size in *Daphnia Pulex* Across a Phosphorus Gradient

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Abstract

The genus *Daphnia* are ideal organisms for laboratory studies of aquatic ecosystems as they exhibit cyclically parthenogenic reproduction, are easily studied as toxicity indicators, and play an integral role in the food webs of their environment. This experiment tested the hypothesis that populations of *Daphnia pulex* will show positive correlations between size and reproductive rate and phosphorus concentration with a negative correlation between instances of sexual reproduction and phosphorus concentration. Significant differences were found between the growth rate of populations at different concentrations as well as the overall population size across a concentration gradient. No significant differences were found between the size of *D. pulex* across the concentrations, nor was any significant difference found between the ratio of ephippia production (sexual reproduction) and growth rate (r) across the concentration gradient. Populations reared at below 0.3 mg P/L were significantly lower than those reared at concentrations higher than the native concentration. This leads to the conclusion that *D. pulex* are adept at handling phosphorus concentrations that are elevated from their natural state with less difficulty than adapting to lower concentrations. With this information, it is possible to further examine the range at which *D. pulex* are able to exhibit similar population characteristics with attention focused on growth rate variations, total population size, and population density.

Introduction

The organisms in the genus *Daphnia (Crustacea: Cladocera)* are well known zooplankton often used as model organisms for ecological studies due to their wide

distribution and adaptability in different ecosystems (Shaw et. al. 2008). *Daphnia* populations inhabit environments ranging from saline to freshwater, oligotrophic to eutrophic, and permanent lakes to temporary ponds (Shaw et. al. 2008). These organisms play a critical role in freshwater food webs, providing a phosphorus source for many secondary consumers (Sterner and Elser 2002, DeMott et al 2004). Although *Daphnia* are considered primary consumers of algae, they have also been known to feed on bacteria and protozoa, placing them in a central role in many food webs (Ojala et al, 2003). With a wide range of habitats and an important role as primary consumers of phytoplankton, *Daphnia* are an important indicator species(Chen and Stillman, 2012. Researchers can examine their populations as diagnostic tools to aid in determining the health of an ecosystem, as their reproductive rate and clear carapace allow investigators more access than many other zooplankton (Shaw et. al. 2008). Their importance in ecosystems combined with their rapid rate of reproduction make various species of *Daphnia* ideal specimens for laboratory work.

The genus is ideal for laboratory study because the many species of *Daphnia,* including *Daphnia pulex,* use a cyclical parthenogenic reproductive technique, only resorting to sexual reproduction when resources or environmental conditions are unfavorable (Innes 1997, Chen and Stillman 2012). As *Daphnia* are commonly used as diagnostic organisms for toxicology and ecology studies, standard rearing methods have been established for the conditions of the organisms in studies of *Daphnia* (Shaw et al. 2008) and were followed as closely as possible in this study. The species of importance in this study, *Daphnia pulex*, is similar to many of the species in the genus in their feeding behavior in that it is a filter feeder. Thus, their size, reproduction rate, and population

makeup are closely tied to the prevalence of this algal food source. Under most conditions, algae's limiting nutrient is phosphorus (Sterner and Elser, 2002), and spikes in phosphate levels have been shown to cause high growth rates and even algal blooms (Huppert et al. 2005, Kleinman et al. 2011, Ojala et al. 2003). Increased runoff of fertilizers from agricultural efforts has been linked to a rise in phosphorus levels in many bodies of water (Kleinman et al. 2011, Liu et al. 2012, Schepers et al. 1980). If this runoff were to have a large scale effect on zooplankton populations, there could likewise be large large scale effects for entire aquatic ecosystems (Chen and Stillman 2012)

These increased global levels of phosphorus represent a major area of concern for ecologists, and developing new simple ways of determining the levels of excess phosphorus contamination has practical purposes. Federal water quality standards issued by the EPA recommend that phosphorus levels should not exceed 0.1mg/L in human non-potable water sources, with even lower standards mandated at 0.05 mg/L for lakes and reservoirs that provide water for human uses. As phosphorus levels increase, algae populations increase, and, as a result, *Daphnia* populations likely also increase. However, in some instances, *Daphnia* and other zooplankton cannot feed on certain types of algae that contain toxins or built in defenses, leading to fish kills and unregulated algal blooms (Huppert et al 2005). *Daphnia pulex* presents an interesting case as its mode of reproduction has been shown to be environmentally triggered by cues such as population density, introduction into new habitat, or reduction or increase in food sources (Innes 1997). *D. pulex* have a very short generation time, with adults able to reproduce every seven to ten days under ideal conditions, while complete reproductive cycles under stressed conditions may take up to 45 days (McCauley et al. 1990) With increased food

availability, the average body size of the *Daphnia* tends to increase and the likelihood of sexual reproduction drops (Innes 1997).

In this experiment, my goal is to examine the effects of phosphorus on *Daphnia pulex* populations through increases in the concentration by studying the reproductive rate, size, and sexual reproduction of *D. pulex* populations. The study system took place in a laboratory setting in order to control the predatory responses that *Daphnia* may exhibit (in both behavior and phenotype) as well as to control the phosphorus levels and initial population size. The specific hypothesis we tested in this experiment is whether populations of *Daphnia pulex* will exhibit an increase in overall size and reproductive rate as well as a reduction of sexual reproduction when phosphorus levels are increased from the natural environment, and that overall size, reproductive rate will decrease and sexual reproduction will increase in environments with lower phosphorus levels than the natural environment when algal supply is even across all trials.

Materials and Methods

Collection and Sorting

In order to test this hypothesis, zooplankton samples were taken from Pond 5 (Figure 1, N 46°13°40.99° W 89°36°36.59°) on the University of Notre Dame Environmental Research Center's property in Northern Wisconsin and Upper Peninsula of Michigan. Pond 5 is a small vernal pond with a basin length of 9m by 7m. Zooplankton were sampled at multiple dates, with the first collection beginning on 30 May, 2012, and the last collection occurring on 22 June, 2012. The populations were integrated. Varying depths of

water were observed at the pond over the length of this time, with many other vernal pools in the area completely dry at times when Pond 5 still contained water.

Samples were collected using an integrated zooplankton tow net (Nitex 153μm) and initially placed into an opaque 1L nalgene container. After samples were taken, the *D. pulex* were sorted from the sample and removed to separate holding jars. Care was taken to ensure only *D. pulex* was taken away from the original pond samples. As trials were not begun immediately, the *Daphnia* were left in 1L containers and fed algae every two days.

SRP Analysis

Water was taken from Pond 5, Pond 32, and Tenderfoot Lake and tested for soluble reactive phosphorus. Pond 5 is a true vernal pond and the source of the *D. pulex* to be used in the trials. Pond 32 is a spring fed pond, and Tenderfoot Lake draws its water from a variety of streams and off-flow from Palmer Lake. Samples for testing from Pond 5 were taken from a variety of sites in the pond to ensure the phosphorus levels that may be observed were evenly dispersed. The mean SRP for Pond 5 was 310.0 μg P/L with SE=10.0, for Pond 32 the mean SRP was 19 μg P/ L with SE=1.0, and for Tenderfoot Lake the mean SRP was <0. 1 μg P/ L with SE<0.51 (Table 1)

Trials

Stock samples at each concentration were created on 27 June, 2012. 115 L of water were taken from Pond 5 with a concentration of .3 mg P/L. Lake water was taken from Tenderfoot at a concentration of < 0.1 μm P/L to serve as the 0 P concentration, the water from Pond 5 was diluted to form 0.1 mg P/ L and 0.2 mg P/ L concentrations. The water from Pond 5 served as the 0.3 mg P/ L concentration. 570mL of 10 mg P/L solution was

added to 19 L of water from Pond 5 to create elevated levels of phosphorus at 0.6 mg P/ L. 1.13 L of 10 mg P/L solution was added to 19 L of water from Pond 5 to create elevated levels of phosphorus at 0.9 mg P/ L.

Modified 500mL containers were set up and 9 bottles were filled with 350 mL at each concentration. From the stock collected, 5 *D. pulex* adults were placed in each bottle and an initial feeding occurred. Feeding was uniform, with each trial receiving .25 mL of algae solution, which was at 95% absorbance at 430nm wavelength. Feeding occurred on 2-day intervals.

The water in the containers was changed every 7 days to prevent die-offs due to oxygen depletion and replenish other nutrients that may have been lowered over the course of the trial. The sample was filtered as to prevent the loss of any *D. pulex* and the used water was discarded. Organic materials were left in the sample, primarily dead algae. The containers were refilled, and the *D. pulex* reintroduced. 4 replicates at the 0.9 mg P/L were lost at day 7, and thus are not included in any data or statistical analysis.

Counting Populations and Measuring Length

At 13 days three of the samples from each concentration were removed and counted. At 21 days, all remaining samples were removed and counted. Within 24 hours of counting, a random subsample of individuals were photographed and measured using the LAS EZ imaging program. Ten individuals were sampled from each population; meaning at minimum 15% of the organisms in each population were measured. The presence and number of ephippia was also recorded during these counts.

Statistical Analysis

We used regressions to test whether there were differences in population size at 13 days and mean total population between each concentration level. A regression was also used to examine the differences between ephippia presence between concentrations. An ANOVA was also used to examine the differences in total population by concentration, with a Tukey Post-Hoc test to check for grouping and to determine the relationship between the different concentrations. An ANOVA was also used to examine the relationship between *D. pulex* length and concentration. The growth rate(r) of the populations was determined using Equation 1:

$$r = \ln(N2/NI) \div T$$

A regression was used to show how the resulting growth rates differed over the range of concentrations.

Results

There was a significant difference between population sizes among the varying concentrations ($p<0.001$, $F=56.51$, df 5,44)(Figure 2, Table 2). The Tukey post-hoc test showed grouping between concentrations 0.1 mg P/L and 0.2 mg P/ L as well as grouping between concentrations 0.3, 0.6, and 0.9 mg P/L. (Table 3). There was a significant positive correlation between growth rate (r) over the concentration gradient. ($p=0.008$, $F=7.77$, df=1,39)(Figure 3, Table 4). No significant relationship was found between population sizes at 13 days across a concentration gradient ($p=0.094$, $F=4.77$, df=1,4)(Figure 4, Table 5). No significant relationship was found between mean total population sizes across a concentration gradient ($p=0.078$, $F= 5.55$, df=1,4)(Figure 5, Table 6). The regression examining the presence of ephippia over the concentration gradient showed no significant

relationship (p=0.101, F=4.49, df=1,4)(Figure 6, Table 7). No significant relationship was found between the ratio of ephippia to growth rate (r) over a concentration gradient (p=0.623, f=0.245, df=1,38) (Figure 7, Table 8). No significant relationship was shown between the lengths of the *D. pulex* over the concentration gradient (p=0.078, F=3.27, df=1,39)(Figure 8, Table 9).

Discussion

The hypothesis was that populations of *Daphnia pulex* will exhibit increased size and reproductive rate combined with a reduction of instances of sexual reproduction when phosphorus levels are increased and food levels are equal throughout the concentration gradient. It was also predicted that overall size and reproductive rate will decrease and sexual reproduction will increase in environments with lower phosphorus levels under equal feeding conditions. The results of the ANOVA comparing population sizes over the concentration gradient shows that *D. pulex* thrive when phosphorus levels are at least at the levels of their native environment. The significant differences between concentrations 0.1 and 0.2 mg P/ L and the higher concentrations illustrate the disadvantage populations at lower phosphorus levels face (Figure 2, Table 2). While we were able to determine that population size was limited by low phosphorus, we were unable to show that populations would be elevated linearly beyond 0.3 mg P/L (Figure 2). It is possible that other factors limited the growth of the *D. pulex* populations, such as population density, but larger volumes of water in the initial environment would have necessitated higher dosages of food as to ensure the organisms would be able to forage effectively (McCauley et al 1990). If larger doses of food were used, the effects of phosphorus may become more difficult to discern from the other factors aiding population growth.

The size of populations on day 13 of the trials in the regression was not shown to be significantly related to the concentration (Figure 4), however, it does show that the trend exhibited by the final population was present for the duration of the study. It is unsurprising that the populations fared better in the water that was the same as their natural habitat, as it suggested that the *D. pulex* in that environment needed less time for adjustment. This is normally the case, as *Daphnia* introduced into new environments need time to adjust to unfamiliar conditions, and it is possible that phosphorus levels could induce such a response (Jeyasingh et al 2011). This adjustment can often result in some of the organisms dying or stress the organisms into sexual reproduction, which, in this case could negatively effect the populations at both the high and low concentrations of phosphorus. This necessary period of adjustment could explain how the *D. pulex* in the elevated levels of phosphorus were unable to take advantage of the higher resources available to them and resulted in a lower mean population than the *D. pulex* at 0.3 mg P/L (Figure 5) .

The instances of sexual reproduction, measured by ephippial production(Figure 6), were higher in the 0.3, 0.6, and 0.9 mg P/L. This would be surprising, for it seemed as though these populations had a gentler environment that promoted the normal partheogenticism exhibited by *D. pulex*. However, there seems to be an easy explanation for this fact, as the higher concentrations also had higher populations. The rate of ephippial production may have been the same or lower in the higher concentrations, but with more organisms in total, the total instances of sexual reproduction would necessarily be higher. To counter act the effect of population size on epphipia production, a ratio of number of ephippia and growth rate (r) was found and run in a regression over the concentration

gradient. This also showed no significant difference (p=0.623, f=0.245, df=1, 38)(Figure 7). It was predicted that the highest instances of sexual reproduction would occur in the lowest concentrations of phosphorus, however, there was almost no sexual reproduction in the trail populations at 0 mg P/L. This is likely due to the shock of being put into a foreign system without the requisite nutrients to survive. As feeding was the same throughout the trials and concentrations, the lack of sexual production by the populations at 0 P is most likely due to the *D. pulex's* reaction to the environment lacking in phosphorus.

The length of *D. pulex* was relatively steady across the concentration gradient, which seems to be a surprising result. However, the regression omits data from the 0 mg P/ L because there were simple no organisms to measure. With the omissions, we can see that there is little change in the size of *D. pulex* across the concentration gradients (Figure 8). Although a positive correlation was predicted to exist between length of *D. pulex* and concentration, it seems to be unlikely that such a correlation would exhibit itself over the course of a 21 day trial, as length a combined product of phenotypic plasticity and feeding resources. Although the feeding resources were the same, the effects of phosphorus would likely have more influence as time passed and more generations reproduced.

The growth rate (r) of the populations were shown to be significant across the gradient of concentrations (p=.008) (Figure 3, Table 4). This growth rate does not take into account the r values at 0 mg P/L, as the population declines lead to negative growth rates. The change in population was changed into (r) *Equation 1*. The significant differences of (r) along the concentration gradient show that phosphorus does have a noticeable effect on growth rates, and while this is similar to the data offered by the ANOVA examining

population, it offers a more accurate means of examining the relationship between the different populations of *D. pulex* at different trial concentrations.

The growth rate (r) examines the relationship between the concentrations, but it is also useful to look at the population's progression over the period in question (Figure 9) This model does provide a glimpse of the population dynamics. In all cases except for the 0 mg P/L trials, the population rose until the 13th day, and then by the 21st day, populations consistently began to decline. It is possible that each population had bred over its carrying capacity in the first 13 days, and was in the process of restoring its balance at the 21st day. If the experiment were to be expanded, it would be valuable to examine the populations at more frequent intervals for a longer period in order to see whether or not the various populations would in fact reach a stable state. Previous studies have shown that when limits are placed on food resources (which is the condition overpopulation would create) the reproductive cycles are affected, with juveniles maturing more slowly, and adults dying from a combination of lack of food and the natural end of the life span (McCauley et al 1990). The slow development of juveniles slows reproduction, meaning that in instances of food stress, population would decrease for a time, then gradually rise to attempt to find the carrying capacity of the environment (McCauley et al 1990).

The phosphate concentrations were shown to have effects on *Daphnia pulex* but not in the exact ways predicted. The lower levels of phosphorus concentration coincided with significantly lower populations of *D. pulex* but the elevated levels of phosphorus produced populations that were in no way significantly different than the populations at the native concentration. The results of this study seem to show that *D. pulex* are adept at surviving at raised phosphorus levels, as they exhibit little change from populations at native

concentrations. Going by this experiment, the potential for *D. pulex* as an indicator organism for phosphorus levels seems low. Future expansions of this experiment should allow the populations of *D. pulex* to adapt to their specific concentration levels for a period of time before the start of trials. Trials should take place at a variety of volumes as to examine the effects of population density on the reaction of *D. pulex* to phosphorus concentrations. A larger variety of concentrations could also be used, for though the highest phosphorus level in this study was nine times greater than the EPA recommended level, there did not seem to be much noticeable difference between the elevated concentrations, with each yielding similar results, and the lower concentrations also showing a tendency to group. Though not the goal of this study, it would also be prudent to examine the reactions of *D. pulex* from a variety of sources, so long as those sources are of a similar SRP level.

The role of *Daphnia* in freshwater ecosystems cannot be downplayed. They are an important primary consumer and play a central role in many food webs. Thus it is vital to have an understanding about what effects the human population may or may not have on *Daphnia* and other zooplankton genus. Phosphorus runoff is of major importance in a world ever more reliant on heavy fertilizer use, and it is important to know how to determine the extent of runoff. If a certain species of zooplankton is tolerant of high levels of phosphorus, such as *Daphnia pulex,* it could be possible to determine the health if a body of water by a simple analysis of the species composition. If the body is dominated by zooplankton who can tolerate higher levels of phosphorus, there has likely been some event introducing the element to the system. With further tests of their competitive

abilities, the trends showing the ability of *Daphnia pulex* to adapt to higher phosphorus could be tailored to create a new diagnostic to determine the health of an ecosystem.

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Tables

Table 1: Soluble Reactive Phosphorus levels for Pond 5, Pond 32, and Tenderfoot Lake

Source	SRP in µg/L	Standard Deviation
Tenderfoot Lake	-0.213	0.509
Pond 32	18.907	1.305
Pond 3	310.88	10.845

Table 2: ANOVA testing for differences in *Daphnia pulex* populations across a phosphorus gradient. Significant differences were found.

Dependent Variable	POPULATION
N	50
Multiple R	0.93019759146512
Squared Multiple R	0.86526755916752

Analysis of Variance

Source	Type III SS	df	Mean Squares	F-Ratio	p-Value
CONCENTRATION	16,754.019999999993005	5	3,350.80399999999870	56.51463354799141	0.00000000001108
Error	2,608.79999999999930	44	59.29090909090908		

Table 3: Tukey post-hoc test checking for grouping among the populations across the phosphorus concentration gradient. Significant differences are found between 0 mg P/L and all other concentrations, between 0.1 mg P/L and 0.3, 0.6, and 0.9 mg P/L, and between

0.2 mg P/L and 0.3, 0.6, and 0.9 mg P/L.

Tukey's Honestly-Significant-Difference Test

CONCENTRATION(i)	CONCENTRATION(j)	Difference	p-Value	95% Confidence Interval	
				Lower	Upper
0	0.1	-26.44444444	5.18152E-06	-37.25759197	-15.63129692
0	0.2	-29.77777778	5.14329E-06	-40.5909253	-18.96463026
0	0.3	-53	5.14233E-06	-63.81314752	-42.18685248
0	0.6	-43.44444444	5.14233E-06	-54.25759197	-32.63129692
0	0.9	-52.46666667	5.14233E-06	-65.26095536	-39.67237798
0.1	0.2	-3.333333333	0.939710208	-14.14648086	7.479814189
0.1	0.3	-26.55555556	5.17713E-06	-37.36870308	-15.74240803
0.1	0.6	-17	0.000375596	-27.81314752	-6.186852478
0.1	0.9	-26.02222222	8.75905E-06	-38.81651091	-13.22793353
0.2	0.3	-23.22222222	6.22321E-06	-34.03536974	-12.4090747
0.2	0.6	-13.66666667	0.00612872	-24.47981419	-2.853519145
0.2	0.9	-22.68888889	5.71046E-05	-35.48317758	-9.8946002
0.3	0.6	9.555555556	0.110773253	-1.257591966	20.36870308
0.3	0.9	0.533333333	0.999995914	-12.26095536	13.32762202
0.6	0.9	-9.022222222	0.30579298	-21.81651091	3.772066467

Table 4: Regression between growth rate (r) and concentration. Significant correlation was found.

Dependent Variable	R_GROWTH
N	41
Multiple R	0.40760272173833
Squared Multiple R	0.16613997876849
Adjusted Squared Multiple R	0.14475895258307
Standard Error of Estimate	0.03523572924314

Analysis of Variance

Source	SS	df	Mean Squares	F-Ratio	p-Value
Regression	0.00964744104966	1	0.00964744104966	7.77043989038103	0.00816455810618
Residual	0.04842070799653	39	0.00124155661530		

Table 5: Regression between population size at day 13 and concentration. No correlation found.

Dependent Variable	DAY_13
N	6

Dependent Variable	DAY_13				
Multiple R	0.73745895459781				
Squared Multiple R	0.54384570971650				
Adjusted Squared Multiple R	0.42980713714562				
Standard Error of Estimate	14.99647300196892				
Analysis of Variance					
Source	**SS**	**df**	**Mean Squares**	**F-Ratio**	**p-Value**
Regression	1,072.51208439198100	1	1,072.51208439198100	4.7689627943956	0.09434351196418
Residual	899.57680999513036	4	224.89420249878259		

Table 6: Regression between total mean population and concentration. No correlation found.

Dependent Variable	DAY_13				
N	6				
Multiple R	0.73745895459781				
Squared Multiple R	0.54384570971650				
Adjusted Squared Multiple R	0.42980713714562				
Standard Error of Estimate	14.99647300196892				
Analysis of Variance					
Source	**SS**	**df**	**Mean Squares**	**F-Ratio**	**p-Value**
Regression	1,072.51208439198100	1	1,072.51208439198100	4.76896279439567	0.09434351196418
Residual	899.57680999513036	4	224.89420249878259		

Table 7: Regression between mean total presence of ephippia and concentration. No correlation found.

Dependent Variable	TOT_MEAN_APHIP				
N	6				
Multiple R	0.72741763272502				
Squared Multiple R	0.52913641239927				
Adjusted Squared Multiple R	0.41142051549909				
Standard Error of Estimate	7.28792485230808				
Analysis of Variance					
Source	**SS**	**df**	**Mean Squares**	**F-Ratio**	**p-Value**
Regression	238.74830897936880	1	238.74830897936880	4.49502935740243	0.10132512882669
Residual	212.45539461155869	4	53.11384865288967		

Table 8: Regression between the ratio of ephippia to growth rate (r) and concentration. No significant difference was found.

Dependent Variable	R_GROWTH
N	40

Dependent Variable	R_GROWTH
Multiple R	0.080032097939
Squared Multiple R	0.006405136701
Adjusted Squared Multiple R	0.000000000000
Standard Error of Estimate	9.3156260761292E+001

Analysis of Variance

Source	SS	df	Mean Squares	F-Ratio	p-Value
Regression	2.125821317551E+003	1	2.125821317551E+003	0.244964223962	0.623493228751
Residual	3.297673789275E+005	38	8.678088919144E+003		

Table 9: Regression between *Daphnia pulex* length across a phosphorus gradient. No significant correlations were found.

Dependent Variable	LENGTH
N	41
Multiple R	0.27852004317550
Squared Multiple R	0.07757341445048
Adjusted Squared Multiple R	0.05392145071844
Standard Error of Estimate	0.12840818421993

Analysis of Variance

Source	SS	df	Mean Squares	F-Ratio	p-Value
Regression	0.05407930639801	1	0.05407930639801	3.27978747681745	0.07784584817734
Residual	0.64305780921175	39	0.01648866177466		

Figures

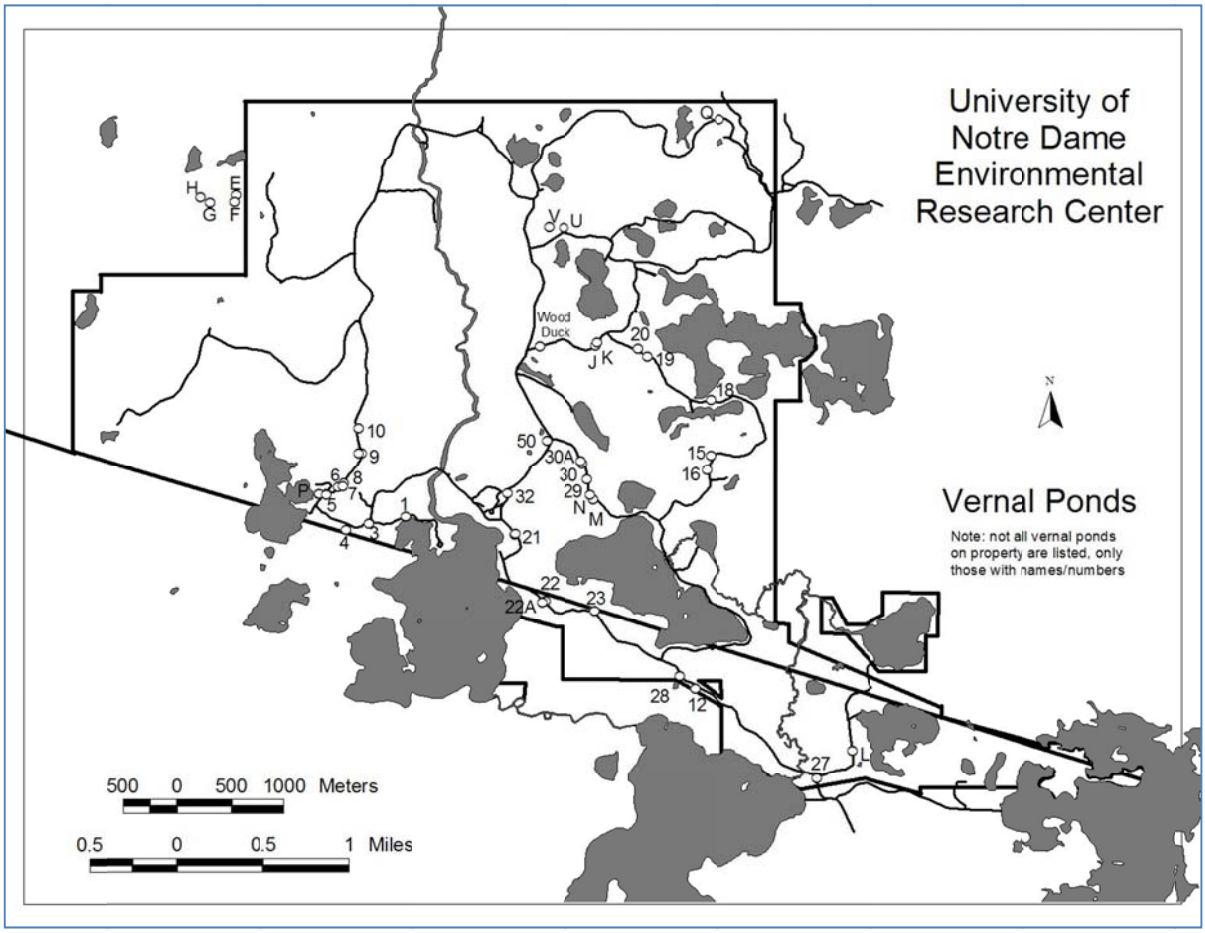

Figure 1: Map of Vernal ponds on the UNDERC property

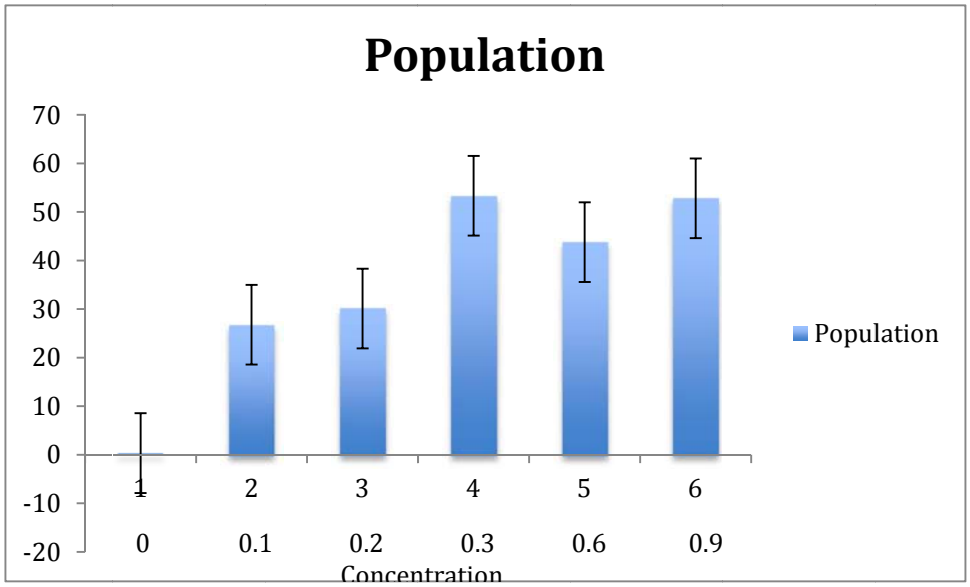

Figure 2: ANOVA comparing *D. pulex* population across a concentration gradient. Significant differences were observed (p<.001) and a Tukey post-hoc test was performed(Table 5).

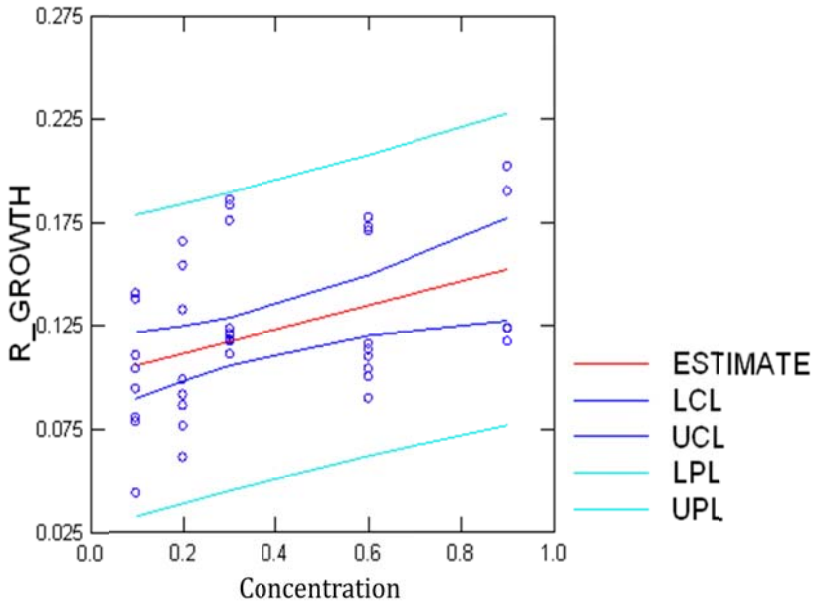

Figure 3: Regression comparing growth rate (r) of *D. pulex* populations across a concentration gradient. Significant differences were observed (p=0.008).

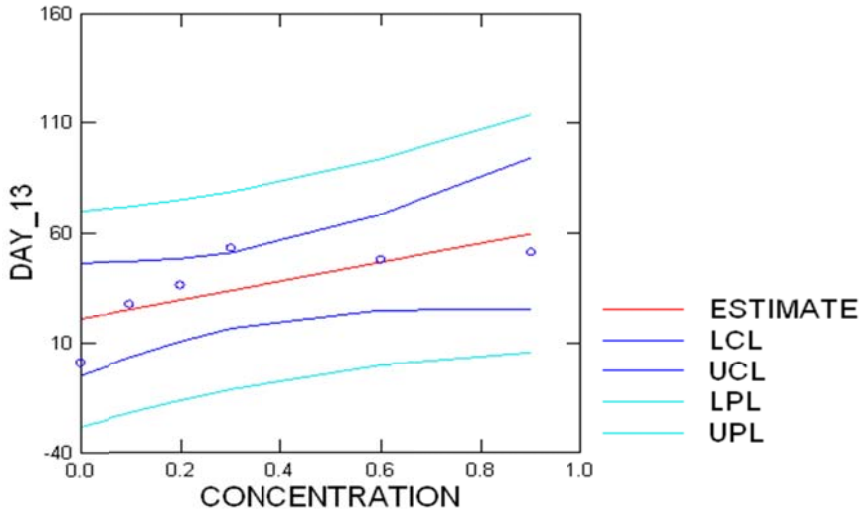

Figure 4: Regression comparing population of *D. pulex* at day 13 across a concentration gradient. No correlation was found (p=0.094).

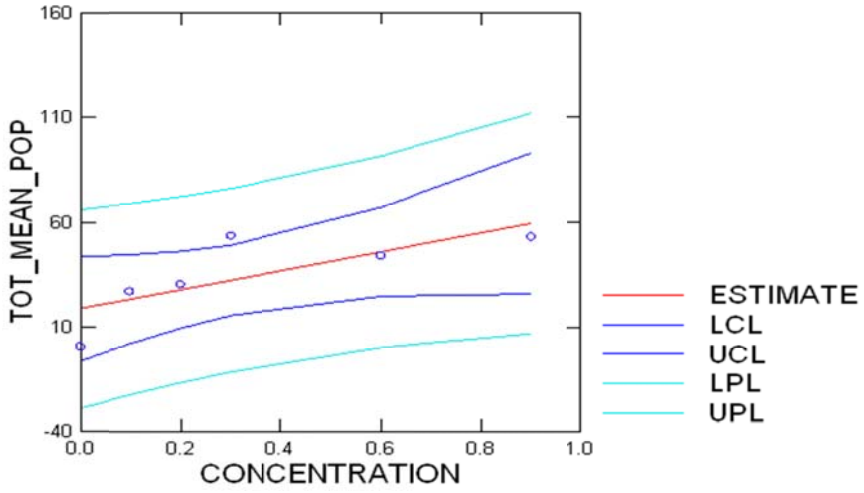

Figure 5: Regression comparing total mean population of *D. pulex* across a concentration gradient. No correlation was found (p=0.078).

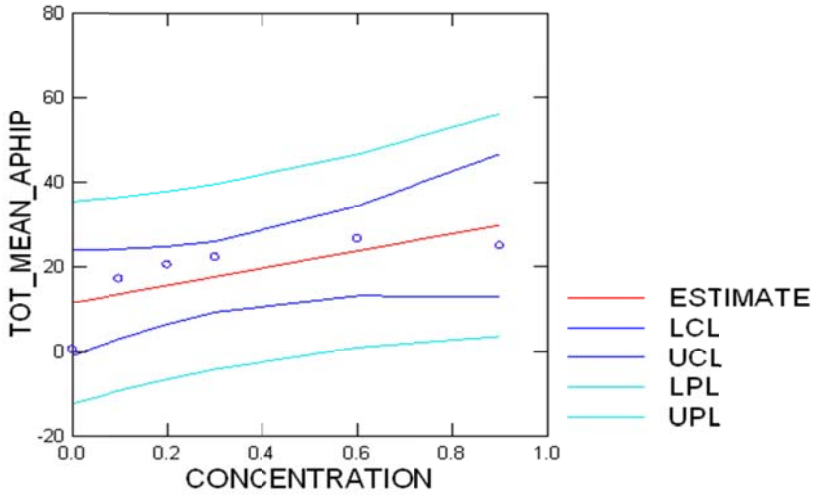

Figure 6: Regression comparing the total mean ephippia presence across a concentration gradient. No correlation was found (p=0.101).

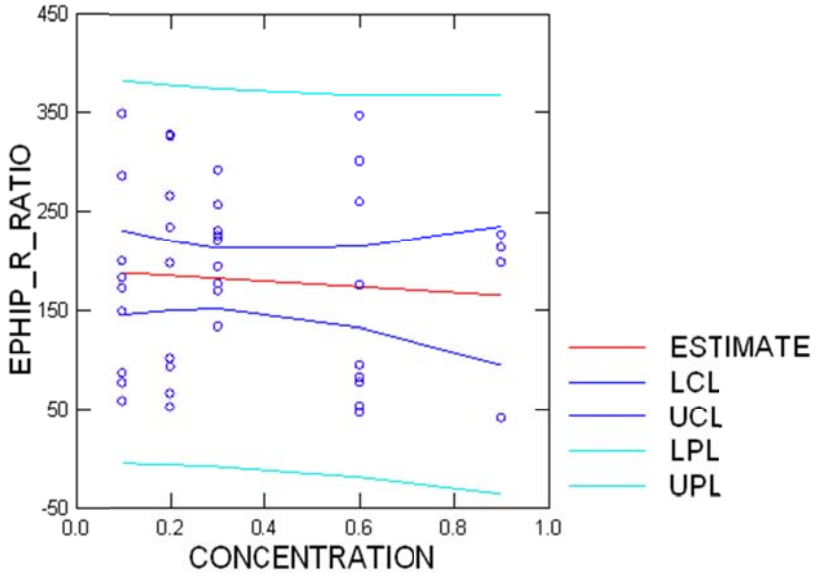

Figure 7: Regression comparing the ratio of ephippia to growth rate (r) across a concentration gradient. No significant differences were found (p=0.623)

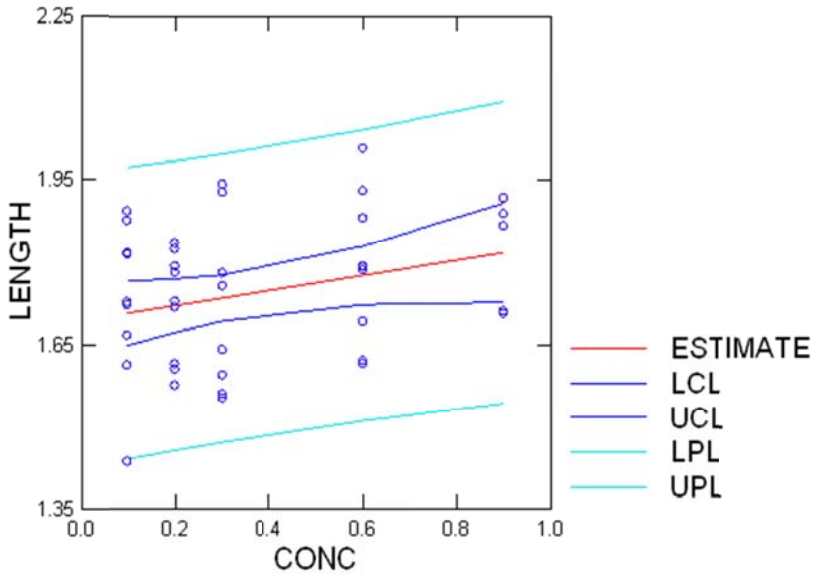

Figure 8: Regression comparing lengths of *D. pulex* across a concentration gradient. No significant differences were found (p=0.0.78).

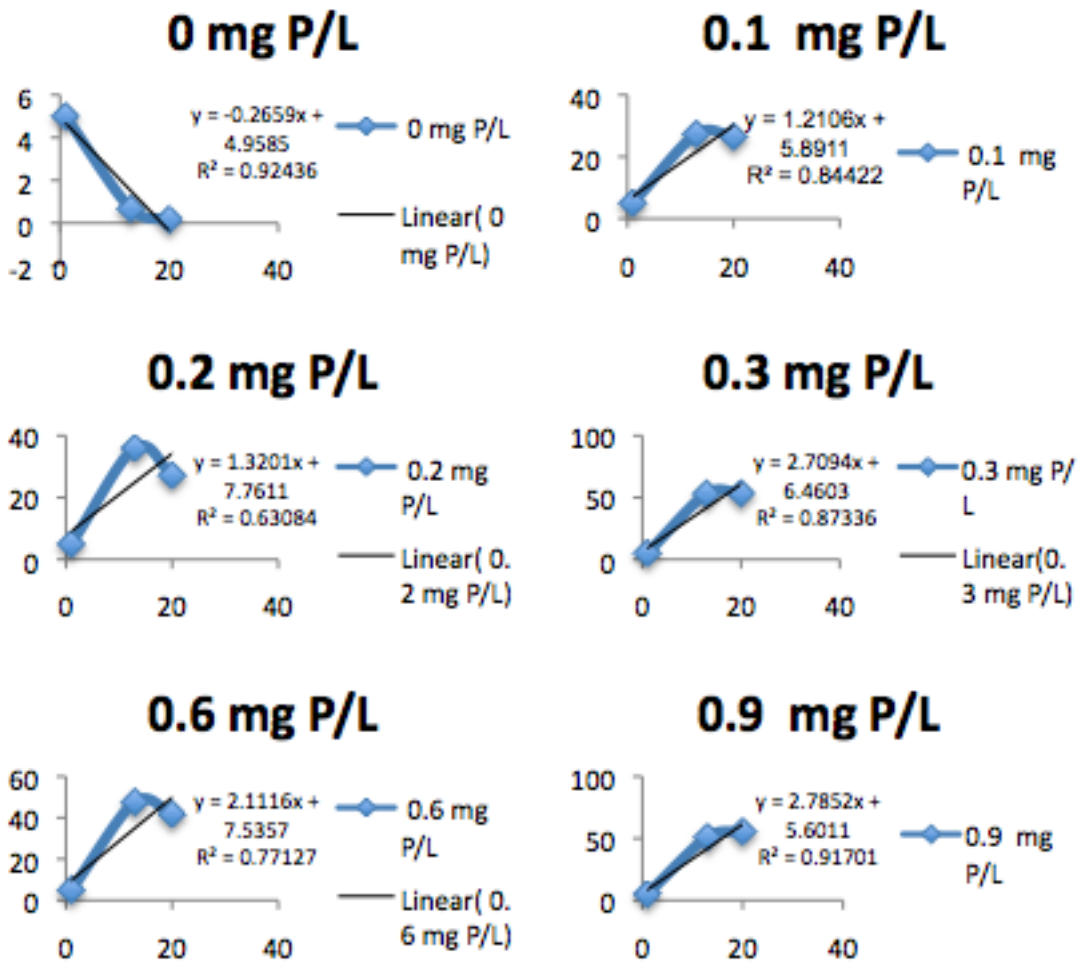

Figure 9: Graphs showing population growth and decline over trial period.