Thermal tolerance of *Daphnia pulex* in relation to their original location and body size.

BIOS 35502: Practicum in Field Biology

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Abstract

The purpose of this experiment was to find whether *Daphnia pulex* showed higher thermal tolerance if they came from a vernal pond that showed large temperature fluctuations. The experiment later began to study the heat tolerance of daphnids in relation to their body size. *Daphnia* were collected from vernal ponds exhibiting different environmental conditions on UNDERC property. They were then heated in a hot water bath to measure their half-life. pH and light intensity readings of each pond was also collected to see if they affect body size. In the end, I found that there was no correlation between a daphnid's heat tolerance and the vernal pools temperature fluctuations. I also found that a more acidic pH and/or higher UV rays have a direct correlation with smaller body size. Lastly, the *Daphnia* were found to show a higher heat tolerance if they were smaller in size. These findings could indicate that the size of *Daphnia pulex* will become smaller over time as areas like this experience higher temperatures due to global warming.

Introduction

The effects of global warming are one of the most important ecological concerns of the 21st century. It has and will have serious effects on the wilderness. As stated in 2002 by Root et al, "Over the past 100 years, the global average temperature has increased by approximately 0.6 °C and is projected to continue to rise at a rapid rate. Although species have responded to climatic changes throughout their evolutionary history, a primary concern for wild species and their ecosystems is this rapid rate of change."

As the earth's atmosphere continues to heat due to anthropogenic causes, some species are affected more than others. Among these species are ones that live in unstable environments such as shallow-water ponds (MacIsaac 1985). In these types of habitats, species must be able to survive in a wide range of conditions throughout the seasons. There are a surprising number of species that live in these impermanent vernal ponds; one species in specific is *Daphnia pulex.*

Daphnia pulex, or commonly known as the water flea, are small aquatic invertebrates that inhibit lakes, ponds, and streams. Over the past few years, daphnids have been widely researched. They have been studied extensively because they have strong ecological importance, such as being parts of aquatic food chains. In particular, they serve as food for small fish and are consumers of algae. Also, daphnids bodies are clear and their beating heart can be seen; therefore they have served as subjects for the study of depressants on the nervous system. Lastly, they can be used to measure the toxicity of water as well as rapid changes in water conditions.

Daphnia have been studied under different pH conditions. In one specific experiment done by Locke and Spurles (1999) they showed that *D. pulex* were "adversely affected by either acidic water or acidic phytoplankton." They found that in acidic water their average body sized decreased from 0.45mm to 0.41mm. Furthermore, they found that the population size in acidic environments was half of that in typical (neutral) water habitats (Locke & Spurles 1999).

Daphnia are filter feeders, meaning they are animals that feed by straining suspended matter and food particles from water. Past experiments have tested how changing environmental conditions affects *Daphnia* filtering rate. An experiment by Carolyn Burns (1969) demonstrated a correlation between temperature and filtering rate in different types of *Daphnia*, showing that the filtering rate at 20°C was higher than that at 15 or 25°C. This change gave indication that as temperature increases, there is a certain point where the filtering rate of daphnids starts to decrease, in this case between 20 and 25˚C(Burns 1969).

Also, the thermal tolerance of *D. pulex* has already been studied at length. One experiment in particular by MacIsaac et al. (1985) tested temperature acclimation and tolerance. Daphnids were able to withstand higher temperatures if they were acclimated at a higher temperature (MacIsaac 1985). This acclimation before testing was done in the lab but can also be applied to daphnids living in ponds with increased temperatures.

Lastly, it has been recognized that an increase in ambient ultraviolet (UV) exposure is a side effect of global warming. This increase in UV rays bouncing back onto the earth can have detrimental effects on many species on earth. There has been an experiment done to directly study the effect that temperature and UV exposure have on *D. pulex* growth. In this specific experiment, daphnids were exposed to high temperatures alone (25°C), high UV exposure alone (30 KJ m^{-2}), and both high temperature and UV exposure together (Williamson et al. 2002). What was found was that as each condition went up, the body size of the daphnids was observed to go down. Also, it was observed that when they were exposed to both stresses, they exhibited a uniform smaller body size as compared to the untreated daphnids (Williamson et al. 2002).

This study was designed to test the thermal tolerance of *Dapnia pulex* coming from multiple vernal ponds, each differing in temperature fluctuations. This will help to determine their response as it relates to climate change. This was done by measuring the temperature changes of a vernal pond over a designated period of time and comparing it to the half-life of each pond's sample population. I hypothesized that *Daphnia* coming from ponds of greater fluctuation will exhibit higher thermal tolerance compared to those from ponds with lower fluctuations. I also hypothesized that smaller daphnids would be able to withstand higher temperatures because they might already be acclimated to higher temperatures. Lastly, I believe that the same findings regarding the effect that pH and light intensity on body size will hold true in the vernal ponds on property.

Materials and Methods

Nine vernal ponds on the University of Notre Dame Environmental Research Center (UNDERC) property were tested (Figure 1). These ponds were chosen for two main reasons:

permanence and difference. I chose ponds that were a bigger or deeper, hopefully meaning they would not dry up in the early summer. I also found ponds that had different canopy coverings so the there would be a range of locations with different temperature fluctuations.

To test the temperature fluctuations of each vernal pond, I suspended a Hobo temperature datalogger just below the surface in each pond. The Hobo dataloggers were set to record the temperature every 12 minutes for 16 days, after which they were removed. For each pond, I also collected information about light intensity by using a LI COR datalogger and pH by using a handheld meter. These were taken only once at a time when most of the trials were being run.

To study daphnid temperature tolerance, I collected daphnids from each vernal pond and tested their half-life. The half-life is the time when exactly half of the organisms from each pond are dead. To collect the daphnids, I used a cup with a phytoplankton net at the bottom. I ran the cup through the water to catch the *Daphnia* in the net and rinsed them into labeled jars. Ten daphnids from each pond were placed in separate labeled medicine cups and floated in the warm water bath (temperatures ranged from 34 and 37°C). I watched each cup until half of the daphnids died and recorded the time. Appearance of daphnids and outside temperature were also taken at each trial. The sizes of the daphnids were measured using a micrometer. *Daphnia* from ponds 6, N, and K were studied secondly because there was extra time to confirm my original findings on the first six vernal ponds.

First, a multiple regression was used to test how pH and light intensity affect the size of daphnids. Then a linear regression was also performed on daphnid size versus half-life. Lastly, another linear regression was performed to determine if pool temperature variance affected the thermal tolerance of the *D. pulex* from that pond.

Results

This study demonstrated that *Daphnia pulex* is affected by both light intensity and pH. As the light intensity increased or pH of a pond became more acidic, the size of the daphnids would decrease (Figure 2). Both of these variables together showed significant results [$F_{df(2), df(5)}$ = 10.52, P = 0.016]. Also, there was an R^2 value of 0.899, meaning the trendline was a good representation of the data-points. When comparing temperature range to the half-life of the daphnids, there was a slight inverse relation, (Figure 3) but there was no significance [$F_{df(1), df(34)}$ = 0.375, P = 0.545]. There was also a R^2 value of 0.011, which further does not support the original hypothesis

To use the half-life data for statistics, it was better to measure the half-life of a certain pond starting after the first pond's half-life was recorded. This helped to correct for the fact that the temperature bath was not a uniform temperature throughout every trial. Before thermal tolerance was tested in each trial, it was observed that at 38°C all the daphnids would die really quickly (less than 4min) regardless of their size, thus trials would not be run this high. Lastly, when the final linear regression was run, there were significant results to show the correlation between daphnid body size and thermal tolerance [$F_{df(1), df(87)}$ = 73.594, P = <0.001]. There was also a R^2 value of 0.458, showing the regression line fits the data pretty well. It was seen that as the body size went down, the *D. pulex* would be able to withstand higher heat for a much longer time (Figure 4). The data for vernal ponds 30, WD, and 6 were the most similar when it came to light intensity(Table 1).

Discussion

Before the experiment started, it was only hypothesized that there would be a correlation between a vernal pools variation and the thermal tolerance of the *Daphnia* from that pond. The size and appearance of the daphnids only started being recorded after it was noticed that this trend was happening during the first few trials.

Previous studies have shown that UV rays and pH affects the size of *Daphnia* (Locke & Spurles 1999) and I found this trend as well in the vernal ponds on the UNDERC property. The vernal ponds that were studied had a wide range of canopy covering. In the ponds WD, 30, and 6 there was no vegetative covering over the pond, resulting in a very high light intensity. This high light intensity may have contributed to the over-all small size of the daphnids. Other vernal pools, such as J or K, had complete vegetative covering over them, which might prevent much of the ultraviolet rays from penetrating the water surface. Also, the pH of the ponds was slightly different (ranging from 4.6 to 6). The ponds that had a noticeably low pH generally had smaller daphnids even if the light intensity wasn't very high.

I hypothesized that daphnids from ponds with less vegetative cover (suggesting higher temperature fluctuations) would be acclimated to temperature extremes. However, after some initial observations, it was obvious that this was not the case. There was no relationship found between higher light intensity and acclimation of temperature extremes. Trends may have been shown if I was able to use an easier controlled water bath to test all the daphnids at the same temperature. It would also have been helpful to keep track of the vernal pond's temperature throughout the whole summer instead of just a few weeks.

However, this study did find that smaller daphnids are able to withstand higher temperatures for a longer period of time. *Daphnia* from ponds WD, 30, and 6 were all much smaller in size compared to the other ponds. They were measured to be almost 1/3 the size of the larger daphnids. Due to these daphnids small size, they were seen to have a longer half-life than bigger daphnids. It might be possible that because the daphnids from these ponds are smaller (from high UV rays) that they became more tolerant of the higher temperature over time. To increase the validity of this result, it might prove valuable to collect daphnids of different sizes all from the same location to make sure that only the size is affecting the thermal tolerance and not the characteristics of the vernal pond itself.

So what do these results mean for the future? It is possible that as time goes on, average *Daphnia* size may decrease in general. Numerous studies have already shown that daphnids in bodies of water with predators are much smaller to be less conspicuous to the predators (Carson *Personal Communication*). So with the pressure to be smaller to avoid predation and having to deal with higher UV exposure, it is possible that only the small daphnids are going to be able to survive and reproduce the next generation.

In conclusion, daphnids were not found to show a higher heat tolerance if they came from a pond that fluctuated greatly. However, they were found to exhibit higher tolerance if they were smaller in size. The small size could have been caused by the high UV radiation or low pH, which was found to effect body size. It will be interesting to see if the size of *Daphnia* decreases over time due to climate change.

Acknowledgements

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Tables and Figures

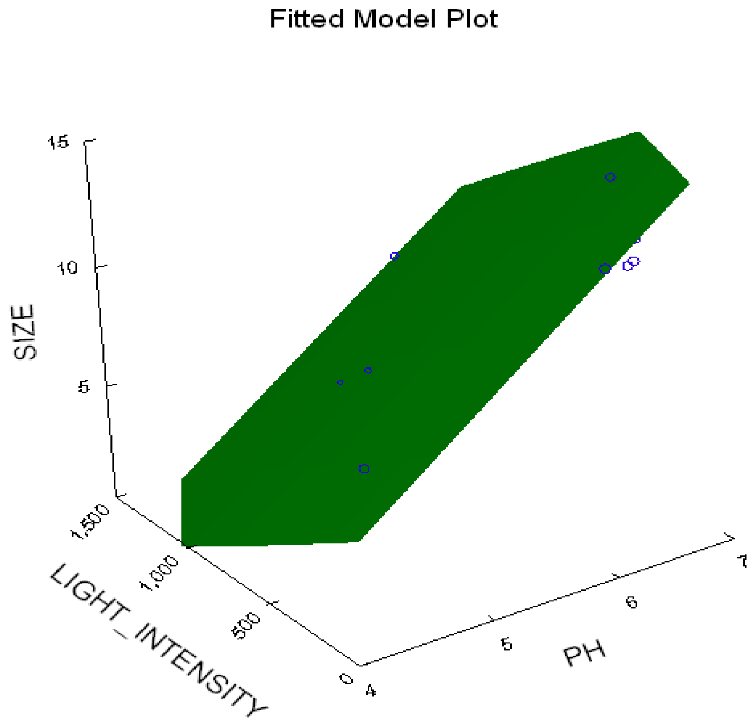

Figure 2: Multiple regression comparing light intensity and pH to *D. pulex* body size. As the light intensity goes up or the pH becomes more acidic, the average size of daphnids in the ponds decreases.

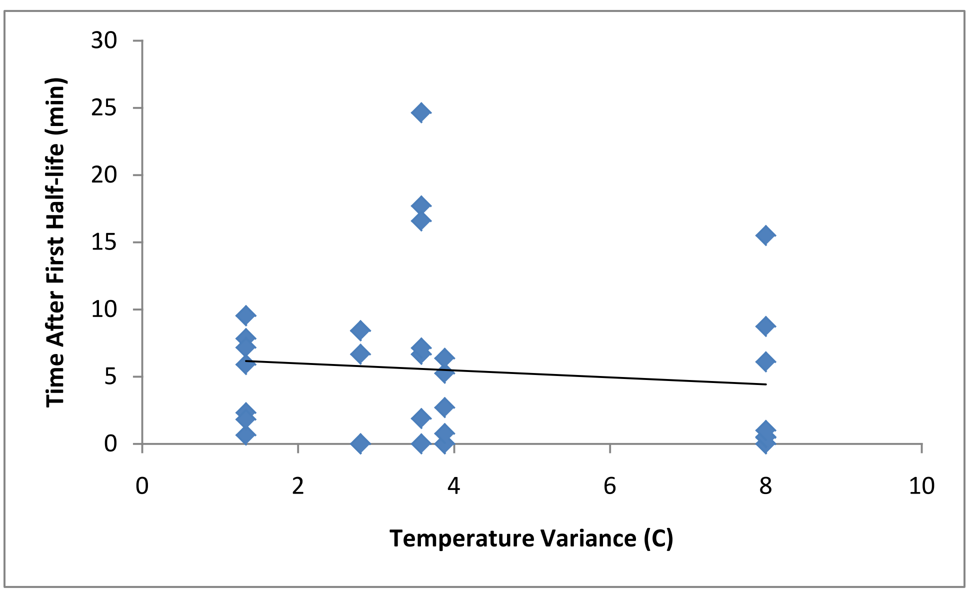

Figure 3: Linear regression comparing half-life of daphnids to vernal pond's temperature variance (range of temperatures a pond exhibits). No relationship was found as seen by the minute slope of the line

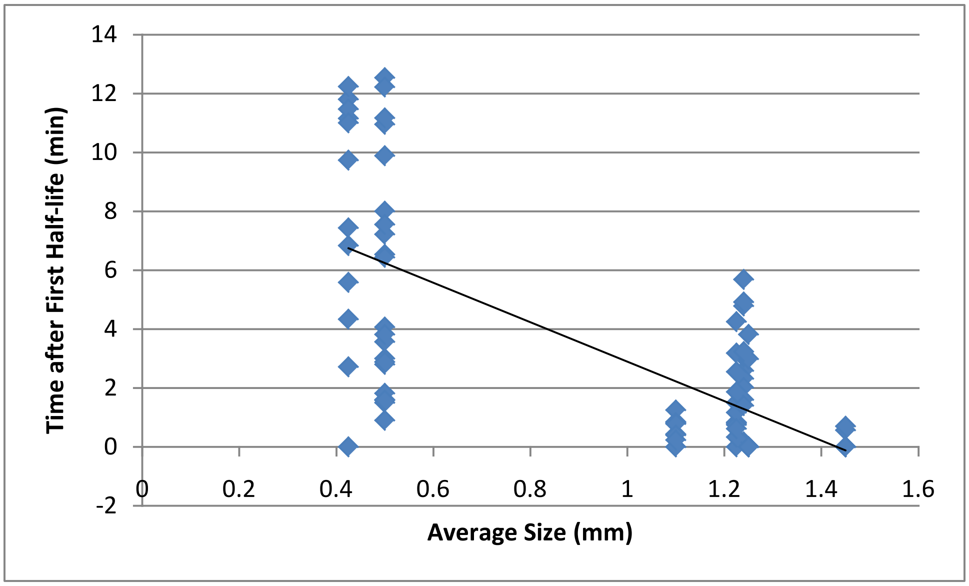

Figure 4: Linear regression showing the relationship between half-life and body size of *D. pulex.* As seen, the half-life of daphnids decreases as their size increases.

Table 1: Information gathered on each vernal pond and the *Daphnia from that location.* The temperature variance of ponds N, K, and 6 were not measured due to time constraints.

Vernal Pond	Average Size	Variance of Temperatures	Range of Temperature	pH	Light Intensity (UM)
30	4.25	8	19.47	5.5	1200
7	12.25	1.33	6.1	6	36
D	12.4	3.88	10.32	5.8	23
J	12.5	2.8	10.7	6	12
WD	5	3.58	9.93	5.6	1100
N	11	N/A	N/A	5.4	800
K	14.5	N/A	N/A	6.3	300
6	5	N/A	N/A	4.6	400

Appendices

Figure 1: Map of vernal ponds studied on UNDERC property.