The Effects of Temperature, Predator Density, Dissolved Oxygen, and Percent Emergent Plants on *Lithobates sylvatica* Tadpole Body Mass

BIOS 35502: Practicum in Environmental Field Biology

Nick Kalejs

Advisor: Dr. Matt J. Michel

2012

Abstract

Phenotypic plasticity is a known occurrence in wood frog (*Lithobates (Rana) sylvatica*) tadpoles. Tadpoles can adjust body morphology based on external environmental pressures such as water temperature and predation. What is less well known is how tadpole body mass responds to external pressures. In this study, I tested the effects of pond temperature, predator density, dissolved oxygen content, and percent emergent plants on the body mass of *L. sylvatica* tadpoles. Tadpoles were collected from nine ponds on the University of Notre Dame Environmental Research Center (UNDERC)-East property. Body mass was analyzed as average body mass per pond and as variability in body mass per pond. Variability in body mass was found to be positively correlated with predator density and with dissolved oxygen content. All other factors showed no correlation with average body mass or with variance in body mass. Though sample size was small, these results indicate that body mass can be affected by certain environmental pressures. They also point to the importance of the environment in shaping life histories.

Introduction

In the natural world, one important way to improve fitness is adaptation. The ability to adapt is often genetically determined, and developed over generations of natural selection. However, some species possess the unique ability to alter their expressed phenotype in response to environmental pressures. This is known as phenotypic plasticity, and is most simply expressed as environmentally-induced phenotypic variation (Stearns 1989). Individuals can use adaptive phenotypic plasticity to respond to dangers and stress in the environment and so grant themselves a better chance of survival (Pigliucci 2001). For example, some *Nicotiana* plant species can produce nicotine as an inducible defense after predation by an herbivore (Baldwin 1999).

Wood frog (*Lithobates (Rana) sylvatica*) tadpoles have been shown to display phenotypic plasticity in response to environmental pressures (Van Buskirk and Relyea 1998). Some of the best-studied and most well-documented pressures on tadpoles are pond temperature and the influence of predators. Both have been shown to have significant effects on the phenotype expressed by *L. sylvatica* tadpoles. The temperature of the pond environment in which tadpoles are raised can influence *L. sylvatica* growth in a variety of ways. For example, higher temperatures during egg incubation have been shown to promote increased hatching success, as well as longer tail lengths in successfully hatched tadpoles (Waldman 1982; Watkins and Vraspir 2006). Higher pond temperatures may also stimulate amphibian metabolic processes, and have shown a positive correlation with juvenile growth rates (Berven 1982). However, despite the faster growth rate associated with warmer water temperatures, warmer temperature waters produce tadpoles that are smaller in body size than those raised in cooler water temperatures (Watkins and Vraspir 2006). Because colder temperatures can prolong the period leading up to metamorphosis (Berven 1982), tadpoles from cooler ponds may simply have a chance to grow for a longer period of time than those from warmer ponds, and thus end up larger than their warm pond counterparts.

The presence of predators has also been shown to have a strong influence on *L. sylvatica* tadpole growth. In particular, an increased risk of predation leads to tadpoles with deeper tailfins, shorter bodies, and lower overall masses than those raised outside of the constant threat of predation (Relyea 2001). These traits have been shown to aid in predator avoidance (Smith and Van Buskirk 1995). Predators can also affect a decrease in activity, which can lead to a long term reduction in development and growth (Lawler 1989; Skelly 1992).

Other factors that could influence tadpole growth in ponds include dissolved oxygen content and the percentage of the pond covered by emergent plants. While little research thus far has focused on the direct effects of dissolved oxygen on tadpole growth, it has been demonstrated that increasingly hypoxic conditions can lead to a reduction in overall metabolic rate in green frog (*Lithobates clamitans*) tadpoles (Moore and Townsend 1998). Even less research has focused on the effects of emergent plant cover on tadpole growth; however, emergent plants do inhibit the growth of algae, an important food source for tadpoles (Songyan et al. 2011).

In this study, I was interested in the effects of temperature, predator density, dissolved oxygen content, and percent emergent plants on *L. sylvatica* tadpole growth. In particular, I aimed to analyze the effects of these four factors by recording both average tadpole mass and distribution of tadpole mass (variability of tadpole mass) in ponds at the University of Notre Dame Environmental Research Center (UNDERC)-East. This property, located on the border of Wisconsin and Michigan, contains a wide variety of vernal and permanent ponds, thereby providing ample habitat for *L. sylvatica* tadpoles. Additionally, *L. sylvatica* is ideal for studies in comparative tadpole growth as all individuals in an area breed synchronously over the span of a few days (Waldman 1982).

Each factor lent itself to two hypotheses: one for average tadpole mass, and the other for tadpole mass variability per pond. First, it is predicted that an increase in temperature will lead to larger tadpoles and increased variance among tadpole mass distribution. Warmer water should stimulate tadpole growth. While this may lead to earlier metamorphosis, in this study all measurements will be taken prior to transformation and within a relatively small time period. The small sampling period will help ensure that all tadpoles will have had roughly the same amount of time to grow, as breeding in *L. sylvatica* is synchronous (Waldman 1982). Measurements taken prior to metamorphosis should eliminate the effects of a longer juvenile growth phase by ending growth at a similar time for all individuals. Warmer water could also remove a factor (cold temperatures) that limits tadpole growth, and so allow certain tadpoles to flourish more than others. This would lead to higher variance among tadpole mass distributions within a pond. Second, it is predicted that an increase in predator density will lead to a reduction in average tadpole mass but an increase in mass variability. Predation has been shown to lower tadpole mass, but only if predators are consistently present. Because ponds can possess high variability in many environmental factors (Alford 1986), such as predator density, it is possible that some tadpoles may experience higher predation risk than other individuals within the same pond. Third, it is predicted that an increase in dissolved oxygen will result in larger tadpoles and higher variance among tadpoles. Higher levels of dissolved oxygen could increase metabolic rate, and so affect larger growth. Similarly to temperature, more oxygen could remove the effects of a limiting resource and may allow for some tadpoles to grow more strongly than others, thus resulting in higher variability of tadpole mass within a pond. Finally, it is predicted that an increase in percent emergent plants will lead to a reduction in both average tadpole mass and variability of mass. Because emergent plants inhibit algae production, and algae are an important food source for tadpoles, they may make food a limiting resource that could set limits on average tadpole growth. Furthermore, a food shortage would only permit the strongest tadpoles to survive. In species similar to *L. sylvatica*, large tadpoles have been shown to outcompete smaller ones (Steinwascher 1979). This would lead to a reduction in mass variability, as only the larger tadpoles would be able to survive a food shortage.

Materials and Methods

To perform this study, I first located viable ponds with *L. sylvatica* tadpoles. Ponds with tadpole populations were found using a variety of techniques, including visual identification, dipnetting, and trapping via minnow traps. Once *L. sylvatica* tadpoles were identified in a pond, I continued sampling at that location until a minimum of seven tadpoles was collected from the pond. Once collected, I recorded mass in grams for each tadpole.

For each pond, a variety of measurements were also performed. I collected temperature (^oC) in three times throughout the summer. Each sampling period was spaced about three weeks apart, to account for natural fluctuation in summer temperatures. Daily temperature fluctuations were also accounted for, as all temperature sampling occurred within a two hour period, between 1400 and 1600. I also sampled on days with similar weather conditions as much as possible, although there were some unavoidable variations. I then took the mean of all three sampling periods for each pond to get an average temperature for each pond over the summer.

Dissolved oxygen (mg/L) readings were also taken from each pond, using a dissolved oxygen meter (Yellow Springs, Inc.). I took three readings per pond, at three separate locations within each pond. I then used the mean of those readings to get a dissolved oxygen value for each pond.

I also measured predator density and percent emergent plants in each pond (following Michel 2010), using a PVC pipe with diameter 31 cm and length 70.5 cm. At each pond, I established a transect across the width of the water. Every five meters, I stopped and inserted the PVC pipe into the water until it rested on the bottom of the pond. Within the pipe, I then estimated the percentage of emergent plants. I also used a dipnet to collect all predators that were captured in the pipe. I recorded the number of predators found in each pipe sample, and then took the mean of all pipe samples in a pond to get an average predator density value for each pond.

After data collection, statistical tests were run using the program SYSTAT (Cranes Software International Ltd.). Eight linear regressions were performed, with two dependent variables tested: average mass per pond and variability of mass per pond (measured as standard deviation from the mean). Each dependent variable was regressed against one of four independent variables: temperature, dissolved oxygen, predator density, and percent emergent plants. Regressions using temperature as a factor used data from all nine ponds sampled, while all other factors used only eight ponds, with one pond being discounted as an outlier.

Results

All four linear regressions comparing average tadpole mass per pond showed no significance for any factor. Temperature (p=0.633, R^2=0.029, $t_{(8)}$=-0.455), predator density(p=0.104, R^2=0.379, $t_{(7)}$=1.914), dissolved oxygen (p=0.137, R^2=0.329, $t_{(7)}$=1.715) and percent emergent plants (p=0.235, R^2=0.225, $t_{(7)}$=1.318) all were not significant. Table 1 demonstrates the lack of any significant trend in these regressions.

All four linear regressions comparing tadpole mass variability (through standard deviation) in each pond to all factors showed slightly different results. Comparisons with temperature (p=0.347, R^2=0.127, $t_{(8)}$=-1.008) and percent emergent plants (p=0.416, R^2=0.113, $t_{(7)}$=0.874) were both not significant. This is clearly shown in Table 2. Predator density (p=0.020, R^2=0.623, $t_{(7)}$=3.149) and dissolved oxygen (p=0.049, R^2=0.501, $t_{(7)}$=2.455), however, both showed significant positive relationships with tadpole mass variability. These significant trends are evident in Table 2, as well as in Figures 1 and 2.

Discussion

There were several factors that did not have a significant relationship with tadpole size, which could be due to a variety of reasons. Temperature was not found to have a significant effect on average tadpole mass. More than likely, this was due to a combination of environmental variation and experimental error. Much of the environmental variation stemmed from issues in weather. Despite attempts to sample ponds during similar weather conditions, sampling had to be performed during specific weeks that did not always provide ideal circumstances. For example, sampling occurred across a range of ambient air temperatures, spanning 18-27 ^{o}C. Also, weather fluctuated between sun, clouds, and rain across all sampling periods. Furthermore, all ponds may not have received their water supply in the same way. As not all sampling sites were vernal ponds, there may have been a wide range of ways in which the ponds gained water. Some may have been largely surface fed, while others may have been spring fed. This may have strongly altered how much weather conditions affected pond temperature, and further skewed measured data. These unavoidable variations likely had some effect on recorded pond temperatures, and may have contributed to the non-significant relationship with average tadpole mass. Temperature also did not have a significant effect on tadpole mass variability per pond. Again, environmental variations likely had a strong influence on data viability. As with all four factors, sample size also provided a large problem for comparisons with temperature. With only nine ponds sampled, there simply was not enough data to definitively say anything about the possible effects of temperature. Individual ponds often had too much influence or leverage on the overall statistical analysis, and so disproportionately affected the outcome.

Predator density also had no significant effect on average tadpole mass. Again, sample size was more than likely a large issue here. However, it is possible that other factors came into play as well. For example, predators may potentially have been more or less active depending on the time or the weather conditions at sampling. This may have altered the total predator count in each pipe sample, and so changed predator density numbers. Predator density did, however, have a significant effect on *L. sylvatica* mass variability per pond. This fit with my hypothesis, which predicted higher mass variability due to pond microhabitats. The data suggest that predators may not have been evenly dispersed throughout the pond, leading to unequal effects on tadpole mass and higher mass variability. While my hypothesis was supported, it would still be valuable to further investigate this relationship with a larger sample size. Also, it would be interesting to study exactly how predators are distributed throughout a pond, and whether they do indeed favor specific microhabitats.

Dissolved oxygen content also did not have a significant effect on average tadpole mass. As with predator density, it is very possible that dissolved oxygen fluctuates between microhabitats in ponds. Oxygen can vary greatly across aquatic environments (Breitburg 1992), and so affect tadpole growth in different, localized ways rather than in a broad trend. Variance in oxygen levels can also distort measurement accuracy. This accuracy could also be changed by levels of plant respiration, which can naturally vary throughout a day. Dissolved oxygen content, however, did significantly affect variability of *L. sylvatica* tadpole mass per pond. This fit with my hypothesis, which predicted higher mass variability due to the removal of a limiting resource for tadpoles. The data suggest that more dissolved oxygen may indeed allow for some tadpoles to capitalize on abundant resources and grow larger than others. However, as with predator density, it would be useful to analyze the effects of dissolved oxygen in a larger sample size and in specific pond microhabitats to further support any correlation with mass variability.

Percent emergent plants also did not significantly affect average tadpole mass. All four factors, then, did not have a significant effect on average tadpole mass. As with the other factors, sample size was simply too small to provide much useful data. However, it is possible that there was not enough emergent plant cover to inhibit algal growth to the point that its function as a tadpole food source was affected. There was also no significant correlation between percent emergent plants and *L. sylvatica* mass variability per pond. Again, if emergent plants failed to inhibit a major tadpole food source, then there may not have been any measureable effects on mass variability per pond.

Overall, this study suffered from a small sample size, but nevertheless yielded some significant results. Predator density and dissolved oxygen were both shown to have positive relationships with variability of tadpole mass per pond. This suggests that predators may be only present in certain areas of ponds, and that dissolved oxygen may serve as a limiting factor in tadpole growth. However, it is unclear if those relationships occurred exactly as hypothesized. Little literature exists on these specific relationships; future studies might choose to emphasize the exact, local effects of predator density and dissolved oxygen. For non-significant data, it is likely that sample size had a pronounced effect on the outcome of all statistical analyses. However, it is also quite likely that each of the four independent variables had a strong influence on all other factors. Interactions between factors may have occurred, and could have further caused deviations from expected results. General linear models were briefly run to investigate this possibility, but small sample size nullified their effectiveness. Another potential issue is that of pond water loss. Some ponds completely dried, while others lost most of their water, before a large rainfall restored water levels. This occurred while sampling was still happening, so data are spread out both before and after the rainfall event. Issues here could include death of the original predator populations due to a lack of water, and potential alterations in independent variable consistency or magnitude upon refilling. Again, this is another variable that makes it difficult to draw definitive conclusions from the results.

Going forward, it would be advantageous to perform further studies with a much larger sample size. I would also suggest searching for microhabitats within each pond. These may provide additional insight into the distribution of each factor within a pond, and how that distribution changes each factor's influence. Furthermore, it may be beneficial for future studies to leave temperature and dissolved oxygen probes in the ponds throughout the entire sampling period. This could help eliminate the high degree of variability that occurred due to isolated and unpredictable sampling episodes. Above all, future studies should continue to emphasize the importance of phenotypic plasticity. It is essential to remember the role of the environmental in all scientific studies. Knowledge of how the environment shapes the biotic world will only provide added insights into the workings of nature, and into nature's effects on our own lives.

Acknowledgements

I would like to thank my advisor, Dr. Matt Michel, who provided the foundation for my entire project and lots of invaluable experience. I would also like to thank Luke DeGroote, Maggie Wisniewska, Matt "Squiggles" Igleski, and Dr. Michael Cramer for answering endless questions about design, statistics, and life. Thanks also go out to Chelsea Gyure for her photography skills and for keeping me sane during intense weeks of research. Finally, I would also like to thank the Bernard J. Hank Family endowment for making the entire UNDERC experience possible.

Literature Cited

Alford, R.A. 1986. Habitat use and positional behavior of anuran larvae in a northern Florida temporary pond. *Copeia* 1986:408-223

Baldwin, I.T. 1999. Inducible Nicotine Production in Native Nicotiana as an Example of Adaptive Phenotypic Plasticity. *Journal of Chemical Ecology* 25(1):3-30.

Berven, K.A. 1982. The Genetic Basis of Altitudinal Variation in the Wood Frog Rana sylvatica. I. An Experimental Analysis of Life History Traits. *Evolution* 36:962-983.

Breitburg, D.L. 1992. Episodic Hypoxia in Chesapeake Bay: Interacting Effects of Recruitment, Behavior, and Physical Disturbance. *Ecological Monographs* 62(4):525-546.

Lawler, S.P. 1989. Behavioural responses to predators and predation risk in four species of larval anurans. *Animal Behaviour* 38(6):1039-1047.

Michel, M.J. 2011. Spatial dependence of phenotype-environment associations for tadpoles in natural ponds. *Evolutionary Ecology* 25(4):915-932.

Moore, M.K. and V.R. Townsend, Jr. 1998. The Interaction of Temperature, Dissolved Oxygen and Predation Pressure in an Aquatic Predator-Prey System. *Oikos* 81(2):329-336.

Pigliucci, M. 2001. Phenotypic Plasticity: Beyond Nature and Nurture. JHU Press, Baltimore, Maryland.

Relyea, R.A. 2001. The Lasting Effects of Adaptive Plasticity: Predator-Induced Tadpoles Become Long-Legged Frogs. *Ecology* 82(7):1947-1955.

Skelly, D.K. 1992. Field Evidence for a Cost of Behavioral Antipredator Response in a Larval Amphibian. *Ecology* 73(2):704-708.

Smith, D.C. and J. Van Buskirk. 1995. Phenotypic Design, Plasticity, and Ecological Performance in Two Tadpole Species. *The American Naturalist* 145(2):211-233.

Songyan, Q., Z. Qixing, Z. Jinxi, and D. Yaqi. 2011. Algae Growth Inhibition by the Combination of Emergent Plant and Submerged Macrophytes. *Advanced Materials Research* 183-185:1349-1353.

Stearns, S.C. 1989. The Evolutionary Significance of Phenotypic Plasticity. *BioScience* 39(7):436-445

Steinwascher, K. 1979. Competitive Interactions among Tadpoles: Responses to Resource Level. *Ecology* 60(6):1172-1183.

Van Buskirk, J. and R.A. Relyea. 1998. Natural selection for phenotypic plasticity: predator-induced morphological responses in tadpoles. *Biological Journal of the Linnean Society* 65:301-328.

Waldman, B. 1982. Adaptive Significance of Communal Oviposition in Wood Frogs (Rana sylvatica). *Behavioral Ecology and Sociobiology.* 10:169-174.

Watkins, T.B. and J. Vraspir. 2006. Both Incubation Temperature and Posthatching Temperature Affect Swimming Performance and Morphology of Wood Frog Tadpoles (Rana sylvatica). *Physiological and Biochemical Zoology* 79:140-149.

Table 1. Statistical results of mass regressions. Mass was compared to temperature, predator density, dissolved oxygen, and percent emergent plants. No data was significant.

Factor	Coefficient	Standard Error	R^2	t	p
Temperature	-0.013301	0.029203	0.028780	-0.455444	0.662580
Predator Density	0.153127	0.079993	0.379160	1.914243	0.104094
Dissolved Oxygen	0.063491	0.037012	0.329061	1.715427	0.137088
Percent Emergent Plants	0.014396	0.010919	0.224633	1.318433	0.235449

Table 2. Statistical results of standard deviation regressions. Standard deviation was compared to temperature, predator density, dissolved oxygen, and percent emergent plants. Significant data is in bold.

Factor	Coefficient	Standard Error	R^2	t	p
Temperature	-0.009445	0.009119	0.126714	-1.007821	0.347100
Predator Density	0.071118	0.022585	0.623003	3.148848	**0.019844**
Dissolved Oxygen	0.028388	0.011564	0.501099	2.454879	**0.049462**
Percent Emergent Plants	0.003697	0.004232	0.112828	0.873534	0.415960

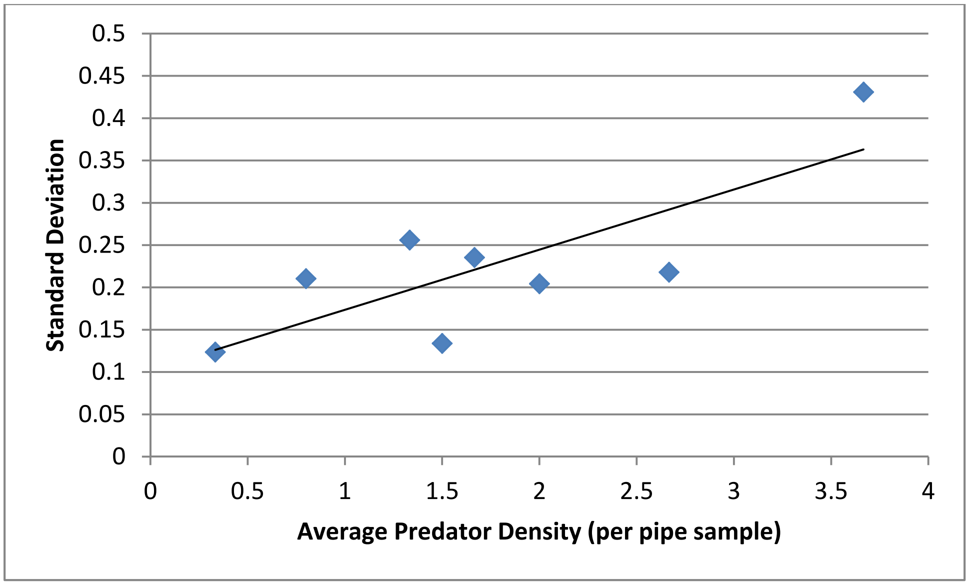

Figure 1. The effects of average predator density on standard deviation of *L. sylvatica* tadpole masses. There was a significant positive correlation between average predator density and standard deviation of masses (p=0.019844, R^2=0.623003).

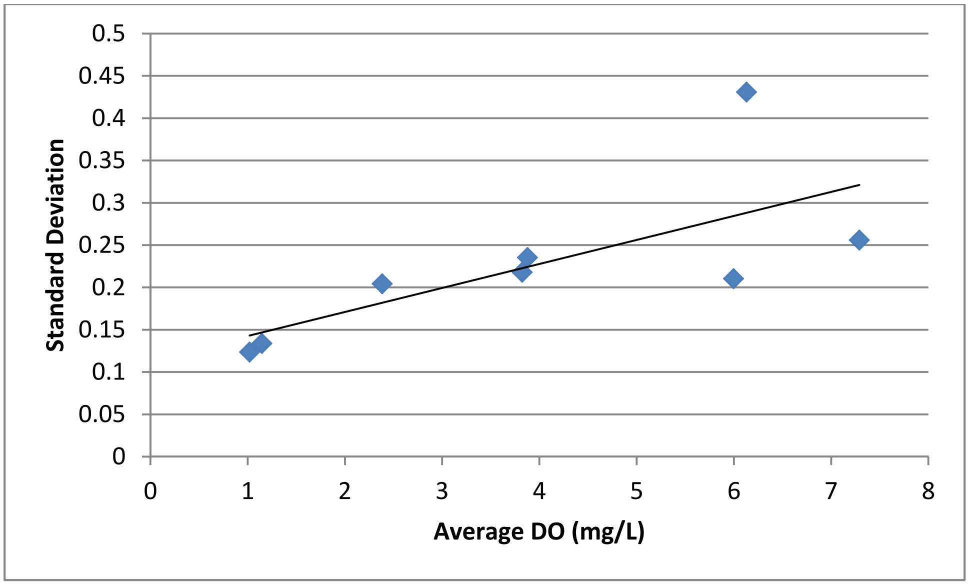

Figure 2. The effects of average dissolved oxygen content on standard deviation of *L. sylvatica* tadpole masses. There was a significant positive correlation between average dissolved oxygen content and standard deviation of masses (p=0.049462, R^2=0.501099).