Plastic responses in juvenile wood frog (*Rana sylvatica*) morphology from predation

BIOS: 569: Practicum in Field Biology

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2013

Abstract-

Phenotypic plasticity is the ability of an organism to modify behavior, morphology, and life history traits in response to various environmental cues. Plasticity influences the structure of population dynamics and also gives insight to future environmental conditions. Previous common garden experiments demonstrate that larval wood frogs (*Rana sylvatica*) alter morphology in response to chemical cues exuded from predators. The purpose of this field experiment is to accentuate the relationship between larval predation risk and juvenile morphology in wood frogs inhabiting heterogeneous vernal ponds. I measured five morphological characters including forelimb length, hind limb length, head width, body width, and body length and correlated each against predator densities of six study sites. Predation did not significantly affect juvenile morphology. However, an overshadowing effect from other complex interactions within the ecosystem can further explain the variance among the data.

Introduction-

Phenotypic plasticity is defined as the ability of a single genotype to exhibit variable phenotypes according to different environments (Whitman 2009). Plasticity can manifest as changes not only in physical appearance, but physiology, behavior, and life history traits. Both biotic and abiotic factors influence these responses, which produce phenotypic changes that can be highly adaptive by enhancing survival or maximizing fitness; furthermore, these changes can lower fitness by increasing energy allocation during developmental stages (Weider and Pijanowska 1993). The fitness of an organism depends, in part, on the interaction between the environment and phenotype. Understanding the habitat, physiological mechanisms, and fitness outcomes is imperative to grasp the effects of plasticity. Phenotypic plasticity facilitates

evolution, biodiversity, structures of ecological communities, and generates novel traits (Whitman 2009). Under certain conditions plasticity is beneficial, yet many scenarios concerning the costs, benefits, and selection of plasticity remain untested.

Prey alter their phenotypes as a method to reduce predation, though antipredator adaptations can sustain costs in predator free environments (Relyea 2001a, 2002a). This tradeoff has caused debate whether these plastic responses are adaptive and retained by natural selection (Relyea 2001b). Recent studies have delved into predator-prey interactions within aquatic systems revealing that prey express phenotypic responses in the presence of chemical cues exuded from predators (Weider and Pijanowska 1993). A previous study has demonstrated that selective pressures imposed by invertebrate predators are a key influence on the cladoceran life histories and resource use; as a result, influencing age and size structures of populations (Weider and Pijanowska 1993). For example, when exposed to invertebrate predator cues Daphnia allocate more energy towards growth (Spitze 1992, Weider and Pijanowska 1993). In this case the larval environment matched environmental conditions during the adult stage and the plastic responses are assumed as adaptive. For many organisms this is not true, amphibians spend the larval stage in an aquatic environment and as adults they inhabit terrestrial environments (Relyea 2001b). Biotic and abiotic factors differ widely between the environments in each development stage. So, larval plasticity could have a positive, negative, or neutral effect on adult fitness in amphibians (Relyea 2001b).

Accordingly, amphibians, and more specifically *[Rana (Lithobates) sylvatica*], provide excellent study subjects for predator-induced plasticity. First, amphibians are known to alter morphology, life histories, and behavior in predator dense environments (Relyea 2002a). Larval

development becomes extended from heightened alertness towards predation risk causing a reduction in foraging activity (Relyea 2001b). Relyea (2003a) found that eastern gray treefrog tadpoles (*Hyla versicolor*) responded to predators by developing deep tails, short bodies, and small mouthparts at a cost of slower development. Likewise, *R. sylvatica* tadpoles become less active and develop large tails with comparably small bodies-- making them better adept to escape larval predators (Relyea 2002a). Secondly, amphibians span across a wide range of habitats which exposes them to a diverse selection of aquatic and terrestrial predators (Relyea 2002a). Predator transition refers to the difference in community structure between each habitat (Welborn et al. 1996). A vernal pond with more than 40% canopy coverage is classified as closed canopy and anything less is open canopy (Garrison and Standiford 1996). Open canopy ponds tend to contain a higher assemblages of invertebrate predators because higher productivity from more sunlight (Relyea 2002a). This enables a higher density of aquatic vegetation which is critical for benthic macroinvertebrates. It provides food resources and refuges from predators (Warfe et al. 2004). Habitat structure attracts benthic macroinvertebrates, breeding amphibians, and other prey, thus increasing the presence of predatory invertebrates (Werner 1999). Additionally, closed canopy habitats have lower temperatures and dissolved oxygen levels, which suppresses larval development (Garrison and Standiford 1996, Warfe et al. 2004); creating a selective pressure for invertebrates to colonize open canopy ponds at the cost of increased predation risk. Lastly, *R. sylvatica* exhibit high philopatry to natal ponds which encourages reproductive isolation (Relyea 2002a, Michel 2011). Therefore, in a spatial scale, individuals remain genetically distinct from other populations when expressing plastic responses (Relyea 2002a). This genetic variation produces a wide array of tadpole phenotypes to determine the correlation between larval plasticity and juvenile morphology (Relyea 2001b).

However, the effect of predators and predator density on phenotypic plasticity across heterogeneous habitats on a regional scale remains unclear (Michel 2011). Thus, I intend to study predator-induced plasticity in larval wood frogs (*Rana sylvatica*) among different vernal ponds using a field experiment. Based on previous studies about the lasting effects of larval predation on juvenile morphology, I hypothesize that an increase in larval period caused by predator presence will cause: (1) juveniles emerging from ponds with high predator densities to exhibit longer forelimb and hind limbs; and (2) narrower head and back widths.

Methods-

*Data Collection*

A field experiment will determine the effect of predators on plasticity in *R. sylvatica*. I raised tadpoles using 2x2 foot enclosures made of a wood frame with fiberglass screening on all sides at 8 different vernal ponds (labeled VP-P, VP-9, VP-Q, VP-Wood Duck, VP-K, VP-J, VP-30A, VP-27; see Fig. 1) located on the University of Notre Dame Environmental Research property. One enclosure was used at each study site and housed 10-20 juveniles (except at VP-30A, VP-P). The enclosures keep the tadpoles isolated, but allow them to receive chemical cues from surrounding predators. The density of aquatic vegetation and canopy cover will be accounted for in each pond. This is important because an open-canopy pond has higher productivity from photosynthetic resources resulting in a higher abundance of macrophytes, predators, and competitors (Werner 1999). I selected the sites based on the amount of canopy coverage which should mimic a gradient of predator densities.  These sites range in predator density, from the small, covered vernal ponds that have very little predators (VP-K, VP-J), to the larger, open ponds (VP-P, VP- Q, VP-30A) that have higher predator diversities and densities.

The remaining vernal ponds (9, Wood Duck, 27) represent the middle of the gradient between predominantly open or closed. I sampled at all study sites to measure the predicted predation risks.

The sampling methods for determining predator abundance included minnow traps and dipnetting. Two minnow traps were set at all study sites for six nights during three non-consecutive weeks and checked every morning. I used dip nets as the primary sampling method. I spent a total of 80 minutes at each site sweeping for predators along the pond edges. *Dytiscus* adults and larvae, Odonate larvae, and Belostomatidae comprised the species of predators found. Densities were calculated by taking the total abundance among all species and dividing that by the area of the pond. Similar methods were used to capture larval *R. sylvatica* and emerging juveniles. I constructed single pit fall traps later in the summer at sites VP-30A and VP-P because no tadpoles were captured by dipnetting or minnow trapping. These traps were constructed out of three 1-liter buckets, two 15-foot aluminum flashing pieces, and wooden stakes for anchoring. I also used visual encounter surveys to capture recently emergent *R. sylvatica* along the pond edges.

Morphological measurements such as forelimb length, hindlimb length, head width, back width, and body length were recorded using 0.1 mm calipers. I omitted tail length and depth because at this stage in metamorphosis some individuals had begun to absorb their tails; therefore tail fin measurements would not be a good indicator of plasticity.

*Statistical Analysis*

A single mean value for each the five different morphological features was calculated by averaging the values measured from 13 individuals at each site. To correct the data for general variability in body size I ran a regression analysis using body length as the independent variable. Only hind limb length ($p$=0.00363, $r^2$= 0.9032) was significantly related with body length. I corrected this measurement for body size by taking the average of the residuals and substituting those values as the dependent variable in my regression analysis. Forelimb length ($p$=0.4801; $r^2$=0.5495), body width ($p$=0.2569, $r^2$=0.3039), and head width ($p$=0.1058, $r^2$=0.5201) were not significantly related with body length and therefore not corrected for body size. To determine significance, I ran a simple linear regression correlating the mean measurements of juvenile morphology against predator density.

Results-

Predator densities calculated for each pond are listed in increasing order per square meter (see Table 1). The gradient among predator densities roughly increased as sites changed from closed to open canopies. However, sites that were neither predominantly open nor closed seemed to score lower in predator densities. These sites produced similar abundances in predators but tend to be larger in area, therefore causing a discrepancy in the density gradient.

At least 15 tadpoles were captured by minnow traps and kept in the screened enclosures at sites 27, J, 9, and K. The minnow traps did not produce as many for sites Q and Wood Duck, so I caught juveniles during visual encounter surveys. Two sites (VP-30A, VP-P) were excluded from collecting morphological characteristics because only one individual was captured at each site by pit fall traps, and would not correctly represent the population.

A regression analysis determined there is no significant correlation between juvenile morphology and predator density. The values produced by running five separate regressions are as follows: Forelimb length (p= .436, r²= .147, Fig. 2), hind limb length (p= .441, r²= .154, Fig. 3), head width (p= .868, r²= .008, Fig. 4), back width (p= .781, r²= .022, Fig. 5) and body length (p= .406, r²= .177, Fig. 6).

Discussion-

Correlations between the morphological phenotypes of tadpoles and predator density failed to uphold the original hypothesis. Predator densities did not significantly increase juvenile limb length or decrease body width.  Previous laboratory experiments (Relyea 2002a) have found relationships in predator-induced responses. These significant results imply that more complex interactions affect juvenile morphology in the field.

The data evidently suggests that larval predation has little to no effect on juvenile morphology. Previous studies (Lardner 1998, Relyea 2001) concluded that larval anurans did not exhibit difference in morphology when in the presence of *Dytiscus* larvae. However, stronger interactions within the community could overshadow predator-induced effects. Habitat heterogeneity plays a large role in the development of larval wood frogs and may explain the variation among the results (Michel 2011). Biotic and abiotic factors such as hydroperiod, canopy coverage, and food resources differ among ponds. For instance, the study system focused on vernal ponds, which in most cases are temporary. Inhabiting a non-permanent habitat presents a new array of issues to adapt to. The life history qualities of species that live in permanent habitats, such as lakes, differ from species that inhabit vernal ponds. Vernal ponds run the risk of drying up, so species are known to hasten development to counteract this issue (Relyea 2001, Relyea and Auld 2005). Habitat permanence places a limit on the time allotted to develop plastic

traits (Relyea 2001, Miner 2005). The accelerated metamorphosis may have created a time lag in the appearance of plastic traits as found in Relyea and Hoverman's (2003) study. The authors found that plastic effects were not displayed until a month after metamorphosis. A study conducted by Weider and Pijanowska (1993) confirmed the occurrence of age dependence on the ability to sense predator-released chemical cues. The juvenile frogs collected for this study were in early post-metamorphic stages and beginning to emerge. It is possible that not enough time had passed for the morphological effects to appear. Future studies should focus on the relationship between development stage and the individuals' affinity to detect chemical cues in wood frogs.

Secondly, individual larval behavior can have similar effects on morphology as habitat heterogeneity and age class. Ephemeral species, such as *R. sylvatica*, may exhibit more behavioral plasticity rather than morphological plasticity. Behavioral plasticity is an effective short-term solution to predation while morphological plasticity is a long-term solution (Relyea 2001). However, wood frogs exhibit several discrete life stages, and may focus on current survival rather than allocating for future predation risk once they emerge from the vernal pond (Relyea 2002b, 2003a). It is less costly, in terms of energy allocation, to hide than to grow larger than optimal size to thwart predation risk (Relyea 2001). In this instance, *R. sylvatica* indirectly decreases body size to avoid predation. Furthermore, the invertebrate predators could directly cause growth suppression as found for *Daphnia* (Weider and Pijanowska 1993). The presence of predators and chemical cues causes increased alertness which could require greater energy costs; consequently, reducing growth and development rates.

Additionally, an individual's phenotype is the combined product of plasticity, genetics, and mechanisms of natural selection. Heritable genotypes vary among local populations. Thus,

genetic effects may cause significant variation in phenotypic morphology among wood frog populations (Michel 2011, Relyea 2002a). Existing results confirm that response to predator-released chemical cues vary according to local genetics (Weider and Pijanowska 1993, Spitze 1992). Likewise, studies conducted on *Oncorhynchus mykiss* morphology shows that 52.7% of variation is attributed to heredity, whereas environmental differences only explain 7.3% of variation (Keeley et al. 2007). The simultaneous use of multiple isolated genotypes over several generations in future studies would clarify the interactions between genes and plastic expression.

Finally, other species of frogs, salamanders, and invertebrates inhabit vernal ponds both permanently and for reproductive purposes. Larval wood frogs must compete interspecifically and intraspecifically for food resources and cover (Relyea 2002c). From personal observations larval spring peepers (*Pseudacris crucifer*), larval central newts (*Notophthalmus viridescens*), adult green frogs (*Rana clamitans*) and northern leopard frogs (*Rana pipiens*) were among the other species present. Wood frogs inhabit a wide range of habitats encompassing high predation/ low competition to low predation/ high competition and these different environments produce different selective pressures on morphology (Relyea and Auld 2005). Important phenotypic tradeoffs arise in light of interactions between competitor-induced and predator-induced plasticity (Relyea and Auld 2005). Competitor-induced plasticity affects similar traits in larval wood frogs but in the opposite directions. For example, ponds with high tadpole densities produce more active foraging individuals to enhance growth rates, and also develop relatively smaller tails and longer bodies as opposed to less activity and smaller bodies produced by predator interactions (Relyea 2002c, Relyea and Auld 2005, Michel 2011). The interplay of these interactions can aid in explaining the lack of significance from my data. Vernal ponds 27 and K

(Fig. 5 & 6) are an outlier of larger wood frogs, especially in body length and back width. Competitor-induced mechanisms could have stunted the effect of predator-induced plasticity.

In this paper, I have taken into account the complex ecosystem interactions that can effect predator-prey chemical communication. These results may elucidate the role of phenotypic plasticity in the role of natural selection and evolution for wood frogs. The juveniles improve their fitness by increasing body size and chances for survival, which has large implications for future community dynamics. More work is needed to clarify the biological pathways that might trigger shifts in energy allocation when introduced to chemical cues in prey (Weider and Pijanowska 1993). It should also be considered whether age class or mutigenerational effects hinder the ability to express plastic changes.

Acknowledgements-

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Tables-

| Site | Canopy Type | Density per m$^2$ |
|------|-------------|-------------------|
| 9 | Closed | 0.074 |
| 27 | Closed | 0.094 |
| J | Closed | 0.111 |
| Wood Duck | Closed | 0.146 |
| K | Closed | 0.24 |
| P | Open | 0.253 |
| Q | Open | 0.266 |
| 30a | Open | 1.06 |

**Table 1.** Table of predator densities of each vernal pond per square meter and whether it is an open or closed canopy site.
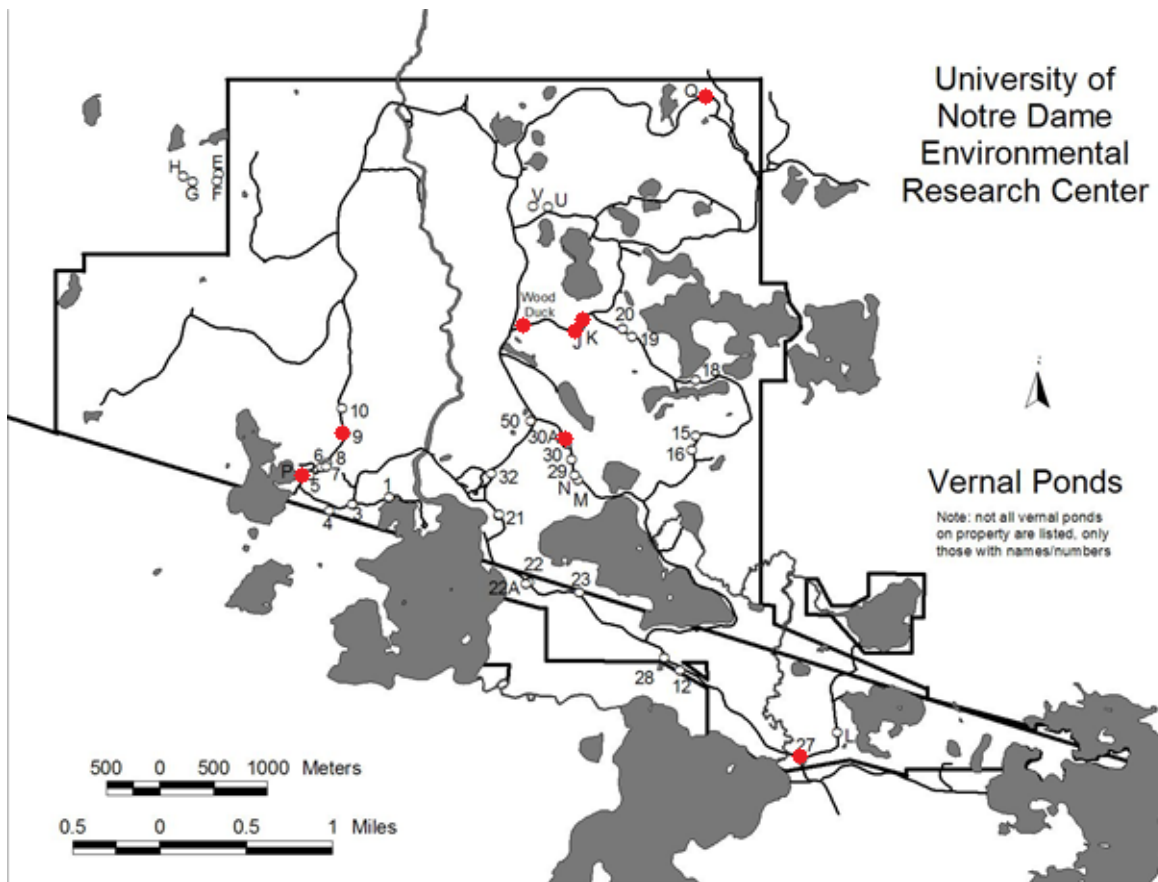
Figures-



**Figure 1.** Map of vernal ponds on UNDERC property. Each of the eight sampling sights is indicated by a red dot (VP-P, VP-9, VP-Q, VP-Wood Duck, VP-J, VP-K, VP-30A, VP-27).

**Figure 2.** The relationship between mean forelimb length and predator density in juvenile wood frogs. There was no significant correlation (*p*= .436, *r²*= .147).
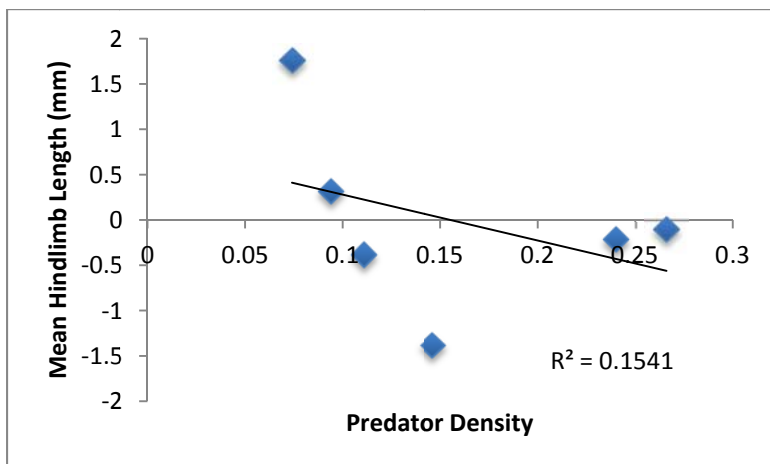


**Figure 3.** The relationship between mean hind limb length and predator density in juvenile wood frogs. There was no significant correlation (p= .441, r²= .154).
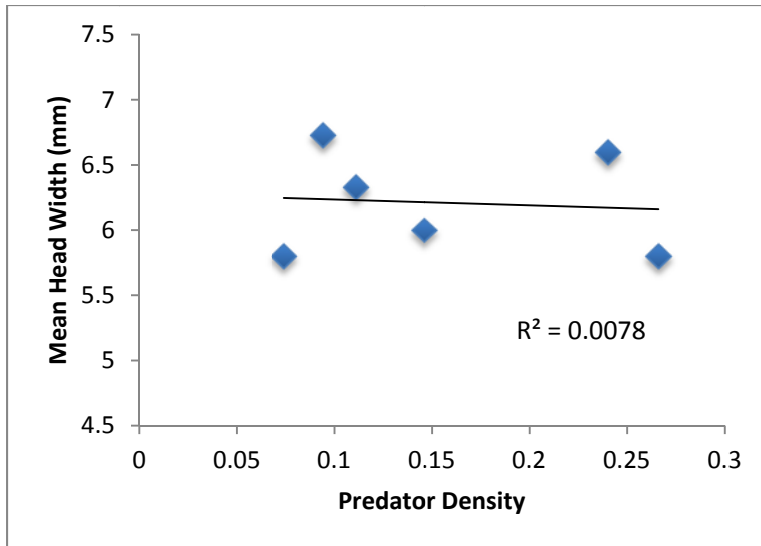
**Figure 4.** The relationship between mean head width and predator density in juvenile wood frogs. There was no significant correlation (p= .868, r²= .008).



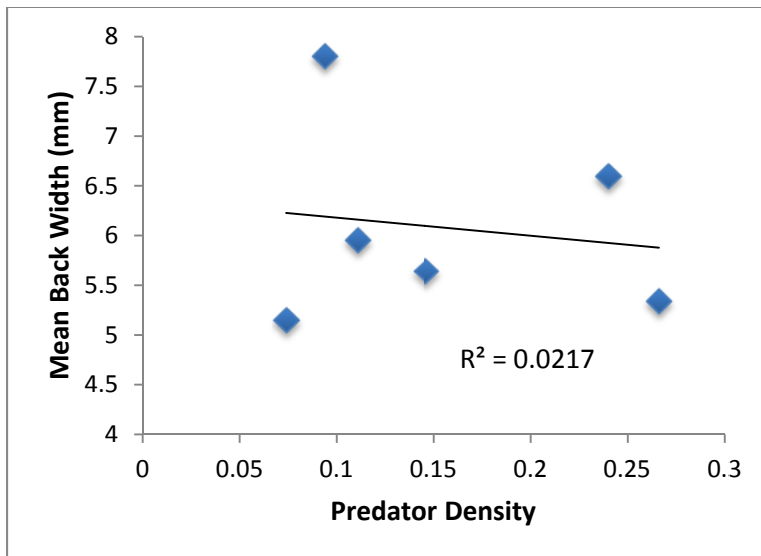**Figure 5.** The relationship between mean back width and predator density in juvenile wood frogs. There was no significant correlation (p= .781, r²= .022).
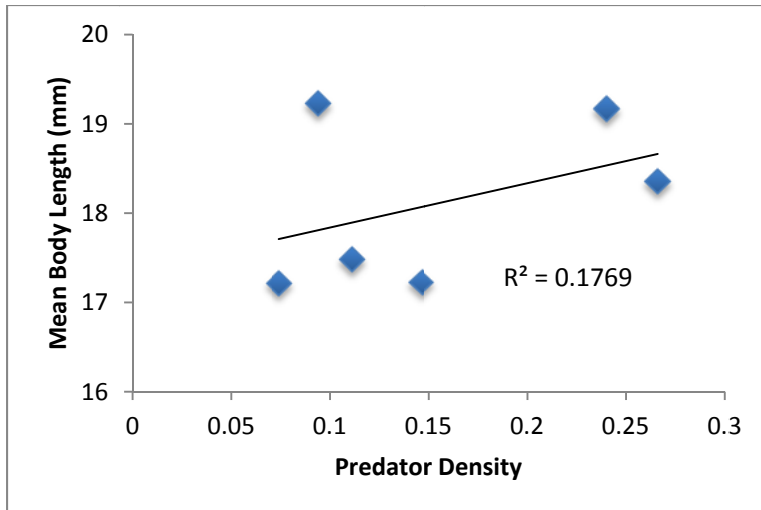
**Figure 6.** The relationship between mean body length and predator density in juvenile wood frogs. There was no significant correlation (p= .406, r²= .177).