

**The effects of larval predation on the morphology of juvenile wood frogs (*Rana sylvatica*)**

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## **Abstract**

Organisms that possess complex life cycles often experience carry-over effects of larval phenotypic plasticity into their juvenile and adult life stages. Predation risk during the larval stage has been shown to affect juvenile wood frog (*Rana sylvatica*) morphology under laboratory conditions. This study sought to determine the effects of predation on juvenile wood frogs emerging from four different vernal ponds. Hind limb length, forelimb length, head width and body width of juveniles were measured and correlated with predator density at each pond site. Predation density was not found to significantly affect juvenile morphology. However, the data suggest that physiological constraints may have prevented the evolution of extreme phenotypes in juvenile frogs in response to carry-over effects of phenotypic plasticity experienced in the larval stage.

## **Introduction**

An organism's phenotype is not solely determined by its genetic code. Individuals with similar genotypes can express a wide range of morphological and behavioral traits depending on differences in environmental conditions (Schlichting 1986). While the phenomenon of phenotypic plasticity has been recognized and confirmed in a wide range of taxa, there have been relatively few studies examining how the plasticity that manifests early in an organism's development affects morphology and behavior later on in life, otherwise referred to as "carry-over" effects of plasticity (Van Allen *et al.* 2010).

A number of species express and benefit from the delayed effects of phenotypic plasticity. For example, *Daphnia pulex* develop different morphologies based on the presence or absence of predators at an early life stage, thereby increasing their chances for survival later on and improving their fitness (Spitze 1992). In this case, carry-over

effects are beneficial because the environment encountered by the individual in earlier developmental stages is indicative of the environment experienced by the individual later in life. Due to the similarity of habitat utilization, individuals are able to benefit from the early effects of phenotypic plasticity throughout the entirety of their life cycle. The same is not necessarily true for all species that exhibit phenotypic plasticity. The incidence of plasticity in organisms with complex life cycles, in which larval and adult morphology and habitat utilization are drastically different, has been confirmed in marine invertebrates, fishes, insects, and amphibians (Pechenik *et al.* 1999). For these individuals, carry-over effects of plasticity do not necessarily have a positive effect on adult fitness. Organisms that go through complex life cycles spend their larval and adult stages in environments that are dissimilar enough to pose unique challenges to the individual's survival. For these organisms, the larval environment may indirectly affect adult morphology by influencing larval traits, which are developmentally tied to adult traits (Relyea 2001). In such a case, traits that are developmentally beneficial to the larvae can have a negative effect on adult fitness (Relyea 2001).

Many studies have examined the effect of carry-over phenotypic plasticity on amphibian development and survival. Most amphibians exhibit complex life cycles and tadpoles are well known for exhibiting phenotypic plasticity in response to a range of environmental pressures (Van Buskirk and Relyea 1998). The environmental factors that influence larval traits and, by extension, post-metamorphic traits have also been explored. A study by Van Allen *et al.* (2010) has shown that an increase in food availability increases juvenile mass and growth efficiency in *Pseudacris crucifer*, while low larval density positively affects juvenile body mass in *Agalychnis callidryas*.

Plasticity in amphibians is not solely affected by food availability and competition. Predation risk also influences metamorphic morphology in *Rana sylvatica*. Relyea (2001) showed that juvenile wood frogs exposed to predation risk as larvae developed larger hind limbs and forelimbs and narrower bodies than juveniles emerging from a predator free environment. In addition, these changes were not attributed to predator-induced changes in larval morphology, but to predator-induced changes in larval development time. In wood frogs, a decrease in juvenile body size is correlated with a longer time to reach reproductive maturity and an overall decrease in fecundity (Berven 1990). Studies such as these have started to shed light on the adaptability of phenotypically plastic traits and their role in natural selection.

More studies on the morphological traits of adult organisms with complex life cycles should take into account the phenotypically plastic traits that influence the larval stage. While these effects have been shown to occur under laboratory conditions, few studies explore the carry-over effects of phenotypic plasticity in amphibians in the field. Thus the goal of the present study was to explore the effects of larval predation pressure on the morphology of juvenile wood frogs (*Rana sylvatica*) in the field. The established incidence of phenotypic plasticity in wood frog tadpoles and previous work on the effects of predation on the morphological traits of wood frogs in the laboratory makes this study subject an ideal one to work with (Relyea 2001, Relyea 2002, Berven 1990).

Based on previous research, I made the following hypotheses about the effect of predator density on the morphological traits of juvenile frogs in the field: (1) juvenile hind limb and forelimb length are greater in individuals emerging from ponds with higher

predator densities; and (2) juvenile head and body width are negatively correlated with predator density.

## **Materials and Methods**

### *Data Collection*

I obtained emerging juvenile wood frogs from four open canopy vernal ponds (designated VP-P, VP-U, VP-30A, and VP-9; see Fig.1) on the property of the University of Notre Dame's Environmental Research Center (UNDERC) in the Upper Peninsula of Michigan. Three primary methods of trapping were used: pitfall traps, visual encounter surveys, and dipnetting. Each pitfall trap was built using three 1-liter buckets, two sections of 15-foot stretches of aluminum flashing, and wooden stakes. Two pitfall traps were placed on opposite sides of each vernal pond. To ensure that only individuals emerging from the ponds were caught, traps were angled toward the pond, with the aluminum flashing flush with the back of the buckets. During visual encounter surveys, I waded around the edges of each pond, catching any wood frog individuals that I saw. At vernal pond 9, I used a dipnet to catch wood frog juveniles that had not yet fully reabsorbed their tails.

I measured the morphological characteristics of each individual juvenile wood frog using 0.1 mm calipers. The following measurements were made: hind limb length, forelimb length, head width, back width, and body length. For those individuals that still retained part of their larval stage tails, I measured body length from the base of the tail to the tip of the nose.

I used minnow traps to measure predator density at each vernal pond. Five traps per pond were set out every night for five consecutive nights. The primary predators

found in the traps were Dytiscid and Odonate larvae. After collection, I determined predator density by adding up the total number of predators found at a particular pond and dividing it by the total number of trap nights.

### *Statistical Analysis*

A mean value for each of the measured morphological characteristics was determined for each pond by taking the average of the values from the individuals from each pond. I determined whether the morphological measurements needed to be corrected for body size by log transforming the data to improve its linearity and then running regression analyses using body length as the independent variable and each of the other measurements as dependent variables. Hind limb length ( $p=0.015426$ ,  $R^2=0.198513$ ), body width ( $p=0.000173$ ,  $R^2=0.412296$ ), and head width ( $p=0.000003$ ,  $R^2=0.565711$ ) were all significantly related with body length. I corrected these three variables for body size by taking the average of the residuals of the regression for each pond and used this value as the mean in my statistical analysis. Forelimb length was not significantly related with body length ( $p=0.433680$ ;  $R^2=0.022860$ ) and therefore was not corrected for body size.

To analyze the data, I ran a correlation analysis for each of the four morphological traits, using SYSTAT 13 (2009) software. For each analysis, predator density was correlated with the mean measurements of the four variables (hind limb length, body width, head width and forelimb width).

### **Results**

Predator densities for the vernal ponds were determined to be as follows: VP-9 had a predator density of 0.20, VP-U had a density of 1.28, VP-30A had a density of 0.267, and VP-P had a density of 0.28.

A total of eleven juvenile wood frog tadpoles with legs were caught using dipnets in VP-9. Six individuals total were caught at VP-U, five of which were caught in the pitfall traps and rest of which were caught in visual encounter surveys. At VP-30A and VP-P, five and seven juveniles respectively were caught in visual encounter surveys.

For the 29 juvenile wood frogs that were caught emerging from vernal ponds, there was no significant correlation between predator density and hind limb length ( $p=0.331$ ,  $r=0.669$ , Fig. 2), forelimb length ( $p=0.531$ ,  $r=0.469$ , Fig. 3), head width ( $p=0.759$ ,  $r=0.241$ , Fig. 4), and body width ( $p=0.665$ ,  $r=0.335$ , Fig. 5).

## **Discussion**

The results of the present study failed to support my original hypothesis that predator density will positively affect juvenile wood frog hind limb and forelimb length, while negatively affecting juvenile body and head width. This suggests that in the field, predation during the larval stage has no effect on the morphology of post-metamorphic juveniles. This is contrary to my initial hypothesis based on previous studies conducted in the laboratory that predicted that wood frog tadpoles exposed to predation risk would result in increased juvenile leg length and decreased body width (Relyea 2001). There are a number of possible explanations for these results.

Relyea and Hoverman (2003) have suggested that an extension in larval period is a prerequisite for juvenile limbs to become elongated and that predation risk influences juvenile morphology indirectly by inducing fluctuations in the time it takes for a tadpole

to reach metamorphosis. They showed that larval predation risk had no effect on the relative growth or morphology of juvenile eastern gray tree frogs (*Hyla versicolor*) despite inducing relatively deep tail fins and short bodies in tadpoles. Thus the present study's failure to find a correlation between predator density and juvenile morphology may be caused by unknown confounding variables that are affecting tadpole morphology and time of metamorphosis. Predation is not the sole influence on plastic traits in frogs. Competition and larval food supply also affect tadpole and, by extension, juvenile morphology (Relyea and Hoverman 2003; Goater 1994; Van Allen *et al.* 2010). These latent environmental effects are hard to identify and control for in a field study. Future field studies on phenotypic plasticity in organisms with complex life histories should therefore focus on abiotic and biotic factors besides predation that may contribute to and influence the development of plastic traits in larval wood frogs.

In addition, Relyea and Hoverman (2003) found that forelimb width and body width were not significantly affected by the plastic effects of larval competition until a month after metamorphosis. This suggests that under certain conditions, there exists a time lag between the influence of larval plasticity and its emergence in the adult phenotype. All of the juvenile frogs measured in this study were in early post-metamorphic stages. It is possible that an insufficient amount of time had passed for plastic traits to develop and be discernible in the juvenile phenotype. Future studies should take care to examine effects of plasticity at all points of an organism's life cycle, encompassing the early tadpole stages and late post-metamorphic stages and all stages in between.

Predation risk has been shown to induce a broad range of metamorphic responses in amphibians, both interspecifically and intraspecifically (Benard 2004). While predictions of predator-induced plasticity in larval morphology are generally met, there is not a universally applicable model that can predict the effect of these morphologies on future, post-metamorphic phenotypes (Benard 2004). More comprehensive studies that explore the interactions between morphological, behavioral, and life history plasticity are required before the variation in phenotypic plasticity can be fully elucidated (Benard 2004).

Finally it is important to keep in mind that the phenotypes reflected in juvenile wood frogs' morphology are a product not only of carry-over effects of plasticity experienced in the larval stage, but also of the mechanism of natural selection acting on populations (Agrawal 2001). If a population of frogs possesses a gene for short legs, the environmental effects that would normally induce the development of long legs in juveniles may be muted. This is yet one more consideration to take into account when studying the effects of phenotypic plasticity on organisms with complex life cycles.

An unexpected trend in the data is worth mentioning here. An observation of the relationship between forelimb and hind limb length and predator density suggests a non-linear response. After a qualitative assessment of the data, these variables seem to asymptote with increased predator density (Fig. 2 & 3). This suggests that there may be morphological constraints on the phenotypic traits of juvenile wood frogs. If this is the case, these results would have larger implications for the adaptability of these traits in wood frog populations (Arnold 1992). Constraints on the adaptations of organisms with complex life cycles have long been of interest to researchers due to the multiple and

discrete life stages on which natural selection can act (Moran 1994). The present study suggests that physiological constraints exist that prevent juvenile wood frogs from evolving more extreme morphologies in response to phenotypic plasticity experienced during the larval stage.

The above considerations reveal much about the complex interactions and factors that must be taken into account when studying the evolution and maintenance of phenotypic plasticity and its far-reaching effects on the morphology and fitness of organisms. Understanding the impact that early stage plastic traits have on late stage morphology is an essential prerequisite for the study of organisms with complex life cycles (Relyea 2001). Thus studies such as the present one allow us to shed light on these processes, while allowing us to figure out how best to design future studies and experiments taking into account the inextricable link between tadpole and juvenile morphology and physiology.

### **Acknowledgments**

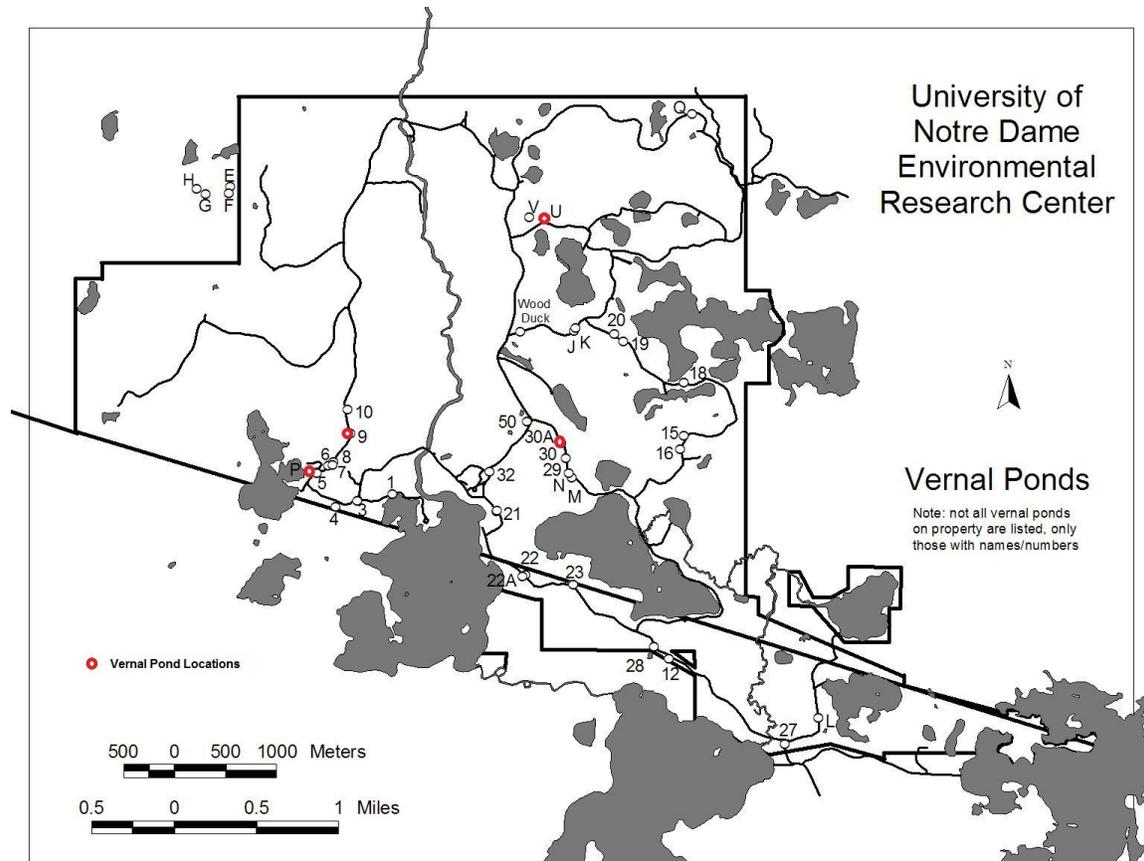
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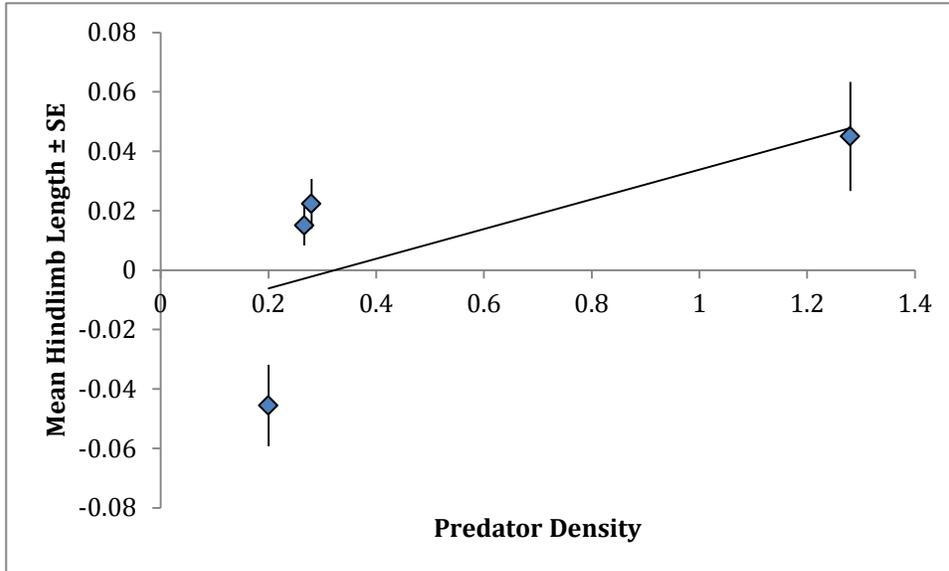
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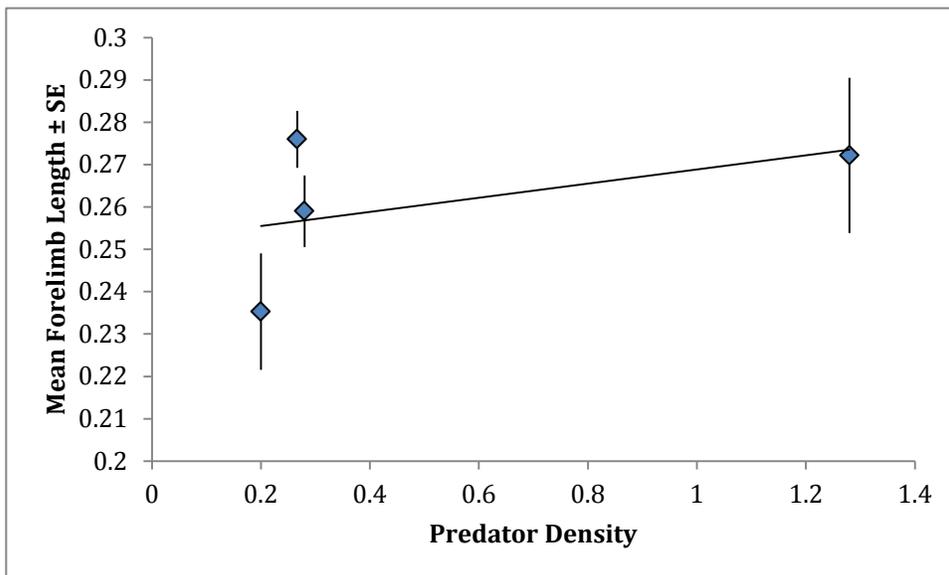
## Figures



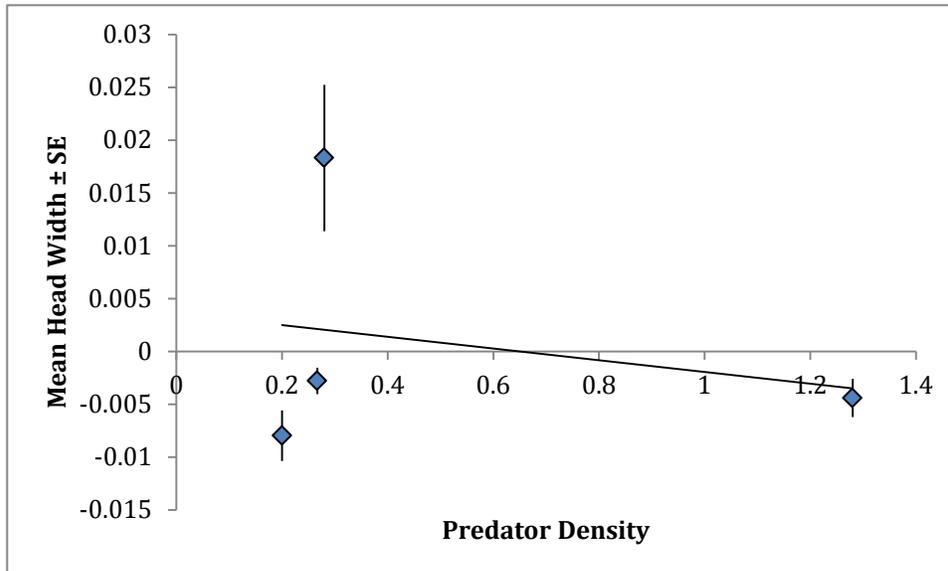
**Figure 1. Map of vernal ponds on UNDERC property.** The red circles indicate the location of the four ponds (VP-P, VP-9, VP-30A, and VP-U) where emerging juvenile frogs were collected and predator density was determined.



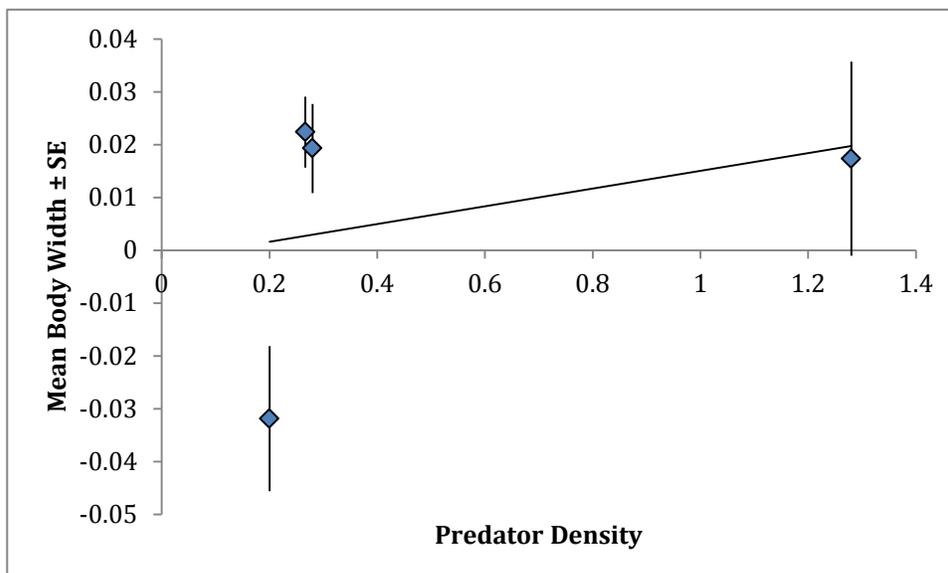
**Figure 2. The relationship between predator density and mean hind limb length  $\pm$  standard error.** There was no significant correlation between predator density and hind limb length in juvenile wood frogs ( $p=0.331$ ,  $r=0.669$ ).



**Figure 3. The relationship between predator density and mean forelimb length  $\pm$  standard error.** There was no significant correlation between predator density and forelimb length in juvenile wood frogs ( $p=0.531$ ,  $r=0.469$ ).



**Figure 4. The relationship between predator density and mean head width  $\pm$  standard error.** There was no significant correlation between predator density and head width in juvenile wood frogs ( $p=0.759$ ,  $r=0.241$ ).



**Figure 5. The relationship between predator density and mean body width  $\pm$  standard error.** There was no significant correlation between predator density and body width in juvenile wood frogs ( $p=0.665$ ,  $r=0.335$ ).