

Effects of the distribution of vegetation and predators on the morphological traits
of tadpoles

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Abstract

In this study I examined the possible correlations between wood frog (*Rana sylvatica*) tadpole abundance, tadpole predator abundance, and aquatic macrophytic cover in seven vernal ponds on UNDERC property. Predators found included dragonfly larvae (Odonata), predaceous diving beetle adults and larvae (Dytiscidae), giant water bugs (*Lethocerus*), and back-swimmers (Notonectidae). I predicted that predator abundance would be negatively correlated to tadpole abundance and both predator and tadpole abundance would be positively correlated to vegetative cover. I also looked at a plastic morphological trait in tadpoles, tailfin depth, as a measure of defensive response to predators and its possible correlation to aquatic vegetation. An additional prediction was that tailfin depth would be positively correlated to predator abundance and negatively correlated to vegetative cover. I sampled points in 2x2m and 3x3m grids on each pond, where I collected, photographed, and later measured tadpole tailfin depth and length. Vegetation percentage and predator abundance and identity were observed at each site. Using linear regressions, I found a negative correlation between tadpole abundance and total predator abundance as well as between tadpole abundance and Odonata abundance and a positive correlation between predator abundance and vegetative cover. The tailfin depth:length used to represent tailfin depth was not significantly related to aquatic macrophyte abundance or predator abundances. It was apparent that predators prefer vegetative cover while tadpoles may disperse more evenly throughout vernal ponds. In these ponds tailfin depth does not appear to be affected by amount of vegetation or predator abundance, perhaps due to tadpole distribution.

Introduction

Some organisms exhibit specific phenotypic responses to biotic and abiotic factors in their environments. These responses include both behavioral and morphological changes in the organism as a result of certain stimuli, such as environmental heterogeneity or the presence of predators (Relyea 2001). When a predator induces a change in morphology or behavior, this is referred to as predator-induced plasticity (Van Buskirk and McCollum 1999). Some of these

phenotypic changes in tadpoles include up to a 15% increase in maximum tailfin depth and a reddening of the tailfin or an appearance of black spots on the tailfin. These changes are thought to act as a type of lure in that deeper, brighter tailfins attract predator attention to the tail, away from the more vulnerable head and body region (Van Buskirk et al. 2003, Van Buskirk et al. 2004). Therefore, the lure effect decreases an individual tadpole's vulnerability to predation because tadpoles survive predator attacks to the tail better than attacks to the head and body (Doherty et al. 1998, Van Buskirk et al. 2003, Van Buskirk et al. 2004).

The degree of this tailfin change varies among tadpole species (Van Buskirk 2002) as well as among predator species present (Relyea 2001). Relyea (2001) concluded that anuran larvae are apparently able to differentiate among predator species, implying that tadpole morphological responses are predator-specific. In this same study wood frogs (*Rana sylvatica*) were shown to discriminate, through varying tailfin depths, the apparent threats of central mudminnows (*Umbra* sp.) and dragonfly larvae (*Anax* sp.) from those of newts (*Notophthalmus* spp.) and predaceous diving beetles (*Dytiscus* spp.).

This study and others also suggest that tradeoffs among morphological and behavioral traits can affect the degree of plasticity shown. Tadpoles in particular appear to favor the least costly defenses possible. In other words, if a tadpole has a very shallow tailfin in a high predator environment, instead of making the costly shift to a deeper tailfin, it may instead alter its behavior and metamorphose faster

to avoid the presence of predators (Van Buskirk and Arioli 2002, Benard 2004). However, induced morphological responses to predators tend to be preferred by tadpoles where predation risk is highly variable (Van Buskirk and Arioli 2005).

Behavioral predator defenses include the utilization of available macrophytic cover in ponds and lakes. Tadpoles generally prefer high levels of environmental heterogeneity (Diaz-Paniagua 1987), but especially so in the presence of predators (Formanowicz and Bobka 1989). These results are conducive to the conclusions of other studies that have found greater tadpole survival from predator attacks when raised in high density aquatic macrophytic cover (Formanowicz and Bobka 1989, Tarr and Babbitt 2002, Baber and Babbitt 2004). Preliminary findings (M. Michel, unpublished data) may point to a possible reduction in the degree of morphological plasticity exhibited by tadpoles found in high aquatic vegetation, which may be related to increased tadpole survival in dense cover.

These findings may be complicated by three major gradients that influence habitat distribution of pond-dwelling tadpoles. Predator density, pond permanence, and extent of forest canopy cover may exert varying degrees of influence on behavioral and morphological traits of anuran larvae (Van Buskirk and Arioli 2005). Pond permanence and extent of forest canopy cover can both affect the distribution of aquatic macrophytic cover in vernal ponds. In relation to

this, tadpoles may vary their distribution and morphological traits depending on the distribution of macrophytes, as well as the distribution of predators.

As demonstrated in the so-called “dosage response,” tadpoles tend to increase their defensive morphological and behavioral responses to predators as the amount of food consumed by predators increases (Van Buskirk and Arioli 2002). Therefore, tadpoles found in areas of high predation risk within a pond may exhibit the highest degree of tadpole predator-induced plasticity, such as the deepest tailfins.

Studies in the field concerning predator-induced morphological plasticity have great ecological importance, because most related studies have been performed in artificial habitats or laboratory experiments. Those few studies that have been done in natural systems show promise that such experiments in artificial environments can at times predict natural patterns but more studies in the field are needed to strengthen this claim (Van Buskirk and McCollum 1999).

In this study I determined the effects of the distribution of aquatic vegetation and predators within vernal ponds on the morphological traits of wood frog tadpoles within that pond. I examined the possible correlations between: predator and tadpole abundances relative to each other and to vegetative cover; and tadpole morphology (maximum tailfin depth) relative to predator abundances and vegetative cover. Predators of larval anuran in these vernal ponds included

the giant water bug (*Lethocerus* spp.), dragonfly larvae (Odonata), predaceous diving beetle adults and larvae (Dytiscidae), and back-swimmers, (Notonectidae).

Given our review of past studies, I predicted that the level of aquatic vegetative cover in vernal ponds would be positively correlated to tadpole abundance and predator abundance. Additionally, I predicted that tadpole tailfin depth relative to tailfin length would be positively correlated to predator abundance and negatively correlated to vegetative cover by percent.

Materials and Methods

I sampled seven vernal ponds on UNDERC property, Gogebic Co., Michigan (Appendix fig. 1). Three of these ponds (the North of Beaver Bog, or NBB, ponds) were shallowly connected to each other but showed themselves to be distinct in several ways, including average water depth, vegetative cover, and tadpole and predator abundances. As a result, they were considered to be three separate ponds: NBB Number (#), NBB City, and NBB Alphabet (Alpha). Due to the sizes of the NBB ponds, a 3x3 meter grid of flagged sampling points was set up on them while a 2x2 meter grid was established on the other four ponds.

NBB # had 25 sampling points and was approximately 75 m². There was a mean aquatic macrophytic cover of 9% and around 85% closed forest canopy over the pond. NBB City had 19 points, 29% mean vegetative cover, and about 10% closed canopy over it. Its area was approximately 57 m². NBB Alpha had 23 points, 41% mean aquatic vegetation, about 5% closed canopy, and was

around 69 m². The vernal pond South of Moccasin Lake (SML) was roadside with 45 points across about 90 m². It was very evenly vegetated with a mean aquatic macrophyte cover of 37% and a closed canopy overhead of about 12%. North Gate Pond (NGP) was the largest pond sampled at 114 m² with 57 points. It had a mean aquatic vegetative cover of 15% and about 40% closed canopy above it. Vernal Pond 9A (VP9A) had 42 points and was around 84 m². Its mean aquatic macrophytic cover is 11% and it has a 30% closed canopy above it. Vernal Pond 9B (VP9B) had 34 points and was about 68 m². Its mean aquatic vegetative cover is 40% and essentially had an open canopy.

At each point on the pond sampling grids, I drove a 12-inch diameter PVC pipe through the water column and into the pond bottom. Within the pipe at every point, I determined by sight and by touch the submerged and emergent vegetative cover (by class: 0-20%, 20-40%, 40-60%, 60-80%, 80-100% and by approximating a percent within each class), and noted water depth. An arcsine square root transformation was applied to the vegetative percent data to better meet the assumptions of the statistical tests performed.

$$\text{“Arcveg”} = \text{Arcsine} [\sqrt{(\text{vegetative cover percentage} \div 100)}]$$

Within the pipe at each point, I dipnetted for tadpoles and predators. If I found neither tadpole nor predator in five scoops of the dipnet, then I moved on to the next sampling point on the grid. Tadpoles were placed in labeled jars of pond water and taken back to the laboratory where they were photographed and then

released back in their pond. Predators were preserved in labeled jars of ethanol and later identified and measured in the laboratory and were only counted if they measured greater than or equal to 1.5 cm.

Using the computer program Image J, for each tadpole I measured tailfin length and depth. A ratio of tailfin depth to tailfin length was calculated as a measure of relative tailfin depth to account for overall variations in tadpole size. In SYSTAT 11, linear and stepwise regressions were run globally on the following variables: arcveg (arcsine-square root transformed vegetative percentages), tailfin depth to length ratio, tadpole abundance, total predator abundance, and individual predator species abundances (Odonata, *Lethocerus*, Dytiscid adult, and Dytiscid larvae). Analysis of variance (ANOVA) was also performed to determine if vegetative cover, tailfin depth:length, total tadpole abundance, and total predator abundance differed among ponds.

Results

I sampled 245 points total in the seven ponds, photographed 212 wood frog tadpoles, and collected 214 predators. I found a significant negative correlation between wood frog tadpole abundance and Odonata larvae abundance (linear regression; $P=0.002$; $r^2=0.0386$; $SE=0.0822$). Total predator abundance showed statistical significance for a negative correlation to tadpole abundance (stepwise regression; $P=0.00292$; $r^2=0.0359$; $SE=0.0752$) and for a positive correlation to the transformed vegetation data (stepwise regression; $P=0.0095$;

$r^2=0.0620$; $SE=0.294$). Tadpole abundance did not show significance with vegetation (stepwise regression; $P=0.667$) while I found that Odonata larvae abundance was significantly positively correlated to vegetation (linear regression; $P=0.05$; $r^2=0.0156$; $SE=0.275$).

Using a stepwise regression, I found that the tailfin depth:length data was not significant with transformed aquatic vegetative cover ($P=0.855$), Odonata larvae abundance ($P=0.621$), Dytiscid larvae abundance ($P=0.284$), Dytiscid adult abundance ($P=0.988$), or *Lethocerus* abundance ($P=0.446$).

I determined that there were statistically significant differences in tadpole abundance between ponds (ANOVA; $P<0.001$; F-ratio = 17.7566; DF = 6; MSE = 1.7604). The post-hoc test showed that VP9A and VP9B were significantly different in tadpole abundance from all other ponds, except between each other (Tukey; $p<0.05$). Total predator abundance was also significantly different between ponds (ANOVA; $P<0.001$; F-ratio = 11.253; DF = 6; MSE = 1.3848). A Tukey's test determined that NGP differed in predator abundance from all other ponds ($P<0.002$).

Analysis of variance (ANOVA) was calculated for tailfin depth to length ratio among ponds, and I found marginally significant differences in tailfin ratio between ponds ($P=0.064$; F-ratio = 2.022; DF = 6; MSE = 0.006). The post hoc test showed NBB # to be significantly different from NBB ALPHA (Tukey; $P=0.052$). Vegetative cover (arcveg) was found to be significantly different

between ponds (ANOVA; $P < 0.001$; $DF = 6$; $F\text{-ratio} = 14.048$; $MSE = 0.059$). See Figure 1 for significant variations in vegetation between ponds and Table 1 for a summary of the mean variables examined at each pond.

Discussion

This study shows support for the hypothesis that total tadpole predator abundance in vernal ponds is positively correlated to level of aquatic vegetative cover. The tadpole predators sampled in vernal ponds on UNDERC property appear to prefer aquatic macrophytic cover, which could be related to their foraging approaches (Tarr and Babbitt 2002). These predators might also use macrophytes as refugia from predators larger than themselves in the vernal ponds.

On the other hand, tadpole abundance is not significantly correlated to level of aquatic vegetation. Wood frog tadpoles may not exclusively prefer high levels of aquatic vegetation because of the higher levels of predators that appear to prefer vegetation. Tadpoles might instead utilize benthic detritus, or leaf litter, as refugia in vernal ponds more often than aquatic vegetation in order to escape predators (M. Michel, personal communication). Another explanation might be that high levels of macrophytes could reduce tadpole foraging efficiency (Tarr and Babbitt 2002). Our results could be possibly skewed because in our analysis of tadpole abundance, I only considered tadpoles which were large enough to photograph for our analysis of morphological traits and ignored tadpoles smaller than this or who were starting to metamorphose with four limbs. This would

make our results for tadpole habitat preference dependent on developmental stage (Formanowicz and Bobka 1989).

This study also determined that wood frog tailfin depth does not appear to be strongly correlated to the amount of vegetative cover present in vernal ponds. A possible explanation could be that tadpoles in these ponds move freely between vegetated and non-vegetated areas, which would be supported by our lack of correlation between tadpole abundance and aquatic macrophytic cover and by our strong correlation between predator preference for vegetative cover. If the tadpoles do not prefer vegetation as refugia from predators, then perhaps a more even spatial distribution across the pond serves as a refugia, which supports Formanowicz and Bobka's work (1989). They found that even distribution optimizes the distance from predator to prey, thus increasing predator foraging time and decreasing attacks on prey.

This could be an important factor in our ponds with high Dytiscid larvae abundance because these predators use chemical and tactile cues rather than visual cues to find prey at short ranges (Formanowicz 1987). It was concluded that individual tadpoles enjoy a lowered risk of predation by scattering themselves throughout the pond even if their predators disperse themselves throughout the microhabitats in a similar way (Formanowicz and Bobka 1989). Although predators prefer vegetative cover, they may also move freely between vegetated and non-vegetated areas in order to hunt for prey.

Tadpole tailfin depth:length was not shown to be correlated to any of the predator abundances in our ponds, which again could be related to tadpole dispersal throughout the ponds and the inverse relationship between tadpole and predator abundance as well as the inverse relationship between tadpole and Odonata abundance. If tadpoles are not heavily concentrated where the predators are, then they do not need the added defense of a deeper tailfin to lure attacks away from the head and body (Van Buskirk et al. 2003). Additionally, I can speculate that the ponds I sampled did not have high variability in predation risk, because then predator-induced deeper tailfins would be preferred (Van Buskirk and Arioli 2005), which they were not. The apparent threats of the predators found in our seven experimental ponds may not have been great enough to provoke the deep tailfins either, which supports Relyea's work (2001, Relyea and Werner 1999). An alternate explanation might be that the predation risks in our vernal ponds, in combination with the transience of these ponds, could cause the tadpoles to metamorphose faster rather than develop much deeper tailfins to avoid predation (Tarr and Babbitt 2002, Bernard 2004).

Because the morphological tailfin depths were not significantly related to vegetation or predator abundance, a possible follow-up to this experiment would be to map the spatial distributions of tadpoles, predators, vegetation, and tailfin depth:length in a Geographic Information System (GIS) with interpolation to examine overlapping or scattered distributions. It would might be helpful to

observe the mean permanence of each vernal pond used over several seasons to examine the effect of pond permanence on tadpole morphological traits. A further study of the question of wood frog spatial distribution might involve observing tadpole activity in a lab study with vegetated and non-vegetated areas to determine if tadpoles move freely in and out of vegetation in the presence and absence of predators. This study might even compare the tailfin depths of tadpoles who preferred vegetation against those that preferred the open and those that moved equally among both the vegetated and open areas. The pursuit of such related studies are crucial for determining possible interactions of behavioral and morphological predator-induced defensive responses in anuran larvae.

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Tables

Table 1. A summary of the variables examined in each pond.

Pond	Number of Wood Frog Tadpoles	Total Number of Predators	Mean Vegetative Cover by Percent	Mean Vegetative Cover by Class	Mean Tailfin Depth: Length
NBB Alpha	3	14	40.67	20-40%	0.305
NBB City	9	14	29.44	20-40%	0.372
NBB #	14	21	9.43	0-20%	0.446
NGP	16	109	14.19	0-20%	0.421
SML	7	37	49.29	20-40%	0.419
VP9A	98	8	11.02	0-20%	0.410
VP9B	65	11	39.62	20-40%	0.403

Figures

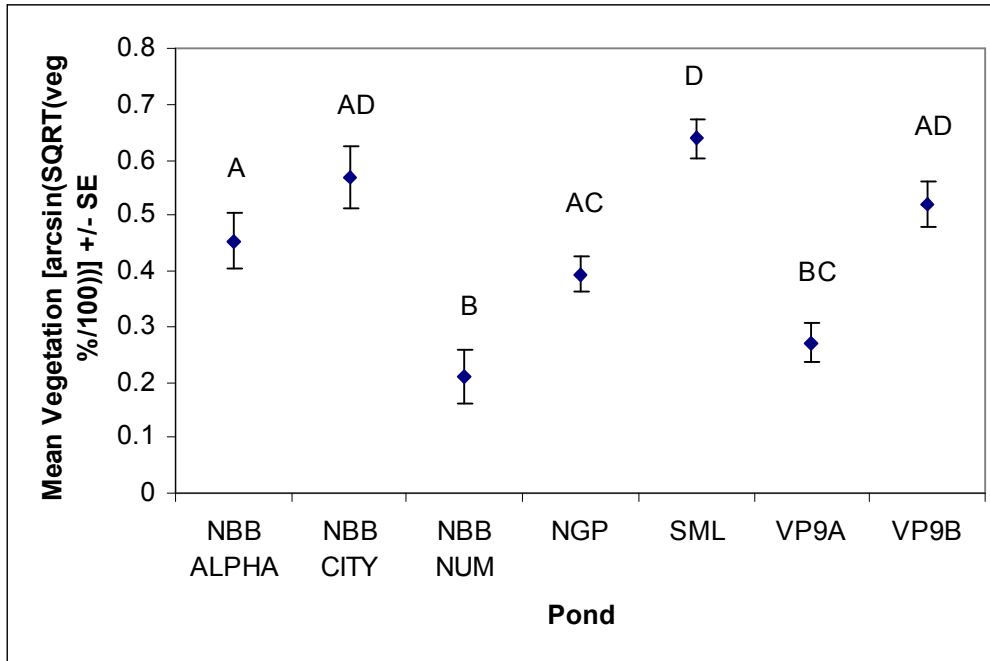
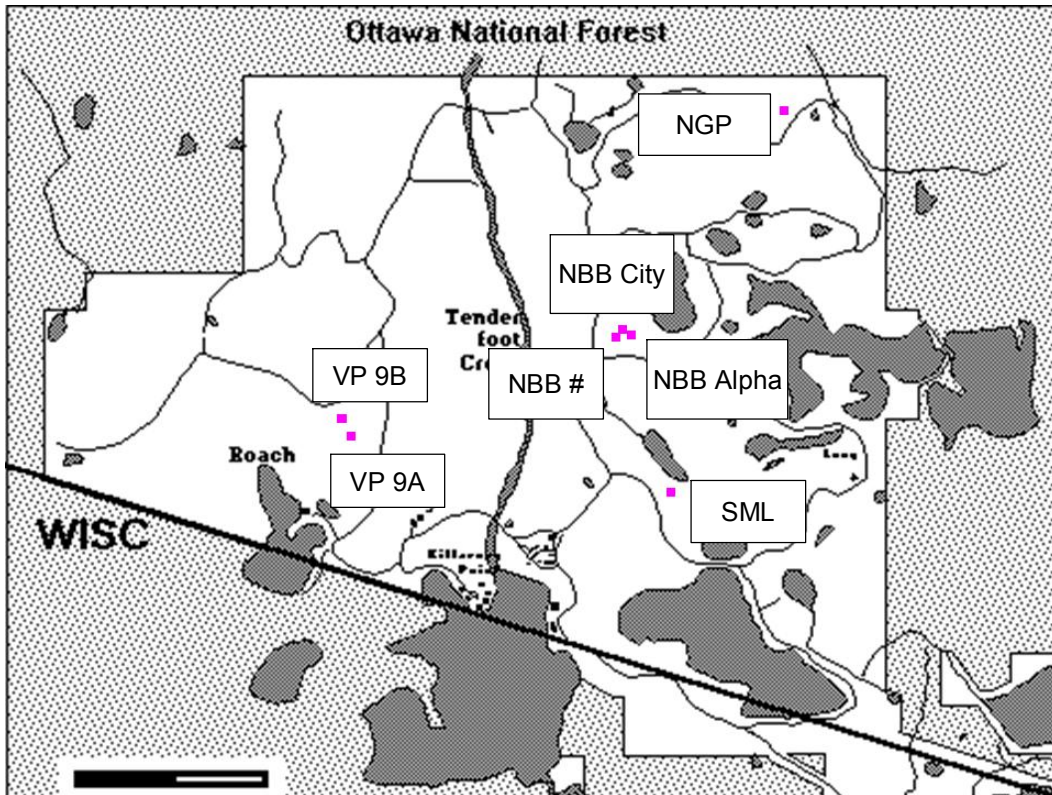


Fig. 1. Means of arcsine-square root transformed vegetative data between ponds as measured by ANOVA and Tukey's test. Letters indicate significant differences in aquatic vegetative cover between ponds. Error bars represent standard error.

Appendix



Appendix Fig. 1. Map of placement of vernal ponds sampled on UNDERC property, Gogebic Co., MI.