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Predator Survival Tactics and Use of Habitat Cover in Rana Catesbeiana.

Tara E. Bergin
Colby College

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Abstract

Predator-prey relationships are an important aspect of the natural world, and, because of its relevance to survival and natural selection, is an interesting relationship to study. In amphibian larvae, level of activity and landscape use are often what determines the survival as prey. I studied the anti-predator behavior of the North American bullfrog (*Rana catesbeiana)* tadpoles when presented with dragonfly (*Aeshna*) larvae, a known predator of tadpoles. Tadpoles were acclimated to four different habitats with varying degrees of habitat cover, and were transferred to a new habitat with a degree of cover equal to one of the acclimation tanks. A restrained predator, and thus its chemical cue, was introduced, and the behavior, particularly the use of the habitat cover to hide from the perceived risk of predation was observed. A significantly higher frequency of inactivity was found in tank I than in II and III, and inactivity followed a general trend of decreasing with increasing habitat cover. Difference in tank cover was not found to have a significant effect on swimming behavior, but did have a significant effect on hiding behavior, which increased with higher availability. Foraging decreased significantly with the addition of a predator, but did not vary significantly with different levels of cover. Hiding behavior and reducing conspicuous behaviors (like foraging) are probably the behaviors that afford the tadpole the most success at eluding a predator in their natural environment.

Introduction

Small aquatic organisms like amphibian larvae are very vulnerable in the first stage of their lives. They are significantly smaller than many of their predators, which include fish, newts, and insect larvae. These organisms must quickly find ways to use their habitat and other mechanisms, such as unpalatability or toxic secretions (Semlitsch and Reyer 2005), in such a way to reduce their risk of predation by terrestrial and aquatic predators. For example, red-eyed green tree frog embryos will hatch up to 30% early to escape predation by snakes and wasps. However, this strategy comes with a developmental cost (Warkentin 2005). Younger hatchlings have more difficulty feeding, remaining neutrally buoyant, and swimming (Warkentin 1999). Warkentin, in this same study showed that while they have escaped the immediate predation risk, they are now at an even greater disadvantage, as they are smaller and less able to defend themselves. At the same time, they are much further behind in the development process, and thus there is a constant balancing act between development and predator evasion, as metamorphosis is a very energy-expensive process.

A prey species may depend on its ability to perceive predators through a variety of cues. For tadpoles these cues are mainly tactile and chemical (Stauffer and Semlitsch 1993). Tadpoles, for example, can sense a chemical cue from the dragonfly larvae, alerting them to the presence of a predator and stimulating anti-predator behavior, which makes them an ideal model for this experiment (Stauffer and Semlitsch, 1993). The chemical cue from the predator prevents the need for a visual stimulus to the anti-predator behavior, since habitats with more cover and murky water will hinder the perception of visual cues. Tactile cues also serve to aid in determining the location of the danger, as that same study demonstrated that even though the chemical cue will diffuse throughout the aquatic environment, tadpoles are still able to determine the direction the cue is coming from through vibrations. Stauffer and Semlitsch (1993) showed that when presented with chemical and tactile cues alone, and without visual contact, tadpoles were more likely to swim on the side of their environment farthest from the source of those cues.

In addition, tadpoles behave as though they retain a memory of an experienced predation environment for several days (Semlitsch and Reyer 1992). Refuge use was higher and swimming activity lower after tadpoles were conditioned with predators, suggesting that past as well as present predator experience make larvae more cautious. In tadpoles in particular, reduced activity (resting or hiding) is a measure of cautiousness, and protects from dragonfly larvae predation (Van Buskirk et al., 2002). Dragonfly larvae are a “sit-and-wait” predator, and rely partly on the movement of their prey in order to find and catch it. To see prey, dragonfly larva have to move their body, which alerts the prey to their presence. Therefore, it is not surprising that tadpoles faced with increased risk of danger, quantified by number of dragonfly larvae present, spent significantly more time in an inactive state (van Buskirk et al 2002).

Habitat structural complexity can also play an important role in predator-prey interactions. Babbit and Tanner (1998) showed that tadpole survival was higher in habitats with high cover than in low cover. The more cover a habitat provides, the better able the tadpoles are able to elude predators. This can be due to a variety of reasons. First, the complexity of the habitat might hinder a predator’s movement, thus providing the tadpole with an advantage. If a predator is blocked from swimming freely throughout the habitat, the tadpole may be able to fit in places the predator cannot. Second, a habitat with more cover should provide more places where tadpoles can hide, thus preventing them from being seen and attacked.

This study was focused on the second hypothesis, that the complexity of the habitat allows the tadpole more places to hide, thus avoiding predation. The more cover a habitat allows, the more places a tadpole will be able to go to escape notice of a predator. Previous experiments have studied the effect of habitat cover and predation on the *development* of tadpoles, but none of them have focused on how the tadpoles respond behaviorally to an introduced predator in an environment with more or less cover than the one to which they were acclimated. Development of the animal itself is an important aspect of the predator-prey interaction, since during periods of higher risk there is a trade-off between eating and predator evasion tactics, which will thus affect development. However I will focus on the behaviors, and how the tadpoles interact in a novel environment.

This paper tests the hypothesis that behavioral patterns of tadpoles will vary relative to the amount of habitat cover in their environment. In environments with more cover, tadpoles may be able to be active even in the presence of the predator, because the increased cover offers more protection. In contrast, tadpoles in more sparse habitats should show more resting behaviors in order to remain unnoticed by the perceived threat. Through this experiment I will demonstrate that tadpoles will use habitat cover more frequently, when it is available, to hide from a perceived threat, and will show different behavioral patterns in environments with less available cover, such as lower activity levels, to avoid detection. In addition, I predict that tadpoles given a choice of both a rock refuge and plant cover will preferentially choose the rock refuge, given the similarity of the color of the rock to their own mottled-brown coloration.

In their natural habitat, this experiment has relevance because the ability to be flexible in changing habitats is important for a tadpoles survival, particularly in bullfrogs. Bullfrogs, *Rana catesbeiana*, are the largest frog in North America, living and over-wintering in ponds, lakes, marshes, and slow-moving streams. As tadpoles, they grow to be about six-inches long, and sometimes take several years to become fully formed, adult bullfrogs. Because of this, bullfrogs are an ideal species in which to observe tadpoles' habitat adaptability in relation to predator cues, because in their natural environments, habitat structure and predator numbers and types are subject to seasonal changes as well as change with a move downstream. To survive rapid changes in environment, the tadpoles must be adept at altering their behavioral patterns to reflect those changes within their habitat.

Materials and Methods

EXPERIMENT SETUP AND DESIGN

All tanks were filled twenty-four hours prior to the experiment with spring water, and all materials added to the tank were soaked for 24 hours in clean water to eliminate any contaminants that may be present. Tank water was filtered with an aquatic filtering system.

Rana catesbeiana (North American bullfrog) tadpoles were acquired from Ward's Natural Science Establishment Inc., and acclimated for one week, living in four different 10-gallon habitats that differ in amount of cover. The different tank setups were as follows: (1) sand material only on bottom; (2) sand on bottom of tank with leafy plants; and (3) sand on bottom plus leafy plants plus and a small, cave-like refuge constructed of larger rocks. The fourth tank was set up as the control, with the same setup as tank (1). Four duplicate trial tanks (denoted with Roman numerals) were created in which the experiments actually took place. In the original setup, the five tadpoles in the control tank were to be transferred to trial-tank four at the same time as the other trials, but with no predator. However, due to unexpected mass death in assimilation-tank one, tank four was instead substituted for tank one in all trials.

Aeshna larvae were introduced into each of the trial tanks in a cage created by clear, plastic boxes, with holes. It is not important that the tadpoles were able to see the predator particularly well, since the chemical cue they produce should be sufficient to stimulate anti-predator behavior. The plastic ventilated boxes prevented the tadpoles from being consumed.

After at least seven days of acclimation to their original habitats, five tadpoles from each group were transferred to a different habitat determined at random for 60 minutes. During each trial period, four restrained dragonfly larva were introduced into the tank in the same ventilated box, thus mimicking one large dragonfly larva, and each tadpole's behaviors was recorded every minute. Behaviors such as hiding in refuge, hiding in or under leaf cover, swimming away near to or far from the predator, foraging, and resting near or far from the predator were recorded as a scan sample of the number of tadpoles doing each behavior. Behaviors were recorded on a data collection sheet. In addition, the same method was used to record the tadpoles in their acclimation habitats, in order to establish baseline activity

The trial periods were at least 48 hours apart in order to allow the tadpoles to re-acclimate to their original predator-less and habitat-cover conditions before transferring them to another tank chosen at random, since tadpoles behave as though they can "remember" a previously experienced predation environment for several days (Van Buskirk et al., 2002).

FOOD

On each day of the experiment, tadpoles were fed JurrassiDiet™ Newt and Aquatic Frog Pellets. *Aeshna* larvae were all housed in separate small containers and fed with mealworms so that they continued to produce the chemical cue that alerts the experimental tadpoles to predation.

DATA ANALYSIS

Data was analyzed using the Kruskal-Wallis Test for k independent samples, and the differences between two individual trials was analyzed using the Mann-Whitney U Test for 2 independent samples. All p-values were considered significant at an $\alpha=0.01$ level.

Results

INACTIVITY

The frequency of inactivity was significantly higher in tank I than in II and III, for Tank 2 (Kruskal-Wallist test for k independent samples, $H=147.932$, $p<0.0001$), Tank 3 ($H=169.830$, $p<0.0001$), and Tank 4 ($H=164.905$, $p<0.0001$, see Figure 1). For all assimilation tanks, inactivity increased with the addition of a predator into Tank I, although for Tank 4 these results were not significant (Mann Whitney U Test, $U=1676.5$, $p=0.3461$). Inactivity followed the general trend of decreasing inactivity with increasing cover, although the difference between Tanks II and III for Assimilation-Tank 4 was not significant (Mann Whitney U Test: $U=1800.5$, $p=0.7587$).

For Trial I, Inactivity was significantly higher when tadpoles from Assimilation-Tank 4 were transferred into Trial-Tank I than for tadpoles from Tanks 2 and 3 (Kruskal-Wallis Test: $H=18.690$, $p<0.0001$). Within Trial-Tank I, inactivity followed the trend of inactivity being lower in tadpoles coming from tanks with more cover.

In Trial II, inactivity was significantly higher in tadpoles from Assimilation-Tank 3 than from tanks 2 and 1, respectively (Kruskal-Wallis Test: $H=41.830$, $p<0.0001$). In this trial tank, inactivity followed the trend of being higher in tadpoles coming from a tank with higher cover, and lower in tadpoles coming from a tank with less cover.

In Trial III, inactivity was not significantly different across all assimilation tanks (Kruskal-Wallis Test: $H=3.035$, $p=0.2193$), however the frequency of inactivity in Trial III was higher in tadpoles from assimilation tanks with less cover, and lower in tadpoles coming from tanks with the same cover.

FORAGING

In all cases, foraging behavior decreased significantly with the addition of a predator (Kruskal-Wallis Test: $H=29.70$, $p<0.0001$). Virtually no foraging was observed during all trial periods.

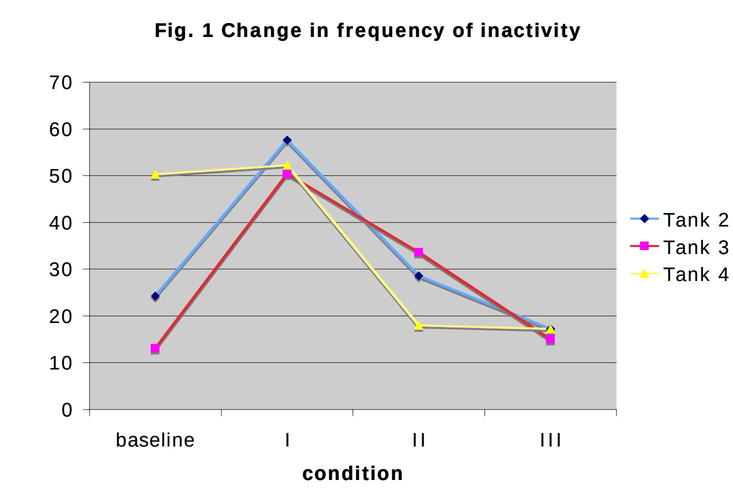
SWIMMING

Swimming frequency did not show a significant increase with increasing habitat cover, and although there were significant differences of swimming behavior in each trial tank, they did not appear to follow any trend. There was no significant decrease in swimming behavior with addition of a predator, in fact in most cases swimming behavior significantly *increased* with the addition of a predator.

HIDING

Hiding behavior significantly increased with more availability of habitat cover in trials for Tank 2 (Mann Whitney U Test: $U=1230.0$, $p=0.0012$), and Tank 3 ($U=422.0$, $p<0.0001$), but not in Tank 4 ($U=1640.5$, $p=0.2600$).

In trials with both plant cover and a rock refuge available, plant cover was significantly preferred overall (Mann Whitney U Test, $U=15224.5$, $p<0.0001$), however in trial III for tank 3, rock refuge was preferred, but with no significance (Mann Whitney U Test, $U=1448.5$, $p=0.0349$, see figure 2).



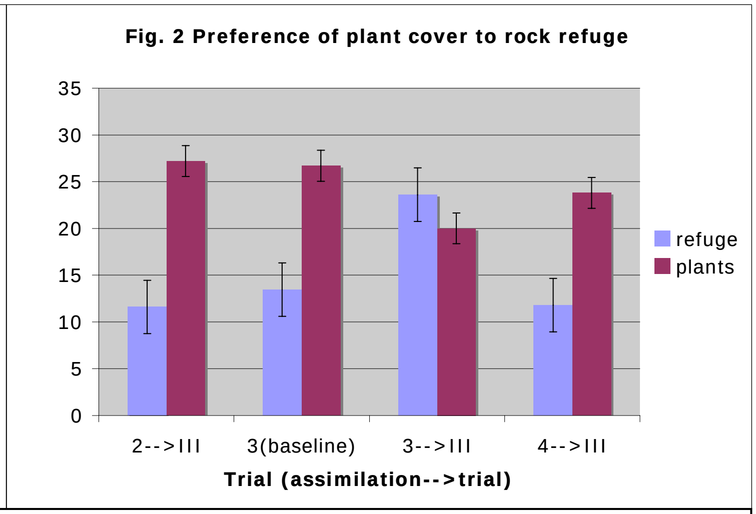


Figure 1. ***Change in frequency of inactivity in relation to tank condition.*** Tadpoles from one of three tanks were transferred from their assimilation (baseline) tank to one of three trial tanks containing increasing amounts of habitat cover (I<II<III), and a dragonfly larva predator. The three trial tanks were identical to one of the assimilation tanks (I=4; II=2; III=3). Each data point represents the frequency/tadpole of inactive behavior.

Figure 2. ***Preference of plant cover to rock refuge in tadpoles.*** Tadpoles in assimilation tanks 2 and 4, in trials where both a rock refuge and plant cover were available, significantly preferred to hide in plant cover. Tadpoles in assimilation tank 3 significantly preferred plant cover to a rock refuge with no influence from a predator. Tank 3 tadpoles, however, did not significantly prefer plant cover to the rock refuge when a predator was introduced.

Discussion

INACTIVITY

The frequency of inactivity was significantly higher in tank I than in II and III for all assimilation tanks. This was as expected, since trial tank I contained no available cover, and thus the only way for the tadpoles to “hide” from the predator was to remain still. For all assimilation tanks, inactivity increased with the addition of a predator, and inactivity followed the general trend of decreasing inactivity with increasing cover. With the addition of a predator, the tadpoles become more cautious, and do less purposeless swimming. Inactivity of tadpoles was able to decrease as the cover in the tank increased because the plant cover, in particular, afforded them more room to be active while still escaping notice by potential predators.

Contrary to my predictions, within trial-tank I and trial-tank 3 (although results for tank III were not significant), inactivity followed the trend of inactivity being lower in tadpoles coming from tanks with more cover. It would make sense for tadpoles coming from tanks with more cover to be even more cautious when they suddenly find themselves without cover, however another possible explanation is that tadpoles that were assimilated in tanks with more cover grew significantly faster and bigger than those tadpoles in tank 4 with no cover (from my own observation), and therefore, the larger tadpoles coming into a tank with no cover and a predator might have less of a need to be cautious because they are physically bigger.

In trial-tank II, however, inactivity followed the predicted trend of being higher in tadpoles coming from a tank with higher cover, and lower in tadpoles coming from a tank with less cover. I am unsure of why this tank differed from the other two trial tanks, however a possible explanation could be that because trial tank II had only a medium amount of cover, inactivity was a much more important factor. In addition, this could simply be due to experimental error. As the plants were very “vertical,” the classification of when a tadpole underneath the plants were actually using the plants as cover was a very difficult distinction to make during a quick scan-sampling.

FORAGING

In all cases, as expected, foraging behavior decreased significantly with the addition of a predator. While these results could have been simply due to less availability of food in trial tanks, it seems unlikely given that this is a behavior that is more conspicuous, and thus more likely to gain notice by a potential predator. In addition, when the tadpoles’ attention is focused on finding food, it is less likely to notice a predator waiting to attack it.

SWIMMING

Swimming frequency did not appear to follow any trend across trial tanks, and in most cases appeared to *increase* with the addition of a predator. This seems counterintuitive, because swimming, like foraging, is a conspicuous behavior, especially in an environment as small as a 10-gallon tank. However, it seems reasonable to suggest that swimming is an important behavior in predator elusion in a natural environment with more space. In general, during trials the swimming behavior was observed more towards the beginning of each trial, and decreasing by the end of each trial. This could explain the increase in swimming behavior, because immediately after sensing a potential predator, the tadpoles first instinctual response could be to swim away. Confined in a 10-gallon tank, however, the tadpoles might have concluded that there was nowhere to go, and thus the next response is to remain as inconspicuous as possible. This suggests a possible hierarchy of behavioral responses to predators. As Teplitsky et al (2004) showed in their study that tadpoles show different responses to different types of predators, and hierarchical responses to multiple predators. Therefore, tadpoles are capable of have different strategies in different situations, and it is a reasonable theory that the tadpoles' behavioral response would change upon discovering that the first tactic is not productive.

HIDING

Hiding behavior increased with availability of habitat cover. The reasoning for this is rather straightforward: when there are more places to hide, and more room within hiding places to fit more tadpoles, more tadpoles will hide. In the one tank where significance was not observed, I believe that with repeated trials, significance would be reached based on the trend that was observed through all trial tanks.

In trials with both plant cover and a rock refuge available, plant cover was significantly preferred overall. The one case where we see no significance might simply be from not enough repetitions and a two-small sample size, since in their assimilation tank (where the sample size was slightly bigger) the plants were significantly preferred.

GENERAL

In almost all cases, frequency of behavior in each assimilation tank was *not* significantly different from the similar experimental tank. This was as predicted, because even with the introduction of a predator during trials, the tadpoles may be more comfortable in the setting most similar to their assimilation tank. Thus their behavior should not change as much as they may feel safer.

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