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Repulsion from Chemical Cues in *Bufo marinus* (Cane Toad) Tadpoles

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ABSTRACT

Predator-prey relationships have been studied relentlessly throughout all different taxa and systems. Larval anurans use the mode of chemosensory to detect and avoid predation. By exposing *Bufo marinus* tadpoles to a variety of natural chemicals (crushed conspecific larvae and metamorphs, and crushed annelid prey) a strong repulsion was demonstrated. Avoidance of the treatments verified that *Bufo marinus* tadpoles are sensitive to changes in the chemical make-up of their environment that could be caused by a predator in close proximity.

RESUMEN

Las relaciones de la Depredador-presa se han estudiado implacablemente a través de todas las diversos taxus y sistemas. Las anuras larvales utilizan el modo de chemosensory para detectar y para evitar la depredación. Exponiendo a los renacuajos de *Bufo marinus* a una variedad de productos químicos naturales (las larvas y los metamorphs conspecifico machacados, y presa anélida machacada) una repulsión fuerte fue demostrada. La evitación de los tratamientos verificó que los renacuajos de *Bufo marinus* sean sensibles a los cambios en el maquillaje químico de su ambiente que se podría causar por un depredador en gran proximidad.

INTRODUCTION

Being able to detect and avoid predators is vital to survival in nature (Lefcort 1998; Petranka & Hayes 1998; Summey & Mathis 1998; Laurila 2000; Laurila *et al* 2002). There are numerous modes of detection such as sight, sound, and even thermosensory. The common mode in which many anuran larvae use to perceive predators is chemosensory (Laurila, Kujasalo, & Ranta 1998; Lefcort 1998; Petranka & Hayes 1998; Summey & Mathis 1998; Chivers *et al* 1999; Gallie, Mumme, & Wissinger 2001; Hagman & Shine 2008; Pearl *et al* 2003). This ability to receive and interpret chemical cues is not only used for predation avoidance; it is, also, used for species identification (Gallie, Mumme, & Wissinger 2001) and prey recognition by some tadpoles (Chivers *et al* 1999; Hagman & Shine 2008). But, when it comes to evading predators, chemosensory is especially useful since chemicals can be perceived while still in hiding, thus, not having to reveal ones self, or with enough of a warning to allow an individual to take hiding or flee before the predator is able to locate the individual being pursuit (Lefcort 1998; Petranka & Hayes 1998; Summey & Mathis 1998; Buskirk & Arioli 2002).

It has been shown in many prior studies that chemical cues induce plastic behavior responses in many aquatic larvae (Werner & Anholt 1996; Laurila, Kujasalo, & Ranta 1998; Laurila 2000; Laurila *et al* 2002; Relyea 2002). Such responses vary greatly; some behaviors are aggregation, dispersion, decreased movement, and increased movement (Anholt, Skelly, & Werner 1996; Lefcort 1998; Petranka & Hayes 1998; Summey & Mathis 1998; Chivers *et al* 1999; Eterovick & Sazima 1999; Laurila 2000;

Relyea 2002; Pearl *et al* 2003; Hagman & Shine 2008). In the case of *Bufo marinus* tadpoles, it has been shown that they react in a repulsive manner to the chemicals released from injured conspecifics and, that several other stimulus do not trigger an avoidance response (Hagman & Shine 2008). Although *Bufo marinus* tadpoles do have poisons in their skin (Crossland & Azevedo-Ramos 1999), it is unknown whether it is the release of poisons or the release of some other “alarm pheromone” that triggers this repulsion response (Laurila, Kujasalo, & Ranta 1998; Summery & Mathis 1998). It is curious though since in the aforementioned experiment (Hagman & Shine 2008) one of the stimulus treatments consisted of lettuce extract as a prey item. Although tadpoles have an extensive list of possible prey items (Savage 2002), lettuce is not one of which they would encounter naturally.

The following experiment further investigates the reaction to different chemicals encountered naturally by *Bufo marinus* tadpoles. Each chemical treatment indicates either a prey item or predator in close proximity feeding on a conspecific. Predator cues released from injured conspecifics, both in the larval and metamorph stage, are expected to cause a repulsion response. Prey cues released from injured annelids are expected to cause a congregating, attraction effect.

METHODS

Natural History

Bufo marinus gestation within the eggs can last between 36 hours and 4 days (Lampa & Leo 1998; Savage 2002). The larvae are not extremely large especially considering the massive adults they will become; in their largest tadpoles stage they have a length of only 24mm. While in their larval form, *B. marinus* are very social and can be found in large aggregations (Savage 2002).

Collection

Collection of the *B. marinus* tadpoles occurred during the late dry season in Monteverde, Costa Rica from an artificial pond located behind the establishment on the property of Marvin Hidalgo. Tadpoles were collected early in the morning of each testing day and released the same day in the afternoon after testing was complete. The larvae were extremely abundant with the estimated population over 1500 (estimations made by observation). The pond had two gentle sloping shorelines; one on the north shore and one on the south shore. The easily accessible shorelines not only made capture relatively simple but they also allowed capture to alternate daily between each shore to reduce the chances of recapture. This was significant because in theory each tadpole was only to be used once. A handled sieve was used to aid in capture as well as a small cooler for transportation from the pond up to the Biological Station. Once at the Biological Station tadpoles were then divided into groups of 50 individuals and each set was contained in a separate plastic bowls.

Set-Up

The testing arena consisted of a large glass tank (70x70x30cm; Fig. 1) with a grid of 100 squares (7x7cm) divided into 10 zones underneath it. Zone 1 was determined by the origin of the treatment being introduced. Thus, Zone 1 had the highest concentration in the gradient of the treatment. Zone 2 was determined as all of the squares that touched

Zone 1, therefore, having the second highest concentration. Zone 3 then was named those squares that touched Zone 2. Each zone followed this pattern sequentially, hence, making Zone 10 those squares that are the very furthest from the point of origin and consequently then having the lowest concentration of the treatment in the gradient.

10	10	10	10	10	10	10	10	10	10
10	9	9	9	9	9	9	9	9	9
10	9	8	8	8	8	8	8	8	8
10	9	8	7	7	7	7	7	7	7
10	9	8	7	6	6	6	6	6	6
10	9	8	7	6	5	5	5	5	5
10	9	8	7	6	5	4	4	4	4
10	9	8	7	6	5	4	3	3	3
10	9	8	7	6	5	4	3	2	2
10	9	8	7	6	5	4	3	2	1

FIGURE 1. Depiction of the Zone layout of the arena grid. Zone 1 had the strongest concentration of treatment; it was the origin point for treatment distribution. Zone 10 had the weakest concentration of treatment in the gradient. The numbers were not written on the grid during any of the trials.

For each trial the tank was emptied, cleaned and filled 6cm deep (approximately 30 liters) with fresh water. Each trial required a new set of 50 tadpoles which were added into the center of the tank and allowed a 5 minute adjusting period. The treatment was introduced in one corner via a 50 ml burette; the tip of which was slightly under the surface of the water to reduce the effect ripples/surface disturbance could have on the results. Each treatment was diluted into 1 liter of water for each trial. The 1 liter of treatment was completely added to the tank over the first 30 minutes of the trial (approximately 160ml every 5 minutes). Three trials were ran per each treatment and each trial lasted for an hour. During that hour pictures were taken every 5 minutes to capture the movement of the *B. marinus* larvae. As a visual aid of the gradient 3 drops of red food coloring were added to each liter of treatment.

Treatments

Test 1 –Larval Conspecific Chemical Cues- To test the effect of chemical cues released from injured conspecifics 2g of *B. marinus* tadpoles were macerated and diluted into 1 liter of water for each of the 3 trials. The individuals that were crushed were casualties from another peers experiment. So, no tadpoles were injured for this experiment.

Test 2 -Dye Control- The first control was to test the effect the food coloring had on the larvae. This was accomplished by adding 3 drops of the dye to 1 liter of fresh water for each of the 3 trials.

Test 3 -Annelid Prey Chemical Cues- This stimulus was found and collected as it was being preyed upon by tadpoles while in the pond. Two individuals were found, they were distributed between 3 trials. Thus, making it so that each trial had approximately 0.7g of annelid that was macerated into 1 liter of water added to it.

Test 4 -Human Control- To ensure that taking pictures had no human induced effect on the movement of the tadpoles this treatment actually consisted of nothing. No actual treatment was added to the tank. Only pictures were taken each 5 minute increment.

Test 5 -Metamorph Conspecific Chemical Cues- This stimulus tested the effect that injured metamorphs of *B. marinus* had on the larvae. To ensure that the mortality rate remained at 0 for this experiment a search was carried out around the ponds edge to find metamorphs that had already drowned; 3 individuals were found. Each trial consisted of approximately 0.33g of crushed metamorphs diluted into 1 liter of water.

Data Analysis

The number of tadpoles per zone was then tallied up for each 5 minute increment. Calculations were made to determine the average number of tadpoles per square per zone to take away the influence the area of each zone has on the results. Next, a linear regression was run for each of the 5 minute intervals to determine the slope between time passed and the average number of individuals per square. This allowed the direction of movement to be determined; a positive slope indicates repulsion from the origin and a negative slope proposes attraction or a lack of concern toward the stimulus (Hagman & Shine 2008). Lastly, a repeated measures ANOVA.

RESULTS

The preliminary linear regressions displayed an interesting trend (Fig. 2). In all of the tests it was observed that as the time progressed any response that had initially occurred became weaker, as in the slopes moved closer to 0. During actual trials it was observed that the integrity of the gradient declined. Meaning that, as time progressed the stimulus became more uniform throughout the tank instead of maintaining a decreasing gradient of concentrations from the point of origin. Congruently with the unification of the gradient the number of tadpoles per zone became more unified. Note that Zone 1 was not calculated into the results or depicted in the Fig. 2. All but one of the stimulus clearly resulted in repulsion (Fig. 3). The conspecific chemical cues, dye control, annelid prey chemical cues, and metamorph conspecific chemical cues all had a positive slope which showed that as the zones move further away from the stimulus point of origin the higher the density of larvae gets. A repeated measures analysis showed that no one stimulus caused a greater repulsion than the others ($F_{(4,9)}=1.5087$, $p=0.2788$; Fig. 3)

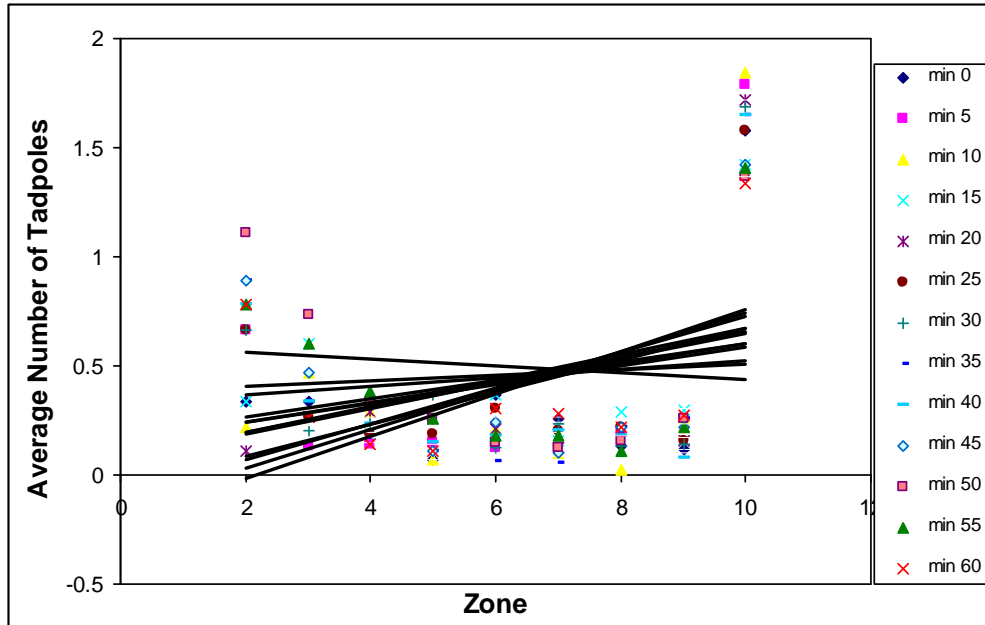


FIGURE 2. The progression of *Bufo marinus* tadpoles' movement over a gradient from high concentration (Zone 2) to low concentration (Zone 10) of larval conspecific chemical cues (Test 1). Each linear regression plots the number of tadpoles per zone per 5 minute increments.

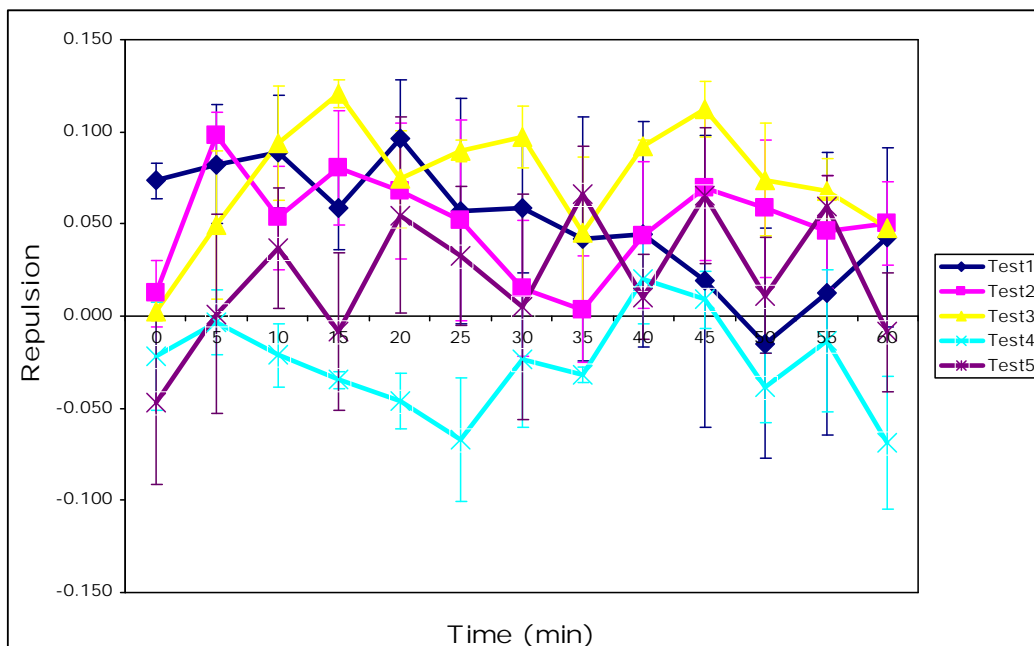


FIGURE 3. This index of repulsion for *Bufo marinus* larvae from 5 different treatments (Test 1- Larval Conspecific Chemical Cues; Test 2- Dye Control; Test 3- Annelid Prey Chemical Cues; Test 4- Human Control; Test 5- Metamorph Conspecific Chemical Cues) depicts the slopes calculated from linear regressions (fig. 3). A positive slope indicates repulsion from the origin and a negative slope proposes attraction or a lack of concern toward the stimulus.

DISCUSSION

Repulsion is an effect of natural chemical cues for *Bufo marinus* tadpoles. It was expected that both the injured larval and metamorph conspecifics would induce a repulsive effect but, it was interesting to see that the injured prey also provoked repulsion. From this prey repulsion it is possible that the same or similar chemical that is released by the conspecifics is universal and also released by the annelids. Another possible explanation is that *B. marinus* is extremely sensitive to any change in the chemical make-up of their surrounding no matter what chemical is being introduced; this would help to explain their response to the food coloring and is, also, backed up with the fact that no one chemical induced a greater response than the rest.

Another factor to consider is that the gradient deteriorated after approximately half the time had passed. This could have had an effect on the magnitude of the response. It would be interesting in future studies to devise a way to continue the gradient completely through the trial. Suggestions to accomplish this are a larger tank or less individuals per trial since the unification of the gradient appeared to be propelled by the movement of the tadpoles.

The final thoughts for a continuation of this project would be to test the effect corners had on the aggregation of the tadpoles. It has been suggested in previous studies that once predator reception has occurred, tadpoles move into smaller spaces or crannies and then reduce all movement (Eterovick & Sazima 1999). This was exhibited in Zone 1, thus is the reason Zone 1 was not calculated into the results.

Predation avoidance via chemosensory is an intricate and interesting behavior that effects anuran larvae movement drastically. Development and further investigation in the understanding of the chemical cues received by larval anurans will give a clearer picture as to what behavior is to be elicited and why.

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