SHORT COMMUNICATION

The influence of tadpole density and predation on the behavioral responses of two Neotropical anurans

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The risk of predation can prompt a variety of responses from the prey species, including changes in morphology, life history, and behavior. Adaptive responses to predators may depend on the amount of resources available or the presence of competitors (Abrams and Vos 2003). The number of conspecifics may influence the perception of predation risk and affect the behavior of individuals (Bohlin and Johnsson 2004, Downes and Hoefer 2004). Therefore, it is expected that because large groups of individuals (i.e., tadpoles) perceive a lower risk of predation, they exhibit "riskier" behavior (i.e., leaving refuges and increasing their rates of activity; Skelly 1994, Awan and Smith 2007) in contrast to an individual tadpole or a small group of larvae (Roberts 1996, Bohlin and Johnsson 2004). However, increased activity of larvae in large groups may be associated with an increase in the number of conspecifics competing for the available resources (Relyea 2002).

The activity rates of anuran larvae change depending on the number of conspecifics present; thus, the greater the number of larvae, the more active the tadpoles. This suggests that the more tadpoles in an aggregation, the less the perceived risk of predation (Relyea 2002, Peacor 2003). Also, tadpoles differ in their natural activity rates and various studies have shown that species that inhabit ephemeral ponds with few predators are more active than species that inhabit more permanent ponds (Skelly 1996, 1997, Dayton and Fitzgerald 2001).

I performed a series of experiments to examine how odor of a predatory fish (*Moenkhausia dichroura* Kner, 1858) influences the behavior (i.e., activity rate) of the larvae of the Mewing Frog, *Physalaemus albonotatus* (Steindachner, 1864), and the Cururu Toad, *Rhinella diptycha* (Cope, 1862). Both anurans are geographically widespread in Brazil, Paraguay, Bolivia, and Argentina, and breed from September–March (Frost 2018). *Physalaemus albonotatus* is commonly found in ephemeral ponds whereas *Rhinella diptycha* usually is found in permanent ponds; however, both may coexist in semi-permanent ponds with vertebrate and invertebrate

predators. The primary goal was to determine the effect of the odor of a predator on larval densities.

I expected that at low densities, the activity rate of tadpoles would decrease in the presence of the odor of a predator. Conversely, I expected that at high densities, the activity rate of tadpoles would increase in response to the odor of a predator, resulting in a display of more risky behavior.

The tadpoles were obtained from three foam nests of *Physalaemus albonotatus* and two egg clutches of *Rhinella diptycha* collected from a semi-permanent pond located 10 km from Corrientes, Argentina (27°28'26" S, 58°43'35" W). Fish were collected from a permanent pond (near the pond where eggs were collected; 27°28'42" S, 58°43'50" W) with dip nets and aquatic funnel traps. Anuran eggs and fish were maintained in the laboratory in containers that were 60 × 40 × 10 cm in size. The eggs were incubated under lab conditions (temperature 26–28°C, photoperiod 13L:11D h) until the tadpoles hatched. The tadpoles were reared in a large aquarium (35 × 25 × 8 cm) filled with tap water and were fed boiled lettuce ad lib. three times a week. Five fish were housed in tanks filled with 3.5 L well water for 5 days prior to the experiments and fed fish-food flakes every 2 days. The well water in which the fish had been placed was then used as an agent of predator odor (Mitchell *et al.* 2017).

Experiments were conducted on tadpoles of each species at developmental Stages 30–32 (Gosner 1960). The larvae were placed in plastic containers holding 1200 mL of well water and the experiments performed on the same day, between 10:00 and 14:00 h. I used tadpoles of intermediate size (body length means ± 1SD; 7.75 ± 0.91) because the evidence suggests that vulnerability to predators decreases with increasing tadpole size (Formanowicz Jr. 1986, Richards and Bull 1990, Jara and Perotti 2010, Asquith and Vonesh 2012) and that some species are palatable to vertebrate and invertebrate predators only at intermediate tadpole stages

(Brodie and Formanowicz Jr. 1987). The experimental design consisted of two larval densities (4 and 8 tadpoles) replicated 6 times, resulting 12 experimental units for each species. Behavioral response to predator was analyzed by estimating tadpole activity—i.e., the proportion of tadpoles active in the containers. Tadpoles were randomly assigned to the containers where they temporarily experienced the relevant density effects and were acclimated for 90 min before their behavior was recorded prior to adding the predator cue. Using a calibrated plastic syringe, 12 mL of water was removed from each container with tadpoles and replaced with 12 mL of water with the predator odor taken from the containers with predators to maintain a constant water volume in each container. Then the activity of the tadpoles was recorded at 15-, 30-, and 45-min intervals following the introduction of the predator odor. The activity of the tadpoles was recorded with a video camera (JVC Everio Gz-ms 120, dual memory, zoom 40 ×). At each of the four time points, the activity was recorded for five 5-s intervals, 1 min apart. The initial 15-min recording following the addition of the predator cue should have allowed enough time for the predator odor to be recognized by the tadpoles, and the successive records were made to observe if the tadpoles changed their behavioral response to the predator through time. A total of 240 videos was recorded in the experiment for each species.

The statistical analysis to determine differences in the activity of the larvae was carried out with data from the 60 videos (5 repetitions × 12 containers) obtained at each of the four time periods for each species. Activity was calculated as the proportion of active larvae during a single 5-s interval and transformed to the arcsine. To compare larval activity among the four time periods, we performed a repeated measured ANOVA using density of tadpoles and time point (the 4 different times period tested) as factors, and the proportion of active larvae as the dependent variable. The statistical tests were carried out using XLSTAT 7.5 (Addinsoft 2006).

In tadpoles of *Physalaemus albonotatus,* the proportions of larvae active at the two different densities were significant (Table 1). Larvae at the high density were more active than those at the low density (Tukey test, Difference = 3.35; Critical value = 1.97; *p* = 0.047). However, the activity of these tadpoles did not differ significantly in the presence or absence of predator odor. These results were consistent regardless of the time point or the density of the larvae in the enclosures (Table 1, Figure 1A).

In contrast, in tadpoles of *Rhinella diptycha,* the proportions of larvae active at the two different densities was not significantly different. There was an effect of different time points (predator odor), but this interacted with tadpole density. A post-hoc test revealed that at high densities before the predator cue was introduced, the proportions of active tadpoles were significantly lower than 15 and 30 min after the cue was introduced (Table 2, Figure 1B).

These results indicate that there are behavioral differences between anuran species in the ways in which their larvae respond to the presence of predator odor and the number of conspecifics present. The activity of tadpoles of *P. albonotatus* did not change in the presence of predators but did change in response to conspecific density. Perhaps the response to increased density reflects intraspecific competition for space and consumable resources (Kehr 1987). *Physalaemus albonotatus* is a common inhabitant of ephemeral ponds; thus, securing resources to grow and metamorphose before the pond dries may be more important than responses to predators.

The activity of tadpoles of *R. diptycha* only changed in response to predator odor at high larval densities; there was no change at low densities. However, before the predator cue was added and after 45 min (at the end of the assay, when the signal may have been less intense), tadpoles in low density were more active than they were at 15 and 30 min following the addition of the cue (Figure 1B) and more active than high-density tadpoles at the same points in time. It has been suggested that tadpoles may decrease their activity in presence of predator signals (Hartmann and Lawer 2014, Preston and Forstner 2015)—a
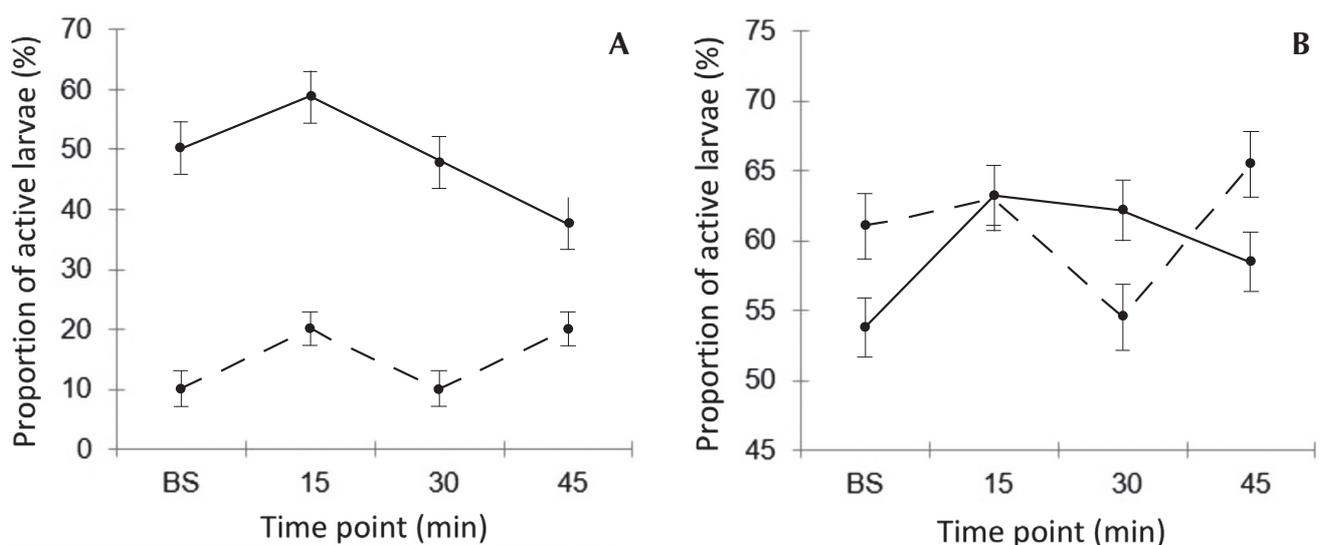

Figure 1. Effect of chemical cue of predator fish (*Moenkhausia dichroura)* on activity rate of *Physalaemus albonotatus* (**A**) and *Rhinella diptycha* (**B**) tadpoles. Continuous line = high density; dashed line = low density; BS = before introducing the predator odor, and 15, 30 and 45 min after stimulus. Each point represents the mean and the bars the standard error.

Table 1. Results of ANOVA test on the influence of density and times point (effect of predator odor) on the activity of tadpoles of *Physalaemus albonotatus* and *Rhinella diptycha.*

Factors	*Physalaemus albonotatus*				*Rhinella diptycha*			
	df	**Den *df***	*F*	**Pr < *F***	*df*	**Den *df***	*F*	**Pr < *F***
Density	1	232	6.925	**0.009**	1	232	0.593	0442
Time point	3	232	0.228	0.877	3	232	1.980	0.118
Density × Time point	3	232	0.248	0.863	3	232	3.216	**0.024**

pattern that is consistent with my observations. Moreover, tadpoles of *R. diptycha* are common in semipermanent ponds; thus, at low densities, tadpoles would tend to be more active because space is not a limiting resource.

These results do support the premise that anuran larvae are more likely to invest in defense behavior when there is less competition (Stav *et al.* 2007, Teplitsky and Laurila 2007). Although tadpoles of *R. diptycha* tend to reduce activity in presence of predator odor, the difference is not statistically different. However, the tadpoles of *R. diptycha* that were maintained at higher densities engaged in riskier behavior, increasing their activity when exposed to predator cues. This

behavior was previously observed in *Scinax nasicus* (Cope, 1862) (Gómez and Kehr 2013) and *Lithobates sylvaticus* (LeConte, 1825) (as *Rana sylvatica;* Relyea 2002); in the presence of predators, the activity of these larvae increased as the number of conspecifics increased. Fernandez and Oliveira (2017) found that the tadpoles of *Rhinella ornata* (Spix, 1824), which show schooling behavior, tended to flee when they detected chemical cues of predator, thereby avoiding predators, as well as other tadpoles. This is consistent with the assumption that increased activity in presence of predators may signal the efforts of the tadpoles flee from the danger zone (Spieler 2005, Jara and Perotti 2010).

Table 2. Results of Post-hoc analysis performed in tadpoles of *Rhinella diptycha* to determine the effect of interaction between density and time point (predator odor) on the activity of tadpoles. BS = before introducing the predator odour.

Comparisons	Low density			High density		
	Difference	**Critical value**	*p*	**Difference**	**Critical value**	*p*
BS vs. 15 min	5.723	2.58	0.066	9.447	2.16	**0.014**
BS vs. 30 min	0.948	2.58	0.977	8.396	2.16	**0.036**
BS vs. 45 min	4.612	2.58	0.193	4.723	2.16	0.358
15 min vs. 30 min	4.776	2.58	0.463	1.051	2.16	0.994
15 min vs. 45 min	1.112	2.58	0.986	4.724	2.16	0.624
30 min vs. 45 min	3.664	2.58	0.677	3.673	2.16	0.784

The tadpole behavior observed in the presence of predator odor at different conspecific densities in these species may indicate that tadpole activity is more closely related to the type of environment and competitors than to the chemical cues of predatory fish.

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