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## BEHAVIORAL RESPONSES OF *BUFO ARENARUM* TADPOLES TO ODONATE NAIAD PREDATION

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**Abstract.**—In the presence of predators, anuran tadpoles often exhibit behavioral plasticity, which in turn reduces predation risk. We evaluated indirect effects of odonate larva predation on activity of *Bufo arenarum* tadpoles. We reared intact and tail-damaged larvae in three different predator treatments: (1) caged predators fed tadpoles; (2) caged predators that were starved; and (3) no predators. Both intact and damaged tadpoles were significantly less active when exposed to predator cues. There were no differences in activity between tadpoles with damaged and intact tails, however significant differences were observed between fed and starved predator treatments. Our results are consistent with other studies that have shown bufonid tadpoles reduce activity when exposed to predator cues and address a novel contribution of southern-hemisphere bufonid, expanding the knowledge of that family in ecological experiments.

**Key Words.**—behavior; *Bufo arenarum*; insect predator; predation; *Sapo común*; tadpoles

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### INTRODUCTION

It is well known that many species of anurans alter their behavior and morphology in the presence of predators (McCullum and Van Buskirk 1996; Relyea 2001a; Van Buskirk 2001; Moore et al. 2004) and that these responses increase the probability of survival (Van Buskirk and McCullum 2000a, 2000b). Common responses of tadpoles when exposed to predators are reduced activity, increased tail depths, and a shorter head to body ratio (e.g., Relyea 2004 and references therein; Miner et al. 2005). These responses can be induced both by predator presence or chemical cues (Stauffer and Semlitsch 1993; Adams and Claeson 1998).

Anuran tadpoles of the genus *Bufo* make good candidates for predator-response studies for several reasons: (1) They are more vulnerable to predators during intermediate stages of development (i.e., Gosner stages 30-33; Gosner 1960) (Brodie and Formanowicz 1987), (2) different degrees of tail damage result from direct encounters with predators (McCullum and Leimberger 1997; Hoff and Wassersug 2000; Van Buskirk and McCullum 2000a), and (3) *Bufo* tadpoles can perceive presence of predators by detecting chemical cues released from conspecifics that were killed by predators (Hews 1988). The alarm substance was described by Pfeiffer (1982) as a pheromone that is secreted by one individual and received by a second individual of the same species, eliciting specific behavior. This type of alarm response is innate in *Bufo* tadpoles (Semlitsch and Gavasso 1992), and indirect in the sense that tadpoles can respond to chemical cues without directly experiencing contact with a predator. Then tadpole responses can be induced by chemical cues from predators (kairomones) and injured prey (alarm cues) (Relyea 2001b; Schoeppner and Relyea 2005).

Here we provide evidence for behavioral plasticity in *Bufo arenarum* (*Sapo Común*) when exposed to the predatory dragonfly *Pantala* sp. *B. arenarum* is a common toad in Argentina that breeds in ephemeral and permanent ponds. There are no data on predator abundance in *B. arenarum* habitats, but it is well known that tadpole predator abundance varies with hydroperiod and size of pools (Skelly 1995; Wellborn et al. 1996). We have observed *B. arenarum* living in ephemeral pools without predators and in permanent pools that harbor invertebrate predators. *Pantala* sp. are voracious tadpole predators that commonly occur with *B. arenarum*. On several occasions we have observed *B. arenarum* tadpoles with varying degrees of tail damage resulting from encounters with predators, presumably *Pantala* sp. Because *B. arenarum* frequently inhabit sites with high numbers of *Pantala*, we hypothesized that tadpoles should elicit changes in behavior when reared with predators. Furthermore, we wanted to examine whether behavioral responses varied when tadpoles were exposed to different levels of predator cues and tail damage.

We designed an experiment to manipulate the tadpole experience in two treatments: "intact tadpoles" were tadpoles with no direct predation experience, and "damaged tadpoles" were tadpoles that experienced simulated encounters with predators by having a portion of the tail excised. We hypothesized that damaged and intact tadpoles may respond differently to the predatory environment particularly, damaged tadpoles may have a strong response in reducing activity to predator presence than intact tadpoles do. Therefore we examined variation in activity between intact and damaged tadpoles in the absence of predators ("no predator"), in the presence of predators that had not eaten tadpoles ("starved"), and in the presence of predators that were fed tadpoles ("fed predator") to determine what cues trigger changes in behavior and whether or not these changes varied between treatments.

## MATERIALS AND METHODS

We collected tadpoles of *B. arenarum* of similar size corresponding to aggregations found in several ephemeral pools in Anillaco, La Rioja, Argentina, on 28 January 2000. Tadpoles were placed in the laboratory on 29 January, and maintained at 29°C on a 12-h light: 12-h dark cycle in plastic containers (20 x 18 x 40 cm) filled with 2 l of tap unchlorinated water. Tadpoles in each tub were fed 5 mg TetraMin Fish flakes every day. A supplement of 5 mg TetraMin Fish flakes per day provided an ample amount of food, minimizing any potential competitive effects. Feces of tadpoles were removed from the containers and water was changed daily, prior to feeding the tadpoles.

Our experimental design consisted of a 2 x 3 full factorial experiment with five tadpoles in each tub exposed to two levels of tail damage, and three levels of predator stimuli. *Pantala* sp. was one of the most voracious predators found at the study site and it commonly co-occurs with *Bufo arenarum* tadpoles. Predators were kept in cages inside the tubs. We used cylindrical cages (10 cm diameter x 20 cm long) constructed of plastic mesh to house predators with tadpoles. Cages allowed for exchange of water while preventing predators from taking tadpoles. On several occasions, the *Pantala* larva died; in these instances it was replaced within 12 h. *Pantala* larvae were randomly assigned to each cage. Predators were maintained in the laboratory and fed with palatable *B. arenarum*, stages 30-31 (Gosner 1960) with weights ranging from 0.05 to 0.15 g. The mass of tadpole used to feed the predator was held constant at 0.1 mg tadpole mass per predator. Predator treatments consisted of: (1) "control" (empty cage); (2) "starved" (*Pantala* larvae that were not fed at least two days prior to the experiment); and (3) "fed" (caged *Pantala* larvae that were fed one *Bufo* tadpole each day). To perform the "damage" treatments, we excised the distal third of the tadpole subject's tail ("damaged"), or left the tail intact ("intact"). All the tadpoles (intact and damaged) were anesthetized before the procedure with 0.2% chlorotone. Tadpoles used in experiments were matched for size, then randomly selected and placed in one of the 6 treatments. Each of the six treatments was replicated three times in a randomized complete-block design, using laboratory tables as blocks. The experiment began on 29 January and was terminated on 11 February when the first tadpoles reached Gosner stage 42; the majority of individuals were in Gosner stage 41 (Gosner 1960).

We used ANOVA to test the hypotheses of no mean difference in activity between treatments. There were no significant block effects and no significant block x treatment interaction effects; therefore, we did not include blocks in subsequent analyses. Two observers (LM and MP) measured tadpole activity by standing over the tubs and counting the number of tadpoles in the tub that were moving (Skelly 1994). Previous observations on activity indicated that observers do not alter activity patterns. We quantified activity through scan observations by counting the number of tadpoles moving in each tub, every other day, totaling 18 activity data in four repeated measurements (4th, 6th, 8<sup>th</sup>, and 10th February). The proportion of tadpoles that were active was arcsine-square root transformed prior to analysis.

To test for differences in mean activity of tadpoles among predator treatments, we used repeated measures ANOVA, followed by Student-Newman-Keuls Post-hoc tests (Zar 1999). Student-Newman-Keuls Post-hoc tests were not performed for damage groups, because the number of replicates was less than three.

## RESULTS

Tadpoles were significantly less active when exposed to predators ( $F_{2,12} = 21.45$ ,  $P < 0.0001$ ; Fig. 1) and there was no significant difference in activity between damaged and intact treatments ( $F_{1,12} = 1.081$ ,  $P = 0.319$ ) (starved intact tadpoles  $X = 0.40$ ,  $SE = 0.03$ ; starved damaged tadpoles  $X = 0.37$ ,  $SE = 0.04$ ; fed intact tadpoles  $X = 0.28$ ,  $SE = 0.06$ ; fed damaged tadpoles  $X = 0.27$ ,  $SE = 0.04$ ). Both damaged and intact tadpoles were significantly less active in predator treatments than control treatments (Post Hoc Student-Newman-Keuls,  $P < 0.05$ ) (control intact tadpoles  $X = 0.58$ ,  $SE = 0.04$ ; control damaged tadpoles  $X = 0.51$ ,  $SE = 0.04$ ). In addition, tadpoles exposed to the fed-predator treatment were significantly less active than tadpoles in starved predator treatment ( $t = -2.92$ ,  $P = 0.004$ ; Fig. 1).

## DISCUSSION

Our results suggest that the presence of dragonfly larvae induces changes in the behavior of *Bufo arenarum* tadpoles and non-contact cues associated with predation are sufficient to induce those changes. Antipredator responses among amphibians may be influenced by the predator's recent diet (Wilson and Letcort 1993) particularly when predators have recently fed on prey conspecifics (e.g., Kats et al. 1988; Semlitsch and Gavasso 1992; Schoepner and Relyea 2005). Several studies have shown that *Bufo* species reduce activity when they are exposed to predators fed with conspecifics (Skelly and Werner 1990; Semlitsch and Gavasso 1992; Anholt et al. 1996; Kiesecker et al. 1996; Summey and Mathis 1998). Our results show that *B. arenarum* tadpoles respond not only to the simple presence-absence of predator cues, but also to additional cues provided by predators when feeding with conspecifics.

Thus, our observations confirm the hypothesis that responses in amphibian larvae are mediated by water-borne cues and particularly *Bufo arenarum* tadpoles could also respond to the presence of excretions related to consumption of conspecifics in addition to the presence of the predator. In addition, this study expands the coverage of the Bufonidae family in ecological experiments, particularly improving the knowledge for southern-hemisphere species and providing further evidence that behavioral plasticity of tadpoles in the genus *Bufo* is widespread and may be deeply rooted within the family.

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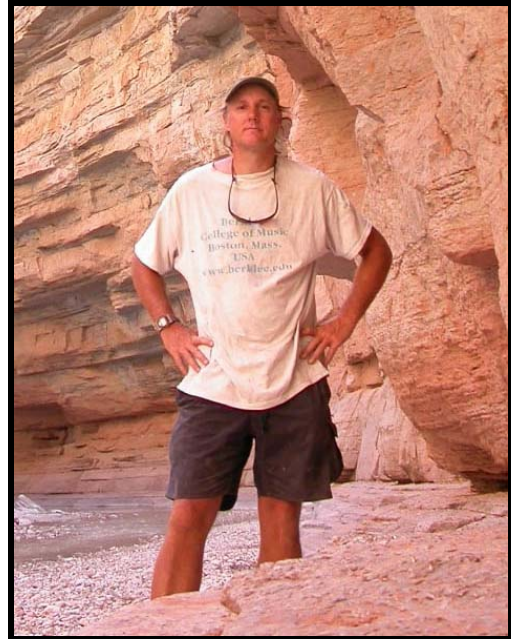
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