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Morphological and developmental plasticity in larvae of *Physalaemus santafecinus* (Anura: Leiuperidae) in response to chemical cues of different predators

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Abstract

Morphological and developmental plasticity in larvae of *Physalaemus santafecinus* (Anura: Leiuperidae) in response to chemical cues of different predators. Many antipredator responses are mediated or induced by the ability of the prey to identify chemical cues of predators. The presence of chemicals produced by predators may alert tadpoles to the presence of the predators, and a heightened response to alarm cues or predator presence may increase the possibility of prey survival. We examined changes in morphology, and growth and development rates of *Physalaemus santafecinus* tadpoles reared in the presence of chemical cues of water beetles (Hydrophilidae) and a fish (Characidae). We recorded the time to metamorphosis, as well as weights of metamorphic individuals to determine if the larval stage is accelerated. The experiments were performed under microcosm conditions, with three treatments—chemical cues from fish, water beetles, and a control group. Each treatment was replicated 30 times. To obtain independent data from different variables, treatments were conducted on individual larvae in separate containers. The principal results were, as follow. (1) Larval morphology was significantly affected by the presence of a predator. (2) Control tadpoles were significantly larger than those subjected to the other two treatments (cues of water beetles and fish). (3) Growth and development rates did not differ significantly among any treatments. (4) Neither time to metamorphosis nor weights of metamorphs varied significantly among treatments. Our results suggest that tadpoles are able to perceive predators by chemical cues released in the water, and *P. santafecinus* tadpoles alter their morphology to chemical cues that indicate predation. However, chemical cues of predators had no detectable effect on growth rate and developmental rates of these tadpoles.

Keywords: Anti-predator response, developmental rate, growth rate, morphological changes, tadpoles.

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Resumo

Plasticidade morfológica e de desenvolvimento em larvas de *Physalaemus santafecinus* (Anura: Leiuperidae) em resposta a estímulos químicos de diferentes predadores. Muitas respostas anti-predação são mediadas ou induzidas pela habilidade da presa de identificar estímulos químicos dos predadores. Substâncias químicas produzidas por predadores podem alertar girinos para a sua presença, e uma resposta aumentada aos estímulos de alarme ou à presença do predador pode aumentar a possibilidade de sobrevivência da presa. Examinamos as mudanças morfológicas e as taxas de crescimento e de desenvolvimento de girinos de *Physalaemus santafecinus* criados na presença de estímulos químicos de besouros aquáticos (Hydrophilidae) e de um peixe (Characidae). Registramos o tempo até a metamorfose e o peso dos indivíduos metamorfoseados para determinar se o estágio larval havia sido abreviado. Os experimentos foram conduzidos em condições de microcosmos, com três tratamentos—estímulos químicos de peixes, de besouros aquáticos e um grupo-controle. Cada tratamento foi replicado 30 vezes. Para a obtenção de dados independentes das diferentes variáveis, os tratamentos foram conduzidos com larvas individuais em recipientes separados. Os principais resultados obtidos foram os seguintes: (1) a morfologia das larvas foi significativamente afetada pela presença de um predador; (2) os girinos do grupo-controle foram significativamente maiores do que aqueles submetidos aos dois tratamentos (estímulos de besouros aquáticos e de peixes); (3) as taxas de crescimento e de desenvolvimento não diferiram significativamente entre os tratamentos; (4) o tempo até a metamorfose e o peso dos indivíduos metamorfoseados não variaram significativamente entre os tratamentos. Nossos resultados sugerem que os girinos de *P. santafecinus* são capazes de perceber a presença de predadores pelos estímulos químicos liberados na água e que alteram sua morfologia em resposta a estímulos que indicam predação. Contudo, os estímulos químicos dos predadores não exercem efeitos detectáveis sobre as taxas de crescimento e de desenvolvimento desses girinos.

Palavras-chave: Girinos, mudanças morfológicas, resposta anti-predação, taxa de crescimento, taxa de desenvolvimento.

Introduction

The composition of many biological communities is determined by interactions among predators and prey, and factors that modify the force of predator-prey interactions (Wellborn *et al*. 1996). Many prey species, especially in aquatic environments, use chemical cues produced during predation events to estimate predation risk (Kats and Dill 1998). Theoretical models of defenses that can be induced hypothesize that selection for plasticity in antipredator defenses should occur given certain ecological conditions (Clark and Harvell 1992), as follow: (1) predation risk is an important, but unpredictable, cause of mortality; (2) prey has constant cues to evaluate predation risk; and (3) the defense induced reduces predator efficiency resulting in a cost of defense. Many antipredator responses are mediated or induced by the ability of the prey to identify chemical cues from predators (Kats and Dill 1998). In prey species, the defense systems that can be induced frequently are activated by cues associated with predators before the attack (Tollrian and Harvell 1999). Thus, the presence of chemicals produced by a predator may provide reliable information concerning its presence to the tadpoles, and it follows that a greater response to alarm cues or predator presence may increase prey survival (Downes 2002).

Defenses often are viewed as produced by constitutive and plastic components; the former refers to the basic phenotype in the absence of a predator stimulus, whereas the latter is an estimate of the variation in phenotypic value with and without predator chemical cues (Schlichting 1986, Tollrian and Harvell 1999). Although the presence of many predators can decrease prey abundance, the consequences of the predators on prey survival can be influenced by prey behavior. Among the strategies used by prey are morphological change (Bronmark and Miner 1992, McCollum and Van Buskirk 1996, Nicieza 2000, Van Buskirk 2002), modification of behavioral responses (Chivers *et al*. 1996, Kiesecker *et al*. 1996), and alteration of the length of life history (Dodson and Havel 1988, Chivers *et al*. 1999, Skelly and Werner 1990). The size-dependent consequences of such adjustments can have a substantial affect on juvenile performance (Nicieza *et al*. 2006).

The results of previous studies have shown that presence of predators can induce a change in tadpole morphology; such changes involve energetic costs (McCollum and Van Buskirk 1996). However, implementation of antipredator strategies often requires time and energy that otherwise would be available for activities such as reproduction and foraging (Lima and Dill 1990).

We studied tadpoles of *Physalaemus santafecinus* because this species breeds in a broad range of habitats from ephemeral to permanent ponds. Thus, tadpoles from the same population could be exposed to predator assemblages dominated by fish, insects, or both. We examined whether tadpoles of *P. santafecinus* use chemical cues from fish (*Moenkhausia dichroura*) and water beetles (Hydrophilidae) to modify their morphology and growth rate in response to the perceived risk of predation. We also recorded the amount of time for the tadpoles to metamorphose and the weights of metamorphic individuals to determine if there are changes, such as the acceleration of larval stages, in the timing of life-history events.

Materials and Methods

Physalaemus santafecinus occurs in eastern Paraguay and northwest Argentina, and breeds from September–April in ephemeral ponds, where the frogs co-occur with beetles (Hydrophilidae), in permanent ponds with bandtail tetra fish (*Moenkhausia dichoroura*), and in semi-permanent ponds with both beetles and tetra fish. Larvae were obtained from two foam nests of *P. santafecinus;* these were collected on 27 October 2009 from a temporary pond located 10 km (27°30' S, 58°45' W) from Corrientes, Argentina, and transported to the laboratory for hatching. The foam nests ware placed in a shallow, plastic wading pool (47 × 33 × 13 cm) filled with well water 10 cm deep. Three days after hatching, the larvae were assigned randomly to the treatments. The water beetles were collected at different sites near Corrientes, and the fish were collected with aquatic dip nets from a permanent pond. To obtain chemical cues, 15 fish were placed in a single plastic container and 20 beetles were placed in another single plastic container (18.5 cm diameter × 17 cm high) with 3.5 l of well water.

The tadpoles were fed boiled lettuce *ad libitum* twice a week. The containers in which the tadpoles were housed were partially cleaned once a week. The captive fish were fed fish food (Shulet brand, Shulet S.A. 108/A/E, Buenos Aires) and the beetles were fed with tadpoles of *Physalaemus santafecinus*. We fed the predators in separate containers to avoid introducing chemical cues of their food in the containers and thereby influence the responses of the tadpoles. The predators kept in the container without food were replaced with predators that had been fed twice a week; we used different predators throughout the experiments. Containers with predator chemical cues were partially cleaned twice a week. We removed tadpoles that died during the first 3 days so that the experimental results would not be influenced by individual deaths caused by initial stress.

Experimental Design

The experiments were conducted under microcosm conditions. Temperature ranged between 26 and 29°C and the photoperiod was L:D = 13:11. The tests were conducted in plastic containers (8 cm diameter × 9 cm high) filled with 300 ml of well water. The experimental design involved three treatments: chemical cues from fish and from water beetles, and a control group.

The experiments were begun on October 30th (Day 0) when tadpoles that had reached developmental Stage 26 (Gosner 1960) were randomly assigned to the treatments, and terminated when the last tadpole metamorphosed. Each container held a single larva and each treatment was replicated 30 times, resulting in a total of 90 experimental units. The containers were arranged in three blocks, each one containing 10 replicates of each treatment; the 30 containers in each block were placed randomly.

We used a calibrated plastic syringe to remove 3 ml of water from each container with tadpoles and then added 3 ml of water with the chemical signals taken from the containers with predators, thereby keeping the water volume in each container constant. This procedure was performed three times a week. In the case of the control treatment, the 3 ml of water extracted was replaced with 3 ml of well water from an isolated container without predators.

On 13 November, 15 days after the start of the experiment, the tadpoles were measured, weighed, and staged according to the developmental table of Gosner (1960). To quantify morphological phenotypic responses, we photographed lateral views of all tadpoles in a glass box with 1-mm grid. Five linear measurements were taken—body length, body height, tail-fin length, tail-fin height, and caudal muscle height. As tadpoles metamorphosed, we recorded the date, along with the weight of the individual.

Statistical Analyses

Each dependent variable in each treatment was tested for normality (Shapiro-Wilk test) to determine if the data were normally distributed before further analyses were undertaken. First, we performed a principal component analysis (PCA) for each treatment to estimate which variables were more important in relation to the total variance explained by each component. The two body dimensions and the three tail-fin dimensions for every individual were entered into the PCA to assess the loading of each variable on the different components and their explained variance.

To compare the morphologies of the tadpoles between treatments with different predators, we performed a two-factor MANCOVA, using treatment as factor and weight as covariable to eliminate the effect of size in the tadpoles. The five morphological variables were included as dependent variables. Subsequently, if Wilks' Lambda indicated significance, we performed a one-way ANOVA for each dependent variable; for significant cases, a posteriori pairwise comparisons were made using Tukey's comparison test.

In addition, an ANOVA test was used to determine the effect of predator treatments on the growth rate, and we used a Kruskall-Wallis test to compare the differences in development rates between treatments; in this case, we used a non-parametric test because the data did not adjust to a normal distribution (Shapiro-Wilk = 0.951; p = 0.003). To determine if there were significant differences among metamorphic individuals, we conducted a MANOVA, with treatments as factor and time to metamorphosis and weight of metamorphs as dependent variables.

All statistical tests were carried out using Systat 7.0 (SPSS 1997) and XLSTAT 7.5 (Addinsoft 2006). The photographs were measured using Image-Pro Plus 4.5. The growth rates of tadpoles for each treatment were calculated by dividing the natural logarithm of final weight by the natural logarithm of total experiment duration in days = (Lnfinal weight/Lndays) (Kehr 1991). The rate of larval development for each treatment was calculated by dividing the difference in size between final and initial stages by number of days (Lnfinal stage – Lninitial stage/ Lndays).

Results

The Principal Component Analysis (PCA) conducted on each treatment indicated that the five morphological variables were important in explaining the variance. PCA 1 accounted for more than 85% of the variance for each treatment (fish = 90.3, beetles = 89.5, control = 92.1). The variables score were < 0.9 for the five variables in the three treatments. Chemical cues of predators significantly affected the morphology of the tadpoles (MANCOVA Wilks's Lambda = 0.560; F10, 150 = 5.051; p < 0.001). The ANOVA tests indicated significant differences in body and tail lengths, and body, tail, and caudal muscle heights between tadpoles in either predator treatment and those in the control group (Table 1). Tadpoles in the control group had higher means values for each of the five variables (Figure 1). Chemical cues of predators had no detectable influence on the growth and developmental rates of tadpoles (ANOVA F2, 80 = 0.349; p = 0.707; Kruskal Wallis = 5.991; df = 2; p = 0.163). There was no significant difference in the number of days to metamorphosis or the weights of metamorphs (MANOVA Wilk`s Lambda = 0.950; F2, 34 = 0.886; p = 0.422).

Discussion

Many prey species can increase the probability of being captured by a predator if the prey does not identify and respond to the predator (Chivers *et al*. 1999). Our results support the findings of other authors who argued that the anuran tadpoles can perceive predators by chemical cues released in the water (Petranka *et al*. 1987, Stauffer and Semlitsch 1993); however, several behavioral studies reveal that prey species respond to chemical cues of predators only if the predators had been fed with conspecifics of the prey (Mathis and Smith 1993, Wilson and Lefcort

Table 1. Results of MANCOVA test considering the influence of treatments of the five morphological variables on *Physalaemus santafecinus.* Weight was used as covariable. An ANOVA test for each variable and its posterior comparison (♦ Tukey test) was tested when the probability was significant. The letters indicated the treatments (f = fish, b = beetles, c = control) and the number indicated differences for the Tukey test (same number = not significant differences; different number = significant differences). The letters of the treatments are ordinates from the smallest (left position) to largest (right position) arithmetic mean registered. ANOVA probabilities according to Bonferroni criteria: $p < 0.001$.

Test	Variable	df	F	*p*
MANCOVA (Wilk's lamda = 0.568)	Treatment	10, 150	5.05	< 0.001
ANOVA	Body length ♦ f^1 b^1 c^2	2, 80	16.45	< 0.0001
	Tail length ♦ b^1 f^1 c^2	2, 80	9.26	< 0.001
	Body height ♦ f^1 b^1 c^2	2, 80	13.26	< 0.0001
	Tail height ♦ f^1 b^1 c^2	2, 80	10.22	< 0.001
	Caudal muscle height ♦ f^1 b^1 c^2	2, 80	7.71	= 0.001

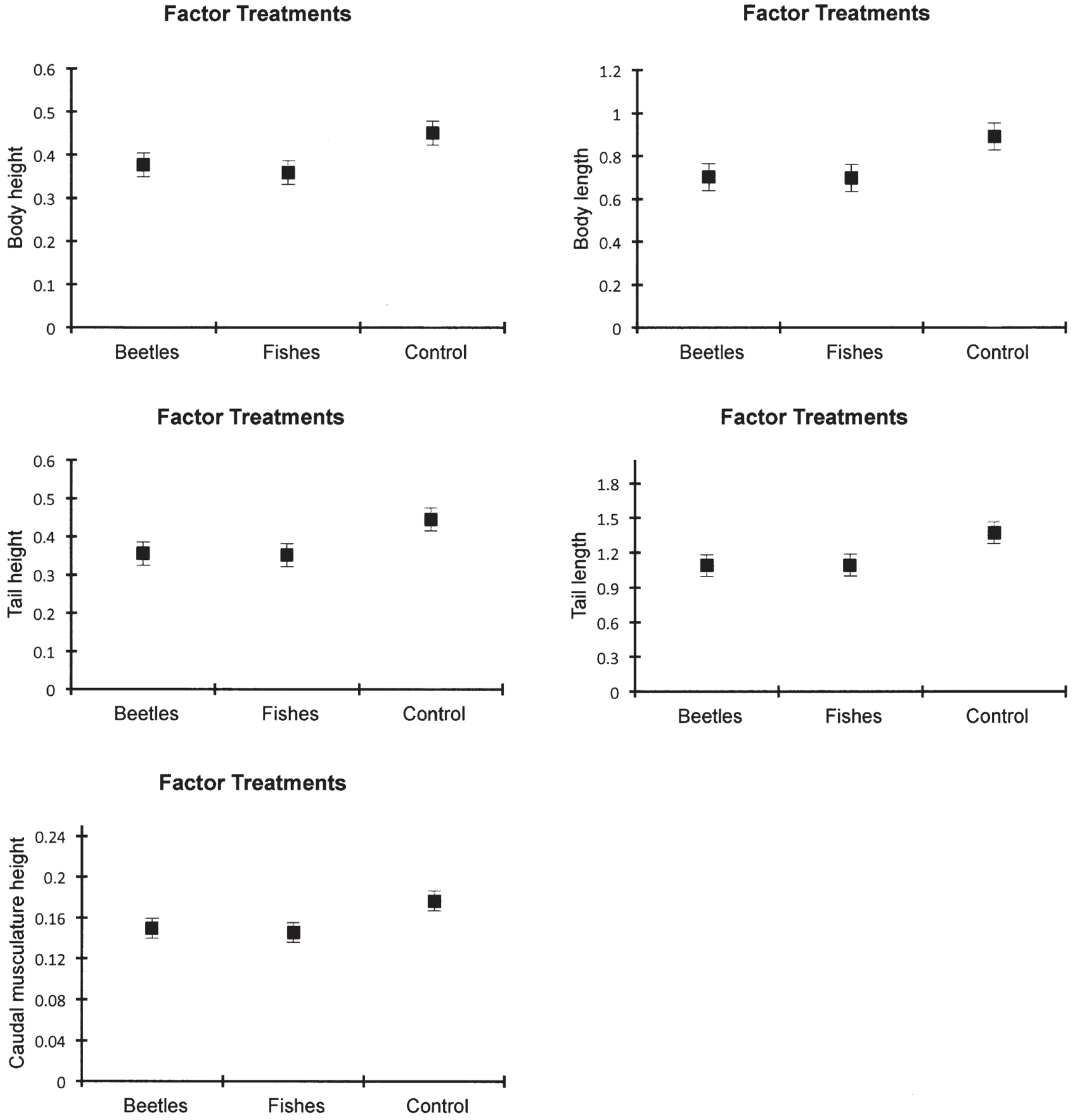

Figure 1. Effects of the presence of predator chemical cues on morphological variables in tadpoles of *Physalaemus santafecinus*. Each point is arithmetic mean; the bars represent standard errors.

1993, Chivers *et al*. 1996). For example, Stabell and Lwin (1997) demonstrated that crucian carp (*Carassius carassius*) displays an adaptive morphological change in response to predators that were fed carp, but they did not respond to predators that were fed a different diet. However, because we fed predators in different containers, there were no conspecific cues in the water that might have served as chemical cues for the tadpoles; therefore, the responses observed are exclusively the result of chemical cues produced by the predators.

This study suggests that tadpoles of *Physalaemus santafecinus* alter their morphology in response to chemical cues signaling predation. The larvae of *P. santafecinus* responded to these cues by not growing as large as control group larvae, as evidenced by the values associated with the morphometric variables recorded. This result is not consistent with the findings of previous studies reporting that tadpoles exposed to invertebrate predators developed higher tails and bodies, whereas those exposed to fish developed longer, more slender tails (Relyea 2001, Teplitsky *et al.* 2004, Kishida and Nishimura 2005, Benard 2006). In a previous study, we found that *P. albonotatus* responded to predators by increasing in size and developmental rate (Gómez and Kehr 2011). The tendency of larvae of *P. santafecinus* to reduce their size may relate to their behavioral traits, which include spatial avoidance and increased use of refuges. A reduction in the body size would be of benefit in this case.

We found no differences between treatments in growth or development rates. Instead, we observed that, despite the smaller sizes the tadpoles exposed to chemical cues of predators, both control and treatment larvae metamorphose at the same weight and in the same amount of time. Perhaps this is because we estimated the growth rate as a function of the total weight of tadpole. It is possible that the tadpoles had smaller measures of height and length, but compensated the weight by increasing a variable that was not considered in this work, such as body width. Or this may be the result of a behavioral shift, such as the predator-exposed group being less active; in an environment with food abundant resources, the larvae might maintain a high growth rate at low levels of activity (Peacor and Werner 2004). However, having applied the same experimental design to *P. albonotatus,* and finding differences in larval growth and development rates, we demonstrated that the larval growth and development rates are accelerated in presence of chemical cues of predators (Gómez and Kehr 2011).

Studies of the size at, and time taken to, metamorphosis have produced variable results. For example, Van Buskirk (1988) and Wilbur and Fauth (1990) showed that in *Bufo americanus,* there was a decrease in both of these variables in response to the presence of dragonfly larvae. In contrast, Skelly and Werner (1990) found that larvae of *B. americanus* metamorphosed at a smaller size in the presence of dragonfly predators, but they found no evidence of a reduction in the time to metamorphosis. In another study, Chivers *et al*. (1999) reported that larvae of *B. boreas* decrease the time taken to reach metamorphosis, but they found no evidence of a difference in size at metamorphosis in presence of predator or alarm cues.

This variability has been summarized by Benard (2004) and Relyea (2007), who observed that in contrast to the prediction of Werner (1986), most previous studies showed that tadpoles exposed to experiments with caged predator exhibit a high growth rate and metamorphose later or at the same time as larvae reared in a non-predator environment. Thus, our results are in line with most previous studies that suggest that larvae usually do not exhibit shifts in the timing of life-history events in response to predators.

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