

## Interaction between competitors and predators and its effects on morphological and behavioural defences in Scinax nasicus tadpoles

### Valeria I. Gómez \* and Arturo I. Kehr

Centro de Ecología Aplicada del Litoral (CECOAL-CONICET), Ruta 5, Km 2.5 Corrientes, C.P. 3400, Argentina \*Corresponding author's e-mail address: valeria\_vig@yahoo.com.ar

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#### Abstract

Predation affects the behaviour of organisms, and the number of conspecifics can influence the perception of predation risk. Two main types of traits, morphological and behavioural, are involved in the induced defences of many organisms. Here, we examined the influence of chemical cues of the predators Belostoma elongatum (Hemiptera, Belostomatidae) and Moenkhausia dichroura (Characiformes, Characidae) on the morphology, growth, development and activity of Scinax nasicus larvae. Our main goal was to determine whether the investment in morphological or behavioural defences varied according to the number of conspecifics and to the chemical cues of predators. To this end, we performed two experiments under microcosm conditions. In experiment 1, we determined morphological defences in tadpoles by analysing changes in morphology, growth rate and development rate, whereas in experiment 2, we determined behavioural defences by analysing changes in the activity of tadpoles. The experimental design consisted of two tadpole densities, with and without chemical cues of predators. We found that: (1) larval morphology, growth and development were significantly affected by density; (2) the chemical cues of the predators did not influence the morphology, growth or development of tadpoles; (3) tadpoles at high densities were more active than those at low densities; (4) tadpoles decreased the activity in the presence of chemical cues of M. dichroura; and (5) tadpoles increased the activity in the presence of chemical cues of B. elongatum. Our results suggest that density is a determinant factor of tadpole morphology of and that *Scinax nasicus* larvae respond to predators with behavioural defences, which vary according to the type of predator.

### Keywords

morphological traits, behavioural traits, conspecifics, predators, tadpoles, Scinax nasicus.

### 1. Introduction

Density-dependent interactions are the main determining factors of the patterns of mortality, rate of ontogeny and individual viability in populations of anuran larvae (Kehr, 1994; Kehr & Marangoni, 1999). Numerous studies have shown that the influence of density on populations is manifested in the effect of group, which involves an increase in the variability of the growth and development rates as well as the suppression of these processes and, subsequently, the viability of individuals (Surova et al., 2009).

Indirect effects on a community are often measured through specific traits of the behaviour of a species in the presence of a competitor, predator, or both, as well as through abiotic factors. The influence of predation on natural communities is complex, because predators reduce the density of the prey and potentially alter the levels of intra- and inter-specific competition (Barnett & Richardson, 2002). These factors (density, predation, competition for food) work together to determine the structure of the community, as well as the abundance and viability of individuals. Simultaneously, the number of conspecifics in an area (i.e., the size of the group) can influence the perception of the risk of predation and affect the behaviour of organisms (Bohlin & Johnsson, 2004; Downes & Hoefer, 2004). Such patterns have been demonstrated in a variety of animals, including fishes (Butler, 1988), lizards (Downes & Hoefer, 2004) and mammals (Fairbanks & Dobson, 2007). In these animals, induced defences involve two principal types of traits: morphological and behavioural.

In anuran larvae, morphological defences involve mainly an increase in the length of the tail. This acts as lure and distracts the attacks towards the tail instead of towards the body, which contains the vital organs (Van Buskirk et al., 2003). Different expressions of defences are sensitive to the environmental conditions and many species exhibit continuous responses to different densities of predators and exhibit specific responses to different types of predators (Van Buskirk & Arioli, 2002; Laurila et al., 2004; Relyea, 2004; Teplitsky et al., 2005).

In contrast, behavioural defences include a decrease in the rate of activity and an increase in the use of refuges, both of which decrease the probability of being detected by the predator (Skelly, 1994). Amphibian larvae seem to be able to adjust the level of activity or behaviour in response to the presence of conspecifics. These responses, which have been observed in several species (Griffiths & Foster, 1998; Relyea, 2002), usually vary according to

the size of the group and suggest that tadpoles use the size of the group as a signal to determine the risk of predation (Peacor, 2003). Some species even respond to the increase in the risk of predation by shaping large groups or more cohesive aggregations (Watt et al., 1997; Nicieza, 1999; Spieler, 2003). However, the increase in activity in large groups may also reflect a response to an increase in the number of conspecifics or potential competitors (Relyea, 2002).

We examined the influence of chemical cues of the predators *Belostoma elongatum* (Hemiptera, Belostomatidae) and *Moenkhausia dichroura* (Characiformes, Characidae) (through the detection of chemical cues of the predator) on the morphology, growth, development and activity of larvae of *Scinax nasicus*. Our main goal was to determine whether the investment in morphological or behavioural defences in *Scinax nasicus* tadpoles varies in relation to the number of conspecifics and the presence of predator chemical cues. We expected that individuals found at low densities respond by investing in structures of defence (growth) — due to the greater availability of resources — and that the individuals at high densities invest in defensive behaviour (reduction of the activity).

### 2. Material and methods

The frog Scinax nasicus breeds from August to May in ephemeral and semipermanent ponds where it co-occurs with water bugs (Belostoma elongatum) and band-tail tetra fish (Moenkhausia dichroura). The tadpoles used in this work were obtained from 50 laid egg clutches collected on 9 October 2011 from a temporary pond located 10 km from Corrientes, Argentina (27°29′33″S; 58°43′45″W) and then transported to the laboratory for hatching. In the laboratory, eggs were placed in shallow plastic wading pools  $(47 \times 33 \times 13 \text{ cm})$  filled with well water up to 10 cm deep. Four days after hatching, the tadpoles were randomly assigned to the treatments. Water bugs were collected at different sites in the vicinity of Corrientes City (from semi-permanent ponds, pools, ditches, etc.), whereas fish were collected using dip nets and aquatic funnel traps, from a permanent pond. The two predator species were kept in separate containers. Ten fish (3.5 cm in standard length) were placed in a plastic container (18.5 cm in diameter × 17 cm high) with 3.5 l of well water to obtain chemical cues. For the same purpose, four water bugs were placed in a similar container with 3.5 l of well water (different number of predators is in accordance with the density observed in natural conditions). Water containing chemical cues of predators was obtained after housing predators in tanks filled with well water for 5 days. The captive predators were fed three times a week; fish were fed fish food (Shulet, Buenos Aires, Argentina), and water bugs were fed with tadpoles. The predators were fed in different containers to avoid introducing chemical cues of their food in the containers and not to influence the tadpole responses. The containers that held the predators were partially cleaned twice a week. The tadpoles were fed boiled lettuce ad libitum three times a week. The tadpoles that died during the first three days of the experiment were removed so that the results of the experiment were not influenced by individual deaths caused by initial stress.

### 2.1. Experimental design

The experiments were performed under microcosm conditions. The temperature ranged between 26–29°C, and the photoperiod was 13 h of light and 11 h of dark. The tests were conducted in plastic containers (22.5 cm long  $\times$  12.5 cm wide  $\times$  6.0 cm high) filled with 1200 ml of well water. Two experiments, which were repeated for both types of predators, were designed.

# 2.1.1. Experiment 1: tadpole morphology, growth and development The experiment began on 17 October 2011 when tadpoles had reached developmental stages 26 (tadpoles with free swimming movement) (Gosner, 1960). The experimental design was a factorial design $(2 \times 2)$ , resulting in four treatments. The factors were: densities of tadpoles (4 and 8 larvae) and chemical cues of predators (with and without chemical cues). Each treatment was replicated 10 times, resulting in a total of 40 experimental units.

Using a calibrated plastic syringe, 12 ml of water was removed from each container with tadpoles, and 12 ml of water with the chemical cues taken from the containers with predators was added to maintain a constant water volume in each container. This procedure was performed three times a week. Similarly, in the case of the treatment without chemical cues of predators, the 12 ml of water extracted was replaced with 12 ml of well water from an isolated container. Twenty days after the start of the experiments, the tadpoles were measured, weighed and staged following Gosner (1960). In order to quantify morphological phenotypic responses, we photographed all tadpoles for each treatment. Tadpoles were photographed in lateral view using a stereomicroscope (Leica DFC 295) equipped with a computer system

to capture and measure the images (LAS V3.7). Five linear measurements describing morphological traits (body length, body depth, tail fin length, tail fin depth and tail fin muscle depth) were taken as reported previously (Gómez & Kehr, 2012).

### 2.1.2. Experiment 2: tadpole behaviour

The experimental design consisted of two larval densities (Group I, low density, 4 tadpoles; Group II, high density, 8 tadpoles) replicated 6 times. The experiments were carried out on 4 November 2011. Tadpole behaviour was analysed by estimating tadpole activity as the proportion of active larvae in the recipient. Tadpole was considered active, when the whole larva was in motion. The behaviour of the larvae was recorded using a video camera (JVG Everio-dual memory, zoom  $40\times$ ). The activity of tadpoles was observed in four different occasions (treatments): (1) before adding chemical cues of predators, and then at (2) 15 min, (3) 30 min and (4) 45 min after introducing the chemical cues. In each occasion (treatment), the behaviour was recorded 5 times (replicates; 5 s each) in each recipient. Once tadpoles were introduced in the containers, they were acclimated for 90 min before recording. After the first record, the chemical cues from predators were added. Then, at 15, 30 and 45 min after adding the chemical cues, the behaviour was recorded 5 times (5 s each) in each recipient again. A total of 240 videos were recorded in every experiment. The behaviour was recorded in larvae that were between stages 28 and 30 of development (Gosner, 1960).

### 2.2. Statistical analysis

# 2.2.1. Experiment 1: tadpole morphology, growth and development Statistical analyses were performed with the arithmetic mean from the larvae of each container. Each dependent variable was tested for normality (Shapiro–Wilk test) to determine if the data were normally distributed before further analyses were undertaken.

We performed a multivariate analysis of covariance (MANCOVA) using the four treatments as factors (low density with chemical cues of predators, low density without chemical cues of predators, high density without chemical cues of predators) and the cube root of the body mass as a covariable (Darlington & Smulders, 2001) to eliminate the size effect within morphological measurement. The five morphological variables were included in the analysis. In

addition, a multivariate analysis of variance (MANOVA) was used to determine the effects of the treatments on the growth and development rates of tadpoles. The growth rates of tadpoles for each treatment were calculated by dividing the natural logarithm of final weight by the natural logarithm of the total experiment duration in days = ln(final weight)/ln(days) (Kehr, 1987). The rate of larval development for each treatment was calculated by dividing the difference in size between the final and the initial stage by the number of days = (ln(final stage) – ln(initial stage))/ln(days) (Acosta, 2010). Subsequently, if Wilk's lambda indicated significance, we performed a one-way analysis of variance (ANOVA) for each dependent variable. For significant cases, a posteriori pairwise comparisons were made using Tukey's comparisons test. Probabilities for the univariate test (ANOVA) were compared with the level of adjusted significance according to that proposed by Bonferroni.

### 2.2.2. Experiment 2: tadpole behaviour

The statistical analysis to determine differences in the activity of the larvae was carried out taking the data recorded in the 60 videos (5 repetitions  $\times$  12 containers) obtained for each treatment.

The data expressed in proportions (proportion of active larvae in the recipient) were transformed to the arcsine. To compare larval activity between treatments, we performed an ANOVA of repeated measurement using density of tadpoles, time (successive records) and treatments (different moments of records) as factors, and the proportion of active larvae as dependent variable.

All statistical tests were carried out using SYSTAT 7.0 (SPSS, 1997) and XLSTAT 7.5 (Addinsoft, 2006).

### 3. Results

### 3.1. Experiment 1: tadpole morphology, growth and development

Tadpole morphology was significantly affected by the treatments in the experiments carried out with both predators: *Belostoma elongatum* (MANCOVA Wilk's lambda = 0.04;  $F_{5,27} = 130.91$ ; p = 0.001) and *Moenkhausia dichoroura* (MANCOVA Wilk's lambda = 0.08;  $F_{5,30} = 65.51$ ; p = 0.001).

Density significantly affected the morphology of tadpoles. In the presence of the chemical cues of *Moenkhausia dichroura* and *Belostoma elongatum*,

**Table 1.** Results of ANOVA test considering factors (density and predator chemical cues), ant its influence of the 5 morphological variables of *Scinax nasicus* tadpoles.

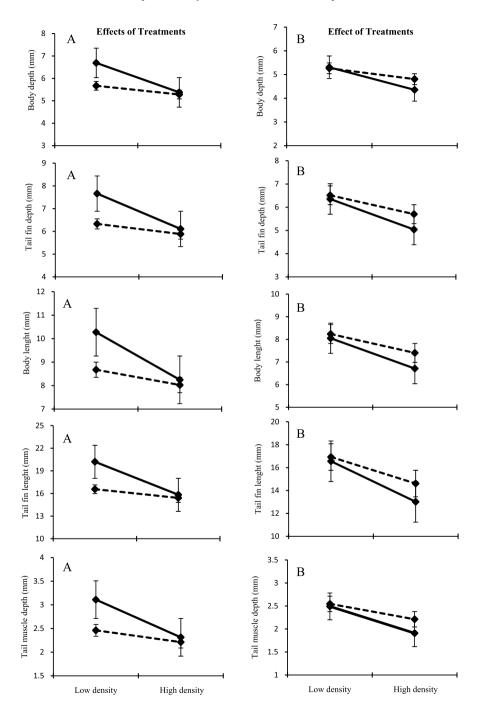
ANOVA	Belostoma elongatum			Moenkhausia dichroura		
	df	F	p	df	F	p
Effect of density						
Body length	1, 34	15.13	0.001	1, 38	9.21	0.004
Tail length	1, 34	18.18	0.001	1, 38	7.21	0.011
Body depth	1, 34	13.18	0.001	1, 38	8.31	0.006
Tail depth	1, 34	14.58	0.001	1, 38	7.14	0.011
Tail muscle depth	1, 34	15.33	0.001	1, 38	8.58	0.006
Effect of predators						
Low density						
Body length	1, 17	0.157	0.697	1, 18	5.72	0.028
Tail length	1, 17	0.096	0.761	1, 18	5.18	0.035
Body depth	1, 17	0.018	0.894	1, 18	4.91	0.040
Tail depth	1, 17	0.117	0.736	1, 18	5.26	0.034
Tail muscle depth	1, 17	0.072	0.792	1, 18	5.22	0.035
High density						
Body length	1, 15	7.38	0.016	1, 18	0.21	0.650
Tail length	1, 15	7.15	0.017	1, 18	0.15	0.703
Body depth	1, 15	5.70	0.031	1, 18	0.09	0.766
Tail depth	1, 15	6.03	0.027	1, 18	0.34	0.657
Tail muscle depth	1, 15	9.91	0.007	1, 18	0.34	0.563

ANOVA probabilities were according to Bonferroni criteria (p = 0.011).

the larvae at low densities increased the length of all the morphological variables (Table 1, Figure 1). Density also significantly influenced the growth and development rates of tadpoles; larvae at low densities showed a significant increase in both variables (Table 2, Figure 2).

The presence of chemical cues of the predators did not influence the morphology of tadpoles significantly. However, we observed different patterns in the responses of the larvae. The presence of the chemical cues of *B. elongatum* led tadpoles at high densities to show a tendency to increase the length of all the morphological variables studied (Figure 1B), but had no influence on the growth and development rates at both low and high densities (Table 2, Figure 2B).

In contrast, in the presence of chemical cues of *M. dichroura*, the larvae tended to reduce the length of all morphological variables when the tadpoles



**Table 2.** Results of the ANOVA tests considering factors (density and predator chemical cues) and its influences on growth rates and developmental rate of *Scinax nasicus* tadpoles.

ANOVA	Belostoma elongatum			Moenkhausia dichroura		
	df	F	p	df	F	p
Effect of density						
Growth rate	1, 34	13.72	0.001	1, 38	6.01	0.018
Development rate	1, 34	11.66	0.001	1, 38	9.01	0.004
Effect of predators						
Low density						
Growth rate	1, 17	0.02	0.875	1, 18	4.50	0.048
Development rate	1, 17	0.74	0.400	1, 18	0.72	0.407
High density						
Growth rate	1, 15	2.77	0.117	1, 18	0.01	0.896
Development rate	1, 15	2.51	0.133	1, 18	0.08	0.771

ANOVA probabilities were according to Bonferroni criteria (p = 0.025).

were at low densities (Figure 1A). In this case, neither the growth rate nor the development rate showed significant differences in tadpoles exposed to high or low densities (Figure 2A).

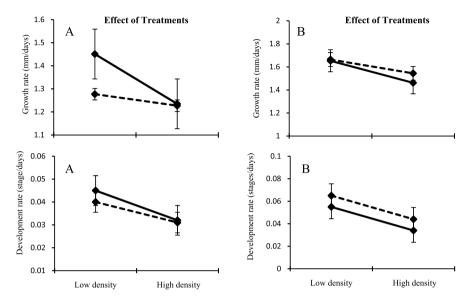
The interaction between density and predator treatment was not significant (p > 0.05) in both *B. elongatum* and *M. dichroura*.

*Scinax nasicus* larvae seemed to be more susceptible to changing the morphology in the presence of *M. dichroura* when they were at low densities. In contrast, at high densities, they seemed to be more susceptible to the presence of *B. elongatum*. Density was a determining factor in the variation of the growth and development rates of *Scinax nasicus* larvae, being both variables little influenced by the presence of the chemical cues of predators (*B. elongatum* and *M. dichroura*).

### 3.2. Experiment 2: tadpole behaviour

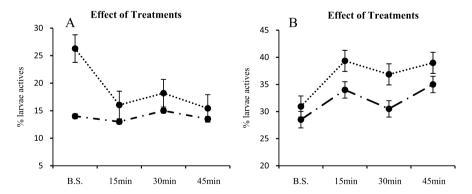
In the experiment performed with chemical cues of *Belostoma elongatum* the statistical analysis showed that the activity of the larvae was significantly

**Figure 1.** Effects of density and predator chemical cues on morphological variables in *Scinax nasicus* tadpoles. Each point represents the arithmetic mean, and the bars indicated the standard error (continuous line, without predators; dashed line, with predators). A, tadpoles exposed to *M. dichoroura*; B, tadpoles exposed to *B. elongatum*.



**Figure 2.** Effects of density and predator chemical cues on growth and developmental rates in *Scinax nasicus* tadpoles. Each point represents the arithmetic mean, and the bars indicated the standard error (continuous line, without predators; dashed line, with predators). A, tadpoles exposed to *M. dichoroura*; B, tadpoles exposed to *B. elongatum*.

influenced by the effect of density (ANOVA  $F_{1,238} = 3.96$ ; p = 0.047). The activity of the larvae of group II (high density) was significantly greater than that of the larvae of group I (low density) (Figure 3B).



**Figure 3.** Effects of density and predator chemical cues on activity in *Scinax nasicus* tadpoles. Each point represents the arithmetic mean, and the bars indicated the standard error (dotted line, high tadpole density; dotted and dashed line, low tadpole density). A, tadpoles exposed to *M. dichoroura*; B, tadpoles exposed to *B. elongatum*; BS, before stimulus.

No significant differences were found in the proportions of active larvae of group I (low density) among treatments (ANOVA  $F_{3,12} = 0.757$ ; p = 0.521) (Figure 3). In contrast, we found significant differences in the proportion of active larvae of group II (high density) before and after introducing chemical cues of B. elongatum (ANOVA  $F_{3,12} = 3.530$ ; p = 0.018). The a posteriori test showed that differences were between treatment 1 (before adding the chemical cues of predators) versus treatment 2 (15 min after the introduction of the chemical cues) (Tukey test: treatments 1–2: Difference = 8.40; Standardized difference = 2.78; Critical value = 2.61; p = 0.033) and treatment 4 (45 min after the introduction of chemical cues) (Tukey test: treatments 1–4: Difference = 8.04; Standardized difference = 2.84; Critical value = 2.61; p = 0.028). In treatment 1 (before adding the stimulus of predators), the proportion of active larvae was significantly lower than that in treatment 2 (15 min after adding the chemical stimulus) and 4 (45 min after the introduction of chemical cues) (Figure 3B).

Larval activity was significantly influenced by the effect of density (ANOVA  $F_{1.228} = 6.36$ ; p = 0.012) in the experiment performed with chemical cues of *Moenkhausia dichoroura*. The activity of the larvae of group II (high density) was significantly greater than that of group I (low density) (Figure 3A). No significant differences were found in the proportion of active larvae of group I (low density) in any of the four treatments performed (ANOVA  $F_{3.11} = 0.231$ ; p = 0.875) (Figure 3A). In contrast, we found significant differences in the proportion of active larvae of group II (high density) before and after introducing chemical cues of M. dichoroura (ANOVA  $F_{3.11} = 5.672$ ; p = 0.001). The a posteriori test showed that differences were between treatment 1 (before adding the chemical cues of predators) and treatment 2 (15 min after adding the chemical cues) (Tukey test: treatments 1–2: Difference = 10.25; Standardized difference = 5.69; Critical value = 2.61; p = 0.001); between treatment 1 and treatment 3 (30 min after adding the chemical cues) (Tukey test: treatments 1-3: Difference = 8.11; Standardized difference = 4.50; Critical value = 2.61; p = 0.001) and between treatment 1 and treatment 4 (45 min after adding the chemical cues) (Tukey Test: treatments  $1 \sim 4$ : Difference = 13.26; Standardized difference = 7.09; Critical value = 2.61; p = 0.001). The proportion of active larvae before adding chemical cues of predators was significantly higher than at 15, 30 and 45 min after adding the chemical cues (Figure 3A).

### 4. Discussion

4.1. Effect of density and chemical cues of predators on the morphology, growth and development rate of tadpoles

The density-dependent competition affects the survival, growth and development rates of organisms (Berven, 1990; Altwegg, 2003) and is critical for any discussion of regulation of populations (Chesson, 1996; Turchin, 1999; Kehr & Marangoni, 1999) and ecology of anuran larvae (Chesson, 1996; Turchin, 1999).

Previous studies have shown that responses to the competition are antagonistic and triggered by the presence of predators. Some researchers have shown intermediate responses to the presence of predators and competitors, such as a decrease in investment in anti-predatory defences when competition is high (Relyea, 2004; Relyea & Hoverman, 2003), whereas others have found the opposite (Parejko & Dodson, 1991) and even, in some cases, none interaction between these factors (Hoverman et al., 2005).

This study shows that the density seems to be a determining factor in *Scinax nasicus* larvae, since they increased the length of all the morphological variables as well as the growth and development rates when maintained at low densities. These responses might be adjusted to the model that proposes that when competition is low, there is an investment in growth, probably due to an abundant availability of resources.

When competition is high, there is a threshold of resources to meet in order to grow and develop; individuals assume more risks by reducing antipredatory behaviour, which increases the number of encounters with potential predators (Teplitsky & Laurila, 2007).

In this work, we observed that the larvae of *Scinax nasicus* exhibit different patterns of responses to different predators. The individuals exposed to chemical cues of *Belostoma elongatum* tended to change the morphology when the density was high, showing an increase in the length of all the morphological variables. However, there were no differences in the growth or development rates. In contrast, in the presence of chemical cues of *Moenkhausia dichroura*, the larvae showed a tendency to reduce the length of the morphological variables. We also observed a reduction in the growth rate when the density was low in tadpoles exposed to this predator.

Barnett & Richardson (2002) found similar results in laboratory experiments and demonstrated that the presence of non-lethal predators does not

significantly influence the mass of larvae of *Rana aurora* and *R. pretiosa* at early stages of development. However, in an experiment in the short term, Van Buskirk & Yurewicz (1998) found that both competition and predation risk determined the growth rate in *Rana sylvatica*. These authors observed that the risk of predation dominated at early stages of development of the larvae, and that competition dominated at later stages.

If we consider the number of surviving individuals, these effects are typically opposite. Mortality decreases the pressure on resources and generates a competitive release, while predation reduces the rate of foraging and may cause changes in the habitat, causing morphological changes, all of which have significant costs. Fauth (1990) studied predation by crayfish in *Hyla chrysoscelis* and found that the negative effects of the cost of avoiding predators were compensated by the positive effects of reducing the size. In another study, Skelly (1992) suggested that predators can have a negative effect in the number of surviving individuals when the competition cannot compensate for the costs of avoiding predators, such as when predators are relatively inefficient or when performance (growth) is not density-dependent.

### 4.2. Effect of density and chemical cues of predators in the activity of the tadpoles

We next investigated the effect of density on the behaviour of the larvae from two perspectives: firstly by analysing changes in activity, considering the size of the group (low density versus high density) without incidence of the treatments, and secondly by recording changes induced by the treatments (predator chemical cues) in each group of individuals.

The results obtained demonstrate that density seems to be an important factor in *Scinax nasicus* larvae, since both experiments with *Moenkhausia dichroura* and *Belostoma elongatum* showed an increase in the proportion of active larvae when the density was higher. This increase can be easily explained considering inter-specific competition for the available space and consumable resources (Kehr, 1987).

We also considered the effect of predators, according to the density, on larval activity. We observed that individuals exposed to chemical cues of predator changed the level of activity when the density was higher, and did not observe changes in the behaviour of these species at low densities. These results contrast with most previous works, which argued that there is an investment in the defence behaviour when the competition is low (Perotti et al., 2006; Stav et al., 2007; Teplitsky & Laurila, 2007; Jara & Perotti, 2010).

In this study, we observed no defence behaviour in larvae at low densities, since there were no differences in the proportion of active larvae before and after introducing the chemical cues from predators. However, we noted that the larvae that were found in larger groups exhibited a more risky behaviour. This may be because the presence of a greater number of conspecifics will dilute or reduce the risk of predation, or because the increase in the number of potential competitors may also influence the activity.

Relyea (2004) found that the activity of *Rana sylvatica* in the presence of predators (odonates) increased according to the increase in the density of conspecifics. On the other hand, it is expected that larvae reduce the activity in the presence of predators. Here, we observed that *Scinax nasicus* larvae exposed to *Belostoma elongatum* increased their activity level 15 min after adding the chemical cues and that those exposed to chemical cues of *Moenkhausia dichroura* decreased the level of activity.

These differences in responses could be due to the fact that the larvae reduced activity only in the presence of an effective predator, which has been observed in other studies with larvae of *Rhinella spinulosa*, *Pleurodema thaul*, *P. bufoninum*, *Bufo viridis* and *B. arenarum* (Perotti et al., 2006; Stav et al., 2007; Jara & Perotti, 2010). In this case, the increase in activity in tadpoles exposed to *Belostoma elongatum* could be due to the fact that the larvae were trying to move away from the danger zone, although it is known that an increase in the activity increases the probability of encounters with the sit and wait predators. In these case *Belostoma elongatum* are predators that need that the prey reaches some dimension to be able to be captured, and in this study maybe the larvae did not reach a size large enough to increase their vulnerability to this specific predator. In previous studies we observed that tadpoles of *Physalaemus albonotatus*, that had a lower size that *Scinax nasicus*, respond to chemical cues of *B. elongatum* in the same way (Gómez, 2012).

Probably the tadpoles change their behavioural response in accordance to their developmental stage and the predator at with are exposed. In some cases, at lower developmental stages is more effective move away from the danger zone, and when the tadpoles increased the developmental stages maybe choose reduce activity.

On the other hand, since the individuals of *Moenkhausia dichroura* are very effective predators, the *Scinax nasicus* larvae are very susceptible to capture in these developmental stages. Therefore, it is understandable that in

this case the larvae decreased the level of activity 15 min after adding the chemical cues, maintaining this behaviour up to 45 min.

The variation in the behaviour observed in the larvae might indicate that the level of activity and the degree of effectiveness of predators through different developmental stages may be related.

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### References

- Acosta, N.R. (2010). Plasticidad fenotípica en la metamorfosis de larvas de Rhinella arenarum del Valle del Lerma, Salta. — Tesis doctoral, Universidad Nacional de La Plata, La Plata.
- Addinsoft (2006). XLSTAT Ver. 7.5 for Excel interface. Addinsoft, Pentraeth.
- Altwegg, R. (2003). Multistage density dependence in an amphibian. Oecologia 136: 46-50.
- Barnett, H.K. & Richardson, J.S. (2002). Predation risk and competition effects on the life-history characteristics of larval Oregon spotted frog and larval red-legged frog. — Oecologia 132: 436-444.
- Berven, K.A. (1990). Factors affecting population fluctuations in the larval and adult stages of the wood frog (*Rana sylvatica*). Ecology 71: 1599-1608.
- Bohlin, T. & Johnsson, J.L. (2004). A model of foraging activity and group size: can the relative importance of predation risk dilution and competition be evaluated experimentally? Anim. Behav. 68: F1-F5.
- Butler, M.J. (1988). In situ observations of bluegill (*Lepomis macrochirus* Raf.) foraging behavior: the effects of habitat complexity, group size, and predators. — Copeia: 939-944.
- Chesson, P. (1996). Matters of scale in the dynamics of populations and communities. In: Frontiers of population ecology (Floyd, R.B., Sheppard, A.W. & De Barro, P.J., eds). CSIRO, Melbourne, VIC, p. 353-368.
- Darlington, R.B. & Smulders, T.V. (2001). Problems with residuals analysis. Anim. Behav. 62: 599-602.
- Downes, S. & Hoefer, A.M. (2004). Antipredatory behavior in lizards: interactions between group size and predation risk. Anim. Behav. 67: 485-492.
- Fairbanks, B. & Dobson, F.S. (2007). Mechanisms of the group-size effect on vigilance in Columbian ground squirrels: dilution versus detection. Anim. Behav. 73: 115-123.

- Fauth, J.E. (1990). Interactive effects of predators and early larval dynamics of the treefrog *Hyla chrysoscelis*. Ecology 71: 1609-1616.
- Gómez, V.I. (2012). Respuestas fenotípicas en larvas de anuros a situaciones cambiantes en los factores bióticos y abióticos. — Tesis doctoral, Universidad Nacional de La Plata, La Plata.
- Gómez, V.I. & Kehr, A.I. (2012). The effect of chemical signal of predatory fish and water bugs on the morphology and development of *Elachistocleis bicolor* tadpoles (Anura: Microhylidae). — Biologia 67: 1001-1006.
- Gosner, K.L. (1960). A simplified table for staging anurans embryos and larvae with notes of identification. Herpetologica 16: 183-190.
- Griffiths, R.A. & Foster, J.P. (1998). The effect of social interactions on tadpole activity and growth in the British anuran amphibians (*Bufo bufo*, *B. calamita*, and *Rana temporaria*). J. Zool. 245: 431-437.
- Hoverman, J.T., Auld, J.R. & Relyea, R.A. (2005). Putting prey back together again: integrating predator induced behavior, morphology, and life history. Oecologia 144: 481-491.
- Jara, F. & Perotti, M.G. (2010). Risk of predation and behavioral response in three anuran species: influence of tadpole size and predator type. Hydrobiologia 644: 313-324.
- Kehr, A.I. (1987). Crecimiento individual en larvas de Hyla pulchella pulchella y Bufo fernandezae en condiciones controladas de coexistencia y densidad (Amphibia Anura). — Cuad. Herpetol. 3: 1-6 (in Spanish).
- Kehr, A.I. (1994). Density-dependent responses in tadpoles of *Bufo arenarum* (Anura: Bufonidae). Physis 49: 31-36.
- Kehr, A.I. & Marangoni, F. (1999). Variación intrapoblacional en el tamaño de la metamorfosis en *Bufo paracnemis*, bajo condiciones naturales. Neotrópica 45: 63-68.
- Laurila, A., Jarvi-Laturi, M., Pakkasmaa, S. & Merila, J. (2004). Temporal variation in predation risk: stage dependency, graded responses and fitness costs in tadpole antipredator defences. — Oikos 107: 90-99.
- Nicieza, A.G. (1999). Context-dependent aggregation in common frog *Rana temporaria* tadpoles: influence of developmental stage, predation risk and social environment. Funct. Ecol. 13: 852-858.
- Parejko, K. & Dodson, S.I. (1991). The evolutionary ecology of an antipredator reaction norm: *Daphnia pulex* and *Chaoborus americanus*. — Evolution 45: 1665-1674.
- Peacor, S.D. (2003). Phenotypic modifications to conspecific density arising from predation risk assessment. — Oikos 100: 409-415.
- Perotti, M.G., Fitzgerald, L.A., Moreno, L. & Pueta, M. (2006). Behavioral responses of *Bufo arenarum* tadpoles to odonate naiad predation. Herpetol. Conserv. Biol. 1: 117-120.
- Relyea, R.A. (2002). Competitor-induced plasticity in tadpoles: consequences, cues, and connections to predator-induced plasticity. Ecol. Monogr. 72: 523-540.
- Relyea, R.A. (2004). Fine-tuned phenotypes: tadpole plasticity under 16 combinations of predators and competitors. Ecology 85: 172-179.
- Relyea, R.A. & Hoverman, J.T. (2003). The impact of larval predators and competitors on the morphology and fitness of juvenile treefrogs. Oecologia 134: 596-604.

- Skelly, D.K. (1992). Field evidence for a cost of behavioral antipredator responses in a larval amphibian. Ecology 73: 704-708.
- Skelly, D.K. (1994). Activity level and the susceptibility of anuran larvae to predation. Anim. Behav. 48: 465-468.
- Spieler, M. (2003). Risk of predation affects aggregation size: a study with tadpoles of *Phrynomantis microps* (Anura: Microhylidae). Anim. Behav. 65: 179-184.
- SPSS (1997). SYSTAT 7.5 for Windows. SPSS, Chicago, IL.
- Stav, G., Kotler, B.P. & Blaustein, L. (2007). Direct and indirect effects of dragonfly (Anax imperator) nymphs on green toad (Bufo viridis) tadpoles. Hydrobiologia 579: 85-93.
- Surova, G.S., Mukhina, T.V. & Bezryadnov, D.V. (2009). Group effect of individual locomotor activity of common toad (*Bufo bufo* L.) and brown frog (*Rana temporaria* L.) tadpoles. Russ. J. Ecol. 40: 280-285.
- Teplitsky, C. & Laurila, A. (2007). Flexible defense strategies: competition modifies investment in behavioral vs. morphological defenses. Ecology 88: 1641-1646.
- Teplitsky, C., Plenet, S. & Joly, P. (2005). Costs and limits of dosage response to predation risk: to what extent can tadpoles invest in antipredator morphology? Oecologia 145: 364-370.
- Turchin, P. (1999). Population regulation: a synthetic view. Oikos 84: 153-159.
- Van Buskirk, J. & Arioli, M. (2002). Dosage response of an induced defense: how sensitive are tadpoles to predation risk? Ecology 83: 1580-1585.
- Van Buskirk, J. & Yurewicz, K.L. (1998). Effects of predators on prey growth rate: relative contributions of thinning and reduced activity. — Oikos 82: 20-28.
- Van Buskirk, J., Anderwald, P., Lupold, S., Reinhardt, L. & Schuler, H. (2003). The lure effect, tadpole tail shape, and the target of dragonfly strikes. J. Herpetol. 37: 420-424.
- Watt, P.J., Nottingham, S.F. & Young, S. (1997). Toad tadpole aggregation behavior evidence for a predator avoidance function. Anim. Behav. 54: 865-872.