

**Intestinal, Body and Tail Plasticity in *Rhinella schneideri* (Bufonidae) Tadpoles Induced by a Predator Insect (*Belostoma elegans*)**

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**Abstract**

According to the adaptive plasticity hypothesis, predator-induced traits in potential prey animals are expected to have costs for those organisms in predator-free environments. In this study, we analysed if the intestinal length, tail length and body length changes in *Rhinella schneideri* tadpoles when the tadpoles were exposed to caged predators (*Belostoma elegans*). We used a randomized block design with a factorial combination of one density of tadpoles and four densities of caged predators. The predators had a significant impact on gut length and tail length but not on body length. We found that tadpoles reared with no predators had relatively longer guts than those reared with caged predators. The reduction in gut length appears to interplay between behaviour, life history, morphology and physiology.

**Keywords:** Inducible defense, phenotypic plasticity, Trade-off, predator-prey interactions.

**1 Introduction**

Tadpoles respond to predator released kairomones (chemical cues released in

the water indicating predation risk) with changes in their behavioral, morphological and life-history responses [32, 33, 2]. These induced responses include reduced feeding and swimming activity, and reduced growth [32, 21, 4, 27, 12, 22, 29].

According to the adaptive plasticity hypothesis [3], predator-induced traits that may be favorable to organisms in helping them escape predation may, in turn, carry costs in predator-free environments. Amphibians are well known for their flexible life history [1, 31], behaviour [8] and morphology [18, 28] in response to different predator and resource environments. Predators commonly induce phenotypic changes that make amphibian larvae better at surviving predation, but at the cost of reduced growth. All of the larval anurans test to date exhibit some form of predator-induced plasticity, but the magnitude and direction depends on the particular predator–prey combination. Often, different prey species respond to the same predator in different ways [16].

Recently, Relyea and Auld [19] combined phenotypic plasticity theory with predictions from optimal digestion theory to demonstrate that intra- and interspecific competition induced relatively long guts in tadpoles while predators induced relatively short guts. In their experiment they used late instar aeshnid dragonfly naiad, *Anax junius* as a predator. The tadpoles that consumed low food quantity or quality should have longer digestive systems [23] such as optimal digestion theory predicts.

Adult water bugs (*Belostoma* sp.) are medium-sized predators (up to 30 mm) that pose a low predation risk to tadpoles due to a low capture efficiency, a long handling time, and a likely smaller gut capacity [16]. Kehr and Schnack [7] showed that the predation rate of adult *Belostoma oxyurum* was higher on smaller tadpoles of *Bufo arenarum* (= *Rhinella arenarum*) (stages 26-29, [5]) than large tadpoles (stages 31-35 and 38-40).

In this paper we combine the predictions from optimal digestion theory with theory on phenotypic plasticity. Relyea [17] hypothesized about the inferior growth abilities of the smaller-bodied. Because a tadpole's body is largely composed of the gut, changes in relative body size might translate into changes in relative gut length and, in consequence translate into differences in growth rate and nutrient assimilation. To analysis this idea, here we test the following hypothesis: *Rhinella schneideri* tadpoles exposed to the predator *Belostoma elegans*, will develop relatively short guts and body length and longer tail length.

## 2 Materials and methods

In this experiment, we tested how *R. schneideri* tadpoles responded to caged water bug predators. The experiment was conducted at the Centro de Ecología Aplicada del Litoral (CECOAL-CONICET, Corrientes, Argentina) in 2005 with the original goal of understanding how tadpoles alter their gut length in response to different predator environments. The *R. schneideri* eggs were collected from

two different egg masses (> 5000 eggs each one) from a large ephemeral pond near to Corrientes City, Argentina (27° 30 ' S, 58° 45 ' W) on 20 October 2005. Eggs were hatched in wading pools containing well water and the tadpoles were fed boiled lettuce *ad libitum* until used in the experiments. The two clutches were mixed and added to the tanks when they were five days old (Stages 25-26, [5]).

We used a randomized block design with a factorial combination of one densities of tadpoles (300) and four densities of caged predators (0, 1, 2 and 4) that were well within relevant natural densities (up to 500 tadpoles m<sup>-2</sup>). The four treatment combinations were each replicated three times (three randomized block) for a total of 12 experimental units. The experimental units were 12 experimental units 500-L tank mesocosms containing 300-L of well water (filled on 24–26 August), 2 g of rabbit chow, and an aliquot of pond water containing phytoplankton and zooplankton from 5 nearby ponds to simulate natural pond. All tanks were covered with 60% shade cloth to prevent colonization by other organisms and all they were equipped with four predator cages constructed of 300 ml. screened plastic bottled. The cages allowed the chemical cues from predators to diffuse through the water while preventing the predators from killing the target animals. Depending on treatment, each predator cage could house a single belostomatids adult (*Belostoma elegans*), but depending on the treatment from zero to four containers had predators in them. Owing to belostomatid's aerial respiratory need, 5 cm at the top of each bottle was above the water line, allowing the *Belostoma* access to air. A slender stick was placed inside each bottle as a substrate for belostomatids. Each belostomatids was fed c. 100 mg of *R. schneideri* tadpoles three times per week. The belostomatids were collected from three large ephemeral ponds near to Corrientes City, one of them where the toads egg masses were obtained.

The experiment was initiated on 28 October 2005. After 26 days (24 November 2005), 20 tadpoles from each tank were euthanized and preserved in 10% formalin. In March 2006, the tadpoles were weighed, extracted and measured the length of the guts with digital calipers (to the nearest 0.01 mm).

### Statistical Analysis

In order to know whether gut length, body length and tail length changed with predation risk, we first had to remove the effect of overall size (i.e. mass). This was done by regressing Ln -transformed gut length, body length and tail length of all individuals against their Ln -transformed mass (Ln -transforming improves the linearity of the relationship). We calculated a mean regression line based on 4 separate regressions by each variable, one for each treatment, because the regression line through all data points is not parallel to the lines for each treatment, which is an essential presumption in size-independent analyses. From the mean regression line for each variable, we obtained the residual values for each tadpole from this line and saved the mean residuals for each tank and variable used [19]. This size-adjustment technique has proven effective in previous studies of morphological changes in tadpoles as they grow [15, 20].

We analyzed the data using MANOVA test. The test were run via computer package RT (V 2.1) [9], based on randomization and Monte Carlo tests. The randomizations number used was 5000.

We used an ANOVA test when the Wilks lambda was significant. Multiple pairwise comparisons using the Dunn's procedure (Two-tailed test) was used when the ANOVA test was significant.

### 3 Results

The tadpoles altered their morphology in the presence of caged predators (Wilk's lambda= 0.0132;  $P= 0.0034$ ). The block effect was not included in the analysis because was not significant (Wilk's lambda= 0.5864,  $P= 0.64$ ).

The predators effect (0, 1, 2, and 4 belostomatids density) on gut length was significant (ANOVA  $F_{3, 8} = 7.42$ ,  $P = 0.009$ ). When we conducted mean comparison across predator treatments, we found that tadpoles reared with no predators had relatively longer guts than tadpoles reared with 1, 2 or 4 caged predators ( $P < 0.05$ ). Mean gut length difference in tadpoles reared with 0 and 1 belostomatids was not significant but these two predator treatments were significantly different with mean gut length in tadpoles reared with 2 or 4 predators. Furthermore, there were no differences in relative gut length among the tadpoles reared with 2 or 4 predators ( $P > 0.05$ ) (Fig. 1).

The predators density also has effect on the tail length (ANOVA  $F_{3, 8} = 17.45$ ,  $P= 0.0066$ ). The difference in the mean tail length of tadpoles reared with 0 and 4 belostomatids was not significant and were greater than tadpoles reared with 1 and 2 predators (Fig. 1).

The body length changes were not induced by the predators treatment (ANOVA  $F_{3,8} = 2.48$ ,  $P= 0.14$ ) (Fig. 1).

### 4 Discussions

The tadpoles of this species live in very fluctuating environments, mainly temporary ponds, where it would be particularly important to reach metamorphosis before the pond dry up. *Rhinella schneideri* is a typical *r*-strategist, where the characteristics related to the productivity and the selection for a rapid development are more important, in contrast to *K*-strategists, here the main features are the efficiency and slower development. Doubtlessly the effect of the abiotic factors, i.e., pond hydroperiod, is of extreme importance in the population dynamics of this species. The adaptative responses of this species would be first toward the abiotic factors and secondary towards the biotic factors (e.g., intra and interspecific competition and predator-prey interaction). The results obtained in our study are analyzed in this context.

Gut plasticity has been frequently observed in other taxa but the emphasis has been on effect of food quality and quantity. In a diversity of taxa (birds, mammals, reptiles and insects) individuals develop relatively longer guts in response to decreased food quality or quantity [34, 24, 14]. In amphibians, gut plasticity has been less extensively studied. Tadpoles of *Rhacophorus arboreus* develop longer intestines when fed lower quality plant diets in relation to high quality animal diets [6]. Similarly, in *Scaphiopus multiplicatus* tadpoles that switch from an omnivorous phenotype to a carnivorous phenotype develop relatively shorter guts [13].

Our experiment offers proof that *Rhinella schneideri* tadpoles are able to adjust the relative gut length in response to the “sit and wait” *Belostoma elegans* predator. In our experiment, the results reflect that the decreasing gut length induced by predators was independent of body length variation because body length changes were not induced by the presence of the predators. Nevertheless, a longer tail length was induced by exposure to queues of a predator’s presence. Our results do not match those of Relyea [16, 18] because our data show shorter gut length induced by predators while the body length was independent of presence of predator and similar into all treatment. However, we did not use some of the combinations of predator and prey that he used.

Tadpoles can react to predator releasing kairomones with morphological, behavioral, and life-historical responses [32, 33, 2]. These induced responses involve reduced feeding and swimming activity, and reduced growth [32, 21, 4, 27, 12, 22, 29]. Principally in systems with sit-and-wait-predators reduced activity by the prey reduces their encounter rate with the predator [32]. Consequently, reduced activity is an effective defense mechanism that reduces mortality due to predation [32]. Reduced activity is commonly associated with a reduced growth rate. Presumably this is because greater activity is related to a high feeding rate, more food ingested, and therefore a high growth rate.

Our data show that predator-exposed tadpoles have shorter guts. Based on optimal digestion theory, the reduction of relative gut length should cause less efficient digestion and therefore slower growth [23, 19]. Nevertheless, Steiner [25] show in *Rana lessonae* tadpoles that despite a reduction in time spent feeding, predator-exposed individuals ingested the same amount of food than tadpoles not exposed and assimilated the food at a higher rate, but did not grow faster. This might indicate that exposed tadpoles convert assimilated food at a lower rate into body mass, that is, they show lower growth efficiency, similar to damselfly larvae [26]. Steiner [25] also observed that tadpoles exposed to predators had a tendency to wider guts increasing the gut volume than tadpoles in the non predator environment.

Induced tadpoles by predators ingest more food and therefore should be heavier. If the presence of predators increased the ingestion rate and possibly the metabolic rate in tadpoles, if the gut length did not change, the tadpoles should be heavier and have more difficulties swimming and escaping predators. Possibly the gut morphology plasticity can be likely explained by a trade-off between gut width and gut length. In relation to ingestion and assimilation of gut content,

however, predator-induced tadpoles ingested 20 % more food than no induce tadpoles based on relative amounts [25].

Predators induce tadpoles to develop relatively large tails improving escape from predators [10]. Studies about predator selection on tadpole morphology have demonstrated that larval anurans with relatively shallow tails and large bodies are preferentially killed by aquatic predators [11, 30]. Our tadpoles responded as expected in relation to tail length induced by predator with a morphological defence that reduces predation [11].

While many studies have been documenting changes in external morphological traits, it is now clear that by examining internal morphological traits (i.e., gut length) we can arrive at a better understanding about the predator response of tadpoles. Furthermore, these tadpoles probably will responses of different ways depending of the life cycles of species. In the opportunistic species developing in highly variable and/or unpredictable environments the abiotic factors will be more important in the tadpole's populations dynamics and the predator-induced responses on tadpoles in external and internal plasticity will change in relation to tadpoles of others species living in more predictable environments. Many plastic responses of tadpoles will depend of time exposition to induction force (e.g., time of exposition to predator). Then, this interplay between behaviour, life history, morphology and physiology adds and counteracts induced costs in different environments.

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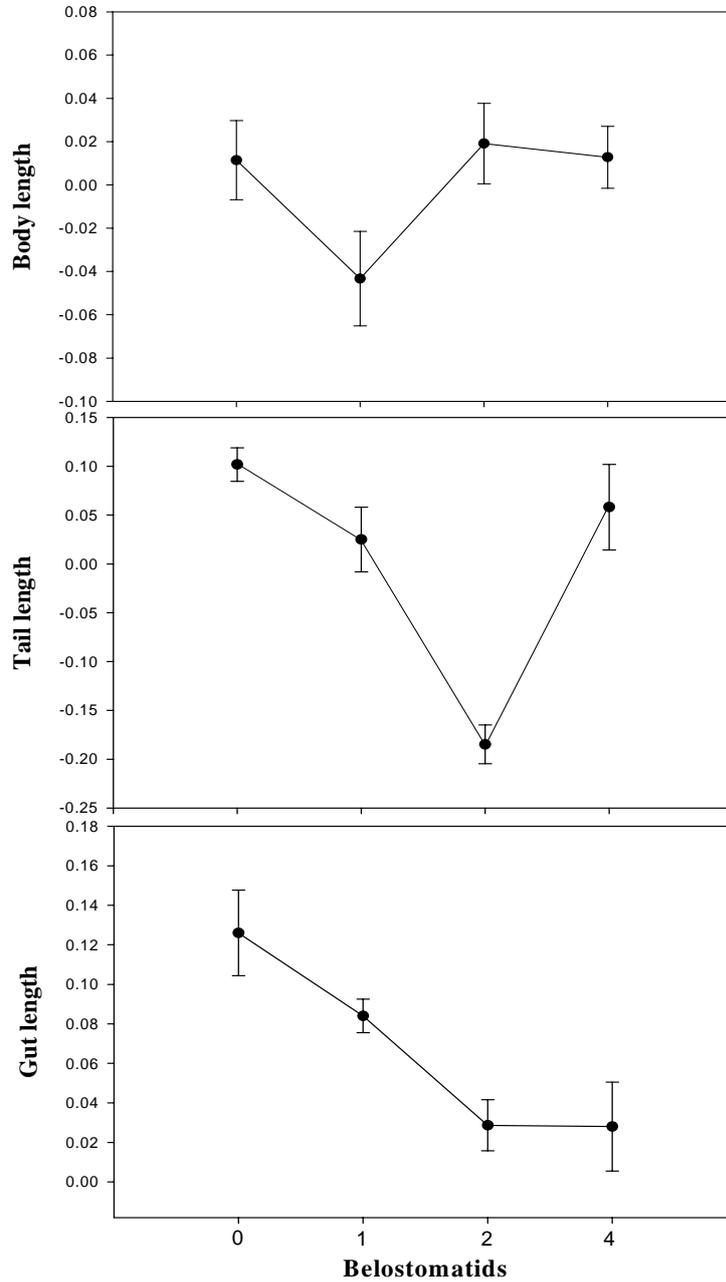


Fig. 1. The change in relative gut length, body length and tail length of *Rhinella schneideri* tadpoles when reared under 4 combinations of caged predators (*Belostoma elegans*). The three variables were made size independent by regressing Ln gut lengths, Ln body lengths and Ln tail lengths against Ln tadpole mass and saving the residuals. Results are mean  $\pm$  SE.